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MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF *ABRAXAS* AND *PALB2* GENES IN HEREDITARY BREAST CANCER PREDISPOSITION

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Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium F202 of the Faculty of Medicine (Aapistie 5 B), on 6 April 2018, at 12 noon

Abstract

Hereditary mutations in DNA damage response (DDR) genes often lead to genomic instability and ultimately tumor development. However, the molecular mechanism of how these DDR deficiencies promote genomic instability and malignancy is not well understood. Thus, the specific aim of this thesis is to identify the functional and molecular framework behind the elevated breast cancer risk observed in heterozygous *PALB2* and *ABRAXAS* mutation carriers.

The heterozygous germline alteration in *PALB2* (c.1592delT) causes a haploinsufficiency phenotype in the mutation carrier cells. Due to PALB2 haploinsufficiency, elevated Cdk activity and consequently aberrant DNA replication/damage response was observed in the *PALB2* mutation carrier cells. Excessive origin firing that is indicative of replication stress was also seen in the *PALB2* mutation carrier cells. In addition to replication stress, *PALB2* mutation carrier cells also experience G2/M checkpoint maintenance defects. The increased malignancy risk in females associated with heterozygosity for the Finnish *PALB2* founder mutation is likely to be due to aberrant DNA replication, elevated genomic instability and multiple different cell cycle checkpoint defects.

The heterozygous germline alteration in *ABRAXAS* (c.1082G>A) causes a dominant-negative phenotype in the mutation carrier cells. Decreased BRCA1 protein levels as well as reduced nuclear localization and foci formation of BRCA1 and CtIP was observed in the *ABRAXAS* mutation carrier cells. This causes disturbances in basal BRCA1-A complex localization, which is reflected by a restraint in error-prone DNA double-strand break (DSB) repair pathway usage, attenuated DNA damage response, deregulated G2/M checkpoint control and apoptosis. Most importantly, mutation carrier cells display a change in their transcriptional profile, which we attribute to the reduced nuclear levels of BRCA1. Thus, the Finnish *ABRAXAS* founder mutation acts in a dominant-negative manner on BRCA1 to promote genome destabilization in the heterozygous carrier cells.

Bose, Muthiah, *ABRAXAS*- ja *PALB2*-geenien molekulaarinen ja funktionaalinen karakterisointi perinnöllisessä rintasyöpäalttiudessa.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Biocenter Oulu; Oulun yliopistollinen sairaala

Tiivistelmä

Perinnölliset muutokset DNA-vauriovasteen geeneissä johtavat usein genomin epävakauteen ja lopulta syövän kehittymiseen. Molekyylitason mekanismeja, joilla vauriovasteen vajaatoiminta ajaa genomin epävakautta ja syöpää, ei kuitenkaan ymmärretä kunnolla. Tämän väitöskirjan tavoitteena on tunnistaa solutoiminnan ja molekyylitason vaikuttajat heterotsygoottisten *PALB2*- ja *ABRAXAS*-geenimuutosten kantajien kohonneen rintasyöpäriskin taustalla.

Heterotsygoottinen ituradan suomalainen perustajamuutos *PALB2*-geenissä (c.1592delT) aiheuttaa haploinsuffisienssin kantajahenkilöiden soluissa. PALB2:n haploinsuffisienssin seurauksena kantajasoluissa havaittiin kohonnutta Cdk-proteiinin aktiivisuutta ja siitä johtuvaa kiihtynyttä DNA:n kahdentumista. *PALB2*-mutaatiota kantavissa soluissa nähtiin myös liiallista replikaation aloituskohtien käyttöä, mikä viittaa replikaatiostressiin. Replikaatiostressin lisäksi *PALB2*-mutaation kantajasoluilla havaittiin vaikeuksia ylläpitää solusyklin G2/M-tarkastuspisteen toimintaa. Näiden solutoiminnan poikkeavuuksien takia heterotsygoottisen *PALB2* c.1592delT -mutaation kantajilla todettiin genomin epävakautta ja kohonnut syöpäriski.

Heterotsygoottinen ituradan mutaatio *ABRAXAS*-geenissä (c.1082G>A) aiheuttaa dominantti-negatiivisen fenotyypin mutaation kantajasoluissa. *ABRAXAS*-mutaatiota kantavissa soluissa havaittiin BRCA1-proteiinitasojen laskua sekä BRCA1- ja CtIP-proteiinien vähentynyttä lokalisaatiota tumaan ja DNA-vauriopaikoille. Tämä aiheuttaa häiriöitä BRCA1-A-kompleksin paikallistumisessa, mikä johtaa häiriöihin virhealttiiden DNA-kaksoisjuoste¬katkoksien korjausmekanismien käytössä, DNA-vauriovasteessa, G2/M-tarkastus-pisteen säätelyssä ja ohjelmoidussa solukuolemassa. Tärkeimpänä löydöksenä havaittiin mutaation kantajasoluissa muuttunut transkriptioprofiili, joka johtunee BRCA1-proteiinitasojen laskusta tumassa. Näin ollen suomalainen *ABRAXAS*-perustajamutaatio toimii dominantti-negatiivisena BRCA1:n suhteen, aiheuttaen genomin epävakautta heterotsygoottisissa kantajasoluissa.

Asiasanat: ABRAXAS, BRCA1, CtIP, DNA-korjaus, DNA-vauriovaste, G2/M-tarkastuspiste, heterotsygoottinen ituradan mutaatio, PALB2, replikaatiostressi, rintasyöpä, syöpäalttius, syöpägenetiikka, syövän kehitys

Dedicated to my Family

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Abbreviations

53BP1	p53-binding protein 1
9-1-1	RAD9–RAD1–HUS1
AA	Amino acids
ADP	Adenosine diphosphate
AML	Acute myeloid leukemia
ATM	Ataxia-telangiectasia mutated
ATR	ATM and Rad3-related protein
ATRIP	ATR interacting protein
BACH1	BTB domain and CNC homolog 1
BBA	Biochimica et Biophysica Acta
BER	Base excision repair
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
BRCC	BRCA1-PALB2-BRCA2-RAD51
BRCC36	BRCA1/BRCA2-containing complex subunit 36
BRCC45	BRCA1/BRCA2-containing complex subunit 45
BRCT	BRCA1 C terminus
BRIP1	BRCA1 interacting protein C-terminal helicase 1
C1QTNF5	C1q and tumor necrosis factor related protein 5
Cas9	CRISPR associated protein 9
CASP8	Caspase 8
CCDC98	Coiled-coil domain containing 98 (alias ABRAXAS, ABRA1, FAM175A)
Cdc25	Cell division cycle 25
Cdc25C	Cell division cycle 25C
CDH1	E-cadherin
Cdk	Cyclin-dependent kinase
CDK4	Cyclin-dependent kinase 4
CDKN1B	Cyclin-dependent kinase inhibitor 1B
CDKN2A	Cyclin-dependent kinase inhibitor 2A
cDNA	Complementary deoxyribonucleic acid
ChAM	Chromatin-association motif
CHEK2	Checkpoint kinase 2
CHK1	Checkpoint kinase 1
CHK2	Checkpoint kinase 2

COS	CV-1 (simian) in origin, and carrying the SV40 genetic material
COX11	Cytochrome C oxidase assembly homolog 11
CRISPR	Clustered regularly interspaced short palindromic repeats
CtIP	C-terminal binding protein 1 (CtBP1) interacting protein
CYREN	Cell cycle regulator of NHEJ
DDR	DNA damage response
DEGs	Differentially expressed genes
D-loop	Displacement loop
DNA	Deoxyribonucleic acid
DSB	Double-strand break
DSBR	Double-strand break repair
DUB	Deubiquitinating
EBV	Epstein-Barr virus
edgeR	Empirical analysis of digital gene expression in R
EdU	Ethynyl-deoxyuridine
eIF4F	Eukaryotic initiation factor 4F
ESR1	Estrogen receptor 1
ETO	Etoposide
EXO1	Exonuclease 1
EZH2	Enhancer of zeste homolog 2
FA	Fanconi anemia
FAM175A	Family with sequence similarity 175, Member A
FAM84B	Family with sequence similarity 84 member B
FANCD2	Fanconi anemia, complementation group D2
FANCM	Fanconi anemia, complementation group M
FANCN	Fanconi anemia, complementation group N
FGFR2	Fibroblast growth factor receptor 2
G1-phase	Gap 1 phase
G2-phase	Gap 2 phase
GRM1	Glutamate receptor, metabotropic 1
HA	Human influenza hemagglutinin
HeLa	Henrietta Lacks cell line
HER2	Human epidermal growth factor receptor 2
HMEC	Human mammary epithelial cells
HR	Homologous recombination
HU	Hydroxyurea
Indels	Insertions and deletions

IPA	Ingenuity pathway analysis
IR	Ionizing radiation
JAMM	JAB1/MPN/Mov34 metalloenzyme
K63	Lysine 63
KEAP1	Kelch-like ECH-associated protein 1
LCLs	Lymphoblastoid cell lines
lncRNA	Long non-coding RNA
LOH	Loss of heterozygosity
LSP1	Lymphocyte-specific protein 1
MAP3K1	Mitogen-activated protein kinase kinase kinase 1
MCF10A	Michigan Cancer Foundation-10A
MCF7	Michigan Cancer Foundation-7
MDC1	Mediator of DNA damage checkpoint 1
MEFs	Mouse embryonic fibroblasts
MERIT40	Mediator of RAP80 interactions and targeting subunit of 40 kDa
MMC	Mitomycin C
MMEJ	Microhomology-mediated end joining
MMR	Mismatch repair
MORF4L1	Mortality factor 4 like 1
M-phase	Mitosis phase
MPN	Mpr1 and PAD1 N-terminal
MRG15	MORF-related gene on chromosome 15
MRN	MRE11–RAD50–NBS1
mRNA	messenger RNA
MRPS30	Mitochondrial ribosomal protein S30
NA	Not applicable
NBS1	Nijmegen breakage syndrome 1 (Nibrin, NBN)
NEK10	NIMA (never in mitosis gene A)-related kinase 10
NER	Nucleotide excision repair
NES	Nuclear export signal
NHEJ	Non-homologous end joining
NLS	Nuclear localization signal or sequence
NPI	Nottingham prognostic index
NRAS	Neuroblastoma RAS viral (V-Ras) oncogene homolog
NRF2	Nuclear factor-erythroid 2-related factor 2
NST	No special type
PALB2	Partner and localizer of BRCA2

PARP	Poly (ADP-ribose) polymerase
PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol 3-kinase
PLA	Proximity ligation assay
Pol η	Polymerase eta
POU4F3	POU class 4 homeobox 3
PP2A	Protein phosphatase 2A
PR	Progesterone
PRC2	Polycomb repressive complex 2
PTEN	Phosphatase and tensin homolog
PTM	Post-translational modification
qPCR	quantitative PCR
RAD51C	RAD51 paralog C
RAD51L1	RAD51-like 1
RAD54L	RAD54 like
RAP80	Receptor-associated protein 80
RB1	Retinoblastoma 1
RET	Rearranged during transfection
RIF1	Rap1-interacting factor 1
RIN	RNA integrity number
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
RNF168	Ring finger protein 168
RNF8	Ring finger protein 8
ROS	Reactive oxygen substances
RPA	Replication protein A
Rpn10	Regulatory particle number 10
Rpn11	Regulatory particle number 11
Rpn8	Regulatory particle number 8
SCE	Sister chromatid exchange
SETD6	SET domain-containing protein 6
SFR	Stalled fork repair
siRNA	Small (or short) interfering RNA
SMAD4	Mothers against decapentaplegic homolog 4
SNP	Single nucleotide polymorphism
S-phase	Synthesis phase
SSA	Single-strand annealing

SSB	Single-strand break
STK11	Serine-threonine protein kinase 11
SUZ12	Suppressor of zeste 12
TAL1	T-cell acute lymphocytic leukemia 1
T-ALL	T cell acute lymphoblastic leukemia
TNBC	Triple negative breast cancer
TOX3	Tox high-mobility group box family member 3
TP53	Tumor protein 53
TRPS1	Trichorhinophalangeal syndrome, type I
U2OS	Human osteosarcoma cell line
Ub	Ubiquitin
UIM	Ubiquitin-interacting motif
UV	Ultraviolet
VWA	von Willebrand factor type A
WB	Western blot
WT1	Wilms tumor 1
XRCC2	X-ray repair cross complementing 2
XRCC3	X-ray repair cross complementing 3
ZP3	Zona pellucida glycoprotein 3
γH2AX	phospho-histone H2AX

Original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:

I   Nikkilä, J., Parplys, AC., Pylkäs, K., Bose, M., Huo, Y., Borgmann, K., Rapakko, K., Nieminen, P., Xia, B., Pospiech, H., & Winqvist, R. (2013). Heterozygous mutations in *PALB2* cause DNA replication and damage response defects. Nat. Commun. 4:2578

II Bose, M., Sachsenweger, J., Laurila, N., Parplys, AC., Willmann, J., Eshraghi, L., Dunlop, TW., Groth, M., Rapakko, K., Nieminen, P., Friedl, TWP., Heiserich, L., Meyer, F., Tuppurainen, H., Devarajan, R., Peltoketo, H., Nevanlinna, H., Pylkäs, K., Borgmann, K., Wiesmüller, L., Winqvist, R., & Pospiech, H. (2017). BRCA1 mislocalization leads to transcriptional misregulation and aberrant DNA damage response in *ABRAXAS* mutation carrier cells. Manuscript.

Contents

1   Introduction

Cancer is not one disease, but a collection of related diseases, which originate from most of the cell types and organs in the human body (Stratton *et al*. 2009). Approximately 90% of human cancers arise from the epithelial cells (Cooper 2000). The risk of suffering any cancer before the age of 40 is ~2%; however, by the age of 80 this risk increases to 50% (Martincorena & Campbell 2015). Cancer is a disease of the genome which arises due to somatically acquired mutations (Fearon 1997, Michor *et al*. 2004, Stratton 2011, Yates & Campbell 2012). Most cancers carry around 1,000 to 20,000 point mutations along with a few to hundreds of insertions and deletions (indels) and rearrangements (Martincorena & Campbell 2015).

Breast cancer is the most commonly diagnosed cancer in women, and most importantly, it is the second leading cause of cancer mortality in women (after lung cancer) (Downs-Holmes & Silverman 2011, Makki 2015). It is estimated that in industrialized countries, approximately one in eight women are expected to develop breast cancer during her lifetime (Ellsworth *et al*. 2010). Worldwide, an estimated 1.7 million breast cancer cases and 521,900 breast cancer related deaths were observed in 2012 (Torre *et al*. 2015). In Finland, 5,161 new breast cancer cases and 841 breast cancer related deaths were reported in 2015 (Finnish Cancer Registry 2017).

Approximately 5–10% of all patients with breast cancer exhibit a strong genetic predisposition to the disease (Claus *et al*. 1996, Romero-Laorden & Castro 2017, Thull & Vogel 2004). The main indicators for a hereditary disease predisposition are: 1) early disease onset, 2) familial clustering of breast cancer, 3) occurrence of multiple primary tumors in the same individual, 4) occurrence of bilateral breast cancer and 5) male breast cancer incidences in the family (Thull & Vogel 2004). Mutations in high-risk breast cancer susceptibility genes such as *BRCA1* and *BRCA2* together account for 15% of hereditary breast cancer cases. Mutations in the rest of the known breast cancer susceptibility genes account for an additional 21% of hereditary breast cancer cases (Couch *et al*. 2014).

Genes that, when mutated, promote cancer development can be classified into dominant-acting proto-oncogenes and recessive tumor suppressor genes, the latter including also the caretaker gene sub-class. However, tumor suppressor gene mutations are not always completely recessive, as they can alternatively be either haploinsufficient or dominant-negative (Payne & Kemp 2005). Haploinsufficiency represents the special circumstance in which one functional

allele is insufficient to maintain the normal cellular process (Berger & Pandolfi 2011, Payne & Kemp 2005). On the other hand, any mutant protein which disrupts the activity of the wild-type protein is classified as dominant-negative mutant (Herskowitz 1987).

It is well known that hereditary mutations in DDR genes associated with important cellular caretaker functions often lead to genomic instability and ultimately tumor development (Negrini *et al*. 2010). However, the molecular mechanisms of how these DDR deficiencies promote genomic instability and malignancy are not well understood. Thus the specific aim of this thesis is to better understand how genomic instability and tumorigenesis arise in the heterozygous *PALB2* and *ABRAXAS* germline mutation carriers.

2 Review of the literature

2.1 Cancer

Cancer encompasses more than 100 distinct diseases which originate from most of the cell types and organs in the human body (Stratton *et al*. 2009). Approximately 90% of human cancers arise from the epithelial cells (Cooper 2000). It is estimated that the risk of suffering any cancer before the age of 40 is ~2%; however, by the age of 80 this risk increases to 50% (Martincorena & Campbell 2015). Cancers are characterized by abnormal proliferation of cells that can invade beyond normal tissue boundaries and metastasize to distant organs (Stratton *et al*. 2009).

Most importantly, cancer is a disease of the genome which arises due to somatically acquired mutations (Fearon 1997, Michor *et al*. 2004, Stratton 2011, Yates & Campbell 2012). Mutation in genes can have both in *cis* and in *trans* effect on cancer development. If a mutant gene directly impacts on cancer development then the mutation is acting in *cis* fashion. However, if a mutation promotes cancer development through some other genes encoded elsewhere then the mutation is acting in *trans* fashion. In addition to genetic changes, cancer can also arise due to epigenetic alterations. Mutation in genes that regulate epigenome as well as epigenetic alteration results in abnormal gene expression, thereby promoting tumorigenesis (You & Jones 2012).

In addition to genetic and epigenetic changes, several studies have recently shown that the tumor microenvironment also plays a critical role during tumor initiation and progression (Chen *et al*. 2015). For tumorigenesis, genes that control critical cellular processes such as cell proliferation and differentiation, DNA replication, cell cycle and DNA repair are frequently targeted. Currently, there are several models available to describe how tumorigenesis arises (reviewed in Meng *et al*. 2011), but in simple terms, biological changes in the epithelium alone or along with changes in stroma/extracellular matrix are necessary for malignancy (Vineis *et al*. 2010).

The human genome is a very dynamic structure with somatic mutations occurring in all cells during fetal development and tissue regeneration (Ju *et al*. 2017, Luning Prak & Kazazian Jr 2000). It is estimated that each normal cell in our body undergoes more than 20,000 DNA-damaging events and over 10,000 replication errors per day (Loeb 2011). Fortunately, most of these mutations are

efficiently repaired by the cellular DNA repair machinery, and it is estimated that only 2–10 mutations per each cell division remain unrepaired (Loeb 2016, Martincorena & Campbell 2015, Rayner *et al.* 2016). Thus, the gradual accumulation of mutations can lead to the transformation of a normal cell into a cancerous one (Loeb 2016).

As shown in Figure 1, for malignant transformation, a normal cell has to acquire the following six essential physiological changes known as hallmarks of cancer: 1) sustaining proliferative signaling, 2) evading growth suppressors, 3) resisting cell death, 4) enabling replicative immortality, 5) inducing angiogenesis and 6) activating invasion and metastasis. Since cancer development is a multistep process, acquirement of one cancer hallmark accelerates the attainment of the remaining hallmarks in succession. Additionally, two emerging hallmarks of cancer, reprogramming energy metabolism and evading immune destruction, are also important for malignant transformation. Furthermore, genome instability and tumor-promoting inflammation are considered as enabling characteristics for malignant transformation (Hanahan & Weinberg 2000, Hanahan & Weinberg 2011).
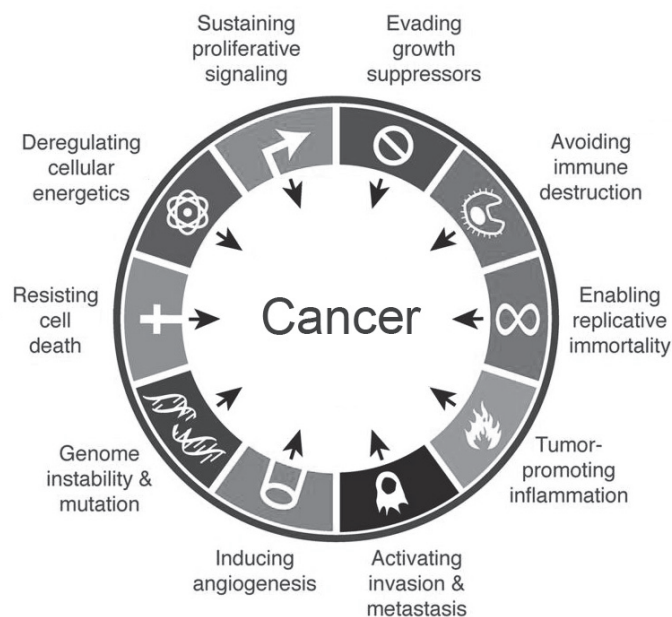
Fig. 1. The hallmarks of cancer. Modified with permission from Elsevier (Cell), Hanahan & Weinberg (2011) (Copyright 2017).

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When compared to the mutagenic processes such as chromosomal rearrangements, copy number changes and indels, point mutations are the most frequent lesion observed in cancer cells (Martincorena & Campbell 2015, Nik-Zainal & Morganella 2017, Vogelstein *et al*. 2013). Although the numbers vary widely, most cancers carry around 1,000 to 20,000 point mutations along with a few to hundreds of indels and rearrangements (Martincorena & Campbell 2015). Among others, leukemias and pediatric brain tumors typically have the lowest numbers of mutations. On the other hand, tumors induced due to exposure to mutagens such as lung cancers (tobacco) or skin cancers (UV rays) have the highest numbers of mutations. Nevertheless, some cancers acquire dramatically increased mutation rates due to the loss of genome maintenance pathways such as DNA repair and cell cycle checkpoints (Martincorena & Campbell 2015, Vogelstein *et al*. 2013).

Using various statistical methods (as reviewed in Vogelstein *et al*. 2013), mutations accumulating during tumorigenesis can be broadly classified into two different groups known as driver mutations and passenger mutations. Mutations that confer a selective growth advantage to the tumor cells are known as driver mutations. Out of all the 20,000 protein-coding genes studied to date, driver mutations have been estimated to be present in 138 genes. Of these, 64 are proto-oncogenes and 74 are tumor suppressor genes. In common adult tumors such as pancreatic, colorectal, breast and brain cancers, mutations are often observed in three to six driver genes. However, in pediatric tumors such as medulloblastomas, the number of driver gene mutations seems to be low (zero to two).

On the other hand, mutations that do not confer any selective growth advantage to the tumor cells are known as passenger mutations. Over 99.9% of the somatic alterations observed in the tumors are due to passenger mutations (Vogelstein *et al*. 2013). In addition to the above-mentioned "driver" and "passenger" genes, a new class of genes known as "epi-driver" has been proposed by Vogelstein *et al*. (2013). Epi-driver genes are not frequently mutated in tumors but are rather expressed aberrantly, thereby providing selective growth advantage to the tumor cells (Vogelstein *et al*. 2013).

2.1.1 Genes involved in cancer development

Genes that promote cancer development when dysregulated can be classified into two major classes known as proto-oncogenes and tumor suppressor genes (anti-oncogenes) (Michor *et al*. 2004). Based on their biological functions, tumor suppressor genes can be further classified into caretakers, gatekeepers and

landscapers (Ashworth *et al*. 2011, Kinzler & Vogelstein 1997, Naik *et al*. 2015). Mutation in one cancer gene alone is not sufficient to give rise to a full-blown cancer (Croce 2008, Michor *et al*. 2004). Loss of tumor suppressor gene function along with overexpression or dysregulation of proto-oncogenes is collectively required for the initiation and development of cancer (Croce 2008, Guo *et al*. 2014).

Proto-oncogenes

A proto-oncogene is a normal cellular gene that encodes a protein which is usually involved in the regulation of cell growth or proliferation. A key genetic alteration in the proto-oncogene results in the activation of the oncogene. At least four distinct genetic mechanisms can produce oncogenes from the corresponding proto-oncogenes: 1) point mutation, 2) gene amplification, 3) chromosomal translocation and 4) hypomethylation. Point mutations in a proto-oncogene may lead to encoding of an oncoprotein, which is constitutively active and differs slightly from that of the normal protein. In contrast, the oncoprotein resulting due to gene amplification, chromosomal translocation and hypomethylation is identical to that of normal protein but expressed at much higher concentrations, thereby conferring the oncogenic potential (Botezatu *et al*. 2016, Croce 2008, Lee & Muller 2010).

Chromosomal translocation can activate oncogenes by the following two mechanisms: 1) by creating an oncogenic fusion protein and 2) by translocating the proto-oncogene in close proximity with an active promoter region. Hypomethylation that usually results in overexpression of oncoprotein has been frequently observed in the promoter and enhancer regions of the proto-oncogene (Botezatu *et al*. 2016). Mutations in proto-oncogenes are a gain-of-function mutation which acts in a dominant fashion, that is, mutation in only one of the two alleles is sufficient for cancer development (Croce 2008, Lee & Muller 2010).

Although a majority of inherited cancer syndromes arise from mutations in tumor suppressor genes, a small number of them also occur due to mutations in proto-oncogenes (Fearon 1997, Frank 2001). So far, inherited mutations in proto-oncogenes such as *RET* (mutated in medullary thyroid carcinoma), *MET* (hereditary papillary renal cell carcinoma and familial colorectal cancer), *NRAS* (Noonan syndrome) and *CDK4* (familial malignant melanoma) have been observed (Ekvall *et al*. 2015, Marsh *et al*. 1996, Neklason *et al*. 2011, Schmidt *et al*. 1997, Zuo *et al*. 1996). Inherited mutations in proto-oncogenes are relatively

rare because such mutations are often lethal during embryogenesis and thus lead to spontaneous termination of pregnancy (Frank 2001).

Tumor suppressor genes

Tumor suppressor genes encode proteins that normally help to prevent unrestrained cellular growth and promote apoptosis, DNA repair and cell cycle checkpoint activation (Lee & Muller 2010). In contrast to proto-oncogenes, defective tumor suppressor genes act recessively, that is, both alleles need to be mutated for cancer development. In addition to being recessive, tumor suppressor genes can also be either haploinsufficient or dominant-negative for cancer development (discussed in more detail in chapter 2.4) (Payne & Kemp 2005). Based on their biological functions, tumor suppressor genes are further classified into caretakers, gatekeepers and landscapers (Ashworth *et al*. 2011, Kinzler & Vogelstein 1997, Naik *et al*. 2015). Caretaker genes (e.g. *BRCA1*, *BRCA2* and *PALB2*) encode proteins that are responsible for maintaining genome stability. The caretaker genes confer efficient repair of DNA damage that arises due to endogenous and exogenous agents (Ashworth *et al*. 2011). If the damaged DNA is not efficiently repaired, the gatekeeper genes (e.g. PP2A, *RB1, PTEN* and *TP53*) that regulate cell growth and apoptosis either stop the cell from proliferating further or eliminate the cell via apoptosis (Kaur & Westermarck 2016, Kotnis *et al*. 2005). Defects in landscaper genes (e.g. *SMAD4*) do not directly affect cellular growth, but generate an abnormal stromal environment that contributes to the neoplastic transformation of cells (Ashworth *et al*. 2011, Soussi & Wiman 2015).

2.1.2 Cancer initiation due to non-genomic mechanisms

In addition to genetic changes, cancer can also be initiated due to non-genomic mechanisms such as aberrant transcription and translation along with aberrant post-transcriptional and post-translational regulation. Overexpression of oncogenic transcription factors can cause changes in the transcriptional regulatory switches, thereby promoting cancer initiation and metastasis. The oncogenic transcription factor known as TAL1 is frequently overexpressed in approximately 50% of T cell acute lymphoblastic leukemia (T-ALL) cases (Lee & Young 2013). TAL1 forms transcriptional regulatory networks with other key transcription factors that control hematopoiesis. Thus, overexpression of TAL1 results in

sustained overactivation of TAL1-mediated transcription, which drives the oncogenic program in T-ALL (Sanda & Leong 2017).

In addition to overexpression of oncogenic transcription factors, suppression of transcription factors that have a tumor suppressor role is also frequently observed during cancer initiation and development (as reviewed in Van Vlierberghe & Ferrando 2012). Besides transcription factors, aberrant expression of long non-coding RNAs (lncRNAs) that regulate transcription can also alter the transcriptome, thereby promoting cancer initiation (Lee & Young 2013). Finally, epigenetic alterations and chromatin modifications also play a crucial role in transcriptional regulation and are frequently targeted during cancer initiation and development (Ellis *et al*. 2009).

Differential regulation in the post-transcriptional mechanisms such as mRNA stability control also plays a pivotal role in cancer initiation and development. Regulation of mRNA half-life plays a central role during normal development as well as during disease progression. Significant changes in the half-life of mRNA encoding growth factors, cell cycle regulators, cytokines and oncogenes are frequently observed in breast cancers. Increased mRNA half-life (or stability) will result in the mRNA being available for longer time for translation, resulting in higher levels of the protein products. lncRNA, microRNA, alternative polyadenylation and mRNA binding proteins have all been shown to regulate the half-life of mRNA (Griseri & Pagès 2014).

Translation is a highly conserved process during which proteins are synthesized using the information present in the mRNA. Translation is one of the most energy-consuming processes in the cell which is frequently targeted during cancer initiation and development. In the vast majority of malignancies, increased protein synthesis is a common feature due to translational dysregulation (Topisirovic & Sonenberg 2015). Several oncogenic pathways such as Myc, Ras and PI3K have been shown to promote cancer development by deregulating the translational machinery (Ruggero 2013). Overexpression of eIF4E (a translation initiation factor) that leads to oncogenic transformation has been frequently found in the majority of human cancers. Moreover, overexpression of eIF4E in cancer patients correlates with a poor prognosis. In addition to eIF4E, another translation initiation factor known as eIF4G was also found to be overexpressed in squamous cell lung carcinoma (Grzmil & Hemmings 2012). These studies collectively emphasize the importance of translational dysregulation for cancer initiation and development.

Post-translational modification (PTM) refers to the enzymatic modification of proteins after their synthesis (Cooper *et al*. 2015). To date, more than 300 different types of PTMs have been identified. Among them, the most common and well-studied ones are ubiquitination, sumoylation, methylation, glycosylation, acetylation and phosphorylation (Lu *et al*. 2016). PTMs are crucial for normal cellular maintenance and they are frequently exploited during cancer initiation and development.

Among the above-mentioned PTMs, protein phosphorylation has been well studied as many enzymes and receptors are activated/deactivated by either phosphorylation (kinases) or dephosphorylation (phosphatases) (Ardito *et al*. 2017). An aberrant phosphorylation-dephosphorylation cascade is frequently observed in various types of malignancies. As reviewed in Kauko & Westermarck (2018), cancer cells inhibit the phosphatase PP2A (tumor suppressor) by non-genomic mechanisms such as PTMs and overexpression of PP2A inhibitor proteins. In human cancers, PP2A is predominantly targeted by non-genomic inhibition and not genetic mutation (Kauko & Westermarck 2018). Thus, taken together, these studies further indicate the prevalence and importance of non-genomic mechanisms for cancer initiation and development.

2.2   Breast cancer

2.2.1 *Epidemiology and risk factors of breast cancer*

Breast cancer is a common term used to address all malignancies that arise from the breast tissue. Breast cancer is the most common form of cancer in women in the developed world, and most importantly, it is the second leading cause of cancer related deaths (after lung cancer) in women (Downs-Holmes & Silverman 2011, Makki 2015). It is estimated that approximately one in eight women will develop breast cancer during her lifetime (Ellsworth *et al*. 2010). Worldwide, an estimated 1.7 million breast cancer cases and 521,900 breast cancer related deaths were observed in 2012. Among women, 25% of all cancer cases and 15% of all cancer deaths are due to breast cancer (Torre *et al*. 2015).

Developed countries alone account for nearly 50% of all breast cancer cases and 38% of breast cancer related deaths (Torre *et al*. 2015). Breast cancer incidence and deaths are generally high in Western and Northern Europe, Northern America, Australia and New Zealand; intermediate in Caribbean, Latin

America, Central and Eastern Europe; and low in most of Africa and Asia (Ferlay *et al*. 2013, Torre *et al*. 2015). International variation in breast cancer incidence is due to the differences in the availability of early malignancy detection programs as well as disease risk factors (Torre *et al*. 2015).

In Finland, 5,161 new breast cancer cases were diagnosed during 2015, which is approximately one-third of all female cancers (16,028) diagnosed that year. Since 1953, breast cancer incidence rates have been rising annually in Finland. Among women, 841 breast cancer related deaths were observed in Finland during 2015, compared to all 5,777 cancer related deaths in that year. Similarly, in Finland during 2015, 30 new breast cancer cases and 7 breast cancer related deaths were observed among men (Finnish Cancer Registry 2017). Although the incidence of breast cancer is increasing, the associated mortality is fortunately declining (Althuis *et al*. 2005, Hery *et al*. 2008, Peto *et al*. 2000). The relative decline in breast cancer mortality is most likely due to improved early diagnosis and efficient use of adjuvant and neoadjuvant therapies (Althuis *et al*. 2005, Hery *et al*. 2008, Karim-Kos *et al*. 2008, Peto *et al*. 2000).

Breast cancer has a complex etiology where susceptibility is influenced by both genetic and environmental factors. Epidemiological evidence suggests that lifetime exposure to endogenous hormones such as estrogens (ER) and androgens promotes breast carcinogenesis (Ellsworth *et al*. 2010). Increased ER exposure throughout a woman's lifetime due to use of oral contraceptives, hormone replacement therapy, early menarche and late menopause has been associated with ~2-fold increase in breast cancer risk among premenopausal women (Ellsworth *et al*. 2010, Kaaks *et al*. 2005a, Kaaks *et al*. 2005b, Key *et al*. 2002).

Late age (>30 years) at first pregnancy or never being pregnant, high breast density and family history are also important in defining the risk for breast cancer (Ellsworth *et al*. 2010). Lifestyle choices such as tobacco use, alcohol consumption, nutrition and physical inactivity also contribute significantly towards breast cancer risk (Ellsworth *et al*. 2010, Torre *et al*. 2015). Most importantly, age is the main risk factor as most breast cancer cases (81%) occur in women aged 50 years or older (Barrett 2010).

Breast cancer is generally treated via surgery, radiotherapy, chemotherapy, hormonal therapy and targeted therapy. The choice of treatment depends on a multitude of factors such as age, hormone receptor status, menopause status, human epidermal growth factor receptor 2 (HER2) status, overall health and various other parameters including personal needs and preferences. Surgery (lumpectomy) is offered to patients with early breast cancer to remove the

primary lesion and the associated regional lymph nodes. However, in patients with advanced disease, total removal of the breast (mastectomy) is often recommended. After lumpectomy and mastectomy, breast reconstruction surgery is offered to the patients to restore the normal shape of the breast (Barrett 2010).

Radiotherapy is usually recommended for patients who have undergone lumpectomy and mastectomy to reduce the risk of disease recurrence. Chemotherapy is usually offered to patients either after surgery (adjuvant chemotherapy) or before surgery (neo-adjuvant chemotherapy). Adjuvant chemotherapy is given to reduce the risk of disease recurrence whereas neo-adjuvant chemotherapy is given to facilitate surgery. Similarly, hormonal therapy is given to breast cancer patients who are hormone receptor positive either after surgery (adjuvant) or before surgery (neo-adjuvant). Finally, using HER2 targeted therapy, breast cancer patients who are HER2 positive can be efficiently treated with high specificity (Barrett 2010).

2.2.2 Classifications of breast cancer

Breast cancers with different biological and histopathological features display distinct behaviors, which results in contrasting therapeutic outcomes. Thus, accurate grouping of breast cancers into clinically relevant subtypes is of paramount importance for efficient therapeutic decision-making (Dai *et al*. 2015). Breast cancers are classified into different categories based on their histopathological type, tumor grade, tumor stage and molecular subtype (expression profile of specific proteins) (Rakha *et al*. 2010).

Breast carcinoma is primarily classified based on the histological appearance of the tumor tissue. Breast cancer originates most commonly in the epithelial cells lining the inside of lobules and ducts. Milk-producing lobules supply the milk via ducts, and cancers originating from the ducts are known as ductal carcinomas (~80%), while those originating from the lobules are known as lobular carcinomas (~10%) (Makki 2015). Ductal and lobular carcinomas are further subdivided into *in situ* or invasive disease depending on the disease progression (Vuong *et al*. 2014). There are more than 21 subtypes of invasive breast carcinoma defined in the latest edition of the WHO classification of tumors of the breast (Lakhani *et al*. 2012). The most frequent among them is the invasive carcinoma of no special type (NST), also known as invasive ductal carcinoma NST, which accounts for 40–75% of the invasive cases (Vuong *et al*. 2014). The remaining invasive cases are morphologically distinct and well defined, including

special subtypes such as invasive lobular, tubular, mucinous, metaplastic carcinoma and carcinoma with medullary, neuroendocrine or apocrine features. When a tumor does not fulfill the criteria for a histological special subtype, it is classified as invasive carcinoma of no special type (Sinn & Kreipe 2013, Vuong *et al*. 2014). For a complete list of breast carcinoma types based on their histological appearance, please see the review by Sinn & Kreipe (2013).

All breast carcinomas irrespective of their histological type should be histologically graded (Vuong *et al*. 2014). The most widely used grading system is based on that of Bloom and Richardson (Bloom & Richardson 1957), which was modified by Elston and Ellis (Elston & Ellis 1991). Scores are assigned for the proportion of tubule formation (score of 1–3, with 3 being poor), the degree of nuclear pleomorphism (1–3, with 3 showing a high degree of pleomorphism) and the mitotic count (1–3, with 3 indicating a high mitotic count). The scores are then combined to give a grade of 1 (total score of 3 to 5), 2 (scores 6 or 7) or 3 (score 8 or 9) (Vuong *et al*. 2014). Tumor grade along with lymph node status is used to predict the 5-year survival of patients using the Nottingham prognostic index (NPI) (Blamey *et al*. 2007). Patients with high-grade breast cancers (which tend to recur and metastasize early) have a poor clinical outcome when compared to patients with low-grade tumors who generally have a very good clinical outcome (Vuong *et al*. 2014).

Determination of tumor stage is most important for effective therapeutic decision-making. Breast cancer is staged using the TNM system, which includes information such as tumor size (T), the status of regional lymph nodes (N), and spread to distant metastatic sites (M). The presence of biomarkers such as ER, progesterone (PR) and HER2 are also routinely evaluated during clinical management of breast cancer patients (Vuong *et al*. 2014).

Using microarray-based gene expression profiling, breast cancers can be divided into five molecular subtypes: luminal A-like, luminal B-like (HER2-negative), luminal B-like (HER2-positive), HER2-type and basal-like (Table 2) (Cho 2016, Goldhirsch *et al*. 2013). The most common molecular subtype of breast cancer is luminal A (71%), with luminal B (8%), HER2-type (6%) and basal-like (15%) subtype accounting for the remaining cases (Wiechmann *et al*. 2009). Genomic complexity, key genetic alterations and prognosis differ between the different subtypes. Patients with luminal A tumors have better survival rates when compared to the other subtypes. On the other hand, the basal-like group cancers are mostly grade 3 and show aggressive clinical behavior (Vuong *et al*. 2014). Basal-like subtype is often referred to as triple-negative breast cancer

(TNBC) due to the absence of ER, PR and HER2. Most importantly, basal-like subtype is more common in individuals carrying mutations in DDR genes such as *BRCA1* and *BRCA2* (Couch *et al.* 2015).

Table 2. Intrinsic subtypes of breast cancer

Intrinsic subtype	ER	PR	HER2	Ki-67
Luminal A-like	+	+	-	Low
Luminal B-like (HER2-negative)	+	- or low	-	High
Luminal B-like (HER2-positive)	+	Any	Over-expressed	Any
HER2-positive	-	-	Over-expressed	NA
Basal-like	-	-	-	NA

NA: not applicable

2.3 Inherited predisposition to breast cancer

Already in the nineteenth century, it was recognized that women from certain families were prone to developing breast cancer (Nielsen *et al.* 2016). In 1866, the French physician Pierre Paul Broca first published a report on a family with increased predisposition to breast cancer (Broca 1866). His wife acquired breast cancer at an early age, and pedigree analysis showed that four generations had breast cancer (Broca 1866). However, it was only in 1948 that two independent studies showed that the transmission of a specific genetic factor is a major cause of breast cancer in certain families (Penrose *et al.* 1948, Smithers 1948). Several twin studies also further confirmed that clustering of breast cancer in certain families is due to strongly penetrant genetic factors (Lichtenstein *et al.* 2000). In general, a monozygotic twin is at an increased risk to develop breast cancer when compared to a dizygotic twin (Mucci *et al.* 2016, Peto & Mack 2000).

Approximately 5–10% of all patients with breast cancer exhibit a strong genetic predisposition to the disease (Claus *et al.* 1996, Romero-Laorden & Castro 2017, Thull & Vogel 2004). Hereditary breast cancer patients are characterized by 1) early onset of the disease, 2) familial clustering of breast cancer, 3) occurrence of several primary tumors in the same patient, 4) occurrence of bilateral breast cancer and 5) incidence of male breast cancer in the family (Thull & Vogel 2004).

As shown in Table 3, breast cancer predisposing factors are classified into three different categories, namely high-penetrance genes, moderate-penetrance genes and low-penetrance variants. The level of penetrance describes the

probability of an individual to develop breast cancer. Usually, an inverse correlation exists between the risk of breast cancer conferred by a particular genetic variant and its prevalence in the population. In other terms, enrichment of mutations in high- and moderate-penetrance susceptibility genes is often rare in the population whereas the low-penetrance variants can be very common.

Table 3. Recognized high-, moderate- and low-risk breast cancer susceptibility genes.

Classification	Gene/Locus	Disease risk (fold)	Population frequency (%)
High-penetrance	*BRCA1, BRCA2, PALB2, TP53, PTEN, STK11, CDH1*	>6	<0.1
Moderate-penetrance	*CHEK2, ATM, BRIP1, NBS1, RAD50*	2 – 4	0.1 – 0.5
Low-penetrance	Multiple common SNPs including: *CASP8, FGFR2, MAP3K1*, *TOX3, LSP1, MRPS30, FAM84B , NEK10, COX11, NOTCH2, RAD51L1, ESR1, CDKN2A*	<1.5	>1.0

This table is compiled from (Antoniou *et al*. 2014, Couch *et al*. 2014, Erkko *et al*. 2007, Foulkes 2008, Hollestelle *et al*. 2010, Lalloo & Evans 2012). SNP: single nucleotide polymorphism

As shown in Figure 2, mutations in high-risk breast cancer susceptibility genes such as *BRCA1* and *BRCA2* are observed in 15% of hereditary breast cancer cases. Additionally, mutations in the rest of the breast cancer susceptibility genes account for 21% of hereditary breast cancer cases (Couch *et al*. 2014). Nevertheless, despite decades of medical research and appreciable technological advancements, currently only 36% of hereditary breast cancer cases have an identified causative gene mutation (Figure 2) (Couch *et al*. 2014, Rousset-Jablonski & Gompel 2017). Thus, further studies are warranted to find new breast cancer susceptibility genes and other factors/mechanisms responsible for missing disease heritability. Regardless of the shortcomings, these genetic studies have already given us valuable information to design a breast cancer specific gene panel for routine use in clinical diagnostics. Panel-based testing enables more women and their family members to be tested efficiently and swiftly for heritable germline mutations. Currently, in Finland, a breast cancer specific gene panel that includes high-risk genes such as *BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11* and *TP53* is available for commercial use.
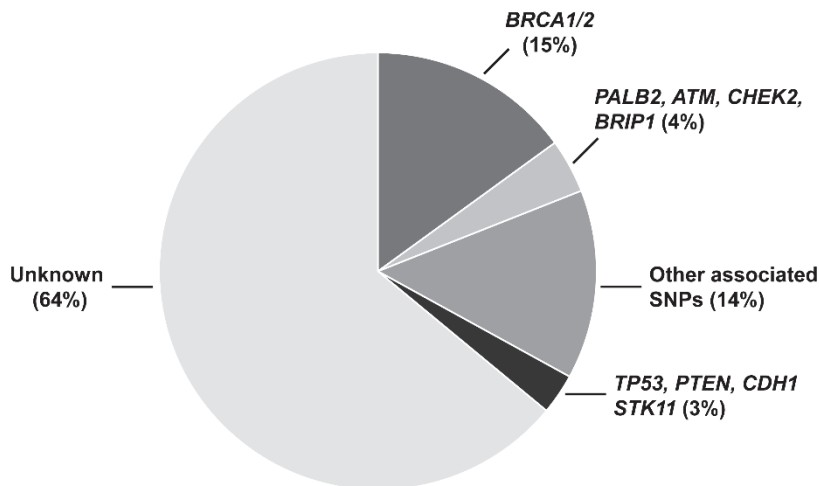
Fig. 2. Contribution of known genes to familial aggregation of breast cancer.

2.4 Tumorigenesis due to inherited mutations in tumor suppressor genes

As discussed in section 2.1.1, cancer genes can be categorized into recessive tumor suppressor genes and dominant-acting proto-oncogenes (Payne & Kemp 2005). Interestingly, most inherited cancer syndromes arise due to genetic alterations in tumor suppressor genes (Fearon 1997). In 1969, using somatic cell hybridization experiments, Harris *et al*. (1969) demonstrated that tumor suppressor genes must be completely inactivated (being recessive) for malignancy to occur. In 1971, by comparing retinoblastoma (unilateral vs. bilateral) patients, Knudson proposed his two-hit hypothesis indicating that both the hereditary and the nonhereditary form of retinoblastoma are mechanistically linked. In the hereditary form of retinoblastoma, one mutant allele is inherited from either of the parents while the second allele is somatically mutated, which significantly accelerates the onset of retinoblastoma. However, in the nonhereditary form, somatic alterations must occur simultaneously in both alleles prior to retinoblastoma initiation (Knudson 1971). Further epidemiological studies on cancer solidified the belief that tumor suppressor genes act recessively, i.e. both alleles need to be inactivated (Payne & Kemp 2005).

However, tumor suppressor gene mutations are not always completely recessive, as they may alternatively be either haploinsufficient or dominant-negative. In diploid (2n) organisms, barring X and Y chromosome genes, two functional copies (or alleles) of all the other genes are present. For a majority of genes, one functional copy is sufficient to maintain normal cellular functions. However, certain genes are sensitive to gene dosage levels (i.e. being haploinsufficient) and require both functional alleles to maintain normal cellular functions (Payne & Kemp 2005). Haploinsufficiency describes a special circumstance in which one functional allele is insufficient to maintain the normal cellular process (Berger & Pandolfi 2011, Payne & Kemp 2005). In 1996, three independent studies identified $p27^{kip1}$ (*CDKN1B*) as a haploinsufficient tumor suppressor gene, with the $p27^{+/-}$ heterozygote mice showing an intermediate size between the small $p27^{+/+}$ and large $p27^{-/-}$ homozygote mice (Fero *et al.* 1996, Kiyokawa *et al.* 1996, Nakayama *et al.* 1996). $p27^{+/-}$ mice also showed an intermediate phenotype in both tumor-free survival and tumor multiplicity when compared to $p27^{+/+}$ and $p27^{-/-}$ littermates (Fero *et al.* 1998, Philipp-Staheli *et al.* 2002).

Under certain circumstances, *TP53* is inactivated as expected by Knudson's two-hit hypothesis; however, in other cases it shows clear evidence for haploinsufficiency (Venkatachalam *et al.* 1998). $p53^{+/-}$ mice also showed an intermediate phenotype in both spontaneous chromosomal aberrations and apoptosis activation when compared to $p53^{+/+}$ and $p53^{-/-}$ littermates (Bouffler *et al.* 1995, Clarke *et al.* 1994). Studies on tumorigenesis due to haploinsufficiency have traditionally been based on murine models or cellular knockdown approaches. However, using patient-derived materials, three recent independent studies have successfully shown genomic instability and tumorigenesis due to *BRCA1* and *PALB2* haploinsufficiency (Nikkilä *et al.* 2013, Pathania *et al.* 2014, Vaclová *et al.* 2015). An extensive list of known haploinsufficient tumor suppressor genes is reviewed in Berger & Pandolfi (2011) and Payne & Kemp (2005).

PTEN is a critical tumor suppressor gene whose function is frequently disrupted in cancer. Like *CDKN1B* and *TP53, PTEN* is also a haploinsufficient tumor suppressor gene. Mice heterozygous for *PTEN* develop autoimmunity disorder and are susceptible to multiple tumor types. Interestingly, although complete loss of *PTEN* promotes tumorigenesis it has been shown to be less tumorigenic than heterozygous loss of *PTEN*. This is because complete loss of *PTEN* activates cellular senescence mediated by p53 that limits the tumorigenic

potential of the cells. In contrast, cells heterozygous for *PTEN* have a high proliferation rate without the activation of cellular senescence. Thus, complete loss of *PTEN* is less tumorigenic than heterozygous loss of *PTEN* due to p53 mediated cellular senescence; this paradigm is known as obligate haploinsufficiency (Berger & Pandolfi 2011).

In addition to haploinsufficiency, mutations in tumor suppressor genes can also lead to dominant-negative effects as described below. By definition, any mutant protein that disrupts the activity of the wild-type protein is classified as a dominant-negative mutant (Herskowitz 1987). By nature, it is difficult to differentiate a dominant-negative mutation from a haploinsufficient mutation. In both these cases, the wild-type allele is retained, with the reason for the retention being different in each case. In the case of a haploinsufficient mutation, the wild-type allele is not lost since the reduced dosage of the wild-type protein is sufficient for tumorigenesis (Payne & Kemp 2005). In the case of a dominant-negative mutation, the wild-type allele is not lost since the mutant protein often disrupts the activity of the wild-type protein (Payne & Kemp 2005, Veitia 2007).

A dominant-negative mutation in *RAP80* was recently shown to promote genomic instability and tumorigenesis in U2OS cells (Nikkilä *et al*. 2009). Similarly, dominant-negative mutations in *SETD6* and *WT1* have been shown to predispose the mutation carriers to familial colorectal cancer type X and Wilms tumor, respectively (Martín-Morales *et al*. 2017, Pelletier *et al*. 1991). Besides cancer, the dominant-negative disease mechanism is also observed in late-onset retinal degeneration (due to mutation in *C1QTNF5*), spinocerebellar ataxia type 44 (*GRM1*) and empty follicle syndrome (*ZP3*) (Chen *et al*. 2017, Stanton *et al*. 2017, Watson *et al*. 2017).

A simple classification of tumor suppressor gene mutations as either recessive, haploinsufficient or dominant-negative is currently being challenged. In addition to being either recessive, haploinsufficient or dominant-negative, mutations in tumor suppressor gene can also have a gain-of-function phenotype (Payne & Kemp 2005). For example, mutations in *TP53* can under certain circumstances also have a gain-of-function phenotype. These gain-of-function *TP53* mutants acquire new oncogenic properties that are independent of wild-type p53 (Zhang *et al*. 2016). Depending upon the mutation, p53 can behave as a classical (Knudson two-hit hypothesis), haploinsufficient, dominant-negative or gain-of-function tumor suppressor gene.

2.5 DNA damage response

Maintenance of genome stability is a fundamental process for cell homeostasis. As mentioned in section 2.1, the genome of a cell is constantly challenged by both exogenous and endogenous agents. In the presence of DNA damage, to maintain genome integrity, cells activate the DNA damage response (DDR) pathway to sense and repair the DNA damage. Upon activation, the DDR pathway coordinates a response that includes activation of DNA repair pathways, cell cycle control, apoptosis and transcription (Figure 3) (Ghosal & Chen 2013).

DNA damaging agents can be categorized into two main classes based on their origin: endogenous and exogenous (Chatterjee & Walker 2017). A majority of the endogenous DNA damage arises due to hydrolysis, exposure to reactive oxygen substances (ROS) and other reactive metabolites (Ali *et al*. 2017, De Bont & van Larebeke 2004). In addition, inherent DNA replication processes are also a frequent source of endogenous DNA damage (Georgoulis *et al*. 2017). On the other hand, exogenous DNA damage occurs when environmental, physical and chemical agents damage the DNA (Chatterjee & Walker 2017). Among the environmental DNA damaging agents, ultraviolet (UV) light causes significant DNA damage in humans. Although the ozone layer absorbs the most hazardous UV-C radiations, residual UV-A and UV-B in strong sunlight can induce ~100,000 lesions per exposed cell per hour. Ionizing radiation (IR) generates the most toxic and lethal DNA damage of all, known as double-strand break (DSB) (Jackson & Bartek 2009).

As shown in Figure 3, conceptually, DDR facilitates a key four-step process. (1) Recognition of the DNA damage; a group of specialized proteins known as sensors sense the DNA damage and activate the appropriate DNA repair system. Next, (2) amplification and transduction of the DNA damage signal; once the DNA damage is sensed, the cell must transduce this signal down to its appropriate effector. A group of proteins known as transducers performs this signal amplification and transduction. (3) Activation of genome maintenance pathways; upon activation, a group of proteins known as effectors activate the DNA repair pathways, transcription, cell cycle control and apoptosis. Finally, (4) reversal of the previous steps; once the DNA is faithfully repaired, the genome maintenance pathways will be turned off for normal cell cycle progression (Georgoulis *et al*. 2017, Harper & Elledge 2007).

40
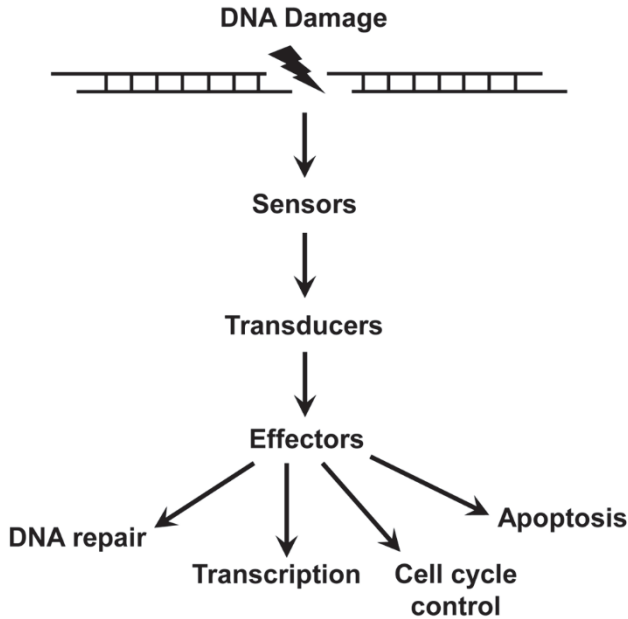
Fig. 3. Outline of the DNA damage response signal transduction pathway

The DNA repair pathways can be broadly classified into two groups based on the type of damage they repair: repair of DNA damage affecting only one strand (single-strand break (SSB) repair) and repair of DNA damage affecting both DNA strands (DSB repair). Nucleotide excision repair (NER), base excision repair (BER) and DNA mismatch repair (MMR) pathways are prominent to repair different types of single-strand DNA damage. Whereas, the DNA DSBs are repaired by non-homologous end joining (NHEJ), homologous recombination (HR), microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA) pathways (Ghosal & Chen 2013, Jackson & Bartek 2009, Tubbs & Nussenzweig 2017). In addition to DNA repair pathways, DDR also activates cell cycle checkpoints in order to delay the cell cycle progression, thus preventing the cells from entering the next cell cycle phase with unrepaired DNA damage (Ghosal & Chen 2013, Houtgraaf *et al*. 2006). If the DNA damage is irreparable, then apoptosis is activated to eliminate the cells carrying DNA lesions and to preserve genomic integrity (Al-Ejeh *et al*. 2010, Houtgraaf *et al*. 2006).

2.5.1 DNA double-strand break repair pathway

DNA DSBs are cytotoxic lesions that threaten genome stability, and failure to repair such a lesion will induce genomic instability and cell death. To repair DSBs, cells employ four main mechanisms known as NHEJ, HR, MMEJ and SSA. NHEJ repairs the DSBs without a need of the homologous sequence by directly ligating the broken ends (Figure 4). NHEJ is active throughout the cell cycle but predominantly in G0/G1 and G2. Alternatively, the DSB end can be resected resulting in 3′ single-stranded DNA overhangs, which can be repaired by three possible mechanisms: HR, MMEJ and SSA (Figure 4). HR predominates during the mid-S and mid-G2 cell cycle phases when the sister chromatids become available. Since HR uses sister chromatid DNA sequence information to repair the DSBs, it requires strand invasion mediated by RAD51 (Figure 4). When compared to NHEJ, HR is an error-free repair pathway with slow repair kinetics (Ceccaldi *et al*. 2016).
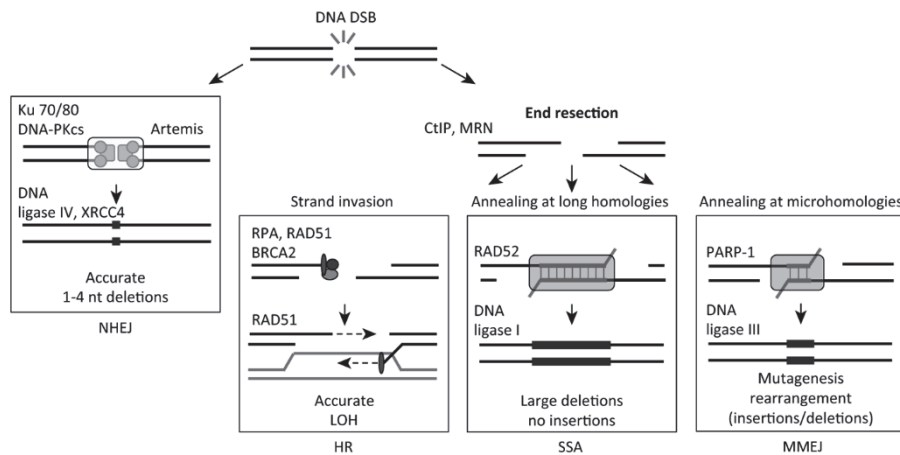
Fig. 4. Simplified representation of the DNA DSB repair pathways. LOH; loss of heterozygosity. Modified with permission from Elsevier (Trends in Cell Biology), Ceccaldi *et al*. (2016) (Copyright 2017).

Besides HR, the resected DSB can also be repaired by mutagenic repair pathways such as SSA and MMEJ. SSA mediates end joining between the interspersed nucleotide repeats in the genome with the help of RAD52 protein (Figure 4). Thus, SSA is a highly mutagenic process resulting in the loss of intervening sequence between the nucleotide repeats (Ceccaldi *et al*. 2016). Finally, MMEJ uses

microhomologous sequences on either side of the DSB to ligate the broken DNA ends (Figure 4). Similar to SSA, MMEJ is also a highly mutagenic process resulting in the deletion of DSB flanking regions (Wang & Xu 2017). Moreover, MMEJ can also join DSBs located on different chromosomes, thereby generating chromosomal translocations and other mutagenic rearrangements (Ceccaldi *et al*. 2016).

Homologous recombination

As mentioned above, HR repair is limited to S and G2 phases of the cell cycle, since it requires sister chromatids as templates to repair the DSB. A simplified model of HR repair mechanism is presented in Figure 5. As shown in Figure 5, there are four main key steps in the HR repair pathway. In the first step, $3'$ single-stranded DNA overhangs are generated by nucleolytic degradation of the $5'$ strands. This first step is catalyzed by endonucleases such as MRE11–RAD50–NBS1 (MRN) complex, CtIP and EXO1. In the second step, the single-stranded DNA ends are coated with replication protein A (RPA). In the third step, RPA-coated ssDNA filaments are replaced by RAD51 in a BRCA1-PALB2-BRCA2 dependent manner, leading to strand invasion into an undamaged homologous DNA template (Krajewska *et al*. 2015). In the final step, after strand invasion, the concerted action of DNA polymerase, ligase, helicase and resolvase results in resolution of HR intermediates to yield an intact and repaired DNA strand.
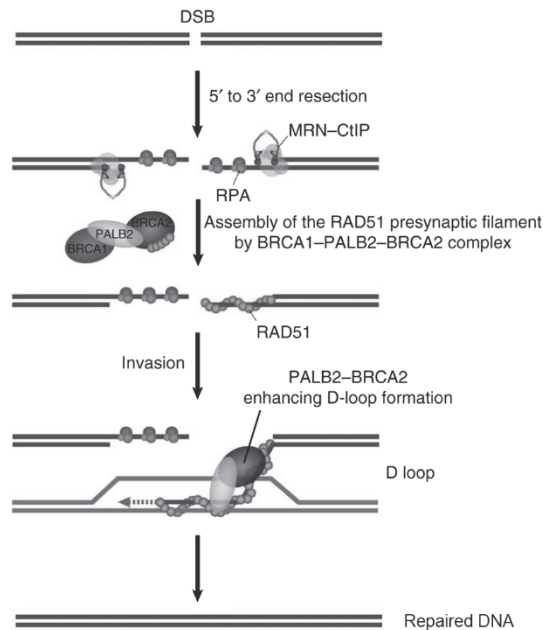
Fig. 5. Simplified representation of HR repair pathway. Modified with permission from Nature Publishing Group (Nature Structural and Molecular Biology), Buisson *et al*. (2010) (Copyright 2017).

The role of PALB2 and ABRAXAS in HR will be discussed in more detail in chapters 2.6.1 and 2.6.2, respectively.

DSB repair pathway choice

For the precise repair of DSBs, the repair pathway that is optimal for the particular condition needs to be selected (Shibata 2017). The specific DSB repair pathway is activated based on the cell cycle stage and resection status. End resection plays an important role in differentiating NHEJ from the other DSB repair pathways. As shown in Figure 4, since NHEJ directly ligates the broken DNA ends, it does not require end resection. Once end resection is activated, the DSB is committed to be repaired by either HR or MMEJ or SSA. The initial phase of end resection known as 'end clipping' is carried out by MRN complex together with CtIP. During this phase, a relatively small number of base pairs (20 bp in mammalian cells) are removed, thereby making the resected DNA ends

available for MMEJ. In the next phase of end resection (known as 'extensive resection'), the concerted actions of helicases and exonucleases generate long stretches of single-stranded DNA, thereby committing the DSBs to be repaired by either HR or SSA (Ceccaldi *et al*. 2016).

The cell cycle also plays a major role in the DSB repair pathway choice. CtIP itself is activated in a cell cycle dependent manner; CDK-dependent phosphorylation of CtIP favors the BRCA1–CtIP interaction (BRCA1-C complex) during the S/G2 phases (Ceccaldi *et al*. 2016). CDK-dependent phosphorylation of EXO1 also promotes end resection during S/G2 phases of the cell cycle. Consistently, inhibition of EXO1 phosphorylation attenuates end resection, HR and cell survival but augments NHEJ activity (Tomimatsu *et al*. 2014). In noncycling cells, DSB end resection is greatly reduced, thereby favoring NHEJ over the major resection-dependent repair pathways (such as HR, MMEJ, and SSA) (Ceccaldi *et al*. 2016).

The balance between 53BP1and BRCA1 also plays a major role in the DSB repair pathway choice by either preventing or promoting end resection. 53BP1 is a pro-NHEJ factor as it blocks end resection by preventing access of CtIP to the broken DNA ends (Ceccaldi *et al*. 2016). Recently, REV7 and RIF1 were also shown to promote NHEJ by inhibiting DNA end resection (Boersma *et al*. 2015, Escribano-Díaz *et al*. 2013, Xu *et al*. 2015). Arnoult *et al*. (2017) has recently identified an S/G2 cell cycle specific inhibitor of NHEJ known as CYREN. The cell cycle also controls the interaction of BRCA1 with PALB2-BRCA2 to restrict HR activity to the S/G2 phases only (Orthwein *et al*. 2015).

2.5.2 Cell cycle checkpoints

The induction of DNA damage in dividing cells results in the activation of cell cycle checkpoints. In contrast, in terminally differentiated cells (such as muscle cells) cell cycle checkpoints are not activated upon DNA damage. Checkpoints generally halt the cell cycle progression in order to give ample time for the DNA repair machinery to efficiently repair the damaged DNA. Thus, cell cycle checkpoints avoid transmission of incorrect genetic information from one cell generation to the next (Houtgraaf *et al*. 2006).

As shown in Figure 6, the eukaryotic cell cycle consists of four phases known as gap (G)1, synthesis (S), G2, and mitosis (M). In G1 phase, the cell increases in size and starts synthesizing RNA and proteins that are required for DNA synthesis. During S phase, DNA is replicated carefully to produce an almost identical copy

of the genome for the subsequent daughter cells. In G2 phase, the cell will grow further and synthesize more proteins to ensure that two viable daughter cells can be formed. Finally, the cell will enter into M phase to accurately distribute (segregate) its chromosomes into two daughter cells. If required, cells can also stop dividing and remain in G0 phase of the cell cycle (Figure 6) (Houtgraaf *et al.* 2006).
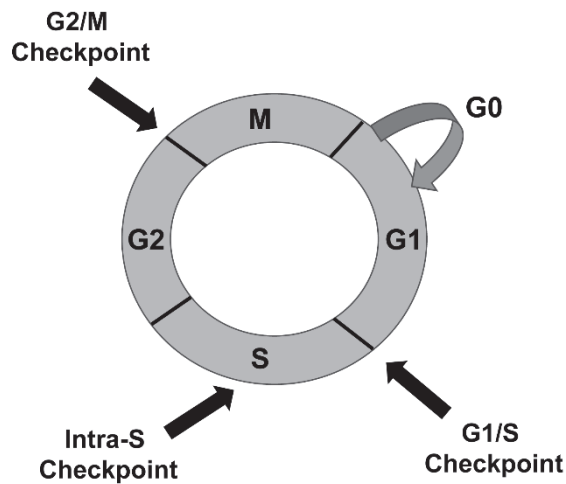
Fig. 6. Schematic representation of the cell cycle and its DNA damage checkpoints

As shown in Figure 6, there are three DNA damage checkpoints in the cell cycle known as G1/S, intra-S, and G2/M (Houtgraaf *et al.* 2006). The G1/S checkpoint ensures that DNA damaged in the G1 phase is not replicated in the subsequent S phase (Dasika *et al.* 1999). The intra-S checkpoint ensures accurate replication of the genome (Gaillard *et al.* 2015). Finally, the G2/M checkpoint ensures that cells do not initiate mitosis with persistent DNA damage (Houtgraaf *et al.* 2006).

Although distinct, all these checkpoints respond to DNA damage in a similar way and share many common proteins. A more simplified image of the DNA damage induced checkpoint response is shown in Figure 7 (Houtgraaf *et al.* 2006). Depending upon the cell cycle stage and DNA lesions, the sensor proteins such as MRN complex and RAD9–RAD1–HUS1 (9-1-1) complex will sense the DNA damage and activate the checkpoint response. ATM and ATR are further activated by the MRN complex and 9-1-1 complex, respectively (Cimprich & Cortez 2008). ATM is primarily activated in response to IR, whereas ATR is primarily activated

in response to UV and hydroxyurea (HU). ATR and ATM carry out the signal transduction to the respective effector kinases such as CHK1 and CHK2. Depending upon the cell cycle phase, CHK1 and CHK2 either activate p53 or inactivate Cdc25A-C, which leads to cell cycle arrest. Finally, cell cycle checkpoints have predominantly evolved to prevent transmission of DNA damage to the daughter cells, and defects in checkpoint responses can thus result in increased genomic instability (Houtgraaf *et al.* 2006).
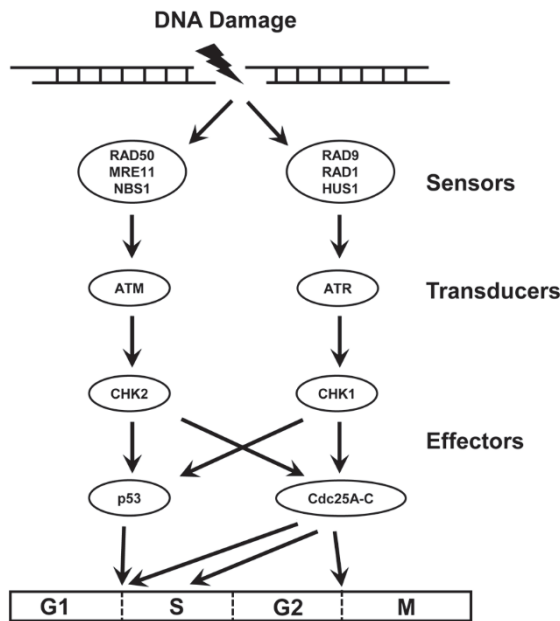
Fig. 7. Simplified representation of the DNA damage induced checkpoint response.

2.5.3 Replication stress response

The DNA replication machinery successfully carries out accurate genome duplication even in the presence of numerous obstacles. Under chronic stress or after loss of key cellular signaling pathways (which help to deal with replication stress), a range of deleterious events can occur (Zeman & Cimprich 2013). In eukaryotes, DNA replication originates at thousands of individual replication origins in a bi-directional way (Técher *et al.* 2017, Zeman & Cimprich 2013).

Already prior to S-phase, each replication origin is licensed by a combination of replication initiation proteins to prepare the chromatin for replication (Gaillard *et al.* 2015, Zeman & Cimprich 2013). Once they enter the S phase, the replication origins will be fired after activation by S phase-specific kinases and DNA replication commences. To complete the replication in an efficient manner, cells need to balance accuracy and the speed of replication. Most importantly, cells need to monitor the consumption and distribution of relevant resources such as nucleotides, energy and replication factors for efficient replication (Zeman & Cimprich 2013).

Interestingly, in unperturbed S phase, most of the licensed replication origins do not fire at all. Instead, these dormant origins are only activated after disturbances of progressing replication forks, and thus represent a back-up resource to ensure the completion of DNA replication (McIntosh & Blow 2012, Woodward *et al.* 2006, Zeman & Cimprich 2013). Thus, presence of surplus replication origins is direct evidence of the presence of slowing or stalling of the replication fork progression. Limitations of replication resources such as nucleotides or damage of the DNA during S phase augments fork slowing and stalling and thus induces replication stress (Mazouzi *et al.* 2014, Zeman & Cimprich 2013). The manifestation of stalled replication forks thus indicates the presence of replication stress.

Upon sensing replication stress, cells will activate the intra-S phase checkpoint (as shown in Figure 7) in order to alleviate the replication stress. ATR activation is initiated in the presence of single-stranded DNA that is generated at stalled replication forks (Gaillard *et al.* 2015). Single-stranded DNA generated at stalled replication forks is generally coated with RPA protein, which independently brings together ATR and its co-factor ATRIP to activate ATR (Mazouzi *et al.* 2014). Upon persistent replication stress, ATM may also be activated by DSBs that are generated by collapse of stalled replication forks (Gaillard *et al.* 2015). As shown in Figure 7, ATM and ATR further activate the effector kinases CHK2 and CHK1, respectively, to activate the intra-S phase checkpoint as described above.

Even in the absence of exogenous challenges, cells that are deficient in various checkpoints or DNA repair proteins display reduced replication fork speed and increased replication origins (Técher *et al.* 2017). Cells deficient in *ATR*, *CHK1*, *RAD51*, *BRCA1* and *BRCA2* display an increased number of replication origins and stalled replication forks (i.e. replication stress) (Eykelenboom *et al.* 2013, Maya‑Mendoza *et al.* 2007, Pathania *et al.* 2014,

Petermann *et al*. 2010, Técher *et al*. 2016, Wilhelm *et al*. 2014, Wilhelm *et al*. 2016). Increasing evidence suggests that several DNA repair and damage response proteins are involved in the replication of the genome. Thereby, defects in the corresponding genes accelerate genomic instability and tumorigenesis. This led to the proposal that DNA replication stress should be considered as a hallmark of cancer in its own right (Macheret & Halazonetis 2015).

2.6   PALB2 and ABRAXAS

2.6.1 *PALB2*

In response to DNA damage, both BRCA1 and BRCA2 are rapidly recruited to the DNA damage sites to facilitate DNA repair (Chen *et al*. 1996, Scully *et al*. 1996, Yuan *et al*. 1999). BRCA1- and BRCA2-deficient cells exhibit similar phenotypes with defects in HR, hypersensitivity to DNA damaging agents and chromosomal instability, indicating that both proteins have similar biological functions despite their lack of structural homology (Moynahan *et al*. 2001a, Moynahan *et al*. 2001b, Nepomuceno *et al*. 2017). In line with this, both BRCA1 and BRCA2 have been shown to facilitate DNA repair through their interaction with RAD51 (Scully *et al*. 1997, Sharan *et al*. 1997). Interestingly, it was later shown that both BRCA1 and BRCA2 coexist in the same RAD51-containing protein complexes (Chen *et al*. 1998). However, it was not clear whether the interaction between BRCA1 and BRCA2 is due to a direct or indirect association (Chen *et al*. 1998, Nepomuceno *et al*. 2017, Park *et al*. 2014a, Tischkowitz & Xia 2010). In 2009, three independent studies reported that BRCA1 and BRCA2 interact indirectly with PALB2 acting as a linker (Sy *et al*. 2009b, Zhang *et al*. 2009a, Zhang *et al*. 2009b).

Shortly after its functional characterization, *PALB2* was identified as a major breast cancer susceptibility gene (Antoniou *et al*. 2014, Erkko *et al*. 2007, Erkko *et al*. 2008, Foulkes *et al*. 2007, Rahman *et al*. 2007, Southey *et al*. 2016, Tischkowitz *et al*. 2007). In addition to inactivating genetic changes, epigenetic modifications such as promoter hypermethylation have also been observed in the *PALB2* gene in both inherited and sporadic breast and ovarian cancer (Potapova *et al*. 2008).

Xia *et al*. (2006) showed that the identified FLJ21816/LOC79728 protein was a major BRCA2-interacting factor and subsequently named it PALB2, for 'partner

and localizer of BRCA2'. The *PALB2* gene, which encodes a protein of 1,186 amino acids (aa, 131 kDa) is located on chromosome 16p12.2 and consists of 13 exons (Pauty *et al*. 2014). As shown in Figure 8, PALB2 contains a coiled-coil and WD40 domain in the N- and C-terminus, respectively. In addition, the central portion of PALB2 contains the chromatin-association motif (ChAM) (Park *et al*. 2014a).
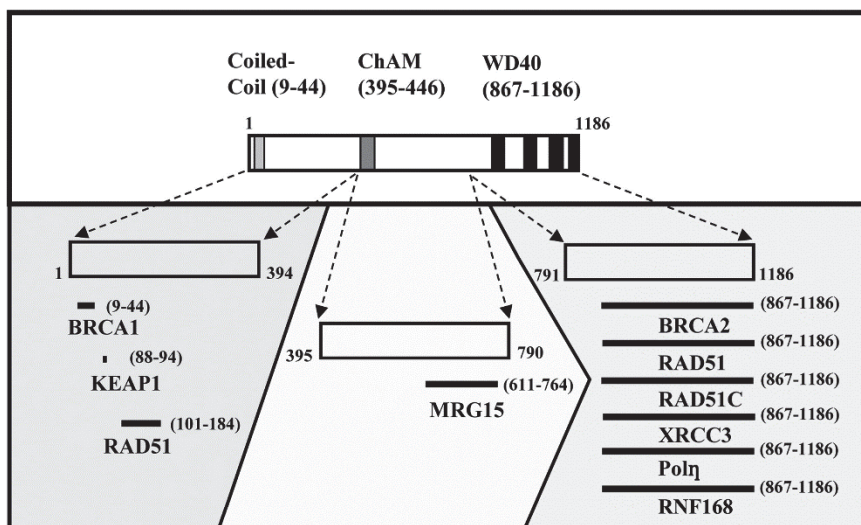
Fig. 8. Schematic protein structure of PALB2. PALB2 contains three structural domains, one each in the N-terminal, central, and C-terminal of the protein. Interacting proteins and the region of PALB2 required for their associations are also depicted. Modified with permission from Elsevier (Biochimica et Biophysica Acta (BBA) – Reviews on Cancer), Park *et al*. (2014a) (Copyright 2017).

The amino-terminal (approximately, aa 1–394) of PALB2 contains one prominent structural element, a coiled-coil domain (aa 9–44), which is known to mediate protein-protein interactions (Park *et al*. 2014a). The coiled-coil motif in PALB2 mediates interactions with BRCA1, disruption of which results in impaired localization of BRCA2, PALB2 and RAD51 to the DNA damage sites (Sy *et al*. 2009b, Zhang *et al*. 2009a, Zhang *et al*. 2009b). Interaction between BRCA1 and PALB2 is crucial for efficient HR-mediated DNA repair and resistance against MMC treatments (Foo *et al*. 2017, Simhadri *et al*. 2014, Sy *et al*. 2009b, Zhang *et al*. 2009a, Zhang *et al*. 2009b). Several studies have shown that BRCA1 acts upstream of PALB2 and facilitates its localization to the DNA damage sites,

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which in turn recruits BRCA2 and RAD51 to sites of DNA damage (Xia *et al*. 2006, Zhang *et al*. 2009a, Zhang *et al*. 2009b).

In addition to interacting with BRCA1, the N-terminal coiled-coil domain of PALB2 also facilitates its oligomerization (Buisson & Masson 2012, Sy *et al*. 2009c). Monomeric PALB2 showed higher efficiency to bind DNA and promote RAD51 filament formation when compared to dimeric PALB2 (Buisson & Masson 2012). Moreover, Buisson & Masson (2012) have shown that there is competition between PALB2 self-interaction and PALB2–BRCA1 interaction after DNA damage. The switch from PALB2–PALB2 to PALB2–BRCA1 interaction allows activation of HR in the presence of DNA damage. Thus, regulation of PALB2 self-interactions could be a critical step in controlling HR to prevent aberrant recombination events (Buisson & Masson 2012).

Ma *et al*. (2012) have recently shown that PALB2 directly interacts with KEAP1, an oxidative stress sensor that binds and targets NRF2 for cytoplasmic degradation. NRF2 is a master transcription factor that induces the expression of several antioxidant genes upon oxidative stress to mitigate oxidative damage. Intriguingly, both PALB2 and NRF2 contain an identical LDEETGE sequence, which enables direct binding to KEAP1. Thus, PALB2 can effectively compete with NRF2 for KEAP1 interaction, thereby promoting NRF2 accumulation and subsequent anti-oxidant response (Ma *et al*. 2012). In addition, Orthwein *et al*. (2015) have shown that the interaction between PALB2 and KEAP1 is critical for cell cycle dependent regulation of HR.

The ChAM domain present in PALB2 promotes its chromatin association in both unperturbed and damaged cells. Deletion of ChAM domain resulted in cellular hypersensitivity to MMC treatments and abrogated PALB2 and RAD51 accumulation at DNA damage sites. Thus, the ChAM domain has a clear role in the function of PALB2 in response to DNA damage (Bleuyard *et al*. 2011).

Additionally, four independent studies have identified interaction between PALB2 and MRG15 both in protein complexes isolated from cells and through yeast-two hybrid screens (Bleuyard *et al*. 2017, Hayakawa *et al*. 2010, Martrat *et al*. 2011, Sy *et al*. 2009a). MRG15, also known as mortality factor 4 like 1 (*MORF4L1*), has been described as a component of the NuA4 histone acetyltransferase complex acetylating histones H4 and H2A. NuA4 has been implicated in transcriptional regulation and may have a direct role in the regulation of HR (Dhar *et al*. 2017, Lu *et al*. 2009). Sy *et al*. (2009a) suggested that abrogation of PALB2 and MRG15 interaction results in hyperrecombination and elevated sister chromatid exchange (SCE) with no change in sensitivity to

MMC. In contrast, Hayakawa *et al*. (2010) have shown that depletion of PALB2 and MRG15 interaction results in a decrease of HR and SCE formation along with hypersensitivity to MMC treatment. Thus, taken together, both of these studies support the role of PALB2-MRG15 interaction in regulating HR, but disagree about whether MRG15 is a positive or negative regulator (Park *et al*. 2014a). Further studies are therefore needed to clarify whether MRG15 is a positive or negative regulator of HR.

The carboxy-terminal of PALB2 (approximately, aa 791–1186) contains a seven-bladed WD40-type domain, which is known to mediate protein interactions (Park *et al*. 2014a). The WD40 domain of PALB2 facilitates direct interactions with many of the key proteins involved in HR, such as BRCA2, RAD51, RAD51C, XRCC3 and RNF168 (Luijsterburg *et al*. 2017, Park *et al*. 2014b). Additionally, the WD40 domain also facilitates direct interaction between PALB2 and Pol η to mediate recombination-associated DNA synthesis (Buisson *et al*. 2014). Recently, a nuclear export signal (NES) was identified to be located in the WD40 domain between amino acids 928 – 945 (Pauty *et al*. 2017). This motif is usually masked and allows export of PALB2 under specific conditions. The WD40 domain is critical for the tumor suppressor function of PALB2 as truncation of even the last four amino acids of the WD40 domain destabilizes the entire PALB2 protein (Oliver *et al*. 2009).

PALB2 in conjunction with BRCA2 plays a crucial role in HR and in resistance to MMC (Xia *et al*. 2006). Additionally, PALB2 together with BRCA2 also regulates both the intra-S phase checkpoint and G2 checkpoint in response to IR (Menzel *et al*. 2011, Xia *et al*. 2006). PALB2 has two different regions that can directly bind to RAD51 and stimulate D-loop formation: 1) at the N-terminus (aa 101–184) and 2) in the WD40 domain (Buisson *et al*. 2010, Dray *et al*. 2010). Of these two RAD51 binding sites in PALB2, the WD40 domain appears to be the primary binding site for RAD51. Further studies are warranted to delineate the roles of these RAD51-binding sites in mediating HR (Park *et al*. 2014a).

It has been shown that PALB2 promotes D-loop formation by inhibiting the association of RPA with the invading DNA, thereby promoting the binding and stabilization of RAD51 to DNA (Buisson *et al*. 2010). The interaction between the full-length PALB2 and APRIN appears to be essential for the PALB2-mediated RPA displacement and RAD51 filament formation during HR (Couturier *et al*. 2016). Moreover, three independent studies have recently shown that ATM/ATR-mediated phosphorylation of PALB2 at S59, S157, and S376 is

necessary for efficient RAD51 foci formation during HR (Ahlskog *et al*. 2016, Buisson *et al*. 2017, Guo *et al*. 2015).

Finally, the phenotype of *PALB2* knockout mice resembles those of *BRCA1* and *BRCA2* knockout mice, suggesting that a key function of PALB2 is to facilitate communication between BRCA1 and BRCA2 (Rantakari *et al*. 2010). In addition, it was recently shown that tumors originating due to *PALB2* mutation displayed a similar mutational signature (signature 3) to that observed in *BRCA1* and *BRCA2* mutant tumors (Alexandrov *et al*. 2013, Nik-Zainal *et al*. 2016, Polak *et al*. 2017).

PALB2 is a Fanconi anemia gene

Shortly after its identification and initial characterization, *PALB2* (*FANCN*) was found to be biallelically mutated in nine Fanconi anemia (FA) families (Reid *et al*. 2007, Serra *et al*. 2012, Xia *et al*. 2007). FA patients are characterized by growth retardation, congenital abnormalities, infertility and progressive bone marrow failure (Auerbach 2009, Kutler *et al*. 2003). All nine *FANCN* patients developed cancer in their early childhood, including five medulloblastomas, four Wilms tumors, two cases of acute myeloid leukemia (AML), one kaposiform hemangioendothelioma and two neuroblastomas. Cancer treatment was unsuccessful in eight *FANCN* patients, all of whom died before four years of age (Reid *et al*. 2007, Serra *et al*. 2012, Xia *et al*. 2007).

The occurrence of multiple pediatric malignancies along with early mortality in the *FANCN* patients is very similar to that of *FANCD1* (*BRCA2*) patients (Reid *et al*. 2007, Serra *et al*. 2012, Skol *et al*. 2016). The phenotypes of *FANCN* and *FANCD1* patients differ greatly from other FA subtypes, in whom progressive bone marrow failure predominates, often in advance of blood malignancies (Howlett 2007, Jacquemont & Taniguchi 2007, Patel 2007). The reason for the occurrence of multiple pediatric malignancies only in *FANC-N/D1* patients, but not in other FA subtypes is unclear and has to be explored further (Ghazwani *et al*. 2016, Hirsch *et al*. 2004, Miele *et al*. 2015, Reid *et al*. 2007, Wagner *et al*. 2004).

Observations of similar clinical phenotypes in FANCN and FANCD1 patients indicate that both PALB2 and BRCA2 define a distinct genetic, clinical and functional entity in the Fanconi anemia pathway (Patel 2007). Of note, although all FANCN patients displayed a severe form of FA, two FANCN patients expressing a hypomorphic PALB2 allele were recently diagnosed with a mild form of FA (Byrd *et al*. 2016). Finally, the recent discovery of *BRCA1* (*FANCS*) as

a new FA gene further exemplifies the importance of BRCA1-PALB2-BRCA2 interaction during DNA DSB repair (Sawyer *et al*. 2015).

2.6.2 ABRAXAS

ABRAXAS (*ABRA1, CCDC98*, or *FAM175A*) is a tumor suppressor gene in the BRCA pathway, mutations or copy number loss of which occur in several human cancers (Castillo *et al*. 2014). ABRAXAS was identified by three independent groups while searching for novel BRCA1 interacting proteins using the BRCA1 C-terminal (BRCT) domain as a bait (Her *et al*. 2016, Kim *et al*. 2007b, Liu *et al*. 2007, Wang *et al*. 2007). As shown in Figure 9, the gene encodes a protein of 409 amino acids (47 kDa) containing four recognized key elements such as a Mpr1 and PAD1 N-terminal (MPN) like domain (aa 11 – 121), a coiled-coil region (aa 206 – 260), a NLS (aa 358 – 361) and a pSPxF motif (aa 406 – 409) (Wang 2012, Wang *et al*. 2007).
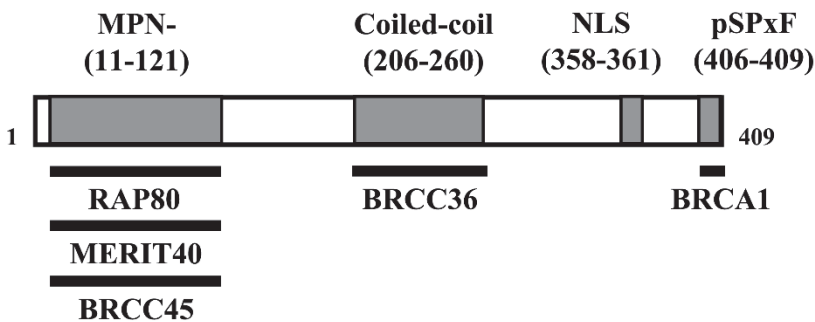
Fig. 9. Schematic protein structure of ABRAXAS. Interacting proteins and the region of ABRAXAS required for their associations are depicted. The NLS domain is important for translocation of BRCA1-A complexes into the cell nucleus and subsequently to DNA damage sites.

ABRAXAS acts as a central scaffold protein of the BRCA1-A complex, which comprises ABRAXAS, BRCA1, BRCC36, BRCC45, MERIT40 and RAP80 (Dong *et al*. 2003, Feng *et al*. 2009, Kim *et al*. 2007a, Kim *et al*. 2007b, Liu *et al*. 2007, Patterson-Fortin *et al*. 2010, Sobhian *et al*. 2007, Wang *et al*. 2009, Wang *et al*. 2007). When phosphorylated, ABRAXAS (p-S406) interacts with the BRCT domain of BRCA1 through its pSPxF motif (Kim *et al*. 2007b, Liu *et al*. 2007, Wang *et al*. 2007). Furthermore, ABRAXAS interacts with RAP80, MERIT40

and BRCC45 through its MPN-like domain and with BRCC36 through its coiled coil domain (Feng *et al.* 2009, Shao *et al.* 2009, Sobhian *et al.* 2007, Wang *et al.* 2009, Wang & Elledge 2007). The NLS domain is important for the translocation of BRCA1-A complex to DNA damage sites in the nucleus (Solyom *et al.* 2012).

As one of the imminent responses upon DNA DSBs, histone H2AX will be phosphorylated at serine 139 (γH2AX) by the DDR sensor kinases ATM/ATR (Burma *et al.* 2001, Rogakou *et al.* 1998). Subsequently, MDC1 is recruited to the DNA damage sites through binding to γH2AX and ATM (Stucki *et al.* 2005). Subsequent phosphorylation of MDC1 by ATM recruits ubiquitin (Ub) ligases, such as RNF8 and RNF168, which generates K63-linked poly-Ub chains on histones right at the damage site (Doil *et al.* 2009, Goldberg *et al.* 2003, Huen *et al.* 2007, Kolas *et al.* 2007, Mailand *et al.* 2007, Stewart *et al.* 2009). These act as a docking site for the BRCA1-A complex, mediated by RAP80 through its ubiquitin-interacting motif (UIM) (Harper & Elledge 2007, Hu *et al.* 2011b, Sobhian *et al.* 2007).

The exact role of the BRCA1-A complex in DNA DSB signaling is still not entirely clear (Wang 2012). It is suggested that the BRCA1-A complex regulates the DNA damage response process by deubiquitinating (DUB) the K63-linked poly-Ub chains (Shao *et al.* 2008, Wang 2012). Interestingly, the BRCA1-A complex shows striking similarity with the lid of 19S proteasome regulatory complex (Wang *et al.* 2009). The 19S proteasome lid cleaves ubiquitin from its substrates and subsequently facilitates the entry of substrates into the proteasome core for degradation (Wang 2012). Like Rpn11 in the 19S proteasome regulatory complex, BRCC36 also contains an active MPN+/JAMM domain that possesses DUB activity (Dong *et al.* 2003, Wang *et al.* 2009). BRCC36 displays DUB specificity towards the K-63 linked poly-Ub chains (Cooper *et al.* 2009, Sobhian *et al.* 2007). Similarly, like Rpn8 in the 19S proteasome regulatory complex, ABRAXAS also contains a MPN domain (Wang *et al.* 2009). Rpn10/S5a in the 19S proteasome regulatory complex contains both a UIM and VWA domain, as here RAP80 possesses a UIM domain and MERIT40 contains a VWA domain (Wang *et al.* 2009, Yan *et al.* 2006).

In addition to ABRAXAS, the BRCA1 BRCT domain also directly interacts with BACH1 and CtIP by binding a pSPxF motif in a CDK-phosphorylation dependent manner, forming BRCA1-B and BRCA1-C complexes, respectively (Cantor *et al.* 2001, Manke *et al.* 2003, Rodriguez *et al.* 2003, Wong *et al.* 1998, Yu *et al.* 1998, Yu *et al.* 2003). In addition to BRCA1-A, B & C complexes, as discussed in section 2.6.1, by direct interaction with PALB2, BRCA1 can also

form the BRCA1-PALB2-BRCA2-RAD51 (BRCC) complex (Savage & Harkin 2015, Sy *et al*. 2009b, Zhang *et al*. 2009a, Zhang *et al*. 2009b). All these four BRCA1-containing complexes compete for the availability of endogenous BRCA1 protein. Disruption of BRCA1-A complex formation by knockdown of RAP80 or ABRAXAS propagates BRCA1-B, BRCA1-C and BRCC complex formation and subsequent assembly at the DNA damage sites (Coleman & Greenberg 2011, Hu *et al*. 2011b, Typas *et al*. 2015).

It is worth noting that the BRCA1-B, BRCA1-C and BRCC complexes promote HR, while several studies indicate that the BRCA1-A complex suppresses HR and promotes end joining pathways (Coleman & Greenberg 2011, Hu *et al*. 2011b, Limbo *et al*. 2007, Litman *et al*. 2005, Sartori *et al*. 2007, Takeda *et al*. 2007, Typas *et al*. 2015). At the same time, ABRAXAS and the BRCA1-A complex seems to be required for the proper execution of HR (Castillo *et al*. 2014, Wang *et al*. 2007, Yan *et al*. 2007). Most importantly, ABRAXAS interaction with BRCA1 and subsequent BRCA1-A complex localization to the DNA damage sites is crucial for DNA repair and genome stability (Castillo *et al*. 2014). Downregulation of any component in the BRCA1-A complex compromises BRCA1 recruitment to the DNA damage sites, which results in aberrant DSB repair pathway choice (Castillo *et al*. 2014, Chen *et al*. 2006, Coleman & Greenberg 2011, Feng *et al*. 2009, Feng *et al*. 2010, Hu *et al*. 2011a, Hu *et al*. 2011b, Kim *et al*. 2007a, Kim *et al*. 2007b, Shao *et al*. 2009, Sobhian *et al*. 2007, Wang *et al*. 2009, Wang *et al*. 2007). Finally, the BRCA1-A complex is also involved in the regulation of G2/M checkpoint, apoptosis and transcription (Castillo *et al*. 2014, Draga *et al*. 2015, Kim *et al*. 2007a, Sobhian *et al*. 2007, Wang *et al*. 2007).

BRCA1-A complex and Cancer

Our team recently reported the presence of a recurrent heterozygous germline alteration in the *ABRAXAS* gene (c.1082G>A) in Northern Finnish breast cancer families (Solyom *et al*. 2012). This specific c.1082G>A (Arg361Gln) alteration resides in the NLS domain of *ABRAXAS* and impairs the nuclear localization of the BRCA1-A complex and thus disrupts the BRCA1-mediated DNA damage response (Solyom *et al*. 2012). Although the appearance of the *ABRAXAS* germline alteration in other populations seems to be relatively rare, c.1082G>A is a founder mutation in the Finnish population (Solyom *et al*. 2012). Interestingly, according to the exome aggregation consortium, the same c.1082G>A alteration

is also observed in non-Finnish Europeans and Latinos (Lek *et al*. 2016). Intriguingly, the NLS domain of *ABRAXAS* is identified as a mutational "hot spot" which is frequently targeted by somatic missense mutations in several types of cancer (Castillo *et al*. 2014).

The average age of disease onset for this particular *ABRAXAS* c.1082G>A mutation carriers is 46 years (variation, 35 to 53 years), which is similar to that of Finnish *BRCA1* (46 years; variation, 32 to 57 years) and *BRCA2* (48 years; variation, 45 to 67 years) mutation carriers (Sarantaus *et al*. 2000, Solyom *et al*. 2012). This indicates that carriers of the *ABRAXAS* c.1082G>A alteration possess a similarly increased cancer risk as that observed for *BRCA1* and *BRCA2* mutation carriers. For comparison, the mean age of disease onset for the *PALB2* c.1592delT mutation carriers is 52.9 years (variation, 39 to 73 years) (Erkko *et al*. 2007).

In addition to breast cancer, families with *ABRAXAS* c.1082G>A mutation also display certain rare types of cancers such as lip and lung cancer along with throat lymphoma (Solyom *et al*. 2012). Similarly, in addition to breast cancer, germline mutations in *ABRAXAS* have also been associated with upper aero-digestive tract and ovarian cancers (Hamdi *et al*. 2016, McKay *et al*. 2011, Pennington *et al*. 2014, Solyom *et al*. 2012).

Members of the BRCA1-A complex are frequently targeted by many clinically important germline mutations. Germline mutations in the BRCA1 BRCT domain disrupt either the binding surface to pSPxF-containing phosphopeptides or the ability of the BRCT domain to dimerize and form BRCA1 complexes (Clapperton *et al*. 2004, Wu *et al*. 2016). Apart from *BRCA1* and *ABRAXAS*, germline mutations in *RAP80* and *MERIT40* are also associated with inherited predisposition to breast and ovarian cancers (Antoniou *et al*. 2010, Bolton *et al*. 2010, Couch *et al*. 2016, Nikkilä *et al*. 2009). These studies collectively emphasize that, in addition to BRCA1, other BRCA1-A members are also frequently targeted for tumorigenesis.

3   Aim of the study

It is well known that hereditary mutations in DDR genes often lead to genomic instability and ultimately tumor development. However, the molecular mechanism of how these DDR deficiencies promote genomic instability and malignancy is not well understood.

Thus, the specific aims of the study were:

1. To identify the functional and molecular framework behind the elevated breast cancer risk observed in heterozygous *PALB2* mutation carriers.
2. To elucidate the molecular mechanisms by which the *ABRAXAS* Finnish founder mutation causes an elevated breast cancer risk for the heterozygous carriers.

4 Materials and methods

4.1 Subjects

4.1.1 Study I

Altogether eight previously identified female individuals carrying the Finnish *PALB2* c.1592delT founder mutation (Erkko *et al*. 2007) were analyzed in the current study. Among them, six individuals had been diagnosed with breast cancer while the other two persons were disease-free, representing two different breast cancer families (Erkko *et al*. 2007). Samples from the breast cancer patients were collected at least 3 years after the initial cancer diagnosis. Six healthy non-carrier female individuals served as controls; three of them were healthy members of two different *PALB2* mutation carrier families and the other three were population controls. All control individuals were ethnically age-matched and originated from the same geographical region as the mutation carriers. Similarly, all control individuals were cancer-free at the time of donation and no follow-up was performed on the health status of the controls. The studied cell lines are presented in Supplementary Table S1 of study I.

4.1.2 Study II

Altogether eight individuals carrying the Finnish *ABRAXAS* c.1082G>A founder mutation were analyzed in this study. Among them, five individuals had been diagnosed with breast cancer and one with lymphoma, and the other two persons were disease-free siblings of one breast cancer patient. Samples from the breast cancer patients were collected at least 3 years after the initial cancer diagnosis. Six healthy non-carrier female individuals used in study I (Nikkilä *et al*. 2013) along with one individual who is a healthy family member of a patient served as controls. All control individuals were cancer-free at the time of donation and originated from the same geographical region as the mutation carriers. No follow-up was performed on the health status of the controls. The studied cell lines are presented in Supplementary Table S1 of study II.

4.2 Methods

4.2.1 Cell culture

In order to generate lymphoblastoid cell lines (LCLs), Epstein-Barr virus (EBV) was used to immortalize B-lymphocytes obtained from fresh peripheral blood samples of the participating individuals. LCLs were cultured in RPMI 1640 medium (Gibco, Invitrogen) supplemented with 20% fetal bovine serum (Gibco, Invitrogen), 1% L-glutamine (Gibco, Invitrogen) and 10 μg/mL gentamycin (B. Braun). The MCF7 breast cancer cell line (ATCC HTB-22™, Manassas, Virginia, U.S.A.) was cultured in Dulbecco's modified Eagle Medium (DMEM), high glucose, with sodium pyruvate (Gibco, Invitrogen) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin (Sigma Aldrich).

4.2.2 Plasmids and transfection

Wild-type and c.1082G>A mutant *ABRAXAS* carrying an N-terminal HA/FLAG-tag were cloned from pOZ-N-*ABRAXAS* vectors into pcDNA3.1/Hygro(-) backbone. MCF7 cells were transiently transfected with wild-type *ABRAXAS*, *ABRAXAS* mutant or empty pcDNA3.1/Hygro(-) vector using Cell Line Nucleofector kit V (Lonza) according to the manufacturer's instructions.

4.2.3 Etoposide and hydroxyurea treatments

Cell lines treated with either 0.5 or 5 μM etoposide (ETO) (Etopofos, Bristol–Myer Squibb and/or Etoposide, Pfizer) and 2 mM hydroxyurea (HU) (Sigma-Aldrich) were cultured for up to 24 h in the presence of the agent. Samples were then collected at different time points (0 h, 0.5 h, 1 h, 2 h, 3 h, 5 h, 7 h and 24 h).

4.2.4 Western blot analysis

A standard western blot (WB) protocol was followed as mentioned in Studies I & II.

4.2.5 RNA extraction, cDNA synthesis and Quantitative PCR

Samples were collected from untreated cells and total RNA was extracted using RNeasy Mini Kit (Qiagen), and reverse transcription was done as described in the iScript™ cDNA synthesis kit (Bio-Rad). Quantitative PCR (qPCR) was performed using iQ™ SYBR® Green Supermix (Bio-Rad) as per the manufacturer's instruction in a CFX96 real-time PCR system (Bio-Rad). *CLK2* and *GUSB* were used as calibrators.

4.2.6 DNA damage signaling PCR array

Samples were collected from untreated cells and total RNA was extracted using the RNeasy Mini Kit (Qiagen). RNA quality was determined using RNA 6000 nanochips (Agilent Technologies) with an Agilent 2100 bioanalyzer, and only samples with an RNA integrity number (RIN score) of 10 were used further. Genomic DNA elimination and reverse transcription was done as described in the RT^2 First Strand Kit (Qiagen). Gene expression was studied using the DNA damage signaling pathway RT^2 profiler PCR array (Qiagen).

4.2.7 RNA sequencing

Samples were collected from untreated cells and total RNA was extracted using the RNeasy Mini Kit (Qiagen). The average RIN score of the samples was 8.7, ranging from 5.1 to 10. Around 1 ug total RNA was used for library preparation using Illumina's TruSeq Stranded mRNA Library Prep Kit (Illumina, CA, USA). The libraries were sequenced (multiplexing five per lane) using an Illumina HiSeq2500 in high-output, single-ended, 51 cycle mode, followed by read extraction (FastQ format) using bcl2fastq v1.8.4 (supported by Illumina). This resulted in an average of 43 million reads per sample (32.8–57 million).

4.2.8 DESeq2 and Ingenuity pathway analysis

DESeq2 (v1.14.1) in Bioconductor/R package was used to identify differentially expressed genes (DEGs) between the two cohorts and by using a negative binomial test. The parameters used in our *DESeq2* analysis were: mode="Union", singleEnd=TRUE, ignore.strand=FALSE. The false discovery rate-adjusted *P*-value (FDR) was estimated based on Benjamini Hochberg correction. Changes in

gene expression with a FDR (*Padj* value) of <0.05 were considered statistically significant. To evaluate the significance of the identified DEGs, Ingenuity Pathway Analysis (IPA®, QIAGEN Bioinformatics) was conducted to find enriched biological, molecular and cellular functions as well as associated diseases and network functions.

4.2.9 edgeR and ENRICHR analysis

In order to find a wider range of DEGs, we performed R package *edgeR* (v3.3.1) analysis. In *edgeR* analysis, default parameters were used (DGEList: remove.zeros=FALSE, lib.size = NULL, norm.factors = NULL, genes = NULL; estimateCommonDisp: tol=1e-06, rowsum.filter=5; exactTest: pair=NULL, dispersion=NULL, common.disp=TRUE). *P*-values were corrected for multiple testing using the Benjamini Hochberg method, and genes with FDR (*Padj* value) <0.01 were considered to be differentially expressed and statistically significant. To evaluate the importance of the DEGs identified by *edgeR*, the official gene names of these DEGs were fed into the web-based gene set enrichment tool service ENRICHR (http://amp.pharm.mssm.edu/Enrichr).

4.2.10   Flow cytometric analysis

Unexposed and 0.5 µM ETO treated cell lines were cultured for 24 h and samples were collected at different time points (0.25 h, 1 h, 7 h and 24 h). Cells were stained with thymidine analogue 5-ethynyl-2´-deoxyuridine (EdU) to measure DNA synthesis and analyze cell cycle distribution as described by the manufacturer of Click-iT® EdU Alexa Fluor® (AF) 647 Flow Cytometry Assay Kit (ThermoFisher Scientific). All measurements were performed with LSRFortessa (BD Biosciences) flow cytometer and FlowJo software was used for quantification.

4.2.11   Immunofluorescence

A standard immunofluorescence protocol was followed as mentioned in Study II to perform high content protein localization analysis and high content foci analysis.

4.2.12 DNA fiber spreads

Exponential growing cells were pulse-labeled with 25 μM CldU (Sigma) followed by 250 μM IdU (Sigma) for the indicated times. For fiber analysis, after damage induction CldU and IdU media was given for the indicated time periods and 10 μM ETO was performed during the last 30 min of the CldU pulse. Labeled cells were harvested and DNA fiber spreads were prepared on microscope glass slides by addition of 200 mM Tris-HCl pH 7.4, 50 mM EDTA and 0.5% SDS. At least 200 replication forks were analyzed.

4.2.13 Caspase activation assay

Untreated cell lines and cell lines treated with 0.5 μM ETO were cultured for 24 h and samples were collected. Furthermore, caspase activation assay was performed using the 660 Polycaspase Assay kit (ImmunoChemistry) according to the manufacturer's instruction. All measurements were done in LSRFortessa (BD Biosciences) flow cytometer and FlowJo software was used for quantification.

4.2.14 Repair pathway analysis

Different DNA mixtures containing expression plasmid for the endonuclease I-SceI (pCMV-I-SceI) together with one of the DSB repair substrates (EJ5SceGFP, EJ-EGFP, 5'EGFP/HR-EGFP, HR-EGFP/3'EGFP, HR-EGFP/5'EGFP) and pBS filler plasmid (pBlueScriptII) or wild-type EGFP expression plasmid (for determination of transfection efficiencies) were introduced into the cells by use of a Gene Pulser with Pulse Controller from Bio-Rad (Munich, Germany). Recombination frequencies were measured by quantification of green fluorescent cells containing reconstituted EGFP within the live cell-population (SSC/FSC gate).

4.2.15 Proximity ligation assay

A standard proximity ligation assay (PLA) protocol was followed as mentioned in Study II.

4.2.16   Cell survival analysis

A standard cytotoxicity assay protocol was followed as mentioned in Study II.

4.2.17   Cytogenetic analysis

The blood samples from the studied breast cancer patients were collected at least 3 years after the initial cancer diagnosis. A minimum of 50 Giemsa-banded metaphases for each sample were evaluated by light microscopy and photographed with an automatic chromosome analyzer (CytoVision version 7.2, Applied Imaging). Chromosomal aberrations were divided into five classes: 1) telomeric associations, 2) chromatid/chromosome breaks and deletions, 3) simple chromosomal rearrangements, 4) complex chromosomal rearrangements, and 5) total rearrangements.

4.2.18   Statistical analysis

Statistical differences for normally and non-normally distributed samples were determined using independent samples Student's t-test and Mann-Whitney U-test, respectively. Mean values are reported for normally distributed samples; in the case of non-normally distributed samples, median values are reported. Correlation analysis was done using Spearman's rank correlation coefficient and rho scores were reported. All P-values were two-sided and statistical evaluations were done with IBM SPSS Statistics (version 20.0).

4.3   Ethical issues (Study I & II)

An informed consent to participate in the study was obtained from each individual. Samples obtained from the participants were used for research purposes only and no information on the outcome of studies was given to the participants or their family members. The studies have been approved by the Ethical Board of the Northern Ostrobothnia Health Care District (Studies I and II) and Ethics Committee of the Helsinki University Hospital (Study II).

5   Results

Key results from both Study I and II are presented below in this section. The results presented here show the mechanistic insight behind the elevated breast cancer risk observed in heterozygous *PALB2* (Study I) and *ABRAXAS* (Study II) mutation carriers.

5.1   *PALB2* c.1592delT mutation carriers display DNA replication and cell cycle checkpoint defects (Study I)

LCLs were established from both controls and heterozygous *PALB2* c.1592delT mutation carriers (Neitzel 1986). A list of the LCLs used is presented in the Supplementary Table S1 of Study I.

5.1.1   *Heterozygous mutation in PALB2 c.1592delT results in PALB2 haploinsufficiency*

Using WB analysis, endogenous full-length PALB2 protein levels were quantified and found to be decreased by 48% in the mutation carriers (carriers: 52.1% vs. controls: 100%, Figure 10, Figure 1a in Study I) indicating *PALB2* haploinsufficiency. Since the *PALB2* c.1592delT allele results in a truncated protein, after long exposure, we were able to detect the truncated PALB2 in all mutation carriers but not in the controls (Figure 1b in Study I). Consistent with the WB results, mRNA analysis also indicated that the transcript of the *PALB2* c.1592delT allele was able to escape nonsense-mediated decay. Thus, taken together, the *PALB2* c.1592delT heterozygous mutation results in *PALB2* haploinsufficiency, which could be responsible for the increased breast cancer risk observed in the mutation carriers.
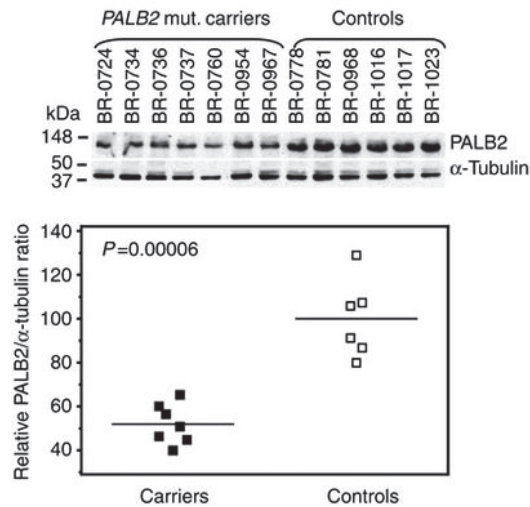
Fig. 10. Heterozygous *PALB2* mutation carriers display reduced PALB2 protein levels under normal growth conditions

5.1.2 PALB2 haploinsufficiency results in elevated replication stress

Cell cycle distribution analysis in a subset of LCLs showed that the carrier cells incorporated larger amounts of EdU, and concurrently, had a smaller fraction of S-phase cells (Supplementary Figure S1 in Study I), indicating that the mutation carrier cells are replicating their DNA faster than the control cells. Due to this, DNA replication phenotypes such as replication origin firing and elongation were studied in the LCLs by using the DNA fiber assay technique. No significant change was observed in the replication elongation rates (Figure 2a in Study I) between the *PALB2* cohorts. However, we noticed closely spaced replication origins in the *PALB2* mutation carrier cells (carriers: 43 kb vs. controls: 62 kb, Figure 2b in Study I). Simultaneously, there was a significant increase in the frequency of 1st pulse replication origins in the mutation carrier cells (carriers: 21.7% vs. controls: 11.8%, Figure 2c in Study I). The observation of closely spaced replication origins and excessive origin firing indicates the presence of replication stress in the mutation carriers due to *PALB2* haploinsufficiency.

5.1.3 Increased replication origin firing is due to elevated Cdk activity

To understand more about the observation of increased replication origin firing in the *PALB2* mutation carrier cells, we studied the regulation of cyclin-dependent kinase (Cdk), as S-phase Cdk activity constitutes a critical regulator of replication firing (Araki 2010, Beck *et al.* 2012). From WB analysis, we found that the inhibitory phosphorylation of Cdk at threonine 14 is significantly reduced in the *PALB2* mutation carrier cells (carriers: 73% vs. controls: 100%, Figure 11, Figure 3a,b in Study I).
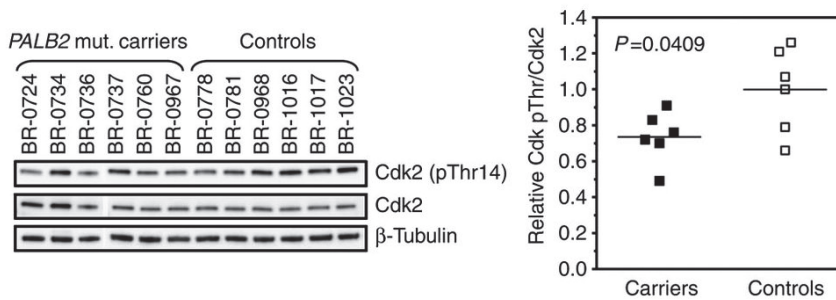
Fig. 11. Heterozygous *PALB2* mutation carriers display increased Cdk activity

In the presence of roscovitine (a Cdk inhibitor), the frequency of 1st pulse origins in the *PALB2* mutation carrier cells dropped dramatically to that of the control levels (carriers: from 26% to 10.5% vs. controls: from 14.2% to 8.8%, Figure 12, Figure 3c in Study I). Taken together, these results indicate that the increased replication origin firing observed in the *PALB2* mutation carrier cells is dependent on increased Cdk activity in the S phase.
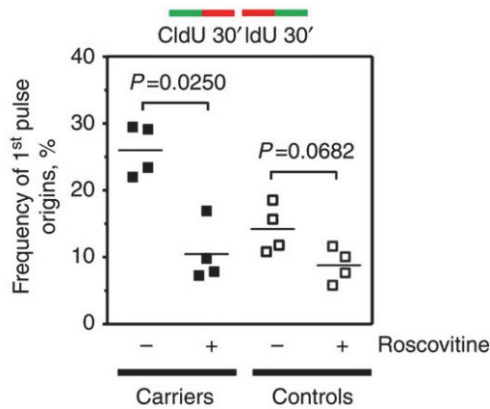
Fig. 12. Increased replication origin firing observed in the *PALB2* mutation carrier cells is due to increased Cdk activity

5.1.4 PALB2 haploinsufficiency results in overexpression of ATR

Since the ATR/CHK1 pathway is essential for the regulation of origin firing (Maya‑Mendoza *et al.* 2007, Petermann & Caldecott 2006, Petermann *et al.* 2010, Syljuåsen *et al.* 2005) changes in its expression were monitored by WB analysis. Though no change was observed in the CHK1 levels (Figure 4a in Study I), ATR levels were significantly increased by almost threefold in the *PALB2* mutation carrier cells (carriers: 275% vs. controls: 100%, Figure 4a,b in Study I). In addition, we also observed a strong inverse correlation between the ATR levels and PALB2 levels (rho = -0.846, Figure 4c in Study I) and inter-origin distance (rho = -0.698, Figure 4d in Study I). These results further validate the presence of aberrant origin firing in the mutation carrier cells, and the overexpression of ATR could be partly related to it.

5.1.5 PALB2 haploinsufficiency results in G2/M checkpoint leakiness

Since PALB2 is a key regulator of G2 checkpoint maintenance (Menzel *et al.* 2011), leakiness in the G2/M checkpoint was assessed in the LCLs in the presence of 0.5 μM ETO (Theunissen & Petrini 2006). After 24 h of ETO addition, nearly all control cells indicated an efficient G2/M checkpoint; however, 57% (4/7) of the mutation carriers had more mitotic cells indicative of G2/M

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checkpoint leakiness (Supplementary Table S2 in Study I). As CHK2 is essential for efficient G2/M checkpoint maintenance (Reinhardt & Yaffe 2009, Zannini *et al*. 2014), phosphorylation of CHK2 at threonine 68 was monitored using WB analysis. CHK2 phosphorylation was significantly reduced in the *PALB2* mutation carrier cells (carriers: 55.0% vs. controls: 108.3%, Figure 6b in study I) after 5 h in the presence of 5 μM ETO. Thus, the observation of reduced CHK2 phosphorylation further supports the G2/M checkpoint leakiness identified in the *PALB2* mutation carrier cells.

5.1.6 Genomic instability due to elevated replication stress and G2/M checkpoint leakiness in the PALB2 mutation carrier cells

Aberration in the maintenance and regulation of DNA replication and cell cycle checkpoint pathways often leads to genomic instability. Therefore, occurrence of genomic instability was assessed in the *PALB2* mutation carriers using untransformed primary cells (fresh peripheral blood lymphocytes). Cytogenetic analysis of the primary cells indicated a significant increase of chromosomal rearrangements in the *PALB2* mutation carriers (carriers: 8.83 vs. controls: 0.67, Table 1 in Study I). The observation of increased genomic instability in the mutation carrier cells is due to defective DNA replication and/or cell cycle checkpoint (Hanahan & Weinberg 2011).

5.2 *ABRAXAS* c.1082G>A mutation carriers display transcriptional misregulation and aberrant DNA damage response (Study II)

LCLs were established from both controls and heterozygous *ABRAXAS* c.1082G>A mutation carriers (Neitzel 1986). A list of the LCLs used is presented in the Supplementary Table S1 of Study II.

5.2.1 Presence of dominant-negative mutant ABRAXAS in the ABRAXAS c.1082G>A mutation carrier cells

To characterize the tumorigenesis pattern, it is important to first establish whether both the mutant ABRAXAS mRNA and protein are stably expressed in *ABRAXAS* c.1082G>A mutation carrier cells by not undergoing nonsense-mediated mRNA decay or proteolysis. From qPCR analysis, we did not observe any significant change in the endogenous ABRAXAS mRNA levels between our case-control

ABRAXAS cohorts (Figure S3A in Study II). RNA sequencing (RNA-Seq) data showed that the *ABRAXAS* c.1082G>A transcripts were stably expressed in the *ABRAXAS* mutation carrier cells and present at levels comparable to those of the wild-type allele ($46.5\% \pm 6.7\%$ of total *ABRAXAS* reads including the c.1082 site; Table S5 in Study II). From WB analysis, we did not observe any significant change in ABRAXAS protein expression between our cohorts (Figure S3B,C in Study II), indicating that the mutant ABRAXAS and wild-type protein have comparable stability and expression levels, as already suggested by previous studies (Solyom *et al*. 2012, Vikrant *et al*. 2015). This indicates that the dominant-negative mutant *ABRAXAS* could be responsible for the increased breast cancer risk observed in the mutation carriers.

5.2.2 BRCA1 mislocalization due to mutant ABRAXAS in the mutation carrier cells

Since the observed *ABRAXAS* single nucleotide variant resides in the NLS domain responsible for nuclear localization, we checked for potential changes in the sub-cellular localization of ABRAXAS and BRCA1 between our cohorts. As expected, we observed significantly decreased nuclear localization of ABRAXAS (z-scores: carriers: -1.97 vs. controls: 0.00, Figure 13A, Figure 1A in Study II) and BRCA1 (z-scores: carriers: -2.12 vs. controls: 0.00, Figure 13B, Figure 1B in Study II) in the *ABRAXAS* mutation carrier cells. Cytoplasmic mislocalization of ABRAXAS and BRCA1 limits the nuclear concentrations of the respective proteins for normal functioning of the DDR pathway.
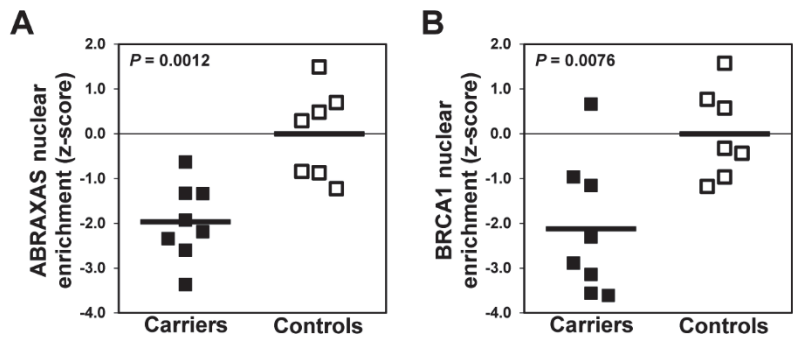
Fig. 13. Heterozygous *ABRAXAS* **mutation carriers display cytoplasmic mislocalization of ABRAXAS and BRCA1**

5.2.3 Reduced ABRAXAS and BRCA1 foci formation in the ABRAXAS mutation carrier cells

During DDR, both ABRAXAS and BRCA1 are known to form foci around the DNA damage sites (Solyom *et al*. 2012, Wang *et al*. 2007). Thus, we compared the foci formation pattern of both ABRAXAS and BRCA1 in our study cohorts. As expected, we observed a reduced number of spontaneous ABRAXAS (z-scores: carriers: -0.57 vs. controls: 0.00, Figure 1C in Study II) and BRCA1 foci (z-scores: carriers: -0.96 vs. controls: 0.00, Figure 1D in Study II) in the *ABRAXAS* mutation carrier cells. In addition, ABRAXAS and BRCA1 foci co-localization was also significantly diminished in the *ABRAXAS* mutation carrier cells (co-localizing foci per nuclei: carriers: 0.83 vs. controls: 2.13, Figure 1E in Study II). PLA also indicated weakened interaction between ABRAXAS and BRCA1, 4 h post IR in the *ABRAXAS* mutation carrier cells (Figure 1F,G in Study II). Observance of fewer ABRAXAS and BRCA1 foci together with diminished co-localization between them is due to the cytoplasmic mislocalization of respective proteins.

5.2.4 Impairment of BRCA1-A complex affects DNA repair pathway choice

Since the BRCA1-A complex is crucial for DSB signaling, we set out to assess differences in the DSB repair pathway usage between our cohorts. No significant changes were noticed in either NHEJ or HR repair pathway usage between our cohorts (Figure 2A,B in Study II). However, we observed a significant decrease in the usage of MMEJ (carriers: 61% vs. controls: 100%, Figure 2C in Study II), SSA (carriers: 86% vs. controls: 100%, Figure 2D in Study II) and SSA+HR (carriers: 76% vs. controls: 100%, Figure 2E in Study II) in the *ABRAXAS* mutation carrier cells.

When analyzing γH2AX (marker for DSBs), whose phosphorylation precedes the choice of DSB repair pathway, we found no significant changes in foci numbers between our cohorts (Figure S5A in Study II). Instead, we observed a significant decrease in γH2AX foci intensity (z-scores: carriers: -2.44 vs. controls: 0.00, Figure 3A in Study II) and global γH2AX levels (carriers: 90.4% vs. controls: 100%, Figure 3B in Study II) in the *ABRAXAS* mutation carrier cells. Cell cycle specific analysis indicated a significant decrease in γH2AX intensity

only in the G1 (carriers: 89.8% vs. controls: 100%, Figure 3C in Study II) and S populations (carriers: 90.1% vs. controls: 100%, Figure 3C in Study II).

5.2.5 Reduced CtIP recruitment in cells from ABRAXAS mutation carriers

Since CtIP is essential for the activation of the MMEJ, SSA and HR pathways (Bennardo *et al*. 2008), we checked for potential changes in its sub-cellular localization and foci formation pattern between our cohorts. Interestingly, CtIP also showed significantly increased cytoplasmic mislocalization (z-scores: carriers: -1.12 vs. controls: 0.00, Figure 3D in Study II) and reduced number of spontaneous foci (z-scores: carriers: -1.17 vs. controls: 0.00, Figure 3E in Study II) in the *ABRAXAS* mutation carrier cells.

5.2.6 Attenuated G2-M checkpoint maintenance in ABRAXAS mutation carrier cells

Since the BRCA1-A complex is a key regulator of G2/M checkpoint maintenance (Solyom *et al*. 2012, Wang *et al*. 2007), leakiness in the G2/M checkpoint was assessed between our cohorts. After 24 h of ETO addition, *ABRAXAS* mutation carriers had significantly more mitotic cells, which is indicative of G2/M checkpoint leakiness (relative M/G2 ratio; carriers: 151% vs. controls: 100%, Figure 4A in Study II). Since ATM and CHK2 play a key role in the regulation of G2/M checkpoint, when analyzing the total protein levels, we found significantly reduced ATM (carriers: 79% vs. controls: 100%, Figure 4B,C in Study II) and CHK2 protein levels (carriers: 72% vs. controls: 100%, Figure 4D,E in Study II) in the *ABRAXAS* mutation carrier cells 24 h post ETO.

5.2.7 DNA damage persistence in ABRAXAS mutation carrier cells

We next looked at the efficiency of DSB repair between our cohorts. At 24 h post ETO treatment in the mutation carrier cells, we found significantly increased numbers of 53BP1 (z-scores: carriers: 3.48 vs. controls: 0.00, Figure 5A in Study II), γH2AX (z-scores: carriers: 1.66 vs. controls: 0.00, Figure 5B in Study II) and RAD51 foci (z-scores: carriers: 2.14 vs. controls: 0.00, Figure 5C in Study II) indicating more residual DNA damage. Apart from a slight increase in the frequency of 1st pulse replication origins fired (carriers: 17.7% vs. controls:

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11.9%, Figure 6D in Study II), no other DNA replication aberrations were observed in the *ABRAXAS* mutation carrier cells. Similarly, apart from a possible slight increase in simple chromosomal rearrangements (carriers: 2.9 vs. controls: 0.0, Table S6 in Study II), no other chromosomal aberrations were observed in the *ABRAXAS* mutation carrier cells.

5.2.8 Cell lines from ABRAXAS mutation carriers display an aberrant transcriptional profile

In unchallenged cells, the BRCA1 (carriers: 80% vs. controls: 100%, Figure 7A,C in Study II), ATM (carriers: 84% vs. controls: 100%, Figure 7B,D in Study II) and BRCC36 (carriers: 81% vs. controls: 100%, Figure 7B,E in Study II) protein levels appeared to be significantly reduced in the mutation carriers. Concurrently, qPCR analysis also indicated downregulation of *ATM* (carriers: 82% vs. controls: 100%, Figure S14B in Study II), *BRCC36* (carriers: 63% vs. controls: 100%, Figure S14C in Study II) and *PALB2* (carriers: 79% vs. controls: 100%, Figure S14D in Study II) mRNA in the mutation carriers. RNA expression analysis using a RT² profiler PCR array also further indicated transcriptional misregulation of several genes functioning in the DNA damage signaling pathway (Table S7 in Study II).

Therefore, we performed RNA-Seq analysis to evaluate differences in the global transcriptome profile between our cohorts. *DESeq2* analysis identified 60 statistically significant DEGs in *ABRAXAS* mutation carriers (Table S8 in Study II). In this set of DEGs, *Ingenuity Pathway Analysis* (IPA) identified "Cancer" to be the top-ranked disease associated with the mutation carriers (Figure 14, Figure 8A and Table S8 in Study II).
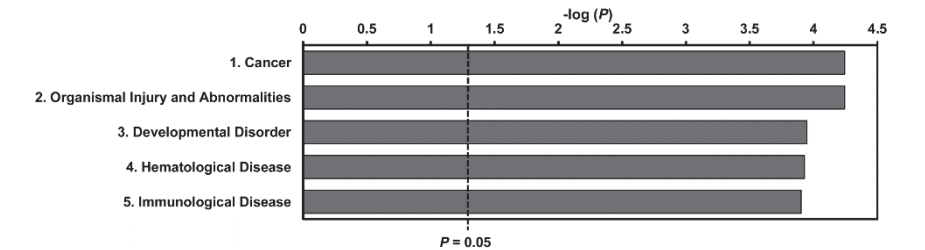
Fig. 14. Top-ranked diseases reported by IPA to be enriched in *ABRAXAS* mutation carrier cells

Next, we performed *edgeR* analysis to find a wider range of DEGs to look for enrichment of particular transcription factor binding/chromatin binding factors and epigenetic modifications in the mutation carriers. *EdgeR* analysis identified 586 genes to be differentially regulated between our cohorts (Table S9 in Study II). ENRICHR analysis in this set of DEGs primarily implicated enriched binding of PRC2 subunits such as SUZ12 and EZH2 to our gene set (Table S9 in Study II). Simultaneously, ENRICHR analysis against the ENCODE histone modification database indicated enrichment of the H3K27me3 epigenetic mark to our dataset (Table S9 in Study II).

6 Discussion

It is well known that hereditary mutations in DDR genes often lead to genomic instability and ultimately tumor development (Negrini *et al.* 2010). However, the molecular mechanism of how these DDR deficiencies promote genomic instability and malignancy is not well understood. It is of paramount importance to learn how genomic instability arises at the cellular level in the carriers of various specific DDR gene mutations. Understanding how genomic instability arises will provide us a greater understanding about cancer development. More importantly, these studies could also provide new targets for therapeutic interventions during cancer treatments.

As discussed in section 2.4, genomic instability and tumorigenesis can, in addition to the well-documented two-hit hypothesis (Knudson 1971), also arise due to haploinsufficiency and dominant-negative phenotype (Payne & Kemp 2005). To better understand how a specific hereditary susceptibility gene mutation results in genomic instability due to haploinsufficiency and/or dominant-negative phenotype, studies have to be performed in patient-derived material or models. However, functional characterization of breast cancer promoting mutations has traditionally been based on analysis of tumor-derived cell lines, cellular knockdown or knockout approaches, mouse models and permanently or transiently induced mutant allele overexpression.

So far, only a small number of studies have attempted to analyze the molecular and biological effects of germline mutations at the heterozygous state using patient-derived material or models. This may be due to genetic heterogeneity of study material or limited availability of suitable human samples along with observation of less pronounced or recessive phenotypes in the heterozygous state. Nevertheless, even in the few studies performed using patient-derived materials, increasing evidence points to genomic instability and tumorigenesis driven by haploinsufficiency (or possibly mild dominant-negative) phenotypes (Nikkilä *et al.* 2013, Pathania *et al.* 2014, Vaclová *et al.* 2015).

6.1 Genomic instability and tumorigenesis driven by haploinsufficiency in *PALB2* c.1592delT mutation carriers (Study I)

Endogenous full-length PALB2 protein levels were found to be decreased by 48% in the mutation carriers when compared to controls. This indicates that the defects

observed in DNA replication and cell cycle checkpoint are indeed due to *PALB2* haploinsufficiency in the mutation carriers. The observation of *PALB2* c.1592delT mRNA and truncated PALB2 protein in the mutation carriers indicates that a dominant-negative phenotype could also be partly responsible for the defects observed in DNA replication and cell cycle checkpoint activities.

Larger amount of EdU incorporation along with smaller fraction of S-phase cells indicates a more rapid overall DNA replication in the *PALB2* mutation carrier cells. Simultaneously, the observation of closely spaced replication origins and excessive origin firing further supports the rapid DNA replication seen in the *PALB2* mutation carrier cells. In an independent study, it has been shown that homozygous inactivation of *FANCM* also results in excessive origin firing without affecting the elongation rates as observed here (Schwab *et al.* 2010). Similarly, cells deficient for *XRCC2* or *RAD51* also show closely spaced replication origins and excessive origin firing as observed in this study (Daboussi *et al.* 2008).

Previously, it has been shown that elevated levels of Cdk activity cause an increase in global DNA replication due to excessive origin firing and reduced inter-origin distances (Beck *et al.* 2012). Similarly, in the current study, we have also observed an increased Cdk activity in the *PALB2* mutation carrier cells. Thus, the increase in Cdk activity might be responsible for the observation of excessive origin firing in the *PALB2* mutation carrier cells. Cdk inhibition using roscovitine in the *PALB2* mutation carrier cells dramatically reduced the number of replication origins fired, further indicating the dependence of origin firing upon Cdk activity.

Thus, taken together, our results indicate that the elevated Cdk activity is responsible for the excessive origin firing, which in turn is responsible for the rapid DNA replication observed in the *PALB2* mutation carrier cells. These results further confirm the presence of replication stress due to *PALB2* haploinsufficiency in the mutation carrier cells. Interestingly, in a later independent study, replication stress was also observed due to *BRCA1* haploinsufficiency in heterozygous *BRCA1* mutation carriers (Pathania *et al.* 2014). Defective stalled fork repair (SFR) and consequently, higher frequency of collapsed forks, indicative of replication stress, was observed in the *BRCA1* mutation carrier cells (Pathania *et al.* 2014).

Notably, these studies (Study I of this thesis and Pathania *et al.* (2014)) used slightly different approaches to uncover the presence of replication stress in the assessed mutation carrier cells. In the current study (Study I), we have analyzed *PALB2* mutation carrier cells for differences in origin activation, whereas Pathania

et al. (2014) analyzed *BRCA1* mutation carrier cells for differences in replication fork protection. Of note, Pathania *et al*. (2014) found replication stress in primary human mammary epithelial cells (HMECs) and fibroblasts, further indicating that the replication stress observed in our current study is not LCLs-specific.

Activation of dormant origins has been observed during replication fork arrest (Karnani & Dutta 2011), which is tightly regulated by ATR and its downstream kinase CHK1 (Liu *et al*. 2000, Zhao & Piwnica-Worms 2001). Both ATR and CHK1 play a key role in the restriction of distant origin firing and simultaneous activation of dormant origins during low levels of replication stress (Ge & Blow 2010). In addition, ATR and CHK1 are also responsible for normal S phase progression and replication timing (Maya-Mendoza *et al*. 2007, Petermann & Caldecott 2006, Petermann *et al*. 2010, Syljuåsen *et al*. 2005). Though we did not observe any significant change in the CHK1 protein levels between our *PALB2* cohorts, ATR levels were significantly increased (by almost threefold) in the *PALB2* mutation carrier cells

A genetic screen performed by Menzel *et al*. (2011) identified both BRCA2 and PALB2 as key regulators of G2 checkpoint maintenance. siRNA-mediated downregulation of PALB2 resulted in the abrogation of G2 checkpoint (Menzel *et al*. 2011). Similarly, in the current study, we have observed G2/M checkpoint leakiness already in the heterozygous *PALB2* mutation carrier cells. Most importantly, ATM and its downstream effector kinase CHK2 are responsible for the efficient activation and maintenance of G2/M checkpoint (reviewed in (Guleria & Chandna 2016, Zannini *et al*. 2014)). In response to DNA damage, CHK2 is rapidly phosphorylated and thereby activated by ATM; active CHK2 further phosphorylates Cdc25C on serine-216 to prevent entry into mitosis (Matsuoka *et al*. 1998). In the *PALB2* mutation carrier cells, we have observed significantly reduced levels of active CHK2 at late time points after DNA damage, further supporting the finding of G2/M checkpoint leakiness.

Abnormalities or maintenance defects in key cellular pathways such as DNA replication and cell cycle checkpoints often lead to increased genomic instability (Hanahan & Weinberg 2011). Since the LCLs used in the functional analysis were transformed using EBV, we used primary untransformed cells to test for the presence of increased genomic instability in the *PALB2* mutation carriers. As expected, indeed both simple and complex chromosomal rearrangements were found to be significantly higher in the *PALB2* mutation carriers. The observation of genomic instability in primary untransformed cells indicates that the described mechanisms of genome destabilization operate at the organism level as well.

In a recent report from our group, we found that the *PALB2* mutation carrier cells use both MMEJ and SSA DSB repair pathways to a significantly higher extent compared to control cells (Obermeier *et al.* 2016). Both MMEJ and SSA are highly mutagenic DSB repair pathways and their augmented use results in increased chromosomal aberrations (reviewed in Bhargava *et al.* 2016, Seol *et al.* 2017). Thus, results from both of these studies indicate that the overuse of the highly mutagenic MMEJ and SSA pathways could be responsible for the observation of increased chromosomal aberration in the *PALB2* mutation carrier cells.

In summary, as shown in Figure 15, *PALB2* c.1592delT causes a haploinsufficiency phenotype in the mutation carrier cells resulting in elevated replication stress and G2/M checkpoint maintenance defects. Due to these aberrations, elevated genomic instability and eventually tumorigenesis are observed. Thus, genomic instability and tumorigenesis are driven by haploinsufficiency in *PALB2* c.1592delT mutation carriers. Therefore, it appears that loss of tumor suppressor/caretaker gene heterozygosity is not always mandatory for tumorigenesis.
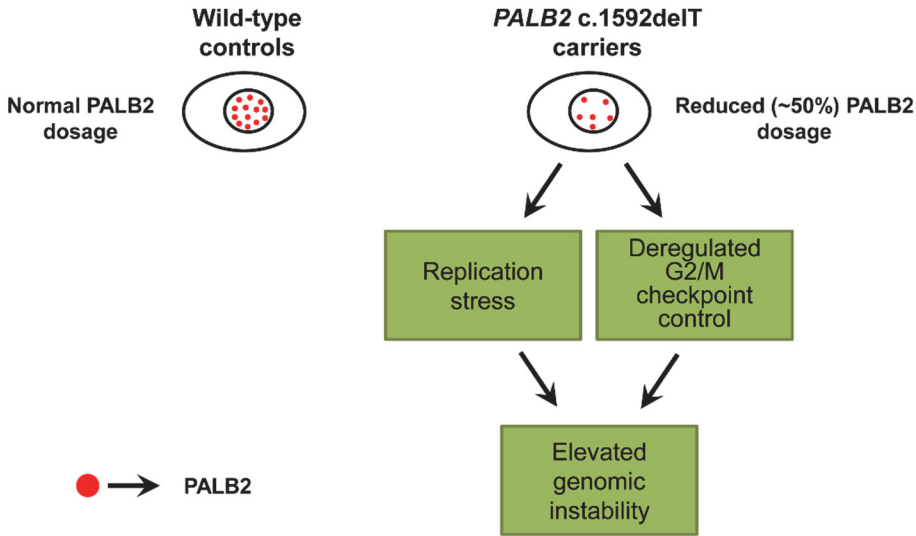

Fig. 15. Proposed model on how the heterozygous Finnish *PALB2* c.1592delT mutation may drive early-stage tumorigenesis in the carrier individuals.

The presence of 'conditional haploinsufficiency' as proposed by Bartek *et al*. (2007) was observed in the heterozygous *BRCA1* mutation carriers (Pathania *et al*. 2014). No significant change in the sensitivity to olaparib (PARP inhibitor) was observed between $BRCA1^{mut/+}$ and $BRCA1^{+/+}$ cells when they are not pre-exposed to DNA damaging agents like UV (Pathania *et al*. 2014). However, after pre-exposure to UV, $BRCA1^{mut/+}$ cells became olaparib-sensitive, indicating 'conditional haploinsufficiency' for HR-DSBR in $BRCA1^{mut/+}$ cells facing sufficient replication stress (Pathania *et al*. 2014). Thus, Pathania *et al*. (2014) reported the existence of innate haploinsufficiency for SFR and 'conditional haploinsufficiency' for HR-DSBR in their $BRCA1^{mut/+}$ cells.

Similar to Pathania *et al*. (2014), 'conditional haploinsufficiency' has also been observed in the *PALB2* mutation carrier cells, where G2/M checkpoint leakiness was only observed after 24 h of ETO treatment, but not in the undisturbed state or at early time points after ETO addition (Supplementary Table S2 of Study I). On the other hand, the observation of replication stress in the *PALB2* mutation carrier cells is due to innate haploinsufficiency as increased origin firing was already observed in the undisturbed state. Thus, taken together, the results of the current study support the existence of innate haploinsufficiency for replication stress and 'conditional haploinsufficiency' for G2/M checkpoint maintenance in the *PALB2* mutation carrier cells.

Genomic instability and tumorigenesis due to haploinsufficiency has previously been shown by several studies using isogenic cell lines, tumor-derived cell lines or mouse models (Barrett *et al*. 2015, Cochran *et al*. 2015, Coschi *et al*. 2014, Gonzalez-Vasconcellos *et al*. 2013, Konishi *et al*. 2011, Savage *et al*. 2014, Tan *et al*. 2017). However, to our best knowledge, this is the first study to show how haploinsufficiency promotes genomic instability and tumorigenesis in a patient-derived cohort. Since PALB2 facilitates the interaction between BRCA1 and BRCA2, we expect to see similar phenotypic changes also in cells heterozygous for mutations in *BRCA1* and *BRCA2*.

6.2   Early-stage tumorigenesis in *ABRAXAS* c.1082G>A mutation carriers due to a dominant-negative effect mutation (Study II)

From RNA sequencing, we found stable expression of mutant *ABRAXAS* mRNA in all the carrier cell lines, indicating that the mutant mRNA is not undergoing nonsense-mediated mRNA decay. WB analysis also showed comparable ABRAXAS protein expression among our cohorts, indicating that the mutant

protein is also not undergoing proteolysis. This suggests that the defects observed in transcription, cell cycle checkpoint and DSB repair pathway choice could be due to the dominant-negative effect of mutant *ABRAXAS* in the carrier individuals.

In a previous report from our group, it was shown that the epitope-tagged mutant ABRAXAS protein expressed at near endogenous levels in HeLa, U2OS and MCF10A cells was stable and did not undergo proteolysis (Solyom *et al.* 2012). In a different study, it was shown that both the ABRAXAS mutant and wild-type protein forms have similar secondary structural patterns with the same number of α-helixes and β-sheets, and also similar tertiary structure with comparable Tm (protein folding pattern) (Vikrant *et al.* 2015).

The ABRAXAS and BRCA1 cytoplasmic mislocalization observed in the *ABRAXAS* mutation carrier cells is due to the missense single nucleotide mutation that resides in the NLS domain of *ABRAXAS*. Similar to this observation, we reported earlier that the HA-tagged mutant ABRAXAS protein predominantly localized in the cytoplasm of HeLa, U2OS and MCF10A cells (Solyom *et al.* 2012). The NLS domain is a short stretch of amino acid responsible for the nuclear import of proteins (Kosugi *et al.* 2009). Recently, a missense mutation in the NLS sequence of *POU4F3* (c.982A>G, p.Lys328Glu) was found to be associated with deafness in a Taiwanese family (Lin *et al.* 2017).

In the *ABRAXAS* mutation carrier cells, the observation of fewer ABRAXAS and BRCA1 foci together with diminished co-localization between them is due to the cytoplasmic mislocalization of the respective proteins. Similar to this, in our previous report, we also observed significantly less ABRAXAS and BRCA1 foci in Hela, U2OS and MCF10A cells overexpressing mutant ABRAXAS protein (Solyom *et al.* 2012). Most importantly, in our earlier report, co-immunoprecipitation experiments revealed that the epitope-tagged mutant ABRAXAS protein formed BRCA1-A complexes as efficiently as the wild-type protein, but the BRCA1-A complex containing the mutant ABRAXAS was localized predominately in the cytoplasm (Solyom *et al.* 2012). This indicates that only the localization, but not the formation of the BRCA1-A complex is altered in the *ABRAXAS* mutation carrier cells.

Several studies have shown that the BRCA1-A complex is responsible for DNA DSB signaling. In addition, this complex plays a major role in the choice of DNA DSB repair pathway (Coleman & Greenberg 2011, Hu *et al.* 2011b, Typas *et al.* 2015). Although in our current study no change was observed in NHEJ and HR repair pathway usage, activation of SSA and MMEJ is suppressed in *ABRAXAS* mutation carrier cells. These results indicate that activation of DSB

repair pathways, which utilize excessive nucleolytic end trimming such as SSA and MMEJ, is suppressed in *ABRAXAS* mutation carrier cells. As reported earlier, this result is exactly the opposite to the repair pathway usage observed in the Finnish heterozygous *PALB2* c.1592delT carriers where a significantly higher usage of the MMEJ, SSA and SSA+HR repair pathways was seen (Obermeier *et al*. 2016).

γH2AX is a well-known marker for DNA DSBs and its phosphorylation precedes the choice of DSB repair pathway. The presence of less γH2AX in the *ABRAXAS* mutation carrier cells was unforeseen to us and the exact cause of it cannot be explained. However, similar to this result, another study reported significantly reduced γH2AX foci in *ABRAXAS* knockdown MCF7 cells transfected with *ABRAXAS* mutants, such as p.Gly39Val and p.Thr141Ile (Renault *et al*. 2016). Results from these two studies indicate that ABRAXAS, by a mechanism yet to be discovered, is responsible for the efficient phosphorylation of H2AX.

Several studies have shown that activation of CtIP and its recruitment to DNA damage sites is essential for the activation of both MMEJ and SSA pathways (Bennardo *et al*. 2008, Clerici *et al*. 2005, Lee-Theilen *et al*. 2011, Rass *et al*. 2009, Zhang & Jasin 2011). Surprisingly, similar to ABRAXAS and BRCA1, we have also observed cytoplasmic mislocalization of CtIP and consequently fewer CtIP foci in the *ABRAXAS* mutation carrier cells. Thus, the presence of reduced CtIP in the nucleus and its compromised recruitment to DNA damage sites could be the reason why both MMEJ and SSA are suppressed in our carrier cells. The compromised CtIP recruitment observed in the *ABRAXAS* mutation carrier cells is due to the compromised recruitment of BRCA1, similarly to that recently shown by Livingston and colleagues in their studies of BRCA1[mut/+] cells (Pathania *et al*. 2014).

Several studies have recognized that the BRCA1-A complex is essential for the regulation of the cell cycle, particularly G2/M checkpoint control (Solyom *et al*. 2012, Wang *et al*. 2007). Similar to the *PALB2* mutation carrier cells in study I, we have observed G2/M checkpoint leakiness also in the *ABRAXAS* mutation carrier cells. In line with this, G2/M checkpoint leakiness was also observed after irradiation in 293T cells transfected with epitope-tagged mutant ABRAXAS protein (Solyom *et al*. 2012). Most importantly, in the *ABRAXAS* mutation carrier cells, we have also observed significant downregulation of ATM and its downstream effector kinase CHK2. As discussed in section 2.5.2, both ATM and

CHK2 play a key role in the efficient activation and maintenance of the G2/M checkpoint.

The presence of significantly higher 53BP1, γH2AX and RAD51 foci indicates persistence of DNA damage in the *ABRAXAS* mutation carrier cells. These results demonstrate that the *ABRAXAS* mutation carrier cells are unable to efficiently repair the DNA DSBs and are therefore left with more residual DNA damage. In an independent study, it was shown that *ABRAXAS*-deleted mouse embryonic fibroblasts (MEFs) had much higher percentage of unrepaired DNA damage after IR (Castillo *et al*. 2014).

In the *ABRAXAS* mutation carrier cells, there is only a marginal increase in the frequency of 1st pulse replication origins fired. It was previously shown that ABRAXAS is not required during replication as no changes were observed in stalled replication forks in *ABRAXAS*-deleted MEFs (Castillo *et al*. 2014). We believe that the observation of marginally increased origin firing in *ABRAXAS* mutation carrier cells is possibly due to the limited availability of BRCA1 as it is critically needed during replication (Schlacher *et al*. 2012).

Similarly, there was a potential increase in simple chromosomal rearrangements in the *ABRAXAS* mutation carrier cells, but limitations in the availability of further study material precluded a conclusive evaluation. Increased numbers of simple chromosomal aberrations have been reported after knockout or knockdown of multiple BRCA1-A complex members (Castillo *et al*. 2014, Coleman & Greenberg 2011, Hu *et al*. 2011b). Taken together, *ABRAXAS* mutation carrier cells display only a moderate increase in origin firing and chromosomal instability. This suggests that the phenotypes observed in the mutation carriers are not primarily driven by DNA replication stress.

As discussed in section 2.5.1, increased DNA repair by MMEJ and SSA as well as extensive replication stress such as that observed in the *PALB2* mutation carriers has been shown to promote gross chromosomal aberration (Burrell *et al*. 2013, Iliakis *et al*. 2015, Nikkilä *et al*. 2013, Obermeier *et al*. 2016). Unlike the situation for *PALB2*, in *ABRAXAS* mutation carrier cells we did not observe any gross chromosomal aberrations which could be due to the reduced MMEJ and SSA pathway usage along with absence of extensive replication stress.

Previously, the BRCA1-A complex has been implicated in transcriptional regulation through its association with RAP80 (Wang *et al*. 2007). WB, qPCR and RT² profiler PCR array analyses indicated transcriptional misregulation of several genes in the *ABRAXAS* mutation carrier cells. Namely, a large number of genes implicated in DNA repair and damage response were found to be moderately, but

significantly downregulated. IPA analysis of the RNA-Seq data identified "Cancer" as the top-ranked disease associated with the *ABRAXAS* mutation carriers, which fits well with the observation of multiple tumors in these families (Solyom *et al*. 2012). The observation of hematological and immunological diseases as high-ranking IPA hits was not surprising because of the hemapoietic origin of the LCLs (Vaclová *et al*. 2015).

On the other hand, *EdgeR* and ENRICHR analysis primarily implicated enriched binding of PRC2 subunits, such as SUZ12 and EZH2 to our gene set in the *ABRAXAS* mutation carrier cells. The EZH2 subunit in the PRC2 complex trimethylates histone H3 at K27 to form H3K27me3, which is a major reversible epigenetic mark for transcriptional silencing (Hansen *et al*. 2008). Interestingly, ENRICHR analysis against the ENCODE histone modification database also simultaneously indicated enrichment of H3K27me3 in the same set of DEGs in *ABRAXAS* mutation carrier cells. Thus, taken together, transcriptional downregulation of multiple genes in the *ABRAXAS* mutation carrier cells could be due to globally increased PRC2 binding and H3K27me3 trimethylation.

Several studies have shown that BRCA1 plays an important role in transcriptional regulation (reviewed in Mullan *et al*. 2006, Savage & Harkin 2015). Most importantly, it was shown that BRCA1 inhibits the methyltransferase activity of PRC2 complex by targeting EZH2. BRCA1 inhibition increased EZH2 targeting and subsequently elevated H3K27me3 levels at PRC2 target loci (Wang *et al*. 2013). In our *ABRAXAS* mutation carrier cells, we have also noticed a moderate but significant downregulation of BRCA1 and this, in connection with the partial cytoplasmic mislocalization of BRCA1, could be the reason for the observation of PRC2 complex enrichment and elevated H3K27me3-mediated epigenetic silencing of several genes.

In summary, as shown in Figure 16, ABRAXAS cytoplasmic mislocalization and its compromised recruitment to the DNA damage sites is due to the mutation in the NLS sequence of the *ABRAXAS* gene. Furthermore, cytoplasmic mislocalization of BRCA1 and its compromised recruitment to the DNA damage sites is due to the dominant-negative effect of mutant ABRAXAS. Reduced BRCA1 localization and foci formation in the *ABRAXAS* mutation carrier cells results in attenuated BRCA1 signaling, altered DSB repair pathway choice, deregulated G2/M checkpoint maintenance, and transcriptional misregulation.
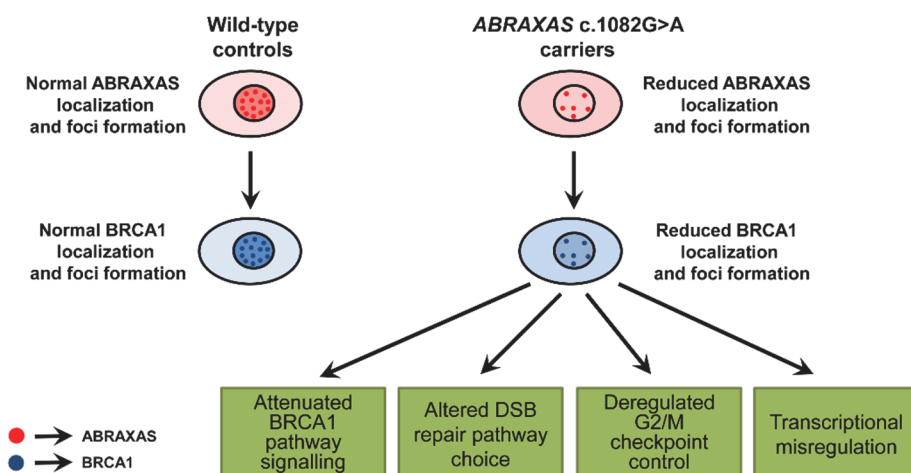
Fig. 16. Proposed model on how the heterozygous Finnish *ABRAXAS* c.1082G>A mutation may drive early-stage tumorigenesis in the carrier individuals.

Complex combinations of rather small changes in the indicated pathways may be collectively required to promote tumorigenesis in the mutation carriers. As observed in the current Study (II), we expect to see similar phenotypic changes in cells heterozygous for mutations in other BRCA1-A members. Finally, the NLS sequence of *ABRAXAS* is frequently targeted in sporadic cancers (Castillo *et al.* 2014), suggesting that also somatic mutations disrupting the NLS are biologically relevant and may thus represent a driver of sporadic cancers.

7 Concluding remarks

Currently, only ~30% of hereditary breast cancer cases have been explained by mutations in the known breast cancer susceptibility genes (Rousset-Jablonski & Gompel 2017). A majority of the known breast cancer susceptibility genes encode proteins with integral roles in the DDR pathways (Mantere *et al*. 2017). It is well established that hereditary mutations in the known DDR genes often lead to genomic instability and eventually to tumorigenesis (Negrini *et al*. 2010). Nevertheless, the molecular mechanism of how these DDR deficiencies promote genomic instability and tumorigenesis is not well studied. Thus, the purpose of this thesis was to acquire mechanistically a better understanding of how genomic instability and tumorigenesis arise in the heterozygous *PALB2* and *ABRAXAS* mutation carriers. Listed below are the major observations and conclusions from studies I and II:

1. *PALB2* c.1592delT causes a haploinsufficiency phenotype in the mutation carrier cells, resulting in elevated replication stress and G2/M checkpoint maintenance defects.
2. Elevated replication stress together with G2/M checkpoint leakiness is responsible for the observation of genomic instability in the *PALB2* mutation carrier cells. Therefore, loss of tumor suppressor/caretaker gene heterozygosity is not always mandatory for tumorigenesis.
3. ABRAXAS cytoplasmic mislocalization and its compromised recruitment to the DNA damage sites is due to the mutation in the NLS sequence of the *ABRAXAS* gene. Furthermore, BRCA1 cytoplasmic mislocalization and its compromised recruitment to the DNA damage sites is due to the dominant-negative effect of mutant ABRAXAS.
4. Reduced BRCA1 localization and foci formation in the *ABRAXAS* mutation carrier cells results in attenuated BRCA1 signaling, altered DSB repair pathway choice, deregulated G2/M checkpoint maintenance and transcriptional misregulation.
5. Finally, the tumorigenesis pattern observed in the *PALB2* mutation carriers is completely different from what was observed in the *ABRAXAS* mutation carriers.

8   Future prospects

1. The observance of genomic instability in the *PALB2* mutation carrier cells due to elevated replication stress offers a new window for therapeutic targeting. Thus, future studies have to be performed to find out whether replication stress can be targeted to delay the onset of tumorigenesis or target the tumor clones in the latter stages.

2. Similarly, since G2/M checkpoint leakiness was observed in both *ABRAXAS* and *PALB2* mutation carrier cells, future studies have to be conducted to find out whether it can be targeted to delay the onset of tumorigenesis, or alternatively, target the tumor clones in the latter stages.

3. Mechanistically, suppression of MMEJ and SSA activation in the *ABRAXAS* mutation carrier cells is not fully explained. Thus, future studies have to be performed to assess why specifically MMEJ and SSA are suppressed in the *ABRAXAS* mutation carrier cells.

4. Both *PALB2* and *BRCA2* define a distinct genetic, clinical and functional entity in the Fanconi anemia pathway. Thus, similar studies have to be performed to check whether the phenotype observed in the heterozygous *BRCA2* mutation carrier cells is similar to that of *PALB2* mutation carrier cells.

5. In addition to *BRCA1* and *ABRAXAS*, germline mutations in other BRCA1-A complex members, such as *RAP80* and *MERIT40,* are also associated with inherited predisposition to breast and ovarian cancers. Thus, similar studies have to be performed to check whether the phenotype observed in the heterozygous *RAP80* and *MERIT40* mutation carrier cells is similar to that of *ABRAXAS* mutation carrier cells.

6. Finally, the tumorigenesis pattern observed in the *PALB2* mutation carriers is completely different from what was observed in the *ABRAXAS* mutation carriers. This indicates that though both ABRAXAS and PALB2 function in the same biological pathway (but in different niches), the pattern of tumorigenesis between them remains starkly different from what was previously envisioned. This observation warrants further investigations to analyze the tumorigenesis pattern both for different mutations in the same gene as well as in different tumor suppressor genes.

List of references

Ahlskog JK, Larsen BD, Achanta K & Sørensen CS. (2016) ATM/ATR-mediated phosphorylation of PALB2 promotes RAD51 function. EMBO Rep 17(5): 671.

Al-Ejeh F, Kumar R, Wiegmans A, Lakhani SR, Brown MP & Khanna KK (2010) Harnessing the complexity of DNA-damage response pathways to improve cancer treatment outcomes. Oncogene 29: 6085.

Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale A, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjord JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinsk M, Jager N, Jones DTW, Jones D, Knappskog S, Kool M, Lakhani SR, Lopez-Otin C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt ANJ, Valdes-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR, Australian Pancreatic Cancer GI, ICGC Breast CC, ICGC MMML-Seq C, PedBrain I, Zucman-Rossi J, Andrew Futreal P, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ & Stratton MR (2013) Signatures of mutational processes in human cancer. Nature 500(7463): 415 – 421.

Ali R, Rakha EA, Madhusudan S & Bryant HE (2017) DNA damage repair in breast cancer and its therapeutic implications. Pathology 49(2): 156 – 165.

Althuis MD, Dozier JM, Anderson WF, Devesa SS & Brinton LA (2005) Global trends in breast cancer incidence and mortality 1973–1997. Int J Epidemiol 34(2): 405 – 412.

Antoniou AC, Wang X, Fredericksen ZS, McGuffog L, Tarrell R, Sinilnikova OM, Healey S, Morrison J, Kartsonaki C, Lesnick T, Ghoussaini M, Barrowdale D, EMBRACE, Peock S, Cook M, Oliver C, Frost D, Eccles D, Evans DG, Eeles R, Izatt L, Chu C, Douglas F, Paterson J, Stoppa-Lyonnet D, Houdayer C, Mazoyer S, Giraud S, Lasset C, Remenieras A, Caron O, Hardouin A, Berthet P, GEMO Study Collaborators, Hogervorst FB, Rookus MA, Jager A, van den Ouweland A, Hoogerbrugge N, van der Luijt RB, Meijers-Heijboer H, Gomez Garcia EB, HEBON, Devilee P, Vreeswijk MP, Lubinski J, Jakubowska A, Gronwald J, Huzarski T, Byrski T, Gorski B, Cybulski C, Spurdle AB, Holland H, kConFab, Goldgar DE, John EM, Hopper JL, Southey M, Buys SS, Daly MB, Terry MB, Schmutzler RK, Wappenschmidt B, Engel C, Meindl A, Preisler-Adams S, Arnold N, Niederacher D, Sutter C, Domchek SM, Nathanson KL, Rebbeck T, Blum JL, Piedmonte M, Rodriguez GC, Wakeley K, Boggess JF, Basil J, Blank SV, Friedman E, Kaufman B, Laitman Y, Milgrom R, Andrulis IL, Glendon G, Ozcelik H, Kirchhoff T, Vijai J, Gaudet MM, Altshuler D, Guiducci C, SWE-BRCA, Loman N, Harbst K, Rantala J, Ehrencrona H, Gerdes AM, Thomassen M, Sunde L, Peterlongo P, Manoukian S, Bonanni B, Viel A, Radice P, Caldes T, de la Hoya M, Singer CF, Fink-Retter A, Greene MH, Mai PL, Loud JT, Guidugli L, Lindor NM, Hansen TV, Nielsen FC, Blanco I, Lazaro C, Garber J, Ramus SJ, Gayther SA, Phelan C, Narod S, Szabo CI, MOD SQUAD, Benitez J, Osorio A,

Nevanlinna H, Heikkinen T, Caligo MA, Beattie MS, Hamann U, Godwin AK, Montagna M, Casella C, Neuhausen SL, Karlan BY, Tung N, Toland AE, Weitzel J, Olopade O, Simard J, Soucy P, Rubinstein WS, Arason A, Rennert G, Martin NG, Montgomery GW, Chang-Claude J, Flesch-Janys D, Brauch H, GENICA, Severi G, Baglietto L, Cox A, Cross SS, Miron P, Gerty SM, Tapper W, Yannoukakos D, Fountzilas G, Fasching PA, Beckmann MW, Dos Santos Silva I, Peto J, Lambrechts D, Paridaens R, Rudiger T, Forsti A, Winqvist R, Pylkäs K, Diasio RB, Lee AM, Eckel-Passow J, Vachon C, Blows F, Driver K, Dunning A, Pharoah PP, Offit K, Pankratz VS, Hakonarson H, Chenevix-Trench G, Easton DF & Couch FJ (2010) A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet 42(10): 885 – 892.

Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkäs K, Roberts J, Lee A, Subramanian D, De Leeneer K, Fostira F, Tomiak E, Neuhausen SL, Teo ZL, Khan S, Aittomäki K, Moilanen JS, Turnbull C, Seal S, Mannermaa A, Kallioniemi A, Lindeman GJ, Buys SS, Andrulis IL, Radice P, Tondini C, Manoukian S, Toland AE, Miron P, Weitzel JN, Domchek SM, Poppe B, Claes KBM, Yannoukakos D, Concannon P, Bernstein JL, James PA, Easton DF, Goldgar DE, Hopper JL, Rahman N, Peterlongo P, Nevanlinna H, King M, Couch FJ, Southey MC, Winqvist R, Foulkes WD & Tischkowitz M (2014) Breast-cancer risk in families with mutations in PALB2. N Engl J Med 371(6): 497 – 506.

Araki H (2010) Cyclin-dependent kinase-dependent initiation of chromosomal DNA replication. Curr Opin Cell Biol 22(6): 766 – 771.

Ardito F, Giuliani M, Perrone D, Troiano G & Muzio LL (2017) The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review). Int J Mol Med 40(2): 271 – 280.

Arnoult N, Correia A, Ma J, Merlo A, Garcia-Gomez S, Maric M, Tognetti M, Benner CW, Boulton SJ, Saghatelian A & Karlseder J (2017) Regulation of DNA repair pathway choice in S and G2 phases by the NHEJ inhibitor CYREN. Nature 549: 548.

Ashworth A, Lord C & Reis-Filho J (2011) Genetic interactions in cancer progression and treatment. Cell 145(1): 30 – 38.

Auerbach AD (2009) Fanconi anemia and its diagnosis. Mut Res/Fundamental and Molecular Mechanisms of Mutagenesis; Fanconi anemia: A paradigm of discovering molecular pathways from patients 668(1): 4 – 10.

Barrett CW, Reddy VK, Short SP, Motley AK, Lintel MK, Bradley AM, Freeman T, Vallance J, Ning W, Parang B, Poindexter SV, Fingleton B, Chen X, Washington MK, Wilson KT, Shroyer NF, Hill KE, Burk RF & Williams CS (2015) Selenoprotein P influences colitis-induced tumorigenesis by mediating stemness and oxidative damage. J Clin Invest 125(7): 2646 – 2660.

Barrett SV (2010) Breast cancer. J R Coll Physicians Edinb 40(4): 335 – 339.

Bartek J, Lukas J & Bartkova J (2007) DNA damage response as an anti-cancer barrier: Damage threshold and the concept of 'conditional haploinsufficiency'. Cell Cycle 6(19): 2344 – 2347.

Beck H, Nähse-Kumpf V, Larsen MSY, O'Hanlon KA, Patzke S, Holmberg C, Mejlvang J, Groth A, Nielsen O, Syljuåsen RG & Sørensen CS (2012) Cyclin-dependent kinase suppression by WEE1 kinase protects the genome through control of replication initiation and nucleotide consumption. Mol Cell Biol 32(20): 4226 – 4236.

Bennardo N, Cheng A, Huang N & Stark JM (2008) Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. PLoS Genet 4(6): e1000110.

Berger AH & Pandolfi PP (2011) Haplo-insufficiency: a driving force in cancer. J Pathol 223(2): 138 – 147.

Bhargava R, Onyango DO & Stark JM (2016) Regulation of single-strand annealing and its role in genome maintenance. Trends in Genetics 32(9): 566 – 575.

Blamey RW, Pinder SE, Ball GR, Ellis IO, Elston CW, Mitchell MJ & Haybittle JL (2007) Reading the prognosis of the individual with breast cancer. Eur J Cancer 43(10): 1545 – 1547.

Bleuyard J, Buisson R, Masson J & Esashi F (2011) ChAM, a novel motif that mediates PALB2 intrinsic chromatin binding and facilitates DNA repair. EMBO Rep 13(2): 135.

Bleuyard J, Fournier M, Nakato R, Couturier AM, Katou Y, Ralf C, Hester SS, Dominguez D, Rhodes D, Humphrey TC, Shirahige K & Esashi F (2017) MRG15-mediated tethering of PALB2 to unperturbed chromatin protects active genes from genotoxic stress. Proc Natl Acad Sci USA 114(29): 7671 – 7676.

Bloom HJ & Richardson WW (1957) Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer 11(3): 359 – 377.

Boersma V, Moatti N, Segura-Bayona S, Peuscher MH, van van der Torre J, Wevers BA, Orthwein A, Durocher D & Jacobs JJL (2015) MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5′ end resection. Nature 521: 537.

Bolton KL, Tyrer J, Song H, Ramus SJ, Notaridou M, Jones C, Sher T, Gentry-Maharaj A, Wozniak E, Tsai YY, Weidhaas J, Paik D, Van Den Berg DJ, Stram DO, Pearce CL, Wu AH, Brewster W, Anton-Culver H, Ziogas A, Narod SA, Levine DA, Kaye SB, Brown R, Paul J, Flanagan J, Sieh W, McGuire V, Whittemore AS, Campbell I, Gore ME, Lissowska J, Yang HP, Medrek K, Gronwald J, Lubinski J, Jakubowska A, Le ND, Cook LS, Kelemen LE, Brooks-Wilson A, Massuger LF, Kiemeney LA, Aben KK, van Altena AM, Houlston R, Tomlinson I, Palmieri RT, Moorman PG, Schildkraut J, Iversen ES, Phelan C, Vierkant RA, Cunningham JM, Goode EL, Fridley BL, Kruger-Kjaer S, Blaeker J, Hogdall E, Hogdall C, Gross J, Karlan BY, Ness RB, Edwards RP, Odunsi K, Moyisch KB, Baker JA, Modugno F, Heikkinenen T, Bützow R, Nevanlinna H, Leminen A, Bogdanova N, Antonenkova N, Doerk T, Hillemanns P, Durst M, Runnebaum I, Thompson PJ, Carney ME, Goodman MT, Lurie G, Wang-Gohrke S, Hein R, Chang-Claude J, Rossing MA, Cushing-Haugen KL, Doherty J, Chen C, Rafnar T, Besenbacher S, Sulem P, Stefansson K, Birrer MJ, Terry KL, Hernandez D, Cramer DW, Vergote I, Amant F, Lambrechts D, Despierre E, Fasching PA, Beckmann MW, Thiel FC, Ekici AB, Chen X, Australian Ovarian

Cancer Study Group, Australian Cancer Study (Ovarian Cancer), Ovarian Cancer Association Consortium, Johnatty SE, Webb PM, Beesley J, Chanock S, Garcia-Closas M, Sellers T, Easton DF, Berchuck A, Chenevix-Trench G, Pharoah PD & Gayther SA (2010) Common variants at 19p13 are associated with susceptibility to ovarian cancer. Nat Genet 42(10): 880 – 884.

Botezatu A, V. Iancu I, Popa O, Plesa A, Manda D, Huica I, Vladoiu S, Anton G & Badiu C (2016) Mechanisms of oncogene activation. InTech.

Bouffler SD, Kemp CJ, Balmain A & Cox R (1995) Spontaneous and ionizing radiation-induced chromosomal abnormalities in p53-deficient mice. Cancer Res 55(17): 3883 – 3889.

Broca PP (1866) Traité des Tumeurs (ed. Asselin, P). (Paris).

Buisson R, Dion-Cote AM, Coulombe Y, Launay H, Cai H, Stasiak AZ, Stasiak A, Xia B & Masson JY (2010) Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. Nat Struct Mol Biol 17(10): 1247 – 1254.

Buisson R & Masson J (2012) PALB2 self-interaction controls homologous recombination. Nucleic Acids Res 40(20): 10312 – 10323.

Buisson R, Niraj J, Pauty J, Maity R, Zhao W, Coulombe Y, Sung P & Masson J (2014) Breast cancer proteins PALB2 and BRCA2 stimulate polymerase η in recombination-associated DNA synthesis at blocked replication forks. Cell Reports 6(3): 553 – 564.

Buisson R, Niraj J, Rodrigue A, Ho CK, Kreuzer J, Foo TK, Hardy EJ, Dellaire G, Haas W, Xia B, Masson J & Zou L (2017) Coupling of homologous recombination and the checkpoint by ATR. Mol Cell 65(2): 336 – 346.

Burma S, Chen BP, Murphy M, Kurimasa A & Chen DJ (2001) ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J Biol Chem 276(45): 42462 – 42467.

Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller M, Shaikh N, Domingo E, Kanu N, Dewhurst SM, Gronroos E, Chew SK, Rowan AJ, Schenk A, Sheffer M, Howell M, Kschischo M, Behrens A, Helleday T, Bartek J, Tomlinson IP & Swanton C (2013) Replication stress links structural and numerical cancer chromosomal instability. Nature 494(7438): 492 – 496.

Byrd PJ, Stewart GS, Smith A, Eaton C, Taylor AJ, Guy C, Eringyte I, Fooks P, Last JI, Horsley R, Oliver AW, Janic D, Dokmanovic L, Stankovic T & Taylor AM (2016) A hypomorphic PALB2 allele gives rise to an unusual form of FA-N associated with lymphoid tumour development. PLoS Genet 12(3): e1005945.

Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA & Livingston DM (2001) BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell 105(1): 149 – 160.

Castillo A, Paul A, Sun B, Huang T, Wang Y, Yazinski S, Tyler J, Li L, You M , Zou L, Yao J & Wang B (2014) The BRCA1-interacting protein Abraxas is required for genomic stability and tumor suppression. Cell Reports 8(3): 807 – 817.

Ceccaldi R, Rondinelli B & D'Andrea AD (2016) Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol 26(1): 52 – 64.

Chatterjee N & Walker GC (2017) Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen 58(5): 235 – 263.

Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G & Sun Y (2015) New horizons in tumor microenvironment biology: challenges and opportunities. BMC Medicine 13(1): 45.

Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM & Scully R (1998) Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol Cell 2(3): 317 – 328.

Chen T, Bian Y, Liu X, Zhao S, Wu K, Yan L, Li M, Yang Z, Liu H, Zhao H & Chen Z (2017) A recurrent missense mutation in ZP3 causes empty follicle syndrome and female infertility. Am J Hum Genet 101(3): 459 – 465.

Chen X, Arciero CA, Wang C, Broccoli D & Godwin AK (2006) BRCC36 is essential for ionizing radiation–induced BRCA1 phosphorylation and nuclear foci formation. Cancer Res 66(10): 5039.

Chen Y, Farmer AA, Chen C, Jones DC, Chen P & Lee W (1996) BRCA1 is a 220-kDa nuclear phosphoprotein that is expressed and phosphorylated in a cell cycle-dependent manner. Cancer Res 56(14): 3168.

Cho N (2016) Molecular subtypes and imaging phenotypes of breast cancer. Ultrasonography 35(4): 281 – 288.

Cimprich KA & Cortez D (2008) ATR: an essential regulator of genome integrity. Nat Rev Mol Cell Biol 9: 616.

Clapperton JA, Manke IA, Lowery DM, Ho T, Haire LF, Yaffe MB & Smerdon SJ (2004) Structure and mechanism of BRCA1 BRCT domain recognition of phosphorylated BACH1 with implications for cancer. Nat Struct Mol Biol 11(6): 512 – 518.

Clarke AR, Gledhill S, Hooper ML, Bird CC & Wyllie AH (1994) P53 dependence of early apoptotic and proliferative responses within the mouse intestinal epithelium following gamma-irradiation. Oncogene 9(6): 1767 – 1773.

Claus EB, Schildkraut JM, Thompson WD & Risch NJ (1996) The genetic attributable risk of breast and ovarian cancer. Cancer 77(11): 2318 – 2324.

Clerici M, Mantiero D, Lucchini G & Longhese MP (2005) The Saccharomyces cerevisiae Sae2 protein promotes resection and bridging of double strand break ends. J Biol Chem 280(46): 38631 – 38638.

Cochran RL, Cidado J, Kim M, Zabransky DJ, Croessmann S, Chu D, Wong HY, Beaver JA, Cravero K, Erlanger B, Parsons H, Heaphy CM, Meeker AK, Lauring J & Park BH (2015) Functional isogenic modeling of BRCA1 alleles reveals distinct carrier phenotypes. Oncotarget 6(28): 25240 – 25251.

Coleman KA & Greenberg RA (2011) The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. J Biol Chem 286(15): 13669 – 13680.

Cooper EM, Cutcliffe C, Kristiansen TZ, Pandey A, Pickart CM & Cohen RE (2009) K63-specific deubiquitination by two JAMM/MPN+ complexes: BRISC-associated Brcc36 and proteasomal Poh1. EMBO J 28(6): 621 – 631.

Cooper GM (2000) The Cell – A molecular approach 2nd Ed., Sunderland (MA): Sinauer Associates.

Cooper J, Maupin K & Merrill N (2015) Origins of cancer symposium 2015: posttranslational modifications and cancer. Genes & Cancer 6(7-8): 303 – 306.

Coschi CH, Ishak CA, Gallo D, Marshall A, Talluri S, Wang J, Cecchini MJ, Martens AL, Percy V, Welch I, Boutros PC, Brown GW & Dick FA (2014) Haploinsufficiency of an RB–E2F1–Condensin II complex leads to aberrant replication and aneuploidy. Cancer Discovery 4(7): 840.

Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, Olson JE, Godwin AK, Pankratz VS, Olswold C, Slettedahl S, Hallberg E, Guidugli L, Davila JI, Beckmann MW, Janni W, Rack B, Ekici AB, Slamon DJ, Konstantopoulou I, Fostira F, Vratimos A, Fountzilas G, Pelttari LM, Tapper WJ, Durcan L, Cross SS, Pilarski R, Shapiro CL, Klemp J, Yao S, Garber J, Cox A, Brauch H, Ambrosone C, Nevanlinna H, Yannoukakos D, Slager SL, Vachon CM, Eccles DM & Fasching PA (2015) Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol 33(4): 304 – 311.

Couch FJ, Kuchenbaecker KB, Michailidou K, Mendoza-Fandino GA, Nord S, Lilyquist J, Olswold C, Hallberg E, Agata S, Ahsan H, Aittomäki K, Ambrosone C, Andrulis IL, Anton-Culver H, Arndt V, Arun BK, Arver B, Barile M, Barkardottir RB, Barrowdale D, Beckmann L, Beckmann MW, Benitez J, Blank SV, Blomqvist C, Bogdanova NV, Bojesen SE, Bolla MK, Bonanni B, Brauch H, Brenner H, Burwinkel B, Buys SS, Caldes T, Caligo MA, Canzian F, Carpenter J, Chang-Claude J, Chanock SJ, Chung WK, Claes KB, Cox A, Cross SS, Cunningham JM, Czene K, Daly MB, Damiola F, Darabi H, de la Hoya M, Devilee P, Diez O, Ding YC, Dolcetti R, Domchek SM, Dorfling CM, Dos-Santos-Silva I, Dumont M, Dunning AM, Eccles DM, Ehrencrona H, Ekici AB, Eliassen H, Ellis S, Fasching PA, Figueroa J, Flesch-Janys D, Forsti A, Fostira F, Foulkes WD, Friebel T, Friedman E, Frost D, Gabrielson M, Gammon MD, Ganz PA, Gapstur SM, Garber J, Gaudet MM, Gayther SA, Gerdes AM, Ghoussaini M, Giles GG, Glendon G, Godwin AK, Goldberg MS, Goldgar DE, Gonzalez-Neira A, Greene MH, Gronwald J, Guenel P, Gunter M, Haeberle L, Haiman CA, Hamann U, Hansen TV, Hart S, Healey S, Heikkinen T, Henderson BE, Herzog J, Hogervorst FB, Hollestelle A, Hooning MJ, Hoover RN, Hopper JL, Humphreys K, Hunter DJ, Huzarski T, Imyanitov EN, Isaacs C, Jakubowska A, James P, Janavicius R, Jensen UB, John EM, Jones M, Kabisch M, Kar S, Karlan BY, Khan S, Khaw KT, Kibriya MG, Knight JA, Ko YD, Konstantopoulou I, Kosma VM, Kristensen V, Kwong A, Laitman Y, Lambrechts D, Lazaro C, Lee E, Le Marchand L, Lester J, Lindblom A, Lindor N, Lindstrom S, Liu J, Long J, Lubinski J, Mai PL, Makalic E, Malone KE, Mannermaa A, Manoukian S, Margolin S, Marme F, Martens JW, McGuffog L, Meindl A, Miller A, Milne RL, Miron P, Montagna M, Mazoyer S, Mulligan AM,

Muranen TA, Nathanson KL, Neuhausen SL, Nevanlinna H, Nordestgaard BG, Nussbaum RL, Offit K, Olah E, Olopade OI, Olson JE, Osorio A, Park SK, Peeters PH, Peissel B, Peterlongo P, Peto J, Phelan CM, Pilarski R, Poppe B, Pylkäs K, Radice P, Rahman N, Rantala J, Rappaport C, Rennert G, Richardson A, Robson M, Romieu I, Rudolph A, Rutgers EJ, Sanchez MJ, Santella RM, Sawyer EJ, Schmidt DF, Schmidt MK, Schmutzler RK, Schumacher F, Scott R, Senter L, Sharma P, Simard J, Singer CF, Sinilnikova OM, Soucy P, Southey M, Steinemann D, Stenmark-Askmalm M, Stoppa-Lyonnet D, Swerdlow A, Szabo CI, Tamimi R, Tapper W, Teixeira MR, Teo SH, Terry MB, Thomassen M, Thompson D, Tihomirova L, Toland AE, Tollenaar RA, Tomlinson I, Truong T, Tsimiklis H, Teule A, Tumino R, Tung N, Turnbull C, Ursin G, van Deurzen CH, van Rensburg EJ, Varon-Mateeva R, Wang Z, Wang-Gohrke S, Weiderpass E, Weitzel JN, Whittemore A, Wildiers H, Winqvist R, Yang XR, Yannoukakos D, Yao S, Zamora MP, Zheng W, Hall P, Kraft P, Vachon C, Slager S, Chenevix-Trench G, Pharoah PD, Monteiro AA, Garcia-Closas M, Easton DF & Antoniou AC (2016) Identification of four novel susceptibility loci for oestrogen receptor negative breast cancer. Nat Commun 7: 11375.

Couch FJ, Nathanson KL & Offit K (2014) Two decades after BRCA: setting paradigms in personalized cancer care and prevention. Science 343(6178): 1466 – 1470.

Couturier AM, Fleury H, Patenaude A, Bentley VL, Rodrigue A, Coulombe Y, Niraj J, Pauty J, Berman JN, Dellaire G, Di Noia J, Mes-Masson A & Masson J (2016) Roles for APRIN (PDS5B) in homologous recombination and in ovarian cancer prediction. Nucleic Acids Res 44(22): 10879 – 10897.

Croce CM (2008) Oncogenes and Cancer. N Engl J Med 358(5): 502 – 511.

Daboussi F, Courbet S, Benhamou S, Kannouche P, Zdzienicka MZ, Debatisse M & Lopez BS (2008) A homologous recombination defect affects replication-fork progression in mammalian cells. J Cell Sci 121(2): 162.

Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J & Shi B (2015) Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 5(10): 2929 – 2943.

Dasika GK, Lin SC, Zhao S, Sung P, Tomkinson A & Lee EY (1999) DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. Oncogene 18(55): 7883 – 7899.

De Bont R & van Larebeke N (2004) Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 19(3): 169 – 185.

Dhar S, Gursoy-Yuzugullu O, Parasuram R & Price BD (2017) The tale of a tail: histone H4 acetylation and the repair of DNA breaks. Philos Trans R Soc London, Biol Sci 372(1731).

Doil C, Mailand N, Bekker-Jensen S, Menard P, Larsen DH, Pepperkok R, Ellenberg J, Panier S, Durocher D, Bartek J, Lukas J & Lukas C (2009) RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell 136(3): 435 – 446.

Dong Y, Hakimi MA, Chen X, Kumaraswamy E, Cooch NS, Godwin AK & Shiekhattar R (2003) Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. Mol Cell 12(5): 1087 – 1099.

Downs-Holmes C & Silverman P (2011) Breast cancer: overview & updates. Nurse Pract 36(12): 20 – 26.

Draga M, Madgett EB, Vandenberg CJ, du Plessis D, Kaufmann A, Werler P, Chakraborty P, Lowndes NF & Hiom K (2015) BRCA1 is required for maintenance of phospho-Chk1 and G2/M arrest during DNA cross-link repair in DT40 cells. Mol Cell Biol 35(22): 3829 – 3840.

Dray E, Etchin J, Wiese C, Saro D, Williams GJ, Hammel M, Yu X, Galkin VE, Liu D, Tsai MS, Sy SM, Schild D, Egelman E, Chen J & Sung P (2010) Enhancement of RAD51 recombinase activity by the tumor suppressor PALB2. Nat Struct Mol Biol 17(10): 1255 – 1259.

Ekvall S, Wilbe M, Dahlgren J, Legius E, van Haeringen A, Westphal O, Anneren G & Bondeson ML (2015) Mutation in NRAS in familial Noonan syndrome--case report and review of the literature. BMC Med Genet 16: 95-015-0239-1.

Ellis L, Atadja PW & Johnstone RW (2009) Epigenetics in cancer: Targeting chromatin modifications. Mol Cancer Ther 8(6): 1409.

Ellsworth RE, Decewicz DJ, Shriver CD & Ellsworth DL (2010) Breast cancer in the personal genomics era. Curr Genomics 11(3): 146 – 161.

Elston CW & Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19(5): 403 – 410.

Erkko H, Dowty JG, Nikkilä J, Syrjäkoski K, Mannermaa A, Pylkäs K, Southey MC, Holli K, Kallioniemi A, Jukkola-Vuorinen A, Kataja V, Kosma V, Xia B, Livingston DM, Winqvist R & Hopper JL (2008) Penetrance analysis of the *PALB2* c.1592delT founder mutation. Clin Cancer Res 14(14): 4667.

Erkko H, Xia B, Nikkilä J, Schleutker J, Syrjäkoski K, Mannermaa A, Kallioniemi A, Pylkäs K, Karppinen S, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma V, Kataja V, Soini Y, Drapkin RI, Livingston DM & Winqvist R (2007) A recurrent mutation in PALB2 in Finnish cancer families. Nature 446(7133): 316 – 319.

Escribano-Díaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JF, Tkáč J, Cook M, Rosebrock A, Munro M, Canny M, Xu D & Durocher D (2013) A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. Mol Cell 49(5): 872 – 883.

Eykelenboom J, Harte E, Canavan L, Pastor-Peidro A, Calvo-Asensio I, Llorens-Agost M & Lowndes N (2013) ATR activates the S-M checkpoint during unperturbed growth to ensure sufficient replication prior to mitotic onset. Cell Reports 5(4): 1095 – 1107.

Fearon ER (1997) Human cancer syndromes: clues to the origin and nature of cancer. Science 278(5340): 1043 – 1050.

Feng L, Huang J & Chen J (2009) MERIT40 facilitates BRCA1 localization and DNA damage repair. Genes Dev 23(6): 719 – 728.

Feng L, Wang J & Chen J (2010) The Lys63-specific deubiquitinating enzyme BRCC36 is regulated by two scaffold proteins localizing in different subcellular compartments. J Biol Chem 285(40): 30982 – 30988.

Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D & Bray F (2013) Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer 49(6): 1374 – 1403.

Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM, Kaushansky K & Roberts JM (1996) A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell 85(5): 733 – 744.

Fero ML, Randel E, Gurley KE, Roberts JM & Kemp CJ (1998) The murine gene p27Kip1 is haplo-insufficient for tumour suppression. Nature 396: 177.

Finnish Cancer Registry (2017) https://syoparekisteri fi/tilastot/tautitilastot/

Foo TK, Tischkowitz M, Simhadri S, Boshari T, Zayed N, Burke KA, Berman SH, Blecua P, Riaz N, Huo Y, Ding YC, Neuhausen SL, Weigelt B, Reis-Filho JS, Foulkes WD & Xia B (2017) Compromised BRCA1-PALB2 interaction is associated with breast cancer risk. Oncogene 36(29): 4161 – 4170.

Foulkes WD (2008) Inherited Susceptibility to Common Cancers. N Engl J Med 359(20): 2143 – 2153.

Foulkes WD, Ghadirian P, Akbari MR, Hamel N, Giroux S, Sabbaghian N, Darnel A, Royer R, Poll A, Fafard E, Robidoux A, Martin G, Bismar TA, Tischkowitz M, Rousseau F & Narod SA (2007) Identification of a novel truncating PALB2 mutation and analysis of its contribution to early-onset breast cancer in French-Canadian women. Breast Cancer Res 9(6): R83.

Frank TS (2001) Hereditary Cancer Syndromes. Arch Pathol Lab Med 125(1): 85 – 90.

Gaillard H, García-Muse T & Aguilera A (2015) Replication stress and cancer. Nat Rev Cancer 15: 276.

Ge XQ & Blow JJ (2010) Chk1 inhibits replication factory activation but allows dormant origin firing in existing factories. J Cell Biol 191(7): 1285.

Georgoulis A, Vorgias EC, Chrousos PG & Rogakou PE (2017) Genome instability and γH2AX. Int J Mol Sciences 18(9).

Ghazwani Y, AlBalwi M, Al-Abdulkareem I, Al-Dress M, Alharbi T, Alsudairy R, Alomari A, Aljamaan K, Essa M, Al-Zahrani M & Alsultan A (2016) Clinical characteristics and genetic subtypes of Fanconi anemia in Saudi patients. Cancer Genetics 209(4): 171 – 176.

Ghosal G & Chen J (2013) DNA damage tolerance: a double-edged sword guarding the genome. Translational Cancer Research; Vol 2, No 3 (June 2013): Translational Cancer Research (DNA Damage and Repair) .

Goldberg M, Stucki M, Falck J, D'Amours D, Rahman D, Pappin D, Bartek J & Jackson SP (2003) MDC1 is required for the intra-S-phase DNA damage checkpoint. Nature 421(6926): 952 – 956.

Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, Senn HJ & Panel members (2013) Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 24(9): 2206 – 2223.

Gonzalez-Vasconcellos I, Anastasov N, Sanli-Bonazzi B, Klymenko O, Atkinson MJ & Rosemann M (2013) *Rb1* haploinsufficiency promotes telomere attrition and radiation-induced genomic instability. Cancer Res 73(14): 4247.

Griseri P & Pagès G (2014) Regulation of the mRNA half-life in breast cancer. World J Clin Oncol 5(3): 323 – 334.

Grzmil M & Hemmings BA (2012) Translation regulation as a therapeutic target in cancer. Cancer Res 72(16): 3891.

Guleria A & Chandna S (2016) ATM kinase: Much more than a DNA damage responsive protein. DNA Repair 39: 1 – 20.

Guo XE, Ngo B, Modrek AS & Lee W (2014) Targeting tumor suppressor networks for cancer therapeutics. Curr Drug Targets 15(1): 2 – 16.

Guo Y, Feng W, Sy SMH & Huen MSY (2015) ATM-dependent phosphorylation of the Fanconi anemia protein PALB2 promotes the DNA damage response. J Biol Chem 290(46): 27545 – 27556.

Hamdi Y, Soucy P, Adoue V, Michailidou K, Canisius S, Lemacon A, Droit A, Andrulis IL, Anton-Culver H, Arndt V, Baynes C, Blomqvist C, Bogdanova NV, Bojesen SE, Bolla MK, Bonanni B, Borresen-Dale AL, Brand JS, Brauch H, Brenner H, Broeks A, Burwinkel B, Chang-Claude J, NBCS Collaborators, Couch FJ, Cox A, Cross SS, Czene K, Darabi H, Dennis J, Devilee P, Dork T, Dos-Santos-Silva I, Eriksson M, Fasching PA, Figueroa J, Flyger H, Garcia-Closas M, Giles GG, Goldberg MS, Gonzalez-Neira A, Grenaker-Alnaes G, Guenel P, Haeberle L, Haiman CA, Hamann U, Hallberg E, Hooning MJ, Hopper JL, Jakubowska A, Jones M, Kabisch M, Kataja V, Lambrechts D, Le Marchand L, Lindblom A, Lubinski J, Mannermaa A, Maranian M, Margolin S, Marme F, Milne RL, Neuhausen SL, Nevanlinna H, Neven P, Olswold C, Peto J, Plaseska-Karanfilska D, Pylkäs K, Radice P, Rudolph A, Sawyer EJ, Schmidt MK, Shu XO, Southey MC, Swerdlow A, Tollenaar RA, Tomlinson I, Torres D, Truong T, Vachon C, Van Den Ouweland AM, Wang Q, Winqvist R, kConFab/AOCS Investigators, Zheng W, Benitez J, Chenevix-Trench G, Dunning AM, Pharoah PD, Kristensen V, Hall P, Easton DF, Pastinen T, Nord S & Simard J (2016) Association of breast cancer risk with genetic variants showing differential allelic expression: Identification of a novel breast cancer susceptibility locus at 4q21. Oncotarget 7(49): 80140 – 80163.

Hanahan D & Weinberg RA (2000) The hallmarks of cancer. Cell 100(1): 57 – 70.

Hanahan D & Weinberg R (2011) Hallmarks of cancer: the next generation. Cell 144(5): 646 – 674.

Hansen KH, Bracken AP, Pasini D, Dietrich N, Gehani SS, Monrad A, Rappsilber J, Lerdrup M & Helin K (2008) A model for transmission of the H3K27me3 epigenetic mark. Nat Cell Biol 10(11): 1291 – 1300.

100

Harper JW & Elledge SJ (2007) The DNA damage response: ten years after. Mol Cell 28(5): 739 – 745.

Harris H, Miller OJ, Klein G, Worst P & Tachibana T (1969) Suppression of malignancy by cell fusion. Nature 223(5204): 363 – 368.

Hayakawa T, Zhang F, Hayakawa N, Ohtani Y, Shinmyozu K, Nakayama J & Andreassen PR (2010) MRG15 binds directly to PALB2 and stimulates homology-directed repair of chromosomal breaks. J Cell Sci 123(7): 1124.

Her J, Soo Lee N, Kim Y & Kim H (2016) Factors forming the BRCA1-A complex orchestrate BRCA1 recruitment to the sites of DNA damage. Acta Biochimica et Biophysica Sinica 48(7): 658 – 664.

Herskowitz I (1987) Functional inactivation of genes by dominant negative mutations. Nature 329: 219.

Hery C, Ferlay J, Boniol M & Autier P (2008) Quantification of changes in breast cancer incidence and mortality since 1990 in 35 countries with Caucasian-majority populations. Ann Oncol 19(6): 1187 – 1194.

Hirsch B, Shimamura A, Moreau L, Baldinger S, Hag-alshiekh M, Bostrom B, Sencer S & D'Andrea AD (2004) Association of biallelic *BRCA2/FANCD1* mutations with spontaneous chromosomal instability and solid tumors of childhood. Blood 103(7): 2554.

Hollestelle A, Wasielewski M, Martens JW & Schutte M (2010) Discovering moderate-risk breast cancer susceptibility genes. Curr Opin Genet Dev 20(3): 268 – 276.

Houtgraaf JH, Versmissen J & van der Giessen WJ (2006) A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovascular Revascularization Medicine 7(3): 165 – 172.

Howlett NG (2007) Fanconi Anemia: Fanconi anemia, breast and embryonal cancer risk revisited. Eur J Hum Genet 15(7): 715 – 717.

Hu X, Kim JA, Castillo A, Huang M, Liu J & Wang B (2011a) NBA1/MERIT40 and BRE interaction is required for the integrity of two distinct deubiquitinating enzyme BRCC36-containing complexes. J Biol Chem 286(13): 11734 – 11745.

Hu Y, Scully R, Sobhian B, Xie A, Shestakova E & Livingston DM (2011b) RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. Genes Dev 25(7): 685 – 700.

Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB & Chen J (2007) RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 131(5): 901 – 914.

Iliakis G, Murmann T & Soni A (2015) Alternative end-joining repair pathways are the ultimate backup for abrogated classical non-homologous end-joining and homologous recombination repair: Implications for the formation of chromosome translocations. Mutation Research/Genetic Toxicology and Environmental Mutagenesis; Insights into formation and consequences of chromosome aberrations: Report on the 11th International Symposium on Chromosomal Aberrations (ISCA 11), Rhodes, Greece, September 12-14, 2014 793: 166 – 175.

Jackson SP & Bartek J (2009) The DNA-damage response in human biology and disease. Nature 461: 1071.

Jacquemont C & Taniguchi T (2007) The Fanconi anemia pathway and ubiquitin. BMC Biochemistry 8(1): S10.

Ju YS, Martincorena I, Gerstung M, Petljak M, Alexandrov LB, Rahbari R, Wedge DC, Davies HR, Ramakrishna M, Fullam A, Martin S, Alder C, Patel N, Gamble S, O'Meara S, Giri DD, Sauer T, Pinder SE, Purdie CA, Borg Å, Stunnenberg H, van dV, Tan BKT, Caldas C, Tutt A, Ueno NT, van 't Veer, Laura J., Martens JWM, Sotiriou C, Knappskog S, Span PN, Lakhani SR, Eyfjörd JE, Børresen-Dale A, Richardson A, Thompson AM, Viari A, Hurles ME, Nik-Zainal S, Campbell PJ & Stratton MR (2017) Somatic mutations reveal asymmetric cellular dynamics in the early human embryo. Nature 543: 714.

Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, Secreto G, Amiano P, Bingham S, Boeing H, Bueno de Mesquita HB, Chang-Claude J, Clavel-Chapelon F, Fournier A, van Gils CH, Gonzalez CA, Gurrea AB, Critselis E, Khaw KT, Krogh V, Lahmann PH, Nagel G, Olsen A, Onland-Moret NC, Overvad K, Palli D, Panico S, Peeters P, Quiros JR, Roddam A, Thiebaut A, Tjonneland A, Chirlaque MD, Trichopoulou A, Trichopoulos D, Tumino R, Vineis P, Norat T, Ferrari P, Slimani N & Riboli E (2005a) Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 97(10): 755 – 765.

Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PH, Biessy C, Dossus L, Lukanova A, Bingham S, Khaw KT, Allen NE, Bueno-de-Mesquita HB, van Gils CH, Grobbee D, Boeing H, Lahmann PH, Nagel G, Chang-Claude J, Clavel-Chapelon F, Fournier A, Thiebaut A, Gonzalez CA, Quiros JR, Tormo MJ, Ardanaz E, Amiano P, Krogh V, Palli D, Panico S, Tumino R, Vineis P, Trichopoulou A, Kalapothaki V, Trichopoulos D, Ferrari P, Norat T, Saracci R & Riboli E (2005b) Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. Endocr Relat Cancer 12(4): 1071 – 1082.

Karim-Kos H, de Vries E, Soerjomataram I, Lemmens V, Siesling S & Coebergh JWW (2008) Recent trends of cancer in Europe: A combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer 44(10): 1345 – 1389.

Karnani N & Dutta A (2011) The effect of the intra-S-phase checkpoint on origins of replication in human cells. Genes Dev 25(6): 621 – 633.

Kauko O & Westermarck J (2018) Non-genomic mechanisms of protein phosphatase 2A (PP2A) regulation in cancer. Int J Biochem Cell Biol 96: 157 – 164.

Kaur A & Westermarck J (2016) Regulation of protein phosphatase 2A (PP2A) tumor suppressor function by PME-1. Biochem Soc Trans 44(6): 1683.

Key T, Appleby P, Barnes I, Reeves G & Endogenous Hormones and Breast Cancer Collaborative Group (2002) Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 94(8): 606 – 616.

Kim H, Chen J & Yu X (2007a) Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science 316(5828): 1202 – 1205.

Kim H, Huang J & Chen J (2007b) CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. Nat Struct Mol Biol 14(8): 710 – 715.

Kinzler KW & Vogelstein B (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 386(6627): 761 – 763.

Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA & Koff A (1996) Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell 85(5): 721 – 732.

Knudson AG (1971) Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 68(4): 820 – 823.

Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, Pelletier L, Jackson SP & Durocher D (2007) Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science 318(5856): 1637 – 1640.

Konishi H, Mohseni M, Tamaki A, Garay JP, Croessmann S, Karnan S, Ota A, Wong HY, Konishi Y, Karakas B, Tahir K, Abukhdeir AM, Gustin JP, Cidado J, Wang GM, Cosgrove D, Cochran R, Jelovac D, Higgins MJ, Arena S, Hawkins L, Lauring J, Gross AL, Heaphy CM, Hosokawa Y, Gabrielson E, Meeker AK, Visvanathan K, Argani P, Bachman KE & Park BH (2011) Mutation of a single allele of the cancer susceptibility gene BRCA1 leads to genomic instability in human breast epithelial cells. Proc Natl Acad Sci USA 108(43): 17773 – 17778.

Kosugi S, Hasebe M, Matsumura N, Takashima H, Miyamoto-Sato E, Tomita M & Yanagawa H (2009) Six classes of nuclear localization signals specific to different binding grooves of importin α. J Biol Chem 284(1): 478 – 485.

Kotnis A, Sarin R & Mulherkar R (2005) Genotype, phenotype and cancer: role of low penetrance genes and environment in tumour susceptibility. J Biosci 30(1): 93 – 102.

Krajewska M, Fehrmann RSN, de Vries EGE & van Vugt MATM (2015) Regulators of homologous recombination repair as novel targets for cancer treatment. Frontiers in Genetics 6: 96.

Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H & Auerbach AD (2003) A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood 101(4): 1249.

Lakhani SR, Ellis IO, Schnitt SJ, Tan PH & van de Vijver MJ (2012) WHO Classification of Tumours of the Breast, Fourth Edition. Lyon, IARC Press.

Lalloo F & Evans DG (2012) Familial breast cancer. Clin Genet 82(2): 105 – 114.

Lee EYHP & Muller WJ (2010) Oncogenes and tumor suppressor genes. Cold Spring Harbor Perspectives in Biology 2(10).

Lee T & Young R (2013) Transcriptional regulation and its misregulation in disease. Cell 152(6): 1237 – 1251.

Lee-Theilen M, Matthews AJ, Kelly D, Zheng S & Chaudhuri J (2011) CtIP promotes microhomology-mediated alternative end joining during class-switch recombination. Nat Struct Mol Biol 18(1): 75 – 79.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna M, Weisburd B, Won H, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG & Exome AC (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536(7616): 285 – 291.

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A & Hemminki K (2000) Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343(2): 78 – 85.

Limbo O, Chahwan C, Yamada Y, de Bruin RA, Wittenberg C & Russell P (2007) Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. Mol Cell 28(1): 134 – 146.

Lin Y, Lin Y, Lu Y, Liu T, Chen C, Hsu C, Chen P & Wu C (2017) A novel missense variant in the nuclear localization signal of POU4F3 causes autosomal dominant non-syndromic hearing loss. Sci Reports 7(1): 7551.

Litman R, Peng M, Jin Z, Zhang F, Zhang J, Powell S, Andreassen PR & Cantor SB (2005) BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer Cell 8(3): 255 – 265.

Liu Q, Guntuku S, Cui X, Matsuoka S, Cortez D, Tamai K, Luo G, Carattini-Rivera S, DeMayo F, Bradley A, Donehower LA & Elledge SJ (2000) Chk1 is an essential kinase that is regulated by Atr and required for the G2/M DNA damage checkpoint. Genes Dev 14(12): 1448 – 1459.

Liu Z, Wu J & Yu X (2007) CCDC98 targets BRCA1 to DNA damage sites. Nat Struct Mol Biol 14(8): 716 – 720.

Loeb LA (2011) Human cancers express mutator phenotypes: origin, consequences and targeting. Nat Rev Cancer 11: 450.

Loeb LA (2016) Human cancers express a mutator phenotype: Hypothesis, origin, and consequences. Cancer Res 76(8): 2057 – 2059.

Lu H, Li G, Zhou C, Jin W, Qian X, Wang Z, Pan H, Jin H & Wang X (2016) Regulation and role of post-translational modifications of enhancer of zeste homologue 2 in cancer development. Am J Cancer Res 6(12): 2737 – 2754.

Lu PYT, Lévesque N & Kobor MS (2009) NuA4 and SWR1-C: two chromatin-modifying complexes with overlapping functions and components. 30th Annual International Asilomar Chromatin and Chromosomes Conference. Biochem Cell Biol 87(5): 799 – 815.

Luijsterburg MS, Typas D, Caron MC, Wiegant WW, van den Heuvel D, Boonen RA, Couturier AM, Mullenders LH, Masson JY & van Attikum H (2017) A PALB2-interacting domain in RNF168 couples homologous recombination to DNA break-induced chromatin ubiquitylation. Elife 6: 10.7554/eLife.20922.

Luning Prak ET & Kazazian Jr HH (2000) Mobile elements and the human genome. Nat Rev Genet 1: 134.

Ma J, Cai H, Wu T, Sobhian B, Huo Y, Alcivar A, Mehta M, Cheung KL, Ganesan S, Kong AT, Zhang DD & Xia B (2012) PALB2 interacts with KEAP1 to promote NRF2 nuclear accumulation and function. Mol Cell Biol 32(8): 1506 – 1517.

Macheret M & Halazonetis TD (2015) DNA replication stress as a hallmark of cancer. Annu Rev Pathol 10: 425 – 448.

Mailand N, Bekker-Jensen S, Faustrup H, Melander F, Bartek J, Lukas C & Lukas J (2007) RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell 131(5): 887 – 900.

Makki J (2015) Diversity of Breast Carcinoma: histological subtypes and clinical relevance. Clin Med Insights: Pathology 8(5287-): 23 – 31.

Manke IA, Lowery DM, Nguyen A & Yaffe MB (2003) BRCT repeats as phosphopeptide-binding modules involved in protein targeting. Science 302(5645): 636 – 639.

Mantere T, Tervasmäki A, Nurmi A, Rapakko K, Kauppila S, Tang J, Schleutker J, Kallioniemi A, Hartikainen JM, Mannermaa A, Nieminen P, Hanhisalo R, Lehto S, Suvanto M, Grip M, Jukkola-Vuorinen A, Tengström M, Auvinen P, Kvist A, Borg Å, Blomqvist C, Aittomäki K, Greenberg RA, Winqvist R, Nevanlinna H & Pylkäs K (2017) Case-control analysis of truncating mutations in DNA damage response genes connects TEX15 and FANCD2 with hereditary breast cancer susceptibility. Sci Rep 7(1): 681.

Marsh DJ, Andrew SD, Eng C, Learoyd DL, Capes AG, Pojer R, Richardson AL, Houghton C, Mulligan LM, Ponder BA & Robinson BG (1996) Germline and somatic mutations in an oncogene: RET mutations in inherited medullary thyroid carcinoma. Cancer Res 56(6): 1241 – 1243.

Martincorena I & Campbell PJ (2015) Somatic mutation in cancer and normal cells. Science 349(6255): 1483.

Martín-Morales L, Feldman M, Vershinin Z, Garre P, Caldés T & Levy D (2017) SETD6 dominant negative mutation in familial colorectal cancer type X. Hum Mol Genet 26(22): 4481 – 4493.

Martrat G, Maxwell CA, Tominaga E, Porta-de-la-Riva M, Bonifaci N, Gómez-Baldó L, Bogliolo M, Lázaro C, Blanco I, Brunet J, Aguilar H, Fernández-Rodríguez J, Seal S, Renwick A, Rahman N, Kühl J, Neveling K, Schindler D, Ramírez MJ, Castellà M, Hernández G, Easton DF, Peock S, Cook M, Oliver CT, Frost D, Platte R, Evans DG, Lalloo F, Eeles R, Izatt L, Chu C, Davidson R, Ong K, Cook J, Douglas F, Hodgson S, Brewer C, Morrison PJ, Porteous M, Peterlongo P, Manoukian S, Peissel B, Zaffaroni D, Roversi G, Barile M, Viel A, Pasini B, Ottini L, Putignano AL, Savarese A, Bernard L, Radice P, Healey S, Spurdle A, Chen X, Beesley J, Rookus MA, Verhoef S, Tilanus-Linthorst M, Vreeswijk MP, Asperen CJ, Bodmer D, Ausems MGEM, van Os TA, Blok MJ, Meijers-Heijboer H, Hogervorst FBL, Goldgar DE, Buys S, John EM, Miron A, Southey M, Daly MB, Harbst K, Borg Å, Rantala J, Barbany-Bustinza G, Ehrencrona H, Stenmark-Askmalm M, Kaufman B, Laitman Y, Milgrom R, Friedman E, Domchek SM, Nathanson KL, Rebbeck TR, Johannsson OT, Couch FJ, Wang X, Fredericksen Z, Cuadras D, Moreno V, Pientka FK, Depping R, Caldés T, Osorio A, Benítez J, Bueren J, Heikkinen T, Nevanlinna H, Hamann U, Torres D, Caligo MA, Godwin AK, Imyanitov EN, Janavicius R, Sinilnikova OM, Stoppa-Lyonnet D, Mazoyer S, Verny-Pierre C, Castera L, de Pauw A, Bignon Y, Uhrhammer N, Peyrat J, Vennin P, Ferrer SF, Collonge-Rame M, Mortemousque I, McGuffog L, Chenevix-Trench G, Pereira-Smith O, Antoniou AC, Cerón J, Tominaga K, Surrallés J & Pujana MA (2011) Exploring the link between MORF4L1 and risk of breast cancer. Breast Canc Res 13(2): R40.

Matsuoka S, Huang M & Elledge SJ (1998) Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282(5395): 1893.

Maya-Mendoza A, Petermann E, Gillespie DAF, Caldecott KW & Jackson DA (2007) Chk1 regulates the density of active replication origins during the vertebrate S phase. EMBO J 26(11): 2719 – 2731.

Mazouzi A, Velimezi G & Loizou JI (2014) DNA replication stress: Causes, resolution and disease. Exp Cell Res 329(1): 85 – 93.

McIntosh D & Blow JJ (2012) Dormant origins, the licensing checkpoint, and the response to replicative stresses. Cold Spring Harbor Perspectives in Biology 4(10).

McKay JD, Truong T, Gaborieau V, Chabrier A, Chuang SC, Byrnes G, Zaridze D, Shangina O, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Bucur A, Bencko V, Holcatova I, Janout V, Foretova L, Lagiou P, Trichopoulos D, Benhamou S, Bouchardy C, Ahrens W, Merletti F, Richiardi L, Talamini R, Barzan L, Kjaerheim K, Macfarlane GJ, Macfarlane TV, Simonato L, Canova C, Agudo A, Castellsague X, Lowry R, Conway DI, McKinney PA, Healy CM, Toner ME, Znaor A, Curado MP, Koifman S, Menezes A, Wunsch-Filho V, Neto JE, Garrote LF, Boccia S, Cadoni G, Arzani D, Olshan AF, Weissler MC, Funkhouser WK, Luo J, Lubinski J, Trubicka J, Lener M, Oszutowska D, Schwartz SM, Chen C, Fish S, Doody DR, Muscat JE, Lazarus P, Gallagher CJ, Chang SC, Zhang ZF, Wei Q, Sturgis EM, Wang LE, Franceschi S, Herrero R, Kelsey KT, McClean MD, Marsit CJ, Nelson HH, Romkes M, Buch S, Nukui T, Zhong S, Lacko M, Manni JJ, Peters WH, Hung RJ, McLaughlin J, Vatten L, Njolstad I, Goodman GE, Field JK, Liloglou T, Vineis P, Clavel-

Chapelon F, Palli D, Tumino R, Krogh V, Panico S, Gonzalez CA, Quiros JR, Martinez C, Navarro C, Ardanaz E, Larranaga N, Khaw KT, Key T, Bueno-de-Mesquita HB, Peeters PH, Trichopoulou A, Linseisen J, Boeing H, Hallmans G, Overvad K, Tjonneland A, Kumle M, Riboli E, Valk K, Vooder T, Metspalu A, Zelenika D, Boland A, Delepine M, Foglio M, Lechner D, Blanche H, Gut IG, Galan P, Heath S, Hashibe M, Hayes RB, Boffetta P, Lathrop M & Brennan P (2011) A genome-wide association study of upper aerodigestive tract cancers conducted within the INHANCE consortium. PLoS Genet 7(3): e1001333.

Meng X, Zhong J, Liu S, Murray M & Gonzalez-Angulo A (2011) A new hypothesis for the cancer mechanism. Cancer Metastasis Rev 31(1-2): 247 – 268.

Menzel T, Nähse-Kumpf V, Kousholt AN, Klein DK, Lund-Andersen C, Lees M, Johansen JV, Syljuåsen R,G. & Sørensen CS (2011) A genetic screen identifies BRCA2 and PALB2 as key regulators of G2 checkpoint maintenance. EMBO Rep 12(7): 705 – 712.

Michor F, Iwasa Y & Nowak MA (2004) Dynamics of cancer progression. Nat Rev Cancer 4: 197.

Miele E, Mastronuzzi A, Po A, Carai A, Alfano V, Serra A, Colafati GS, Strocchio L, Antonelli M, Buttarelli FR, Zani M, Ferraro S, Buffone A, Vacca A, Screpanti I, Giangaspero F, Giannini G, Locatelli F & Ferretti E (2015) Characterization of medulloblastoma in Fanconi Anemia: a novel mutation in the BRCA2 gene and SHH molecular subgroup. Biomarker Res 3(1): 13.

Moynahan ME, Cui TY & Jasin M (2001a) Homology-directed DNA Repair, Mitomycin-C Resistance, and Chromosome Stability Is Restored with Correction of a *Brca1* Mutation. Cancer Res 61(12): 4842.

Moynahan ME, Pierce AJ & Jasin M (2001b) BRCA2 is required for homology-directed repair of chromosomal breaks. Mol Cell 7(2): 263 – 272.

Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, Graff RE, Holst K, Möller S, Unger RH, McIntosh C, Nuttall E, Brandt I, Penney KL, Hartman M, Kraft P, Parmigiani G, Christensen K, Koskenvuo M, Holm NV, Heikkilä K, Pukkala E, Skytthe A, Adami HO, Kaprio J & Nordic Twin Study of Cancer (NorTwinCan) Collaboration (2016) Familial risk and heritability of cancer among twins in Nordic countries. JAMA 315(1): 68 – 76.

Mullan PB, Quinn JE & Harkin DP (2006) The role of BRCA1 in transcriptional regulation and cell cycle control. Oncogene 25(43): 5854 – 5863.

Naik V, Adhyaru P & Gudigenavar A (2015) Tumor suppressor genes in oral cancer. Clinical Cancer Investigation Journal 4(6): 697 – 702.

Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY & Nakayama K (1996) Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 85(5): 707 – 720.

Negrini S, Gorgoulis VG & Halazonetis TD (2010) Genomic instability [mdash] an evolving hallmark of cancer. Nat Rev Mol Cell Biol 11(3): 220 – 228.

Neitzel H (1986) A routine method for the establishment of permanent growing lymphoblastoid cell lines. Hum Genet 73(4): 320 – 326.

Neklason DW, Done MW, Sargent NR, Schwartz AG, Anton-Culver H, Griffin CA, Ahnen DJ, Schildkraut JM, Tomlinson GE, Strong LC, Miller AR, Stopfer JE & Burt RW (2011) Activating mutation in MET oncogene in familial colorectal cancer. BMC Cancer 11(1): 424.

Nepomuceno CT, De Gregoriis G, de Oliveira, Francisco M. Bastos, Suarez-Kurtz G, Monteiro NA & Carvalho AM (2017) The Role of PALB2 in the DNA Damage Response and Cancer Predisposition. Int J Mol Sci 18(9).

Nielsen FC, van OH & Sorensen CS (2016) Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer 16(9): 599 – 612.

Nikkilä J, Coleman KA, Morrissey D, Pylkäs K, Erkko H, Messick TE, Karppinen SM, Amelina A, Winqvist R & Greenberg RA (2009) Familial breast cancer screening reveals an alteration in the RAP80 UIM domain that impairs DNA damage response function. Oncogene 28(16): 1843 – 1852.

Nikkilä J, Parplys AC, Pylkäs K, Bose M, Huo Y, Borgmann K, Rapakko K, Nieminen P, Xia B, Pospiech H & Winqvist R (2013) Heterozygous mutations in PALB2 cause DNA replication and damage response defects. Nat Commun 4: 2578.

Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, Alexandrov LB, Martin S, Wedge DC, Van Loo P, Ju YS, Smid M, Brinkman AB, Morganella S, Aure MR, Lingjærde OC, Langerød A, Ringnér M, Ahn S, Boyault S, Brock JE, Broeks A, Butler A, Desmedt C, Dirix L, Dronov S, Fatima A, Foekens JA, Gerstung M, Hooijer GKJ, Jang SJ, Jones DR, Kim H, King TA, Krishnamurthy S, Lee HJ, Lee J, Li Y, McLaren S, Menzies A, Mustonen V, O'Meara S, Pauporté I, Pivot X, Purdie CA, Raine K, Ramakrishnan K, Rodríguez-González FG, Romieu G, Sieuwerts AM, Simpson PT, Shepherd R, Stebbings L, Stefansson OA, Teague J, Tommasi S, Treilleux I, Van dE, Vermeulen P, Vincent-Salomon A, Yates L, Caldas C, Veer Lv, Tutt A, Knappskog S, Tan BKT, Jonkers J, Borg Å, Ueno NT, Sotiriou C, Viari A, Futreal PA, Campbell PJ, Span PN, Van Laere S, Lakhani SR, Eyfjord JE, Thompson AM, Birney E, Stunnenberg HG, van dV, Martens JWM, Børresen-Dale A, Richardson AL, Kong G, Thomas G & Stratton MR (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 534(7605): 47 – 54.

Nik-Zainal S & Morganella S (2017) Mutational Signatures in Breast Cancer: The Problem at the DNA Level. Clin Cancer Res 23(11): 2617 – 2629.

Obermeier K, Sachsenweger J, Friedl T,W.P., Pospiech H, Winqvist R & Wiesmuller L (2016) Heterozygous PALB2 c.1592delT mutation channels DNA double-strand break repair into error-prone pathways in breast cancer patients. Oncogene 35(29): 3796 – 3806.

Oliver AW, Swift S, Lord CJ, Ashworth A & Pearl LH (2009) Structural basis for recruitment of BRCA2 by PALB2. EMBO Rep 10(9): 990.

Orthwein A, Noordermeer SM, Wilson MD, Landry S, Enchev RI, Sherker A, Munro M, Pinder J, Salsman J, Dellaire G, Xia B, Peter M & Durocher D (2015) A mechanism for the suppression of homologous recombination in G1 cells. Nature 528(7582): 422 – 426.

Park J, Zhang F & Andreassen PR (2014a) PALB2: The hub of a network of tumor suppressors involved in DNA damage responses. Biochim et Biophys Acta (BBA) – Reviews on Cancer 1846(1): 263 – 275.

Park J, Singh TR, Nassar N, Zhang F, Freund M, Hanenberg H, Meetei AR & Andreassen PR (2014b) Breast cancer-associated missense mutants of the PALB2 WD40 domain, which directly binds RAD51C, RAD51 and BRCA2, disrupt DNA repair. Oncogene 33(40): 4803 – 4812.

Patel KJ (2007) Fanconi anemia and breast cancer susceptibility. Nat Genet 39(2): 142 – 143.

Pathania S, Bade S, Le Guillou M, Burke K, Reed R, Bowman-Colin C, Su Y, Ting DT, Polyak K, Richardson AL, Feunteun J, Garber JE & Livingston DM (2014) BRCA1 haploinsufficiency for replication stress suppression in primary cells. 5: 5496.

Patterson-Fortin J, Shao G, Bretscher H, Messick TE & Greenberg RA (2010) Differential regulation of JAMM domain deubiquitinating enzyme activity within the RAP80 complex. J Biol Chem 285(40): 30971 – 30981.

Pauty J, Couturier AM, Rodrigue A, Caron M, Coulombe Y, Dellaire G & Masson J (2017) Cancer-causing mutations in the tumor suppressor PALB2 reveal a novel cancer mechanism using a hidden nuclear export signal in the WD40 repeat motif. Nucleic Acids Res 45(5): 2644 – 2657.

Pauty J, Rodrigue A, Couturier A, Buisson R & Masson J (2014) Exploring the roles of PALB2 at the crossroads of DNA repair and cancer. Biochem J 460(3): 331.

Payne SR & Kemp CJ (2005) Tumor suppressor genetics. Carcinogenesis 26(12): 2031 – 2045.

Pelletier J, Bruening W, Li FP, Haber DA, Glaser T & Housman DE (1991) WT1 mutations contribute to abnormal genital system development and hereditary Wilms' tumour. Nature 353(6343): 431 – 434.

Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, Thornton A, Norquist BM, Casadei S, Nord AS, Agnew KJ, Pritchard CC, Scroggins S, Garcia RL, King MC & Swisher EM (2014) Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res 20(3): 764 – 775.

Penrose LS, Mackenzie HJ & Karn MN (1948) A genetical study of human mammary cancer. Annals of Eugenics 14(1): 234 – 266.

Petermann E & Caldecott KW (2006) Evidence that the ATR/Chk1 pathway maintains normal replication fork progression during unperturbed S phase. Cell Cycle 5(19): 2203 – 2209.

Petermann E, Woodcock M & Helleday T (2010) Chk1 promotes replication fork progression by controlling replication initiation. Proc Natl Acad Sci USA 107(37): 16090 – 16095.

Peto J & Mack TM (2000) High constant incidence in twins and other relatives of women with breast cancer. Nat Genet 26(4): 411 – 414.

Peto R, Boreham J, Clarke M, Davies C & Beral V (2000) UK and USA breast cancer deaths down 25% in year 2000 at ages 20–69 years. The Lancet 355(9217): 1822.

Philipp-Staheli J, Kim K, Payne SR, Gurley KE, Liggitt D, Longton G & Kemp CJ (2002) Pathway-specific tumor suppression: Reduction of p27 accelerates gastrointestinal tumorigenesis in Apc mutant mice, but not in Smad3 mutant mice. Cancer Cell 1(4): 355 – 368.

Polak P, Kim J, Braunstein LZ, Karlic R, Haradhavala NJ, Tiao G, Rosebrock D, Livitz D, Kubler K, Mouw KW, Kamburov A, Maruvka YE, Leshchiner I, Lander ES, Golub TR, Zick A, Orthwein A, Lawrence MS, Batra RN, Caldas C, Haber DA, Laird PW, Shen H, Ellisen LW, D'Andrea AD, Chanock SJ, Foulkes WD & Getz G (2017) A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. Nat Genet 49(10): 1476 – 1486.

Potapova A, Hoffman AM, Godwin AK, Al-Saleem T & Cairns P (2008) Promoter hypermethylation of the *PALB2* susceptibility gene in inherited and sporadic breast and ovarian cancer. Cancer Res 68(4): 998.

Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF & Stratton MR (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet 39(2): 165 – 167.

Rakha EA, Reis-Filho J, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt SJ, Schmitt FC, Tan P, Tse GM, Badve S & Ellis IO (2010) Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res 12(4): 207.

Rantakari P, Nikkilä J, Jokela H, Ola R, Pylkäs K, Lagerbohm H, Sainio K, Poutanen M & Winqvist R (2010) Inactivation of Palb2 gene leads to mesoderm differentiation defect and early embryonic lethality in mice. Hum Mol Genet 19(15): 3021 – 3029.

Rass E, Grabarz A, Plo I, Gautier J, Bertrand P & Lopez BS (2009) Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. Nat Struct Mol Biol 16(8): 819 – 824.

Rayner E, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I & Church DN (2016) A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer 16: 71.

Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, Neveling K, Kelly P, Seal S, Freund M, Wurm M, Batish SD, Lach FP, Yetgin S, Neitzel H, Ariffin H, Tischkowitz M, Mathew CG, Auerbach AD & Rahman N (2007) Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat Genet 39(2): 162 – 164.

Reinhardt HC & Yaffe MB (2009) Kinases that control the cell cycle in response to DNA damage: Chk1, Chk2, and MK2. Curr Opin Cell Biol 21(2): 245 – 255.

Renault A, Lesueur F, Coulombe Y, Gobeil S, Soucy P, Hamdi Y, Desjardins S, Le Calvez-Kelm F, Vallée M, Voegele C, The Breast Cancer FR, Hopper JL, Andrulis IL, Southey MC, John EM, Masson J, Tavtigian SV & Simard J (2016) ABRAXAS (FAM175A) and breast cancer susceptibility: no evidence of association in the Breast Cancer Family Registry. PLOS One 11(6): e0156820.

Rodriguez M, Yu X, Chen J & Songyang Z (2003) Phosphopeptide binding specificities of BRCA1 COOH-terminal (BRCT) domains. J Biol Chem 278(52): 52914 – 52918.

Rogakou EP, Pilch DR, Orr AH, Ivanova VS & Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273(10): 5858 – 5868.

Romero-Laorden N & Castro E (2017) Inherited mutations in DNA repair genes and cancer risk. Curr Probl Cancer 41(4): 251 – 264.

Rousset-Jablonski C & Gompel A (2017) Screening for familial cancer risk: Focus on breast cancer. Maturitas 105: 69 – 77.

Ruggero D (2013) Translational Control in Cancer Etiology. Cold Spring Harbor Perspectives in Biology 5(2): a012336.

Sanda T & Leong WZ (2017) TAL1 as a master oncogenic transcription factor in T-cell acute lymphoblastic leukemia. Exp Hematol 53: 7 – 15.

Sarantaus L, Huusko P, Eerola H, Launonen V, Vehmanen P, Rapakko K, Gillanders E, Syrjäkoski K, Kainu T, Vahteristo P, Krahe R, Pääkkönen K, Hartikainen J, Blomqvist C, Löppönen T, Holli K, Ryynänen M, Bützow R, Borg A, Wasteson Arver B, Holmberg E, Mannermaa A, Kere J, Kallioniemi OP, Winqvist R & Nevanlinna H (2000) Multiple founder effects and geographical clustering of BRCA1 and BRCA2 families in Finland. Eur J Hum Genet 8(10): 757 – 763.

Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J & Jackson SP (2007) Human CtIP promotes DNA end resection. Nature 450(7169): 509 – 514.

Savage KI & Harkin DP (2015) BRCA1, a 'complex' protein involved in the maintenance of genomic stability. FEBS J 282(4): 630 – 646.

Savage KI, Matchett KB, Barros EM, Cooper KM, Irwin GW, Gorski JJ, Orr KS, Vohhodina J, Kavanagh JN, Madden AF, Powell A, Manti L, McDade SS, Park BH, Prise KM, McIntosh SA, Salto-Tellez M, Richard DJ, Elliott CT & Harkin DP (2014) BRCA1 deficiency exacerbates estrogen-induced DNA damage and genomic instability. Cancer Res 74(10): 2773.

Sawyer SL, Tian L, Kähkönen M, Schwartzentruber J, Kircher M, University of Washington Centre for Mendelian Genomics, FORGE Canada Consortium, Majewski J, Dyment DA, Innes AM, Boycott KM, Moreau LA, Moilanen JS & Greenberg RA (2015) Biallelic mutations in *BRCA1* cause a new Fanconi anemia subtype. Cancer Discovery 5(2): 135.

Schlacher K, Wu H & Jasin M (2012) A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer Cell 22(1): 106 – 116.

Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJ, Walther MM, Tsui LC, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Storkel S, Lerman MI, Linehan WM & Zbar B (1997) Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 16(1): 68 – 73.

Schwab RA, Blackford AN & Niedzwiedz W (2010) ATR activation and replication fork restart are defective in FANCM-deficient cells. EMBO J 29(4): 806.

Scully R, Ganesan S, Brown M, De Caprio JA, Cannistra SA, Feunteun J, Schnitt S, Livingston DM, Chen Y, Chen P, Riley DJ, Lee W, Allred DC & Osborne CK (1996) Location of BRCA1 in human breast and ovarian cancer cells. Science 272(5258): 123.

Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T & Livingston DM (1997) Association of BRCA1 with Rad51 in Mitotic and Meiotic Cells. Cell 88(2): 265 – 275.

Seol J, Shim EY & Lee SE (2017) Microhomology-mediated end joining: Good, bad and ugly. Mutation Res/Fundamental and Molecular Mechanisms of Mutagenesis.

Serra A, Eirich K, Winkler AK, Mrasek K, Göhring G, Barbi G, Cario H, Schlegelberger B, Royer-Pokora B, Liehr T, Leriche C, Henne-Bruns D, Barth TF & Schindler D (2012) Shared copy number variation in simultaneous nephroblastoma and neuroblastoma due to Fanconi anemia. Mol Syndromol 3(3): 120 – 130.

Shao G, Lilli DR, Patterson-Fortin J, Coleman KA, Morrissey DE & Greenberg RA (2008) The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. Proc Natl Acad Sci USA 106(9): 3166 – 3171.

Shao G, Patterson-Fortin J, Messick TE, Feng D, Shanbhag N, Wang Y & Greenberg RA (2009) MERIT40 controls BRCA1–Rap80 complex integrity and recruitment to DNA double-strand breaks. Genes Dev 23(6): 740 – 754.

Sharan SK, Morimatsu M, Albrecht U, Lim D, Regel E, Dinh C, Sands A, Eichele G, Hasty P & Bradley A (1997) Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature 386(6627): 804 – 810.

Shibata A (2017) Regulation of repair pathway choice at two-ended DNA double-strand breaks. Mutation Res/Fundamental and Molecular Mechanisms of Mutagenesis 803-805: 51 – 55.

Simhadri S, Peterson S, Patel DS, Huo Y, Cai H, Bowman-Colin C, Miller S, Ludwig T, Ganesan S, Bhaumik M, Bunting SF, Jasin M & Xia B (2014) Male fertility defect associated with disrupted BRCA1-PALB2 interaction in mice. J Biol Chem 289(35): 24617 – 24629.

Sinn H & Kreipe H (2013) A brief overview of the WHO classification of breast tumors, 4th ed., Focusing on issues and updates from the 3rd ed. Breast Care 8(2): 149 – 154.

Skol AD, Sasaki MM & Onel K (2016) The genetics of breast cancer risk in the post-genome era: thoughts on study design to move past BRCA and towards clinical relevance. Breast Cancer Res 18(1): 99.

Smithers DW (1948) Family histories of 459 patients with cancer of the breast. Br J Cancer 2(2): 163 – 167.

Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B, Livingston DM & Greenberg RA (2007) RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science 316(5828): 1198 – 1202.

Solyom S, Aressy B, Pylkäs K, Patterson-Fortin J, Hartikainen JM, Kallioniemi A, Kauppila S, Nikkilä J, Kosma V, Mannermaa A, Greenberg RA & Winqvist R (2012) Breast cancer–associated *Abraxas* mutation disrupts nuclear localization and DNA damage response functions. Sci Transl Med 4(122): 122 – 23.

Soussi T & Wiman KG (2015) TP53: an oncogene in disguise. Cell Death Differentiation 22: 1239.

Southey MC, Goldgar DE, Winqvist R, Pylkäs K, Couch F, Tischkowitz M, Foulkes WD, Dennis J, Michailidou K, van Rensburg E,J., Heikkinen T, Nevanlinna H, Hopper JL, Dörk T, Claes KBM, Reis-Filho J, Teo ZL, Radice P, Catucci I, Peterlongo P, Tsimiklis H, Odefrey FA, Dowty JG, Schmidt MK, Broeks A, Hogervorst FB, Verhoef S, Carpenter J, Clarke C, Scott RJ, Fasching PA, Haeberle L, Ekici AB, Beckmann MW, Peto J, dos-Santos-Silva I, Fletcher O, Johnson N, Bolla MK, Sawyer EJ, Tomlinson I, Kerin MJ, Miller N, Marme F, Burwinkel B, Yang R, Guénel P, Truong T, Menegaux F, Sanchez M, Bojesen S, Nielsen SF, Flyger H, Benitez J, Zamora MP, Perez JIA, Menéndez P, Anton-Culver H, Neuhausen S, Ziogas A, Clarke CA, Brenner H, Arndt V, Stegmaier C, Brauch H, Brüning T, Ko Y, Muranen TA, Aittomäki K, Blomqvist C, Bogdanova NV, Antonenkova NN, Lindblom A, Margolin S, Mannermaa A, Kataja V, Kosma V, Hartikainen JM, Spurdle AB, Investigators K, Australian Ovarian Cancer Study Group, Wauters E, Smeets D, Beuselinck B, Floris G, Chang-Claude J, Rudolph A, Seibold P, Flesch-Janys D, Olson JE, Vachon C, Pankratz VS, McLean C, Haiman CA, Henderson BE, Schumacher F, Le Marchand L, Kristensen V, Alnæs GG, Zheng W, Hunter DJ, Lindstrom S, Hankinson SE, Kraft P, Andrulis I, Knight JA, Glendon G, Mulligan AM, Jukkola-Vuorinen A, Grip M, Kauppila S, Devilee P, Tollenaar RAEM, Seynaeve C, Hollestelle A, Garcia-Closas M, Figueroa J, Chanock SJ, Lissowska J, Czene K, Darabi H, Eriksson M, Eccles DM, Rafiq S, Tapper WJ, Gerty SM, Hooning MJ, Martens JWM, Collée JM, Tilanus-Linthorst M, Hall P, Li J, Brand JS, Humphreys K, Cox A, Reed MWR, Luccarini C, Baynes C, Dunning AM, Hamann U, Torres D, Ulmer HU, Rüdiger T, Jakubowska A, Lubinski J, Jaworska K, Durda K, Slager S, Toland AE, Ambrosone CB, Yannoukakos D, Swerdlow A, Ashworth A, Orr N, Jones M, González-Neira A, Pita G, Alonso MR, Álvarez N, Herrero D, Tessier DC, Vincent D, Bacot F, Simard J, Dumont M, Soucy P, Eeles R, Muir K, Wiklund F, Gronberg H, Schleutker J, Nordestgaard B, Weischer M, Travis RC, Neal D, Donovan JL, Hamdy FC, Khaw K, Stanford JL, Blot WJ, Thibodeau S, Schaid DJ, Kelley JL, Maier C, Kibel AS, Cybulski C, Cannon-Albright L, Butterbach K, Park J, Kaneva R, Batra J, Teixeira MR, Kote-Jarai Z, Olama AAA, Benlloch S, Renner SP, Hartmann A, Hein A, Ruebner M, Lambrechts D, Van Nieuwenhuysen E, Vergote I, Lambretchs S, Doherty JA, Rossing MA, Nickels S, Eilber U, Wang-Gohrke S, Odunsi K, Sucheston-Campbell L, Friel G, Lurie G, Killeen JL, Wilkens LR, Goodman MT, Runnebaum I, Hillemanns PA, Pelttari LM, Butzow R, Modugno F, Edwards RP, Ness RB, Moysich KB, du Bois A, Heitz F, Harter P, Kommoss S, Karlan BY, Walsh C, Lester J, Jensen A, Kjaer SK, Høgdall E, Peissel B, Bonanni B, Bernard L, Goode EL, Fridley BL, Vierkant RA, Cunningham JM, Larson MC,

Fogarty ZC, Kalli KR, Liang D, Lu KH, Hildebrandt MAT, Wu X, Levine DA, Dao F, Bisogna M, Berchuck A, Iversen ES, Marks JR, Akushevich L, Cramer DW, Schildkraut J, Terry KL, Poole EM, Stampfer M, Tworoger SS, Bandera EV, Orlow I, Olson SH, Bjorge L, Salvesen HB, van Altena AM, Aben KKH, Kiemeney LA, Massuger LFAG, Pejovic T, Bean Y, Brooks-Wilson A, Kelemen LE, Cook LS, Le ND, Górski B, Gronwald J, Menkiszak J, Høgdall CK, Lundvall L, Nedergaard L, Engelholm SA, Dicks E, Tyrer J, Campbell I, McNeish I, Paul J, Siddiqui N, Glasspool R, Whittemore AS, Rothstein JH, McGuire V, Sieh W, Cai H, Shu X, Teten RT, Sutphen R, McLaughlin JR, Narod SA, Phelan CM, Monteiro AN, Fenstermacher D, Lin H, Permuth JB, Sellers TA, Chen YA, Tsai Y, Chen Z, Gentry-Maharaj A, Gayther SA, Ramus SJ, Menon U, Wu AH, Pearce CL, Van DB, Pike MC, Dansonka-Mieszkowska A, Plisiecka-Halasa J, Moes-Sosnowska J, Kupryjanczyk J, Pharoah PDP, Song H, Winship I, Chenevix-Trench G, Giles GG, Tavtigian SV, Easton DF & Milne RL (2016) *PALB2*, *CHEK2* and *ATM* rare variants and cancer risk: data from COGS. J Med Genet 53(12): 800.

Stanton CM, Borooah S, Drake C, Marsh JA, Campbell S, Lennon A, Soares DC, Vallabh NA, Sahni J, Cideciyan AV, Dhillon B, Vitart V, Jacobson SG, Wright AF & Hayward C (2017) Novel pathogenic mutations in C1QTNF5 support a dominant negative disease mechanism in late-onset retinal degeneration. Scientific Reports 7(1): 12147.

Stewart GS, Panier S, Townsend K, Al-Hakim AK, Kolas NK, Miller ES, Nakada S, Ylanko J, Olivarius S, Mendez M, Oldreive C, Wildenhain J, Tagliaferro A, Pelletier L, Taubenheim N, Durandy A, Byrd PJ, Stankovic T, Taylor AM & Durocher D (2009) The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. Cell 136(3): 420 – 434.

Stratton MR (2011) Exploring the genomes of cancer cells: Progress and promise. Science 331(6024): 1553.

Stratton MR, Campbell PJ & Futreal PA (2009) The cancer genome. Nature 458: 719.

Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ & Jackson SP (2005) MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 123(7): 1213 – 1226.

Sy SMH, Huen MSY & Chen J (2009a) MRG15 is a novel PALB2-interacting factor involved in homologous recombination. J Biol Chem 284(32): 21127 – 21131.

Sy SMH, Huen MSY & Chen J (2009b) PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proceedings of the National Academy of Sciences 106(17): 7155 – 7160.

Sy SMH, Huen MSY, Zhu Y & Chen J (2009c) PALB2 regulates recombinational repair through chromatin association and oligomerization. J Biol Chem 284(27): 18302 – 18310.

Syljuåsen RG, Sørensen CS, Hansen LT, Fugger K, Lundin C, Johansson F, Helleday T, Sehested M, Lukas J & Bartek J (2005) Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. Mol Cell Biol 25(9): 3553 – 3562.

Takeda S, Nakamura K, Taniguchi Y & Paull TT (2007) Ctp1/CtIP and the MRN complex collaborate in the initial steps of homologous recombination. Mol Cell 28(3): 351 – 352.

Tan SLW, Chadha S, Liu Y, Gabasova E, Perera D, Ahmed K, Constantinou S, Renaudin X, Lee M, Aebersold R & Venkitaraman AR (2017) A class of environmental and endogenous toxins induces BRCA2 haploinsufficiency and genome instability. Cell 169(6): 1105 – 1118.e15.

Técher H, Koundrioukoff S, Carignon S, Wilhelm T, Millot G, Lopez B, Brison O & Debatisse M (2016) Signaling from Mus81-Eme2-dependent DNA damage elicited by Chk1 deficiency modulates replication fork speed and origin usage. Cell Reports 14(5): 1114 – 1127.

Técher H, Koundrioukoff S, Nicolas A & Debatisse M (2017) The impact of replication stress on replication dynamics and DNA damage in vertebrate cells. Nat Rev Genet 18: 535.

Theunissen JF & Petrini JHJ (2006) Methods for studying the cellular response to DNA damage: Influence of the Mre11 complex on chromosome metabolism. Meth Enzymol 409: 251 – 284.

Thull DL & Vogel VG (2004) Recognition and management of hereditary breast cancer syndromes. Oncologist 9(1): 13 – 24.

Tischkowitz M & Xia B (2010) PALB2/FANCN: Recombining cancer and Fanconi anemia. Cancer Res 70(19): 7353.

Tischkowitz M, Xia B, Sabbaghian N, Reis-Filho JS, Hamel N, Li G, van Beers EH, Li L, Khalil T, Quenneville LA, Omeroglu A, Poll A, Lepage P, Wong N, Nederlof PM, Ashworth A, Tonin PN, Narod SA, Livingston DM & Foulkes WD (2007) Analysis of PALB2/FANCN-associated breast cancer families. Proc Natl Acad Sci USA 104(16): 6788 – 6793.

Tomimatsu N, Mukherjee B, Catherine Hardebeck M, Ilcheva M, Vanessa Camacho C, Louise Harris J, Porteus M, Llorente B, Khanna KK & Burma S (2014) Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. Nature Commun 5: 3561.

Topisirovic I & Sonenberg N (2015) Translation and cancer. Biochim Biophys Acta (BBA) – Gene Regulatory Mechanisms; Translation and Cancer 1849(7): 751 – 752.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J & Jemal A (2015) Global cancer statistics, 2012. CA: A Cancer Journal for Clinicians 65(2): 87 – 108.

Tubbs A & Nussenzweig A (2017) Endogenous DNA damage as a source of genomic instability in cancer. Cell 168(4): 644 – 656.

Typas D, Luijsterburg MS, Wiegant WW, Diakatou M, Helfricht A, Thijssen PE, van de Broek B, Mullenders LH & van Attikum H (2015) The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. Nucleic Acids Res 43(14): 6919 – 6933.

Vaclová T, Gómez-López G, Setién F, Bueno JMG, Macías JA, Barroso A, Urioste M, Esteller M, Benítez J & Osorio A (2015) DNA repair capacity is impaired in healthy BRCA1 heterozygous mutation carriers. Breast Cancer Res Treat 152(2): 271 – 282.

Van Vlierberghe P & Ferrando A (2012) The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 122(10): 3398 – 3406.

Veitia RA (2007) Exploring the molecular etiology of dominant-negative mutations. Plant Cell 19(12): 3843 – 3851.

Venkatachalam S, Shi YP, Jones SN, Vogel H, Bradley A, Pinkel D & Donehower LA (1998) Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. EMBO J 17(16): 4657 – 4667.

Vikrant, Kumar R, Siddiqui Q, Singh N, Waghmare SK & Varma AK (2015) Mislocalization of BRCA1-complex due to ABRAXAS Arg361Gln mutation. J Biomol Structure and Dynamics 33(6): 1291 – 1301.

Vineis P, Schatzkin A & Potter JD (2010) Models of carcinogenesis: an overview. Carcinogenesis 31(10): 1703 – 1709.

Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA,Jr & Kinzler KW (2013) Cancer genome landscapes. Science 339(6127): 1546 – 1558.

Vuong D, Simpson PT, Green B, Cummings MC & Lakhani SR (2014) Molecular classification of breast cancer. Virchows Archiv 465(1): 1 – 14.

Wagner JE, Tolar J, Levran O, Scholl T, Deffenbaugh A, Satagopan J, Ben-Porat L, Mah K, Batish SD, Kutler DI, MacMillan ML, Hanenberg H & Auerbach AD (2004) Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. Blood 103(8): 3226.

Wang B (2012) BRCA1 tumor suppressor network: focusing on its tail. Cell Biosci 2(1): 6-3701-2-6.

Wang B, Hurov K, Hofmann K & Elledge SJ (2009) NBA1, a new player in the Brca1 A complex, is required for DNA damage resistance and checkpoint control. Genes Dev 23(6): 729 – 739.

Wang B & Elledge SJ (2007) Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brcc36 complex in response to DNA damage. Proc Natl Acad Sci USA 104(52): 20759 – 20763.

Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP & Elledge SJ (2007) Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science 316(5828): 1194 – 1198.

Wang H & Xu X (2017) Microhomology-mediated end joining: new players join the team. Cell & Bioscience 7(1): 6.

Wang L, Zeng X, Chen S, Ding L, Zhong J, Zhao JC, Wang L, Sarver A, Koller A, Zhi J, Ma Y, Yu J, Chen J & Huang H (2013) BRCA1 is a negative modulator of the PRC2 complex. EMBO J 32(11): 1584.

Watson LM, Bamber E, Schnekenberg RP, Williams J, Bettencourt C, Lickiss J, Jayawant S, Fawcett K, Clokie S, Wallis Y, Clouston P, Sims D, Houlden H, Becker EBE & Németh AH (2017) Dominant mutations in GRM1 cause spinocerebellar ataxia type 44. Am J Hum Genet 101(3): 451 – 458.

Wiechmann L, Sampson M, Stempel M, Jacks LM, Patil SM, King T & Morrow M (2009) Presenting features of breast cancer differ by molecular subtype. Ann Surg Oncol 16(10): 2705 – 2710.

Wilhelm T, Magdalou I, Barascu A, Técher H, Debatisse M & Lopez BS (2014) Spontaneous slow replication fork progression elicits mitosis alterations in homologous recombination-deficient mammalian cells. Proc Natl Acad Sci USA 111(2): 763 – 768.

Wilhelm T, Ragu S, Magdalou I, Machon C, Dardillac E, Técher H, Guitton J, Debatisse M & Lopez BS (2016) Slow replication fork velocity of homologous recombination-defective cells results from endogenous oxidative stress. PLoS Genet 12(5): e1006007.

Wong AK, Ormonde PA, Pero R, Chen Y, Lian L, Salada G, Berry S, Lawrence Q, Dayananth P, Ha P, Tavtigian SV, Teng DH & Bartel PL (1998) Characterization of a carboxy-terminal BRCA1 interacting protein. Oncogene 17(18): 2279 – 2285.

Woodward AM, Göhler T, Luciani MG, Oehlmann M, Ge X, Gartner A, Jackson DA & Blow JJ (2006) Excess Mcm2–7 license dormant origins of replication that can be used under conditions of replicative stress. J Cell Biol 173(5): 673.

Wu Q, Paul A, Su D, Mehmood S, Foo TK, Ochi T, Bunting EL, Xia B, Robinson CV, Wang B & Blundell TL (2016) Structure of BRCA1-BRCT/Abraxas complex reveals phosphorylation-dependent BRCT dimerization at DNA damage sites. Mol Cell 61(3): 434 – 448.

Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, Pals G, Errami A, Gluckman E, Llera J, Wang W, Livingston DM, Joenje H & de Winter JP (2007) Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. Nat Genet 39(2): 159 – 161.

Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ & Livingston DM (2006) Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. Mol Cell 22(6): 719 – 729.

Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, Bouwman P, Bartkova J, Gogola E, Warmerdam D, Barazas M, Jaspers JE, Watanabe K, Pieterse M, Kersbergen A, Sol W, Celie PHN, Schouten PC, van den Broek B, Salman A, Nieuwland M, de Rink I, de Ronde J, Jalink K, Boulton SJ, Chen J, van Gent DC, Bartek J, Jonkers J, Borst P & Rottenberg S (2015) REV7 counteracts DNA double-strand break resection and affects PARP inhibition. Nature 521(7553): 541 – 544.

Yan J, Kim YS, Yang XP, Li LP, Liao G, Xia F & Jetten AM (2007) The ubiquitin-interacting motif containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response. Cancer Res 67(14): 6647 – 6656.

Yan J, Kim Y, Yang X, Albers M, Koegl M & Jetten AM (2006) Ubiquitin-interaction motifs of RAP80 are critical in its regulation of estrogen receptor α. Nucleic Acids Res 35(5): 1673 – 1686.

Yates LR & Campbell PJ (2012) Evolution of the cancer genome. Nat Rev Genet 13: 795.

You JS & Jones PA (2012) Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 22(1): 9 – 20.

Yu X, Chini CC, He M, Mer G & Chen J (2003) The BRCT domain is a phospho-protein binding domain. Science 302(5645): 639 – 642.

Yu X, Wu LC, Bowcock AM, Aronheim A & Baer R (1998) The C-terminal (BRCT) domains of BRCA1 interact *in vivo* with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. J Biol Chem 273(39): 25388 – 25392.

Yuan SF, Lee S, Chen G, Song M, Tomlinson GE & Lee EY-P (1999) BRCA2 is required for ionizing radiation-induced assembly of Rad51 complex *in vivo*. Cancer Res 59(15): 3547.

Zannini L, Delia D & Buscemi G (2014) CHK2 kinase in the DNA damage response and beyond. J Mol Cell Biol 6(6): 442 – 457.

Zeman MK & Cimprich KA (2013) Causes and consequences of replication stress. Nat Cell Biol 16: 2.

Zhang F, Fan Q, Ren K & Andreassen PR (2009a) PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. Mol Cancer Res 7(7): 1110 – 1118.

Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B & Yu X (2009b) PALB2 links BRCA1 and BRCA2 in the DNA-damage response. Curr Biol 19(6): 524 – 529.

Zhang Y, Coillie SV, Fang J & Xu J (2016) Gain of function of mutant p53: R282W on the peak? Oncogenesis 5: e196

Zhang Y & Jasin M (2011) An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. Nat Struct Mol Biol 18(1): 80 – 84.

Zhao H & Piwnica-Worms H (2001) ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. Mol Cell Biol 21(13): 4129 – 4139.

Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N & Dracopoli NC (1996) Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet 12: 97.

Original publications

I   Nikkilä, J., Parplys, AC., Pylkäs, K., Bose, M., Huo, Y., Borgmann, K., Rapakko, K., Nieminen, P., Xia, B., Pospiech, H., & Winqvist, R. (2013). Heterozygous mutations in *PALB2* cause DNA replication and damage response defects. Nat. Commun. 4:2578

II   Bose, M., Sachsenweger, J., Laurila, N., Parplys, AC., Willmann, J., Eshraghi, L., Dunlop, TW., Groth, M., Rapakko, K., Nieminen, P., Friedl, TWP., Heiserich, L., Meyer, F., Tuppurainen, H., Devarajan, R., Peltoketo, H., Nevanlinna, H., Pylkäs, K., Borgmann, K., Wiesmüller, L., Winqvist, R., & Pospiech, H. (2017). BRCA1 mislocalization leads to transcriptional misregulation and aberrant DNA damage response in *ABRAXAS* mutation carrier cells. Manuscript.

Original publications are not included in the electronic version of the dissertation.

Contribution by the doctoral candidate:

For Study I – I was responsible for performing the lab works for Figure 3 and preparing that figure for publication. Additionally, I significantly contributed in making parts of the manuscript revision needed for its publication.

For Study II – I conducted the following experiments: WB analyses, quantitative PCR, flow cytometry analyses, protein localization analysis, DNA damage signalling PCR array, caspase activation assay, statistical analyses and the foci analysis. In addition, I was also actively involved in analysing the RNA-sequencing data. In terms of Figures included in the publication, I have generated the following main figures; Figure 1 A – E, Figure 3, 4, 5, Figure 6 A & B, Figure 7, 8 and 9. And also the following supplementary figures: S2, S3, S4, S5, S6 A – F, S7 A – D, S8, S9, S10, S11, S12, S13 C, S14 and S15. Additionally, I drafted the initial manuscript and upon Prof. Robert Winqvist and Adj. Prof. Helmut Pospiech suggestions, I prepared the final manuscript.

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