OULU 2006
D 868

ACTA

UNIVERSITATIS OULUENSIS

Mari Leppilampi

FUNCTIONAL AND IMMUNOHISTOLOGICAL STUDIES ON CANCER-ASSOCIATED CARBONIC ANHYDRASE IX

FACULTY OF MEDICINE,
DEPARTMENT OF CLINICAL CHEMISTRY,
DEPARTMENT OF PATHOLOGY,
UNIVERSITY OF OULU

ACTA UNIVERSITATIS OULUENSIS
D Medica 868

MARI LEPPILAMPI

FUNCTIONAL AND IMMUNOHISTOLOGICAL STUDIES ON CANCER-ASSOCIATED CARBONIC ANHYDRASE IX

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 7 of Oulu University Hospital, on February 17th, 2006, at 12 noon

OULUN YLIOPISTO, OULU 2006

Acta Univ. Oul. D 868, 2006

Supervised by
Professor Seppo Parkkila
Docent Tuomo Karttunen

Reviewed by
Docent Teuvo Hentunen
Professor Claudiu Supuran

ISBN 951-42-7993-X (nid.)
ISBN 951-42-7994-8 (PDF) http://herkules.oulu.fi/isbn9514279948/

ISSN 0355-3221 http://herkules.oulu.fi/issn03553221/

OULU UNIVERSITY PRESS
OULU 2006

Leppilampi, Mari, Functional and immunohistological studies on cancer-associated carbonic anhydrase IX

Faculty of Medicine, Department of Clinical Chemistry, Department of Pathology, University of Oulu, P.O.Box 5000, FI-90014 University of Oulu, Finland

Acta Univ. Oul. D 868, 2006
Oulu, Finland

Abstract

The carbonic anhydrases (CAs) catalyze the reversible hydration of carbon dioxide. In mammals, there are 13 active isoenzymes, which clearly differ in their cell localisation, tissue distributions and functions.

CA IX, a unique transmembrane member of the CA gene family, is a tumour-associated protein which is thought to be involved in malignant cell invasion, adhesion and the regulation of cell proliferation. The main focus in the present study was on elucidating the function and expression of CA IX in normal and malignant tissues, especially in the alimentary tract. The functional studies also included CA II, which is regarded as another important CA isoenzyme in the alimentary tract.

CA IX immunostaining showed a decrease in the staining intensity of gastric adenomas with increasing dysplasia grade. Well differentiated carcinomas of the intestinal type showed expression comparable to that in the normal mucosa, while expression was decreased in the less differentiated tumours. CA IX deficiency (*Car9*$^{-/-}$) genotype and C57/BL6 strain were the main factors which increased the susceptibility of CA IX deficient mice fed on either a normal or high-salt diet to histological abnormalities, including foveolar hyperplasia and glandular atrophy in the gastric body mucosa, while CA II deficiency was associated with only minor histological abnormalities. In a physiological analysis, CA IX played only a minor role in duodenal bicarbonate secretion (DBS), whereas absence of CA II in mice completely abolished the stimulatory effect of E-type prostaglandin 2 (PGE_2) on duodenal alkalisation.

The results demonstrate that CA IX expression is diminished in most gastric tumours. The variations observed in its expression support the concept that gastric adenomas and carcinomas do not emerge as progressive steps on a single pathway but may instead represent distinct entities with heterogenic genetic backgrounds. In the stomach, CA IX is mainly involved in the regulation of tissue morphogenesis in the body mucosa, while CA II has a major role in maintaining the gastroduodenal acid/base balance.

Keywords: atrophy, bicarbonate, cancer, carbonic anhydrase, hyperplasia, knockout, pH, stomach

Knowledge is a process of piling up facts;
wisdom lies in their simplification.

- Harold Fabing & Ray Marr

Acknowledgements

The present study was carried out at the Departments of Clinical Chemistry and Pathology, University of Oulu, and the Laboratory of Oulu University Hospital. I would like to thank Professor Arto Pakarinen, M.D., Ph.D., Chief of the Laboratory of Oulu University Hospital, Professor Aimo Ruokonen, M.D., Ph.D., Head of the Department of Clinical Chemistry, and Professors Juha Risteli, M.D., Ph.D., and Eeva-Riitta Savolainen, M.D., Ph.D., in the Department of Clinical Chemistry, for kindly providing me with research facilities.

I owe my deepest and warmest thanks to my supervisor Professor Seppo Parkkila, M.D., Ph.D. His expert scientific knowledge together with his patience, optimism and encouragement have been extremely important to me. Seppo, I am greatly indebted to you for all the support that you have given me during this time. Also my second supervisor Dr. Tuomo Karttunen, M.D., Ph.D. is greatly acknowledged for his patient and expert guidance, especially during the microscopy sessions. I am deeply grateful to Dr. Jyrki Kivelä, D.D.S., Ph.D., for the statistical analyses in articles II and III. My special thanks go to Dr. Jokke Hannuksela, M.D., Ph.D. Jokke, it has been a pleasure to work with you.

I want to thank my co-authors Dr. Anna-Kaisa Parkkila, M.D., Ph.D., Dr. Juha Saarnio, M.D., Ph.D., Dr. Silvia Pastoreková, Ph.D., Professor Jaromir Pastorek, Ph.D., Professor William S. Sly, M.D., Professor Abdul Waheed, Ph.D., Professor Hannu Rajaniemi, M.D., Ph.D., Professor Gunnar Flemström, M.D., Ph.D., Dr. Markus Sjöblom, Ph.D., Dr. Pirjo Koistinen, M.D., Ph.D., Professor Onni Niemelä, M.D., Ph.D., Dr. Marta Ortova Gut, Ph.D., and Dr. Gerolf Gros, M.D., Ph.D., for their invaluable collaboration to the original papers.

I thank the official examiners of this thesis, Professor Claudiu Supuran, Ph.D., and Dr. Teuvo Hentunen, Ph.D., for their valuable criticism and comments on this thesis. They helped me to improve the quality of this thesis. I am grateful to Mr Malcolm Hicks, M.A., for his careful revision of the language of the manuscript of this thesis.

I thank Ms Lissu Hukkanen, Ms Pirkko Peronius, Ms Erja Tomperi, Ms Mirja Vaheri, Ms Aulikki Lehmus, Ms Sirpa Tausta, Ms Eija Nissinen, Ms Annemari Lyytinen, and Ms Tanja Sankala for their skilful technical assistance.

I am for ever indebted to my parents Aila and Arvi Korpinen. They have always supported me and believed in me. I want to express my warmest thanks to my brothers Kari-Pekka, Juha and Jani, and my sister Anu, and their families, for so many unforgettable and joyful moments.

Above all, I wish to express my loving thanks to my husband Heikki and to our wonderful children Sami, Mika and Kati. You are the most important thing in my life.

Oulu, December 2005 Mari Leppilampi

Abbreviations

aa	Amino acid
AE	Anion exchanger
AIP	Autoimmune pancreatitis
AML	Acute myeloid leukaemia
ALL	Acute lymphoblastic leukaemia
APC	Adenomatous polyposis coli protein
APC	Adenomatous polyposis coli gene
BSA	Bovine serum albumin
CA	Carbonic anhydrase
CA	Carbonic anhydrase gene
CAI	Carbonic anhydrase inhibitor
cAMP	3',5'-cyclic adenosine monophosphate
Car2$^{-/-}$	Carbonic anhydrase II deficient
Car9$^{-/-}$	Carbonic anhydrase IX deficient
CARP	Carbonic anhydrase-related protein
cDNA	Complementary deoxyribonucleic acid
CMML	Chronic myelomonocytic leukaemia
DAB	3,3'diaminobenzidine tetrahydrochloride
DBS	Duodenal bicarbonate secretion
ECM	Extracellular matrix
EST	Expressed sequence tag
FAB	French, American, British classification
FIH	Factor inhibiting hypoxia inducible factor
GPI	Glycosyl phosphatidylinositol
HIF	Hypoxia inducible factor
HRE	Hypoxia response element
IHC	Immunohistochemistry
kDa	kiloDalton
Mab	Monoclonal antibody
mRNA	Messenger ribonucleic acid

nonO	non-Pit-Oct-Unc domain-containing octamer-binding protein
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PG	Proteoglycan
PGE_2	E-type prostaglandin 2
RNA	Ribonucleic acid
RPTP	Receptor-type protein tyrosine phosphatase
RT-PCR	Reverse transcriptase polymerase chain reaction
SDS	Sodium dodecyl sulphate
TBST	Tris-buffered saline with Tween-20
VHL	Von Hippel-Lindau
WT	Wild type

List of original publications

This thesis is based on the following original papers, which are referred to in the text by the Roman numerals I-IV:

I Leppilampi M, Koistinen P, Savolainen ER, Hannuksela J, Parkkila A-K, Niemelä O, Pastoreková S, Pastorek J, Waheed A, Sly WS, Parkkila S, Rajaniemi H (2002). The expression of carbonic anhydrase II in hematological malignancies. Clin Cancer Res 8: 2240-2245.

II Leppilampi M, Saarnio J, Karttunen TJ, Kivelä J, Pastoreková S, Pastorek J, Waheed A, Sly WS, Parkkila S (2003) Carbonic anhydrase isozymes IX and XII in gastric tumors. World J Gastroenterol 9: 1398-1403.

III Leppilampi M, Karttunen TJ, Kivelä J, Ortova Gut M, Pastoreková S, Pastorek J, Parkkila S (2005) Gastric pit cell hyperplasia and glandular atrophy in carbonic anhydrase IX knockout mice: Studies on two strains C57/BL6 and BALB/c. Transgenic Res 14: 655-663.

IV Leppilampi M, Parkkila S, Karttunen TJ, Ortova Gut M, Gros G, Sjöblom M (2005) Carbonic anhydrase isozyme II-deficient mice lack the duodenal bicarbonate secretory response to prostaglandin E2. Proc Natl Acad Sci USA 102: 15247-15252.

Contents

1 Introduction

The carbonic anhydrases are enzymes that catalyze the reversible hydration of carbon dioxide. There are at least 13 active CA isoenzymes in mammals, and they have different cell localisations, tissue distributions and functions. They are involved in many biological processes, such as the regulation of pH homeostasis, ion transport, respiration, gluconeogenesis, bone resorption and renal acidification and the formation of cerebrospinal fluid and gastric acid (Sly & Hu 1995, Hewett-Emmett & Tashian 1996, Parkkila & Parkkila 1996, Hewett-Emmett 2000, Lehtonen *et al.* 2004a).

CA II is a very efficient enzyme and the most widely distributed of the CA isoenzymes (Khalifah 1971, Sanyal & Maren 1981, Tashian 1992). It is expressed in most organs of the alimentary tract, with high expression in the gastric and intestinal epithelia (Lönnerholm *et al*. 1985, Parkkila S *et al*. 1994, Parkkila & Parkkila 1996, Parkkila 2000). Recent studies have shown that two membrane-associated isoenzymes are also abundantly expressed in various gastrointestinal tissues. Interestingly, both of them have been functionally linked to certain cancers. Of these two isoenzymes, CA IX is a transmembrane glycoprotein composed of four parts: an N-terminal signal peptide, a large extracellular part, a transmembrane anchor and a short intracellular C-terminal tail (Opavský *et al*. 1996). It is rendered distinct from the other isoenzymes by its extracellular part, which has proteoglycan-like (PG-like) and CA domains. Normally CA IX is expressed mainly in the mucosa of the stomach, intestine and gallbladder (Pastoreková *et al*. 1992, 1997, Pastorek *et al*. 1994, Saarnio *et al.* 1998b). Abnormally high CA IX expression has been observed in many carcinomas originating from CA IX-negative tissues (Turner *et al*. 1997, Vermylen *et al*. 1999, Giatromanolaki *et al*. 2001, Liao *et al*. 1994, 1997, McKiernan *et al*. 1999, Saarnio *et al*. 1998b, 2001, Chia *et al*. 2001, Bartošová *et al*. 2002, Beasley *et al*. 2001, Hoogsteen *et al*. 2005). CA IX has been implicated in the regulation of cell proliferation, adhesion, and malignant cell invasion (Saarnio *et al*. 1998a, Svastova *et al*. 2003, 2004, Ivanov *et al*. 1998, Helmlinger *et al.* 2002). The other cancer-associated transmembrane CA isoenzyme is CA XII, which has been demonstrated in various normal tissues, including the colonic mucosa, and it is overexpressed in certain cancers and tumour cell lines (Ivanov *et al.* 1998, 2001, Türeci *et al.* 1998, Kivelä *et al*. 2000a,b, Parkkila *et al.* 2000a,b).

The main aim of this work was to elucidate the function and expression of CA IX in normal and malignant tissues, especially in the alimentary tract. Attempts were also made to ascertain whether CA IX deficiency induces the formation of neoplasias in the gastric mucosa of mice fed on a procarcinogenic high salt diet and to compare phenotypic changes and susceptibility to pathogenic processes in two mouse strains, C57/BL6 and BALB/c. Since CA II is another important isoenzyme in the alimentary tract, this was also included in the investigations. Mice lacking one or both of these enzymes were studied to see whether there are significant changes in stomach morphology or the regulation of duodenal bicarbonate secretion (DBS).

2 Review of the literature

2.1 Carbonic anhydrases

The carbonic anhydrases are zinc-containing proteins that catalyze the reversible hydration of carbon dioxide, $CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+$. There are α-, β-, γ-, δ- and ε-gene families, which are evolutionally unrelated (Hewett-Emmett & Tashian 1996, Hewett-Emmett 2000, Scozzafava *et al*. 2004). It was thought earlier that the α-CAs occur in animals and plant green algae, the β-CAs in many plants, lower eukaryotes and invertebrates, and the γ-CAs in archaebacteria and eubacteria, but this concept was challenged by Hewett-Emmett & Tashian (1996), because the plant Arabidopsis has homologues of all three families. The α-CAs are evidently dominant in vertebrates, however (Hewett-Emmett 2000).

The α-CAs have different cell localisations, tissue distributions and functions. They are involved in many biological processes, such as pH homeostasis, ion transport, respiration, gluconeogenesis, bone resorption, renal acidification, and in the formation of cerebrospinal fluid and gastric acid. So far 13 active isoenzymes have been identified in mammals: five cytoplasmic (CA I, CA II, CA III, CA VII and CA XIII), five membrane-associated (CA IV, CA IX, CA XII, CA XIV and CA XV), two mitochondrial (CA VA and CA VB) and one secreted (CA VI). In addition, a non-classical form NonO/p54^{nrb} has been described (Sly & Hu 1995, Hewett-Emmett and Tashian 1996, Parkkila & Parkkila 1996, Hewett-Emmett 2000, Lehtonen *et al.* 2004a, Hilvo *et al.* 2005, Karhumaa *et al*. 2000b) (Fig. 1, Table 1). There are also three carbonic anhydrase-related proteins (CARPs) that belong to the CA gene family (Tashian *et al.* 2000).

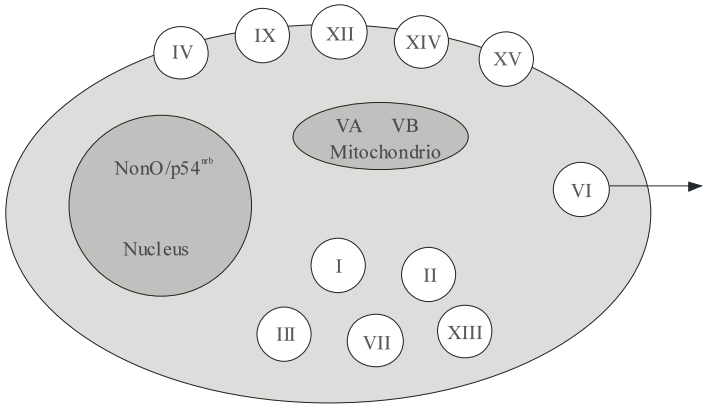

Fig. 1. Locations of active CA isoenzymes in a schematic cell model. CA I, II, III, VII, and XIII are cytoplasmic isoenzymes, VA and VB mitochondrional, CA VI secretory and CA IV, IX, XII, XIV and XV membrane-bound. NonO/p54^{nrb} is a nuclear non-classical form.

Table 1. Distribution of CA isoenzymes in the human alimentary tract.

Isoenzyme	Example(s) of positive tissue(s)	Literature
I	Epithelial cells of the colon, subepithelial capillary endothelium, α-cells of Langerhans islets	Lönnerholm *et al*. 1985, Parkkila S *et al*. 1994
II	Epithelial cells of the salivary glands, oesophagus, stomach, small intestine, colon, pancreatic duct, biliary tract, hepatocytes	Lönnerholm *et al*. 1985, Parkkila S *et al*. 1994
III	Liver	Carter *et al.* 1989, Kelly *et al*. 1991
IV	Epithelial cells of the duodenum, colon and biliary tract, subepithelial capillary endothelium, jejunum, ileum, pancreas	Fleming *et al*. 1995, Parkkila S *et al*. 1996, Fujikawa-Adachi *et al*. 1999a, Saarnio *et al.* 1998a
V	β-cells of Langerhans islets, hepatocytes	Nagao *et al*. 1993, Parkkila *et al*. 1998
VI	Salivary glands, pancreas	Parkkila *et al*. 1990, Fujikawa-Adachi *et al*. 1999a, Leinonen *et al.* 2001
IX	Epithelial cells of the oesophagus, stomach, small intestine, colon, rectum, biliary tract, pancreas	Pastorek *et al*. 1994, Turner *et al*. 1997, Fujikawa-Adachi *et al*. 1999a, Saarnio *et al.* 1998a, Ivanov *et al.* 2001
XII	Epithelial cells of the salivary glands, stomach, colon, pancreatic acini	Türeci *et al*. 1998, Ivanov *et al*. 1998, Fujikawa-Adachi *et al*. 1999a, Kivelä *et al*. 2000a
XIV	Small intestine, colon	Fujikawa-Adachi *et al*. 1999c
XV	Not expressed in human tissues	Hilvo *et al.* 2005

There are two main classes of CA inhibitors (CAIs): metal-complexing anions and unsubstituted sulphonamides (Supuran & Scozzafava 2000, Kim *et al*. 2000, Bertini *et al*. 1982, Briganti *et al*. 1996, Lindahl *et al*. 1991). As with their catalytic activities, the

isoenzymes also differ in their affinity for CAIs (Table 2). These CAIs are widely used as therapeutic agents in the prevention or management of many diseases (Supuran & Scozzafava 2000, 2002), and the CAs and their inhibitors offer interesting opportunities for the development of novel drugs and new diagnostic tools and for understanding in greater depth the fundamental processes in which the actions of different CA isoenzymes are involved (Supuran *et al*. 2003).

Table 2. Higher vertebrate α-CAs and CARPs, their relative CO_2 hydration activity and their affinity for sulphonamide inhibitors (Pastorekovà et al. 2004, Nishimori et al. 2005b,c, Lehtonen et al. 2004a, Whittington et al. 2004, Ulmasov et al. 2000)

Isoform	Catalytic activity	Affinity for sulphonamides
CA I	low-moderate	medium
CA II	high	very high
CA III	very low	very low
CA IV	high	high
CA VA	moderate	high
CA VB	high	high
CA VI	moderate	medium-low
CA VII	high	very high
CARP VIII	acatalytic	-
CA IX	high	high
CARP X	acatalytic	-
CARP XI	acatalytic	-
CA XII	low-moderate	high
CA XIII	low-moderate (mouse)	high
CA XIV	low (human) / high (mouse)	high (human)

2.1.1 Carbonic anhydrase isoenzyme I

CA I is a well-known cytoplasmic CA isoenzyme with a molecular weight of about 30 kDa (Bundy 1977, Lindskog *et al*. 1984). Its gene has been cloned from a human genomic cDNA library (Tashian 1989, Butterworth *et al.* 1991) and has a total length of 50 kb, of which about 36 kb consists of a large intron separating the erythroid-specific promoter from the coding region. Occasionally a small (54 bp) non-coding exon is found within this intron in transcripts (Lowe *et al*. 1990).

CA I has been detected in erythrocytes, where it is one of the most abundant proteins. It is also expressed in the capillary and corneal endothelium, the lens of the eye, the islets of Langerhans, foetal membranes and placenta (Lönnerholm *et al*. 1985, Venta *et al*. 1987, Sasaki *et al*. 1993, Mühlhauser *et al*. 1994). In the gastrointestinal tract it is present in the epithelium of the oesophagus, jejunum, ileum and colon, in the A-cells of the pancreatic Langerhans's islets and in the cryptal enterocytes of the small intestine (Lönnerholm *et al.* 1985, Venta *et al.* 1987, Mühlhauser *et al.* 1994, Parkkila S *et al.* 1994, Christie *et al*. 1997, Saarnio *et al.* 1998a).

2.1.2 Carbonic anhydrase isoenzyme II

CA II is a 30 kDa protein, and the human 17 kb long *CA2* gene is located on chromosome 8, like the *CA1* and *CA3* genes. The protein coding region of the human *CA2* gene is 64-65% identical to those of human *CA1* and *CA3*, which are thought to have arisen by gene duplication (Venta *et al*. 1983, Nakai *et al*. 1987, Murakami *et al*. 1987, Tashian 1989).

CA II was originally found in erythrocytes (Meldrum & Roughton 1932, 1933) and is one of the most efficient enzymes known (Khalifah 1971, Sanyal & Maren 1981) and the most widely distributed of the CA isoenzymes, being present in practically every human tissue or organ (Tashian 1992). In the alimentary tract it is thought to participate in bicarbonate secretion in the exocrine glands (Lönnerholm *et al*. 1985, Parkkila & Parkkila 1996, Parkkila 2000). In the stomach, CA II is expressed in the surface epithelial and parietal cells of the gastric mucosa, where its main physiological function is to regulate the acidity of the gastric juice (Davenport & Fisher 1938, Davenport 1939, O'Brien *et al*. 1977, Parkkila S *et al*. 1994, Parkkila & Parkkila 1996, Parkkila 2000). It is expressed in the surface epithelial cells of the duodenum, jejunum, ileum, caecum, colon and rectum (Lönnerholm *et al*. 1985, Parkkila S 1994), and also those of Brunner's glands. The gastric and duodenal surface epithelial cells secrete mucus and bicarbonate to form a HCO_3^--containing mucous gel layer that covers the epithelium and protects it from digestion (Kivilaakso & Silen 1981, Flemström 1986, Parkkila & Parkkila 1996, Parkkila 2000).

CA II has been demonstrated in the hepatocytes of the liver and in the epithelium of the bile ducts (Dodgson *et al.* 1984, Carter *et al.* 1989, Parkkila S *et al.* 1994). In the pancreas, positive signals for CA II in immunohistochemical stainings have been observed in the epithelial duct cells (Kumpulainen & Jalovaara 1981, Spicer *et al.* 1982, Kumpulainen 1984). CA II is expressed in the renal tubular cells and in the intercalated cells of the collecting ducts of the kidney, where it contributes to urinary acidification (Wistrand 1980, Wåhlstrand & Wistrand 1980, Kumpulainen 1984, Sly & Hu 1995, Lai *et al.* 1998).

CA II is expressed in various other tissues and organs in addition to those described above, including the central nervous system (Kumpulainen *et al.* 1985, Parkkila A-K *et al.* 1994, 1995, 1997), the human adrenal and pituitary glands (Parkkila A-K *et al.* 1993, 1996), alveolar pneumocytes (Fleming *et al.* 1994), human placenta, foetal membranes, several tissues of the reproductive tract (Mühlhauser *et al.* 1994, Kaunisto *et al.* 1990, 1995, Parkkila *et al.* 1991a, Ichihara *et al.* 1997) and osteoclasts (Lehenkari *et al*. 1998). CA II is essential in osteoclast differentiation and bone resorption by regulating the steady state intracellular pH and $Ca2^+$ (Lehenkari *et al*. 1998). It is needed also for osteoclast formation *in vitro* (Laitala-Leinonen *et al*. 1999).

2.1.2.1 CA II deficiency

Carbonic anhydrase II deficiency is the primary defect in a recessive inherited human syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification (Sly *et al*. 1983, Sly & Hu 1995). Other features are growth failure, dental malocclusions and

mental retardation (Strisciuglio *et al*. 1990). Twenty-three mutations have so far been reported to result in this severe but relatively rare disease (Venta *et al*. 1991, Roth *et al*. 1992, Hu *et al*. 1992, 1994, 1997, Strisciuglio *et al*. 1998, Soda *et al*. 1995, 1996, Shah *et al*. 2004) (Table 3). Although CA II is the most abundant isoenzyme in the alimentary tract, no gastrointestinal symptoms have been reported in CA II-deficient patients.

The long-term features of carbonic anhydrase II deficiency syndrome have been described in 35 Saudi Arabian children. Clinically, these patients had typical facial features, and biochemically they all had renal tubular acidosis. Five of them were blind secondary to optic nerve entrapment, and two developed anaemia and secondary erythropoiesis caused by bone marrow involvement. Nineteen patients had attained final adult height. Two patients were married and had normal children. It can be concluded that this syndrome is compatible with long-term survival but can progress and involve the cranial nerves. Thorough clinical and neurological evaluation of such patients is necessary for the early detection and management of potentially serious complications (Awad *et al*. 2002).

Table 3. Mutations in CA II-deficient patients (Shah et al. 2004). The cDNA numbering starts with A of the ATG translation initiation codon as nucleotide +1

Mutation base change	Ethnic group	Reference
82C>T	Turkish	Shah *et al*. 2004
99delC	Italian	Hu *et al*. 1997
120T>G	Japanese	Soda *et al*. 1995
142_145delTCTG	American	Hu *et al*. 1997
145_148delGTTT	American	Shah *et al*. 2004
157delC	Brazilian	Hu *et al*. 1997
191delA	Egyptian	Hu *et al*. 1997
220_221delCA	Ecuadorian	Shah *et al*. 2004
232+1G>A	Arabian	Hu *et al*. 1992
275A>C	Gypsy (Czech)	Hu *et al*. 1997
280C>T	Canadian	Shah *et al*. 2004
290G>A	Italian	Shah *et al*. 2004
319C>T	Belgian	Venta *et al*. 1991
	Italian	Roth *et al*. 1992
	Japanese	Soda *et al*. 1996
430G>C	Canadian	Shah *et al*. 2004
505delA	Afghani	Shah *et al*. 2004
507-1G>C	German	Roth *et al*. 1992
535_536insGT	Italian	Shah *et al*. 2004
621delC	Turkish	Shah *et al*. 2004
630_641del12insCACA	Irish traveller	Shah *et al*. 2004
663+1G>T	Italian	Hu *et al*. 1997
679delA	Caribbean	Hu *et al*. 1994
	Hispanic	
696_697delGG	Indian (Asian)	Shah *et al*. 2004
735delG	Mexican	Hu *et al*. 1997

Mice homozygous for the null allele in the *Car2* gene are runted and have renal tubular acidosis, but the osteopetrosis found in humans with CA II deficiency could not be detected even in very old homozygous null mice (Lewis *et al.* 1988). CA II-deficient (*Car2*$^{-/-}$) mice differ from controls in that they show an age-dependent medial calcification of the small arteries in a number of organs. The male genital tract reveals the most extensive arterial calcinosis, and males may be more seriously affected in general than females. *Car2*$^{-/-}$ mice occasionally show additional changes in the uterus, small bowel, lymph nodes or renal pelvis (Spicer *et al.* 1989). No major morphological abnormalities at either the cellular or subcellular level have been found in the brains of CA II-deficient mice, even though CA II is the main isoenzyme in the nervous system. This suggests that these mice utilize other enzymatic or physiological pathways to compensate for absence of the enzyme (Ghandour *et al.* 1989). Interestingly, type A intercalated cells disappear postnatally from the kidneys of CA II-deficient mice, possibly as a result of apoptosis, loss of differentiation or interconversion to principal cells (Brion *et al*. 2001).

2.1.3 Carbonic anhydrase isoenzyme III

CA III is a 30 kDa protein with a gene architecture very similar to *CA2*, but it has the lowest activity of all the isoenzymes, only about 1% of that of isoenzyme II, and is relatively resistant to acetazolamide (Tashian 1989, Engberg *et al*. 1985, Edwards 1991). Despite the low activity, it has a well-defined binding site for bicarbonate (Eriksson *et al.* 1993), which may lead to its own oxidative modification (Stadtman *et al*. 1990, Stadtman & Berlett 1991, Cabiscol & Levine 1995).

CA III is abundantly present in type I fibre red skeletal muscle, where it comprises about 8% of the cytosolic protein (Holmes 1976, Register *et al.* 1978, Väänänen *et al*. 1985). It has also been detected at lower levels in the human and rat liver (Carter *et al.* 1989, Kelly *et al*. 1991), in the epithelium of the salivary gland ducts, colon, bronchi and male genital tract and in adipocytes, where it accounts for almost a quarter of the total cytosolic protein (Lynch *et al*. 1993, Spicer *et al*. 1990). The human uterus, urine bladder, lung and myoepithelial cells also contain CA III (Jeffery *et al.* 1980, Väänänen & Autio-Harmainen 1987).

The functional roles of isoenzyme III are not clear. Recently published results on *Car3*$^{-/-}$ knockout mice showed no apparent morphological or functional abnormalities linked to the gene defect (Kim *et al*. 2004). In contrast, previous cell biological studies have proposed a role for CA III in protecting cells from oxidative damage, and it could thus also affect growth-signalling pathways (Räisänen *et al.* 1999). Excessive ethanol consumption induces CA III expression in the perivenous hepatocytes, where reactive aldehyde products are simultaneously induced, from which it can be concluded that CA III may protect the hepatocytes from oxidative damage (Niemelä *et al*. 1991, 1994, Chen & Chesler 1992, Halsted *et al*. 1993, Niemelä 1993, Parkkila *et al*. 1999). It may also serve as a clinical biomarker of muscle damage. Interestingly, measurement of the myoglobin/CA III ratio during the first hours after the initiation of thrombolysis may be

useful for evaluating the success of reperfusion after acute myocardial infarction (Vuotikka *et al*. 2003).

2.1.4 Carbonic anhydrase isoenzyme IV

CA IV was the first membrane-associated CA isoenzyme to be described (Whitney & Briggle 1982, Wistrand & Knuuttila 1989). The molecular weight of the human protein is 35 kDa (Zhu & Sly 1990) and the gene is located on chromosome 17 (Okuyama *et al.* 1992, 1993). CA IV is anchored to the apical plasma membranes of epithelial cells through a glycosyl phosphatidylinositol (GPI) link (Zhu & Sly 1990, Ghandour *et al*. 1992, Fleming *et al*. 1995, Okuyama *et al*. 1995).

In the gastrointestinal tract CA IV is expressed abundantly in the apical plasma membrane of the colonic epithelium and at lower levels in the epithelial cells of the small intestine. It is also prominent in the subepithelial capillary endothelium of all segments of the gastrointestinal canal (Fleming *et al.* 1995) and has been observed in the human gallbladder and the biliary epithelial cells (Parkkila S *et al.* 1996).

CA IV has been detected in the lung and kidney (Whitney & Briggle 1982, Wistrand & Knuuttila 1989, Zhu & Sly 1990, Brown *et al.* 1990) and is also expressed in the eye (Hageman *et al*. 1991), brain, skeletal muscle, heart, liver (Waheed *et al.* 1992, Ghandour *et al.* 1992, Sender *et al.* 1994), male reproductive tract (Parkkila S *et al.* 1993, Kaunisto *et al.* 1995) and pancreas (Fujikawa-Adachi *et al.* 1999a). As the presence of serum antibodies to CA IV has shown a significant correlation with the immune response in autoimmune pancreatitis (AIP) patients, CA IV may be a target antigen that is commonly expressed in the epithelial cells of specific tissues involved in AIP and related diseases (Nishimori *et al*. 2005a). CA IV is also functionally linked to another disease, the RP17 form of retinitis pigmentosa. Rebello *et al*. (2004) have recently described a signal sequence mutation in the *CA4* gene that induces apoptosis in the endothelial cells of the choriocapillaris, leading to ischaemia in the overlying retina and producing an autosomal dominant form of retinitis pigmentosa.

2.1.5 Carbonic anhydrase isoenzyme V

CA V is unique among the CAs in its localisation in the mitochondrial matrix (Väänänen *et al.* 1991, Nagao *et al*. 1993, Heck *et al*. 1994). The cDNA for human CA V has been cloned from human liver, and the *CA5* gene has been mapped to chromosome 16 (Nagao *et al.* 1993). Since another mitochondrial CA was later characterised (Fujikawa-Adachi *et al.* 1999b, Shah *et al.* 2000), these two isoenzymes have been termed CA VA and CA VB. The cDNA clone for CA VB was isolated from human pancreas and salivary glands, and the *CA5B* gene is located on chromosome X (Fujikawa-Adachi *et al.* 1999b).

Expression of the CA VA protein has been demonstrated in the liver and skeletal muscle, whereas CA VB has been detected in the brain, heart, liver, lung, kidney, spleen, intestine, testis, skeletal muscle and pancreas. These different distribution patterns suggest that CA VA and CA VB are two genetically distinct isoforms of human CA V

having different physiological roles (Fujikawa-Adachi *et al.* 1999b, Shah *et al.* 2000). It has been proposed that CA VA may participate in two metabolic processes in the mitochondria of hepatocytes: ureagenesis and gluconeogenesis (Dodgson 1991), while CA VB may have a role in the regulation of insulin secretion in the pancreas (Parkkila *et al*. 1998). Mitochondrial CA has also been demonstrated in adipocytes, where it is involved in lipogenesis (Hazen *et al*. 1996). In addition, the expression of CA VB in astrocytes and neurons suggests that it has a physiological role in the nervous system, it may be involved in gluconeogenesis in the astrocytes, and neuronal CA VB could have a role in regulation of the intramitochondrial calcium level. CA VB may also participate in bicarbonate ion-induced GABA responses by regulating bicarbonate homeostasis in the neurons (Ghandour *et al*. 2000).

2.1.6 Carbonic anhydrase isoenzyme VI

CA VI is the only secretory CA isoenzyme. It was originally found in the ovine parotid gland and saliva by Fernley *et al.* (1979) and was purified from human saliva almost simultaneously by two groups (Murakami & Sly 1987, Kadoya *et al.* 1987). The cDNA sequence of human CA VI was determined by Aldred *et al*. (1991), and the *CA6* gene was mapped to chromosome 1 (Aldred *et al.* 1991, Sutherland *et al*. 1989, White *et al.* 1998). The molecular weight of CA VI is about 42 kDa in several species (Feldstein & Silverman 1984, Kadoya *et al.* 1987, Murakami & Sly 1987, Fernley 1991, Parkkila *et al.* 1991b, Ogawa *et al.* 1992). Its catalytic domain is highly homologous to four transmembrane CAs which have an extracellular CA domain (CA IV, CA IX, CA XII and CA XIV) (Fujikawa-Adachi *et al.* 1999b, Mori *et al.* 1999).

CA VI is expressed in the serous acinar cells of the parotid and submandibular glands, from where it is secreted into the saliva (Kadoya *et al*. 1987, Parkkila *et al*. 1991b, Ogawa *et al*. 1992, 1993) under the control of the autonomic nervous system (Fernley 1991), so that its concentration follows a circadian periodicity, being very low during sleep and higher in the daytime and after meals (Parkkila S *et al.* 1995). The gastric mucus contains CA VI, but because it is not expressed in the gastric surface epithelial cells, it must be of salivary origin (Parkkila S *et al*. 1994, 1997). In addition, CA VI has been demonstrated in serum, lacrimal glands, pancreas, mammary glands and milk (Kivelä *et a*l. 1997b, Ogawa *et al*. 1995, Fujikawa-Adachi *et al*. 1999a, Karhumaa *et al*. 2001b).

The exact physiological functions of CA VI are not completely clear. It was originally thought to have a highly specialised role in the maintenance of bicarbonate levels in the saliva (Fernley 1988), but it was later demonstrated that the salivary enzyme is not directly involved in that function but may rather play a role in the enamel pellicle, which is a thin layer of proteins covering the teeth, where it may protect the teeth from caries by neutralising locally the acid produced by cariogenic bacteria (Kivelä *et al*. 1997a,b, 1999, Leinonen *et al*. 1999). It is also thought to neutralise the excess acid in the upper gastrointestinal tract (Parkkila S *et al.* 1997). The high concentration of CA VI in the colostrum suggests that it may have a role in the development or maturation of the infant

gastrointestinal canal (Karhumaa *et al.* 2001b), and it is also involved in taste functions (Thatcher *et al.* 1998, Henkin *et al.* 1999a,b, Law *et al.* 1987).

Another form of CA VI has also been identified, a stress-inducible enzyme (type B) that is thought to take part in the intracellular pH changes induced by stress (Sok *et al.* 1999), but its exact role has never been investigated in detail.

2.1.7 Carbonic anhydrase isoenzyme VII

The cytoplasmic CA VII is the most highly conserved isoenzyme in mammals, with its gene located on chromosome 16 (Montgomery *et al.* 1991, Tashian 1992, Lakkis *et al.* 1997, Earnhardt *et al*. 1998). CA VII mRNA has been detected in the human salivary gland, rat and mouse lung and mouse brain neurons (Montgomery *et al*. 1991, Ling *et al*. 1994, Lakkis *et al*. 1996, 1997). The recombinant CA VII protein shows high enzyme activity that is inhibitable by sulphonamides, but its tissue expression at the protein level has not yet been described (Earnhardt *et al.* 1998, Vullo *et al*. 2005).

2.1.8 Carbonic anhydrase isoenzyme IX

2.1.8.1 Gene and protein

CA IX, initially described as a tumour-associated membrane antigen MN, was first identified in the cervical carcinoma cell line HeLa (Pastoreková *et al*. 1992). Human CA IX cDNA was cloned and sequenced by Pastorek *et al*. (1994), and the genomic structure was subsequently characterized by Opavský *et al*. (1996). The *CA9* gene consists of eleven exons and ten introns, and is located on the chromosome 17 (Opavský *et al*. 1996, Ivanov *et al.* 1998). *CA9* cDNA codes for a protein sequence of 459 amino acids (aa).

CA IX is a transmembrane glycoprotein that is present on Western blots as a twin band of 54 and 58 kDa. It is composed of four parts: 1) an N-terminal signal peptide (aa 1-37), 2) a large extracellular part (aa 38-414), 3) a transmembrane anchor (aa 415-434), and 4) a short intracellular C-terminal tail (Fig. 2). The extracellular part consists of a proteoglycan-like (PG-like) region (aa 53-111) and a carbonic anhydrase domain (aa 135-391). Exon 1 encodes both a signal peptide and a proteoglycan-like region, exons 2-8 code for a CA domain with a highly conserved active site, exon 10 encodes a transmembrane anchor, and exon 11 an intracellular tail (Opavský *et al*. 1996). Under non-reducing conditions, it is able to form homo-oligomers (most likely trimers) with a molecular weight of 153 kDa (Pastoreková *et al*. 1992, Zàvada *et al*. 1993, 2000). *CA9* is considered to be a chimeric gene, assembled by exon shuffling, because its N- and C-terminal parts are unrelated to those of other CA isoenzymes (Opavský *et al*. 1996).

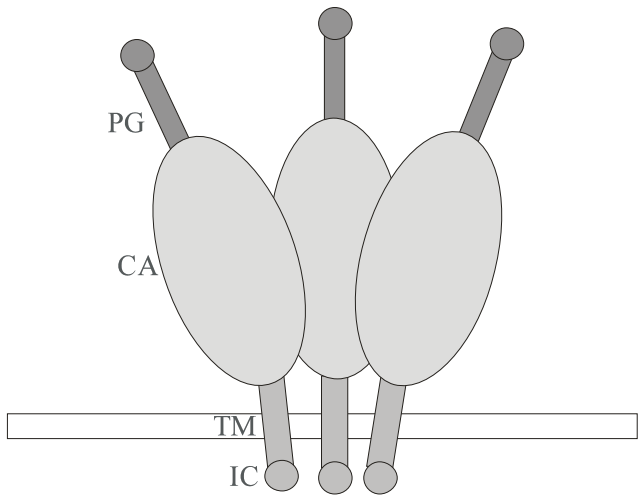

Fig. 2. Schematic model of the CA IX trimer and its location on the plasma membrane. PG = proteoglycan-like region, CA = carbonic anhydrase domain, TM = transmembrane anchor, and IC = intracellular tail. CA IX forms homotrimers via disulphide bonds of cysteine residues.

2.1.8.2 Expression, regulation and function of CA IX

CA IX is normally expressed mainly in the basolateral plasma membrane of the epithelial cells of the gastrointestinal tract (Pastoreková *et al*. 1992, 1997, Pastorek *et al*. 1994). The protein is most abundant in the mucosa of the stomach and gallbladder. All major cell types in the gastric epithelium are positive for it. The most intense expression in the intestinal epithelium has been found in the duodenum and jejunum, decreasing towards the rectum (Saarnio *et al.* 1998b). In contrast to the stomach epithelium, protein expression in the intestinal epithelium is confined to the rapidly proliferating area, the crypts of Lieberkühn. As the cells migrate along the intestinal villus, they differentiate and coincidentally lose their CA IX expression. These observations suggest that CA IX may have a role in cell proliferation and the differentiation of intestinal epithelial cells (Saarnio *et al*. 1998a). In addition to the stomach, gallbladder and intestine, CA IX expression has been detected in the biliary epithelium of the liver and in the pancreatic ducts, male reproductive organs and the lining cells of the body cavities, called the mesothelium (Pastoreková *et al*. 1997, Kivelä *et al*. 2000b, Karhumaa *et al*. 2001a, Ivanov *et al.* 2001).

Abnormally high CA IX expression has been observed in many carcinomas originating from CA IX-negative tissues. A remarkably high proportion of CA IX-positive specimens have been detected among cervical, lung and renal cancers (Table 4), but interestingly, CA IX is usually absent or diminished in tumours originating from CA IX-positive tissues (Pastoreková & Zàvada 2004).

Table 4. Carcinomas in which CA IX is often expressed

Type of carcinoma	Reference
Oesophagus	Turner *et al*. 1997
Lung	Vermylen *et al*. 1999, Giatromanolaki *et al*. 2001
Renal	Liao *et al*. 1997, McKiernan *et al*. 1999
Colon and rectum	Saarnio *et al*. 1998b
Biliary	Saarnio *et al*. 2001
Breast	Chia *et al*. 2001, Bartošová *et al*. 2002
Cervical	Liao *et al*. 1994, Liao & Stanbridge 1996
Head and neck	Beasley *et al*. 2001
Bladder	Hoogsteen *et al*. 2005

A renal cell carcinoma-related antigen, G250, has been sequenced and found to be fully homologous to CA IX (Grabmaier *et al*. 2000). This protein was detected on the surface of renal carcinoma cells with the monoclonal antibody (Mab) G250 (Oosterwijk *et al*. 1986), which is directed against a conformational epitope and thus can be used on living cells or frozen sections in immunohistochemistry (IHC). By contrast, M75 Mab raised against CA IX (Pastoreková *et al.* 1992) recognizes both native and denatured CA IX on tissue sections and is also useful in immunoblotting.

CA IX has been proposed as a promising tumour biomarker on the grounds of its notable association with tumours of different types and of the clear division between its normal and ectopic expression (Pastoreková & Zàvada 2004). One-third of epithelial bone marrow metastases are CA IX-positive (De Raeve *et al*. 2004). CA IX may also serve as a valuable marker to predict the prognosis for the disease. In some studies its expression has been found to be associated with a poor prognosis for brain (Haapasalo *et al*. 2005) or lung (Kim *et al*. 2005) cancer, for instance. Maseide *et al*. (2004) have also noticed recently that CA IX predicts a poor prognosis for patients with soft tissue sarcoma, although elsewhere higher CA IX expression levels have been found to correlate with a better prognosis in cases of renal (Bui *et al*. 2003) and breast cancer (Watson *et al*. 2003).

CA9 gene expression is tightly controlled by a repressor binding to a proximal silencer element under normal circumstances, whereupon specific activation mechanisms are required to override this repression and achieve activation of *CA9* expression *in vitro* or in tumours *in vivo* (Kaluz *et al.* 1999). *CA9* is strongly inducible by hypoxia in a broad range of tumour cells. The hypoxia response element (HRE), which is present close to the transcriptional initiation site, in the *CA9* gene, is the most important element regulating the *CA9* promoter (Wykoff *et al*. 2000). Hypoxia inducible factor (HIF), a transcriptional complex, plays a central role in mammalian oxygen homeostasis (Semenza 2000, Wenger 2000). HRE is recognized by HIF-1, which consists of an oxygen-regulated α-subunit and a constitutive β-subunit. In normoxia HIF-1α is modified by the proline hydroxylases, generating a binding site for a ubiquitin ligase complex containing the von Hippel-Lindau (VHL) tumour suppressor protein, which results in HIF-1α destruction (Kaelin 2005). When the oxygen level is lowered, HIF-1α escapes hydroxylation, dimerizes with HIF-1β, and becomes transcriptionally active. Thus *CA9*, having a HRE element, is a transcriptional target of HIF-1 and becomes regulated by hypoxia (Epstein *et al*. 2001,

Semenza 2003). On the other hand, mutations in *VHL* alleles disrupt the regulatory HIF pathway and result in hereditary VHL disease or the development of sporadic renal cell carcinomas (RCCs) (Latif *et al*. 1993, Kim & Kaelin 2004). Tumour hypoxia is associated with aggressive growth, metastasis and poor response to treatment (Höckel 1996, Brizel 1996). As CA IX is strongly induced by hypoxia in a broad range of tumour cells, it can also be considered to be a promising hypoxia marker (Wycoff *et al.* 2000). Besides hypoxia regulation, the methylation status of the *CA9* gene represents a further mechanism for regulating CA IX expression levels, although its exact physiological relevance is poorly known (Cho *et al*. 2000, Ashida *et al*. 2002).

Like the other CAs, CA IX is believed to be involved in pH regulation. It has been shown to be a highly active enzyme with catalytic activity comparable to CA II (Wingo *et al*. 2001), and its enzyme activity may be of potential significance for tumour progression, because it is thought to be active in the acidification of the extracellular microenvironment surrounding cancer cells, thus facilitating tumour growth and invasion (Ivanov *et al*. 1998, Helmlinger *et al*. 2002, Svastova *et al.* 2004). By analogy with another transmembrane isoenzyme CA IV, CA IX is very likely to interact with anion exchangers (AE) in tumour cells by neutralising the intracellular space, and the protons produced by it may remain outside and increase acidosis of the tumour microenvironment (Fig. 3) (Sterling *et al*. 2002, Pastoreková & Zàvada 2004).

Due to its proteoglycan (PG)-like domain, CA IX may serve another important function by regulating cell adhesion. This hypothesis is supported by previous findings that recombinant CA IX reduces E-cadherin-mediated cell-cell contacts (Svastova *et al*. 2003) and that CA IX deficiency in mice causes disruption of the normal gastric morphology (for details, see the following section) (Ortova Gut *et al.* 2002).

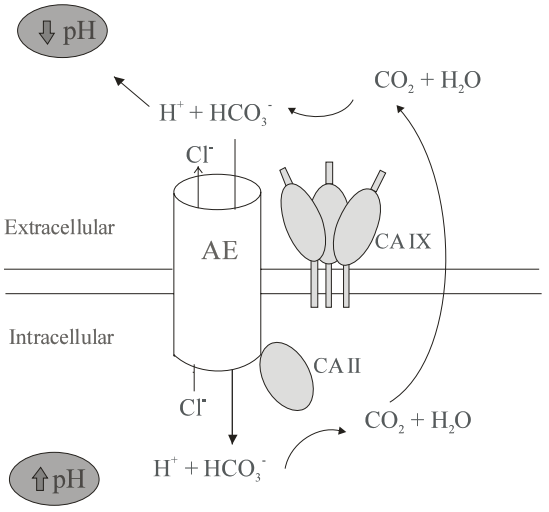

Fig. 3. The proposed interaction of CA IX with an anion exchanger and CA II to produce acidosis of the extracellular tumour microenvironment (Pastoreková & Zàvada 2004).

CA IX also has another form, a soluble protein s-CA IX of 50/54 kDa which is released into the culture medium or into the body fluids, most likely by proteolytic cleavage of the extracellular part. Media of CA IX-expressing tumour cell lines or cultures of tumour explants contain relatively high concentrations of s-CA IX, but the level of this protein in the blood serum and urine of renal clear cell carcinoma patients is about 1000 times lower. s-CA IX is cleared from the blood within a few days after nephrectomy, indicating that the protein largely originated from the neoplasm. Extremely low concentrations of CA IX have been detected in control sera and urine (Zàvada *et al*. 2003).

2.1.8.3 CA IX deficiency in mice

Ortova Gut *et al.* (2002) obtained CA IX-deficient (*Car9*$^{-/-}$) mice by targeted disruption of the *Car9* gene. The first exon was interrupted by replacement of a 14 bp region with a pgk-neo cassette in the reverse orientation. Deletion of the 14 bp was designed to cause a shift in the reading frame of the murine *Car9* mRNA to prevent its correct translation.

Heterozygous mice were healthy and fertile, but histopathological examination of stomach specimens from the *Car9*$^{-/-}$ mice regularly showed considerable hyperplastic changes in the gastric mucosa relative to their heterozygous and WT littermates. No changes were visible in the mutant mice at embryonic ages of E15.5 and E17.5, but a mild increase in mucosal thickness was seen on postnatal day P0.5 and hyperplasia was prominent in 4-week-old animals. Nevertheless, all of the major cell types, parietal cells, chief cells and the mucus-secreting epithelial cells of the gastric mucosa, were present in the area affected by hyperplasia. Deregulated proliferation resulted in excessive branching of the gastric units and largely distorted the architecture of the whole epithelium. These phenotypic changes in the *Car9*$^{-/-}$ mucosa were accompanied by numerous large pathological cysts lined with a single layer of low, cuboidal epithelial cells. No morphological signs of dysplasia were observed in any of the stomach sections studied, and the structure of the subepithelial lamina propria was normal at all stages of development.

The functional involvement of CA IX enzyme activity in the gastric mucosa is still unclear, but these phenotypic consequences demonstrate that CA IX is clearly required for normal gastric morphogenesis and the coordination of dynamic homeostasis within the gastric epithelium.

2.1.9 Carbonic anhydrase isoenzyme XII

Another cancer-associated isoenzyme, CA XII, was cloned and characterized independently by two groups (Türeci *et al.* 1998, Ivanov *et al.* 1998). The human *CA12* gene is located on chromosome 15 (Türeci *et al.* 1998). *CA12* cDNA codes for a protein sequence of 354 amino acids, including a signal sequence (29 aa), a CA domain (261 aa), an additional short extracellular segment, a hydrophobic transmembrane domain (26 aa) and a hydrophilic C-terminal cytoplasmic tail (29 aa). The extracellular CA domain

shows 30-42% homology with other known CAs and contains all three Zn-binding histidine residues found in the active CAs.

The molecular mass of human CA XII is 39 kDa (Ivanov *et al.* 1998, Türeci *et al*. 1998), and the reported molecular weight of CA XII produced in transfected COS cells is 43-44 kDa. This can be reduced to 39 kDa by PNGase F digestion, which is consistent with the removal of two oligosaccharide chains (Türeci *et al*. 1998). The three-dimensional structure of the extracellular catalytic domain of human CA XII, as determined by Whittington *et al*. (2001) using x-ray crystallographic methods, reveals a typical CA fold, although two CA XII domains associate to form an isologous dimer. The helix-helix association observed is also consistent with a dimeric architecture. The dimer interface is situated so that the active site clefts of each monomer are clearly exposed on one face of the dimer, and the C-termini are located together on the opposite face of the dimer to facilitate membrane interaction.

CA12 mRNA has been demonstrated in various tissues. It has been shown in the kidney, colon, pancreas, prostate, ovary, testis, lung and brain by northern blotting (Ivanov *et al.* 1998, Türeci *et al.* 1998), but RT-PCR analysis has revealed a much wider tissue distribution (Türeci *et al.* 1998). Later IHC studies have shown that CA XII is also expressed in reproductive tissues, notably in the epithelial cells of the normal human endometrium, the uterine cervix, efferent ducts and the sporadic cells of the epididymal duct (Karhumaa *et al*. 2000a, 2001a, Ivanov *et al.* 2001). An intense immunoreaction for CA XII has been also reported in the epithelial cells of the colon and rectum, while the duodenum, jejunum and ileum remained negative (Kivelä *et al*. 2000a).

Like CA IX, CA XII is over-expressed in certain cancers and tumour cell lines (Türeci *et al.* 1998, Ivanov *et al.* 1998, Kivelä *et al.* 2000a,b, Parkkila *et al.* 2000a,b, Ivanov *et al.* 2001). In 10% of patients with renal cell carcinoma the CA XII transcript was expressed at much higher levels in the tumour than in the surrounding normal kidney tissue (Türeci *et al.* 1998, Ivanov *et al.* 1998, 2001, Parkkila *et al.* 2000a), and most colorectal tumours have shown abnormal expression (Kivelä *et al.* 2000a). The changes were greatest in the deep parts of the adenomatous mucosa, the positive immunoreaction increasing with the grade of dysplasia. An even, broad staining pattern was detected in adenomas with severe dysplasia and in carcinomas.

The exact function of CA XII in both normal and malignant tissues is so far unknown. Since both CA IX and XII are expressed in several types of tumour, the same functions have been proposed for them. It has been suggested that CA XII may contribute to tumour growth and invasion by acidifying the immediate extracellular space around the cancer cells, thus creating a microenvironment conducive to tumour growth and spreading (Ivanov *et al.* 1998). The *CA12* gene has also been identified as a von Hippel-Lindau target gene, as it is inducible by hypoxia (Ivanov *et al.* 1998, 2001). The WT VHL protein strongly down-regulates CA XII mRNA, indicating a potential role for CA XII in VHL-mediated carcinogenesis (Türeci *et al*. 1998, Ivanov *et al*. 1998).

2.1.10 Carbonic anhydrase isoenzyme XIII

CA XIII is a recently characterized cytosolic isoenzyme. In human tissues, it has been observed in the thymus, small intestine, spleen, prostate, ovary, colon, and testis. In mouse it is expressed in the spleen, lung, kidney, heart, brain, skeletal muscle, and testis. Because CA XIII is a very widely expressed isoenzyme, it may compensate other CAs. In addition, it may have a role in embryogenesis (Lehtonen *et al*. 2004a). Because CA XIII is an active isoenzyme expressed in several tissues, its inhibition by sulfonamides may lead to novel therapeutic applications (Lehtonen *et al*. 2004b).

2.1.11 Carbonic anhydrase isoenzyme XIV

CA XIV is a 37.6 kDa transmembrane isoenzyme with a structure that is most closely related to CA XII. Human and mouse cDNAs have been reported, and the human *CA14* gene has been mapped to chromosome 1 (Mori *et al.* 1999, Fujikawa-Adachi *et al.* 1999c). The activities of human and murine CA XIV are different (Table 2), and the affinity of human CA XIV for sulphonamides is high (Nishimori *et al*. 2005b, Özensoy *et al*. 2005).

CA XIV was initially isolated from mouse kidney, and its mRNA was also detected in the heart, skeletal muscle, liver, brain and lung (Mori *et al*. 1999). In humans, its mRNA has been demonstrated in the heart, brain, liver and skeletal muscle, and at lower levels in the small intestine, colon, kidney and urinary bladder (Fujikawa-Adachi *et al*. 1999c). Kaunisto *et al.* (2002) demonstrated CA XIV immunohistochemically in the mouse and rat kidney, where it was localised to the proximal tubules and thin limbs of Henle. Its presence in the proximal tubules suggested a role in renal acidification. In the mouse liver, it has been detected in the plasma membrane of hepatocytes (Parkkila *et al.* 2002). CA XIV is present in the neuronal membranes and axons of both the human and mouse brain (Parkkila *et al.* 2001), and it was therefore hypothesized that it may have an important role in modulating synaptic transmission in the brain. In addition, CA XIV is expressed in the retinal Müller cells, astrocytes and pigment epithelium (Nagelhus *et al*. 2005).

Whittington *et al*. (2004) have recently produced a truncated form of murine CA XIV which they used for detailed structural and kinetic analyses. The kinetic studies indicated that the specific activity of CA XIV (3284 enzyme units/mg) is higher than that of any of the other isoenzymes investigated so far, including CA II. X-ray crystallography showed a monomeric glycoprotein with a topology relatively similar to that of the other mammalian CA isoenzymes.

2.1.12 Carbonic anhydrase isoenzyme XV

The recently characterised novel CA XV (Hilvo *et al*. 2005) is a low-activity enzyme anchored to plasma membrane via a GPI anchor in the same manner as CA IV.

Phylogenetic studies have suggested that it is most closely related to CA IV, and the properties of these enzymes are quite similar. CA XV mRNA is abundantly expressed in the kidney and has been observed in lower amounts in the brain, testis, 7-day embryo and 17-day embryo. At least eight species, including the mouse, rat, dog, zebra fish and chicken, have a *CA15* gene which most probably encodes an active enzyme. Interestingly, humans have three copies of the *CA15* gene in their genome and chimpanzees at least two, but these copies represent pseudogenes, as they contain several point mutations, insertions and frame shifts, and no CA XV mRNA has been found in human tissues.

2.1.13 Inactive isoforms and non-classical carbonic anhydrases

In addition to the active CAs, the mammalian CA gene family contains three carbonic anhydrase-related proteins (CARP VIII, X, and XI) and two subtypes of the receptor-type protein tyrosine phosphatases (RPTP-β and γ). These proteins have a CA-like domain but lack CA activity (Tashian *et al.* 2000). In addition, a putative colonic CA (Kleinke *et al*. 2005) and a non-O/p54^{nrb} protein (Karhumaa *et al*. 2002b) are categorized under this heading.

2.1.13.1 Carbonic anhydrase-related proteins VIII, X and XI

CARP VIII was initially detected in the mouse brain, and later in the human salivary gland, testis, placenta and lung (Kato 1990, Skaggs *et al.* 1993). It lacks CO_2 hydration activity and is also known as glutathione-S-transferase fusion protein (Bergenhem *et al*. 1998). Small amounts of CARP VIII have been observed in the normal human lung and colonic mucosa. In contrast, it was strongly expressed in almost all archival lung cancer specimens and in colorectal carcinomas. Thus CARP VIII probably plays a role in some malignant tissues (Akisawa *et al*. 2003, Lu *et al*. 2004, Miyaji *et al*. 2003). It may also be an autoantigen involved in the pathogenesis of melanoma-associated paraneoplastic cerebellar degeneration (Bataller *et al*. 2004). Interestingly, a recent study has indicated that CARP VIII deficiency leads to a movement disorder without pathological abnormalities of either the central or the peripheral nervous systems (Jiao *et al*. 2005).

CARP X cDNA was first found in a cDNA library by comparing overlapping partial human cDNA sequences deposited in public databases of EST (Hewett-Emmett & Tashian 1996). A full-length cDNA clone of human CARP X has been obtained, sequenced and mapped to chromosome 17 (Okamoto *et al.* 2001, Tashian *et al.* 2000). Its mRNA has been observed in almost all parts of the human central nervous system, but no expression has been detected in the foetal brain (Okamoto *et al.* 2001).

CARP XI cDNA was first identified in a sheep brain cDNA library, and the human *CA11* gene was mapped to chromosome 19 (Lovejoy *et al.* 1998). CARP XI protein has a theoretical molecular mass of 36 kDa. The mRNA has been detected by Northern blotting only in the human brain (Fujikawa-Adachi *et al*. 1999d), while the more sensitive RT-PCR method produced positive signals in the pancreas, kidney, liver, salivary gland and spinal cord. The strong signal sequence and the existence of several potential

phosphorylation sites and binding motifs suggest that it has a role in signal transduction (Lovejoy *et al*. 1998, Fujikawa-Adachi *et al*. 1999d, Okamoto *et al*. 2001). Like CARP X, CARP XI has also been demonstrated in almost all parts of the human central nervous system but not in the foetal brain (Okamoto *et al.* 2001).

The exact functions of the CARPs are still undefined, but as the human CARPs are highly conserved and consistently observed in the brain, they may have important roles in the central nervous system (Taniuchi *et al*. 2002a,b).

2.1.13.2 Receptor-type protein tyrosine phosphatases

The receptor-type protein tyrosine phosphatases (RPTPβ and γ) have CA-like domains (Krueger & Saito 1992, Levy *et al.* 1993, Barnea *et al.* 1993, Maurel *et al.* 1994). RPTPβ has been shown to be identical to the chondroitin sulphate proteoglycan called phosphacan that binds to neurons and neural cell-adhesion molecules (Barnea *et al.* 1994a,b, Shitara *et al*. 1994). It is unlikely, however, that phosphacan could function as a catalytically active CA, because it has only one of the three histidines required for CA activity (Maurel *et al.* 1994). Similarly, RPTPγ has only one histidine residue, which suggests that it may not function in CO_2 hydration (Barnea *et al.* 1993).

The exact functions of the RPTPs are not fully understood, but RPTPβ is now known to regulate the morphogenesis of Purkinje cell dendrites in the developing cerebellum (Tanaka *et al*. 2003). In addition, neurons are able to express a shorter receptor form of RPTPβ (sRPTPβ) on their cellular surface and to secrete phosphacan. Neuronal expression of sRPTPβ may modulate neuronal differentiation, including neuritogenesis and synaptogenesis (Hayashi *et al*. 2005).

2.1.13.3 Non-O/p54^{nrb} and putative colonic CA

NonO/p54^{nrb}, a non-POU (Pit-Oct-Unc) domain-containing octamer-binding protein, is a 54 kDa RNA and DNA-binding nuclear factor (Dong *et al.* 1993, Yang *et al.* 1993). Karhumaa *et al.* (2000b) purified a 66 kDa polypeptide recognized by CA II antibodies from several rat tissues by CA inhibitor affinity chromatography, and this appeared after characterisation to be identical to nonO/p54^{nrb}. NonO binds both RNA and DNA and is involved in transcriptional regulation (Yang *et al.* 1993, Dong *et al.* 1993, Yang *et al.* 1997, Basu *et al.* 1997, Hallier *et al.* 1996).

NonO exhibits CA activity *in vivo* and binds acetazolamide (Karhumaa *et al.* 2000b), an interesting finding in the light of the fact that only classical CAs have been found to have CA catalytic activity and specific CA inhibitor binding capacity. NonO is catalytically more active than CA III. The significant immunological cross-reactivity seen between nonO and CA II can be explained by the minor sequence homology between them (Karhumaa *et al*. 2000b). NonO has none of the structural elements required for conventional CA activity, i.e. it lacks all the conserved histidines that have been considered essential for CA activity (Kannan *et al*. 1977, Eriksson & Liljas 1991). A polyglutamine stretch, Q29-Q38, in the nonO protein is a potential site for Zn binding,

and thus also a potential site for CA activity. Because nonO has CA activity, it may function in the maintenance of pH homeostasis in the nucleus. In addition, it is possible that CA activity may be an important factor in transcriptional regulation, which is a novel function for CA (Karhumaa *et al*. 2000b).

A distinct CA has recently been observed in the colonic mucus of mammals, including humans. Amino acid sequencing of the isolated protein showed that it is not identical with any other known CA isoenzyme (Kleinke *et al*. 2005).

2.2 Role of carbonic anhydrases in the mucosa of the alimentary canal

As pointed out in section 2.1.2., CA II is widely expressed in the organs of the alimentary tract. As a high activity isoenzyme it is believed to represent a key enzyme for several functions in which pH regulation is important. It is expressed in the epithelial cells of the oesophagus, where it participates in bicarbonate secretion (Meyers & Orlando 1992, Parkkila S *et al*. 1994, Christie *et al*. 1997). This is physiologically important because endogenous bicarbonate secretion is capable of raising the pH of the gastro-oesophageal reflux-derived residual acid from 2.5 to close to neutral, a mechanism that protects the oesophageal mucosa against acidity. In the stomach, CA II is expressed in the parietal cells of the gastric glands, where it regulates the acidity of the gastric juice by proton secretion (Davenport & Fisher 1938, Davenport 1939, O'Brien *et al*. 1977, Sato *et al*. 1980, Lönnerholm *et al*. 1985, Parkkila S *et al*. 1994, Parkkila & Parkkila 1996, Parkkila 2000). It is also located in the surface epithelial cells of the stomach, duodenum and jejunum, where it probably contributes to bicarbonate secretion (Parkkila & Parkkila 1996, Saarnio *et al.* 1998b, Parkkila 2000). In the large intestine, CA II is involved in the electroneutral NaCl reabsorption that also drives water absorption.

Metabolism-dependent bicarbonate secretion was detected in the intact gastric mucosa in the mid-1970s (Flemström 1977, Flemström & Garner 1982) and in the duodenum a few years later (Flemström 1980, Flemström *et al*. 1982). The epithelial surface has to maintain an almost neutral pH in spite of a very acidic luminal solution, and the bicarbonate barrier, which is a stable gel layer, has been shown to be involved in this pH regulation (Florey *et al*. 1955, Bahari *et al*. 1982, Quigley *et al*. 1987, Williams *et al*. 1981). Mucosal bicarbonate secretion neutralises the acid diffusing into the mucus gel layer and maintains a near-neutral pH at the mucus-mucosal surface interface (Allen & Flemström 2005). The mucus gel has a physically unique form: it consists of well-defined stable viscoelastic gels composed of multimeric mucins (Allen *et al*. 1976, Bell *et al*. 1985, Sellers *et al*. 1988). The thickness of the mucus layer is the result of a balance between secretion and erosion (Allen & Carroll 1985). In the rat stomach, this thickness remains unchanged after exposure to acid pH *in vivo*, whereas in the duodenum it increases markedly after acidic exposure (Allen *et al*. 1991, Sababi *et al*. 1995). Furthermore, E-type prostaglandin 2 (PGE_2) increases the thickness of the mucus layer in the stomach (Nishizaki *et al*. 1994).

Studies on several species have demonstrated that epithelial bicarbonate secretion occupies a key role in protecting the duodenal mucosa against luminal acid (Flemström &

Kivilaakso 1983, Kivilaakso & Flemström 1984). The presence of acid in the lumen strongly stimulates gastric and duodenal bicarbonate secretion in several species, including humans (Crampton *et al*. 1987, Isenberg *et al*. 1986, 1987), an effect that is controlled by the central nervous system (Konturek *et al*. 1984, Ballesteros *et al*. 1991). E-type prostaglandins also increase both alkaline secretion in the duodenal mucosa and mucosal resistance to luminal acid (Flemström 1980, Isenberg *et al*. 1986, Yao *et al*. 1993, Takeuchi *et al*. 1997, 1999).

2.3 Cell adhesion

Cell adhesion plays a key role in the morphogenesis of the epithelium and the maintenance of its architecture. The morphogenesis of the epithelium is a result of interplay between various adhesion receptors, the cytoskeleton and the coordination of transduction pathways. The physical aspect of adhesion in the maintenance of the epithelial structure is coupled with the capacity of adhesion molecules to respond to cell-cell or extracellular matrix (ECM)-cell signalling events and to transduce signals into the cells. The adhesion molecules are usually glycoproteins located on the extracellular surface. To maintain the architecture of the epithelium, four kinds of stable adhesion element are important: adherens, desmosomal and occluding junctions and attachment to the ECM (Kolega 1986, Gumbiner 1996). Complex interaction between all the adhesion molecules is needed for precise regulation of the pathways leading to tissue morphogenesis.

Adherens junctions are among the most important adhesive elements that play a focal role in the establishment and maintenance of the unique tissue architecture. Their presence is required for tight association among the epithelial cells, and this is mediated mostly by E-cadherin (Danjo & Gibson 1998), a Ca^{2+}-dependent cell adhesion molecule which functions in the establishment and maintenance of epithelial cell morphology during embryogenesis and adulthood (Takeichi 1991, Behrens *et al.* 1989, McNeill *et al.* 1990). E-cadherin is linked to the actin cytoskeleton by the cytoplasmic catenins, with α-catenin linking it to the actin cytoskeleton through β- or γ-catenin. The cell-cell adhesion mediated by the E-cadherin-catenin complex is strong, but permissive to cell rearrangement and tissue morphogenesis in response to growth factors. E-cadherin is sensitive to local signalling, but also participates in long-range developmental patterning processes in the embryo through the cytoplasmic β-catenin (Cowin & Burke 1996, Miller & Moon 1996). E-cadherin-catenin complex adhesion has been found to be regulated by several direct and indirect mechanisms (Kuroda 1998, Gumbiner 2000). Functionally interfering anti-E-cadherin antibodies cause the early embryo to decompact, indicating that E-cadherin is important for the adhesion of blastomers (Hyafil *et al.* 1980, Vestweber & Kemler 1984). Heterozygous E-cadherin deficient mice, as developed by Riethmacher *et al.* (1995), carry one copy of a shut-down gene but are healthy and fertile, while homozygotes develop normally up to the morula stage and compact properly, so that the morula cells become initially adherent and polarized, but the polarized state is not sustained and the embryos become seriously distorted. This demonstrates that E-cadherin

has an essential function in normal development (Sefton *et al.* 1992, Riethmacher *et al.* 1995).

γ-catenin is also present at desmosomal junctions, interacting with desmogleins and desmocollins, the adhesion receptors of desmosomes, which are important elements in epithelia. The role of desmosomal junctions is that of maintaining tissue integrity (Ben-Ze'ev & Geiger 1998).

Occluding (tight) junctions form a selectively permeable barrier separating two functionally and biochemically distinct regions, i.e. the basolateral and apical plasma membranes. These are typical of a single-layered epithelium. The level of permeability of tight junctions depends on tissue specificity and ranges from whole cells to ions and protons (Gumbiner 1996).

Attachment to the ECM and to its more distinct sheath - a basement membrane - crucially contributes to the tissue integrity, cell polarization and branching morphogenesis of the epithelium (Hynes 1999, Gumbiner 1996, Drubin & Nelson 1996). The most prominent molecules mediating cell adhesion to the ECM are the integrins, a family of transmembrane, heterodimeric proteins. Like the cadherins, integrin-mediated adhesions induce the localized assembly of specialized cytoskeletal and signalling networks at the contacting cell surface. Integrins are linked to actin filaments and actin-associated proteins (Gumbiner 1996).

The presence of a PG-like domain is the feature that distinguishes CA IX from the other CAs and appears to be responsible for its role in cell-cell communication and cell-matrix adhesion (Pastorek *et al*. 1994, Lieskovska *et al*. 1999, Zàvada *et al*. 2000). In polarized epithelial MDCK cells transfected with human CA IX cDNA, the enzyme produced has been localized to the cell-cell contacts and its distribution in lateral membranes has been found to overlap with a key adhesion molecule, E-cadherin. In addition, it reduces E-cadherin-mediated cell-cell contacts via competitive interaction with β-catenin (Svastova *et al*. 2003). The disruption of gastric morphogenesis in CA IX deficient mice also supports the hypothesis that CA IX is indeed functionally involved in cell adhesion, at least in the gastric mucosa (Ortova Gut *et al*. 2002).

2.4 Gastric cancer development

Gastric cancer is a serious health problem worldwide, being the second most common type of fatal cancer (Terry *et al*. 2002). Human gastric carcinogenesis is multifactorial, involving various dietary and non-dietary factors as well as genetic susceptibility (González *et al*. 2002). In view of the considerable variations in the incidence of gastric cancer between generations and between geographical areas, environmental factors may be more important than genetic ones (Chan *et al*. 1999). A high intake of smoked, salted or nitrated food and carbohydrates and a low intake of fruits, vegetables and milk would seem to increase the risk of stomach cancer markedly. Nevertheless, many studies have demonstrated that multiple genetic alterations are responsible for the development and progression of the disease. The two main histological types of gastric cancer can be recognised, a diffuse and an intestinal type, arising from different genetic pathways

(Ebert *et al*. 2002a, Tamura *et al*. 2000, Fiocca *et al*. 2001, El-Rifai & Powell 2002, Laurèn 1965).

The exact molecular mechanisms of gastric tumorigenesis are still uncertain. It is possible that a differentiated adenocarcinoma may arise from a pre-existing adenoma, as in colorectal tumours (Uchino *et al*. 1993), but some studies have suggested that the adenoma-carcinoma sequence may not be the major pathway for gastric carcinogenesis (Maesawa *et al*. 1995, Tamura *et al*. 1995, Tamura 1996, Tsukashita *et al*. 2001).

Inactivation of the tumour suppressor genes caused by loss of heterozygosity and/or mutations is also common in gastric carcinogenesis. Inactivation of the tumour suppressor gene *p53*, for example, has been detected in gastric cancers of both the diffuse and intestinal type and has been reported in up to 80% of gastric cancers regardless of the histological subtype and in about 10% of early dysplastic and metaplastic lesions (Wang *et al*. 2001, Hollstein *et al*. 1996, Fenoglio-Preiser *et al*. 2003, Imazeki *et al*. 1992, Ranzani *et al*. 1995, Kim *et al*. 1991, Yamada *et al*. 1991). Tumour suppressor adenomatous polyposis coli gene (*APC*) mutations are common in intestinal-type gastric cancers, being involved in up to 60% of tumours and in about 25% of adenomas (Tahara 1995, Horii *et al*. 1992, Hofler & Becker 2003, Yokozaki *et al*. 2001). This somatic *APC* mutation seems to have an important role in gastric adenoma and dysplasia, but a limited one in the progression to adenocarcinoma (Lee *et al*. 2002). APC protein is also important for the degradation of β-catenin (Munemitsu *et al*. 1995), and β-catenin mutations have been identified in intestinal-type gastric cancers, but not in diffuse-type ones (Park *et al*. 1999). Both APC and β-catenin belong to the Wnt/wingless signal pathway, which is altered in over 90% of colon cancer cases (Zheng *et al*. 2004). The Wnt proteins are small secreted glycoproteins which play key roles in both embryogenesis and mature tissues (Uusitalo *et al*. 1999). Alterations of Wnt signalling in animal models lead to abnormal morphogenesis and, in humans, germline mutations of genes involved in the Wnt signalling pathway lead to congenital defects. The role of disrupted Wnt signalling in tumourigenesis is not limited to specific tissue types, and can therefore be regarded as a generic step to the process of carcinogenesis (Ilyas 2005). Also Hedgehog signalling pathway has a role in development, especially in embryonic patterning. Aberrant activation of Hedgehog signalling pathway leads to pathological consequences in a variety of human tumours, including gastric cancer (Saldanha 2001, Katoh & Katoh, 2005).

Activation of oncogenes, mostly growth factors and their receptors, can also induce gastric carcinogenesis. The *c-met* proto-oncogene encoding a tyrosine kinase receptor for the hepatocyte growth factor, for example, has been found to be overexpressed in both intestinal and diffuse-type gastric cancers and indicates a poor prognosis on account of its increasing invasiveness and poor differentiation (Yokozaki *et al*. 2001, Hara *et al*. 1998, Carneiro & Sobrinho-Simoes 2000, Amemiya *et al*. 2000). Another proto-oncogene, c-erB2, is a transmembrane tyrosine kinase receptor which is overexpressed or its gene is amplified in about 25% of all gastrointestinal tract malignancies (Ross & McKenna 2001).

Genes that regulate programmed cell death, or apoptosis, e.g. *FAS*, *Bcl-2* and tumour necrosis factor (*TNF*), contribute to cancer development (Werner *et al*. 2001, Vollmers *et al*.1997), and alterations in cell-cycle regulators, specifically cyclins and their kinases,

also participate in the development and progression of gastric cancers (Yasui *et al*. 2001, Akama *et al*. 1995).

In addition, abnormalities in adhesion molecules such as E-cadherin are critical for gastric cancer development (Joo *et al*. 2002, Takeichi 1991). Abnormal E-cadherin seems to be a possible marker of submucosal invasion in early differentiated-type gastric cancer and lymph node metastasis (Tanaka *et al*. 2002), and a dysfunction in the E-cadherin-catenin complex has been observed in the early stage of gastric carcinogenesis (Joo *et al*. 2002). Somatic mutations in E-cadherin have occurred in 50% of diffuse-type cancer cases, resulting in markedly reduced cell adhesion, enhanced cellular motility and altered morphology (Oda *et al*. 1994, Becker *et al*. 1994, Handschuh *et al*. 1999).

Helicobacter pylori apparently plays a causative role in gastric cancer, but the exact mechanism is still unknown (Terry *et al*. 2002). It induces both cell proliferation and apoptosis during early inflammation of the gastric mucosa and significantly increases the numbers of apoptotic cells in gastric tissues, but further evidence is needed to explain *H. pylori* infection as an aetiological factor for gastric carcinogenesis (Ebert *et al*. 2002b, Moss *et al*. 1996, Jones *et al*. 1997, Houghton & Wang, 2005). In a recent study, Houghton *et al*. (2004) have described how bone marrow–derived cells can home onto and repopulate the gastric mucosa in response to chronic *Helicobacter pylori* infection and contribute to metaplasia, dysplasia and cancer in the course of time. There are also other external factors which contribute to the neoplastic process in the gastric mucosa, including dietary factors such as high salt intake, which has been considered an important causative factor for many years (Correa *et al.* 2004).

3 Aims of the research

1. To study the expression of various CA isoenzymes in malignant haematopoietic cell lines and malignant blast cells of bone marrow samples collected from patients with different leukaemias.
2. To analyse a series of specimens from the non-neoplastic gastric mucosa and from various dysplastic and neoplastic gastric lesions for the expression of CA IX and XII.
3. To study whether CA IX deficiency causes a preneoplastic induction in the gastric epithelial cells of mice fed a high-salt diet, which could initiate a neoplastic process. Two mouse strains, C57/BL6 and BALB/c, were compared in terms of phenotypic changes and susceptibility to pathogenic processes.
4. To examine mice lacking CA II and/or CA IX to see if there are significant changes in gastric morphology or the regulation of duodenal bicarbonate secretion (DBS) and to evaluate their regulatory role in the DBS response to E-type prostaglandin 2 (PGE_2).

4 Materials and methods

4.1 Leukaemia cell lines (I)

Six cell lines were studied. Five of them were obtained from the American Tissue Culture Collection (Manassas, VA): RPMI 8226 (multiple myeloma, B-lymphoid/plasmacytic lineage, plasma blast), U-937 (histiocytic lymphoma, monocytic lineage, monoblast), Jurkat (acute lymphoblastic leukaemia, T-lymphoblast), MOLT-3 (acute lymphoblastic leukaemia, T-lymphoblast) and HL-60 (acute promyelocytic leukaemia, myeloid blast). The OCI-AML-2 cell line, representing myeloid blasts from a patient with acute myelomonocytic leukaemia, has been described earlier (Wang *et al*. 1989).

CAs were purified from the cell lines by centrifuging cultured cells at 200 x g for 5 min and suspending them in ice-cold 0.1 mol/l Tris-SO_4-buffer, pH 8.7, containing 1 mmol/l benzamidine, 1 mmol/l o-phenatroline and 0,05% TritonX-100. They were then sonicated and incubated on ice for 30 min, and the suspension was centrifuged at 15000 x g for 5 min and the supernatant subjected to affinity purification. Inhibitor affinity chromatography was performed using CM Bio Gel A coupled to p-aminomethyl benzenesulphonamide according to Parkkila *et al*. (1990).

4.2 Antibodies (I, II)

The polyclonal rabbit antibodies against human CA I, II and XII had been produced and characterized earlier (Parkkila A-K *et al*. 1993, Kivelä *et al*. 2000a). The monoclonal antibody M75 against human CA IX had also been described earlier (Pastorekovà *et al*. 1992).

4.3 SDS-polyacrylamide gel electrophoresis (I)

The proteins were transferred from the gel to a nylon membrane (Millipore; Bedford, MA) electrophoretically in a Novex Blot Module. After transblotting, the sample lanes were first incubated with TBST buffer (20 mM Tris, 500 mM NaCl, 0,3% Tween-20, pH 7,5) containing 10% cow colostral whey (Biotop Ltd, Oulu, Finland) for 30 min and then with the primary antibody diluted 1:2000 in TBST buffer for 60 min. The membranes were washed five times for 5 min with TBST buffer and incubated for 30 min with a peroxidase-conjugated secondary antibody (Bio-Rad Laboratories) diluted 1:3000 in TBST buffer. After washing four times for 5 min in TBST buffer, the polypeptides were visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB). All the steps were carried out at room temperature.

4.4 Western blotting (I)

10 µg of purified proteins obtained from the inhibitor affinity chromatography were analysed by SDS-PAGE under reducing conditions according to Laemmli *et al*. (1970). The protein standards for SDS-PAGE were from Bio-Rad Laboratories (Richmond, CA). The electrophoreses were performed in a Novex XCell SureLock unit (Invitrogen Corp/NOVEX, Carlsbad, CA) using Novex NuPAGE 10% Bis-Tris gels.

4.5 Bone marrow samples (I)

Bone marrow samples were collected during the diagnostic blastic phase (at diagnosis or relapse) from 43 patients, of whom 26 had acute myeloid leukaemia (AML), 15 acute lymphoblastic leukaemia (ALL) and 2 chronic myelomonocytic leukaemia (CMML). The survival rates for the patients were 8 out of 26 (30.8 %), 5 out of 15 (33.3 %) and 0 of 2 (0 %) in these three categories, respectively (range of follow-up 1.5-8 years). The samples were taken during routine diagnostic and treatment evaluation at Oulu University Hospital and classified on the basis of their lymphoid or myeloid morphological appearance according to the French American British (FAB) classification criteria (Bennett *et al*. 1976, 1982, 1985). The FAB subtypes of the AML patients were: M0 (n=1), M1 (n=5), M2 (n=5), M3 (n=1), M4 (n=9), M5 (n=2), M6 (n=1), M7 (n=1) and unknown (n=1). In addition, the diagnoses were based on the immunophenotype as assessed by flow cytometry using standard lineage immunophenotype markers. The invstigation was approved by the Ethics Committee of Oulu University Hospital and performed in accordance with the guidelines of the Declaration of Helsinki. Mononuclear cells were isolated from the bone marrow samples by Lymphoprep density gradient centrifugation (Nycomed Pharma AS, Oslo, Norway) and washed with FACSFlow electrolyte sheath fluid (Becton Dickinson, Franklin Lakes, NJ). The cells were then fixed and permeabilized with a Fix and Perm kit (Caltag Laboratories, Burlingame, CA) according to the manufacturer’s protocol and spread onto microscope slides.

4.6 Immunocytochemistry of bone marrow samples (I)

The bone marrow samples were immunostained for CA II using a Histostain-Plus kit (Zymed, So. San Francisco, CA) according to the instructions provided in the kit insert. After this immunocytochemical staining the cell nuclei were slightly counterstained with haematoxylene. The staining was scored in terms of extent and intensity by two of the investigators (ML and SP). A negative score (0%) was given to a sample which had no evidence of specific immunostaining, and the other categories were < 10%, 10-50%, and 50-100% positive cells. The intensity of staining was scored on a scale of 0 to 3 as follows: 0 = no reaction, 1 = weak reaction, 2 = moderate reaction, 3 = strong reaction.

4.7 Double immunofluoresence staining of bone marrow cells (I)

For double immunofluoresence staining, the cells were first fixed, permeabilized and spread onto microscope slides. They were then rinsed with PBS and pretreated with a solution containing 0.1% BSA and 0.05% saponin in PBS (BSA-PBS-saponin) for 30 min and incubated with the primary antibodies (rabbit anti-human CA II serum diluted 1:50 in BSA-PBS-saponin and mouse anti-human CD34 antibody (Zymed) diluted 1:10) for 60 min. The cells were rinsed three times for 5 min with BSA-PBS-saponin and incubated for 60 min with the secondary antibodies (fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG and tetramethylrhodamine isothiocyanate (TRITC)-conjugated swine anti-rabbit IgG (Dakopatts, Glostrup, Denmark)) diluted 1:30 with BSA-PBS-saponin. After washing twice for 5 min in BSA-PBS-saponin buffer and once with PBS, the cells were mounted (Mounting medium, Inova Diagnostics, San Diego, CA) and analysed by confocal laser scanning microscopy (Carl Zeiss, Göttingen, Germany).

4.8 Stomach tissue samples (II)

Tissue samples taken from the non-neoplastic, benign and/or malignant neoplastic gastric mucosa for routine diagnostic purposes at Oulu University Hospital (Oulu, Finland) were used. The protocol was approved by the Ethics Committee of Oulu University Hospital and carried out according to the guidelines of the Declaration of Helsinki.

4.9 Stomach tissue staining (II)

The tissue specimens were fixed in 4% neutral-buffered formaldehyde for 24-48 h, dehydrated, embedded in paraffin in a vacuum oven at 58°C, and 5 μm sections were placed on microscope slides. The CA isoenzymes were immunostained by the biotin-steptavidin complex method, employing the following steps: (1) pretreatment of sections with undiluted cow colostrum (Biotop Ltd) for 30 min and rinsing in phosphate-buffered saline (PBS), (2) incubation for 1 h with anti-CA XII serum (1:100), normal rabbit serum

(1:100) or hybridoma medium with M75 antibody (1:10) in 1% BSA-PBS, (3) incubation for 1 h with biotinylated swine anti-rabbit IgG (Dakoplatts) or goat anti-mouse IgG (Dakopatts) diluted 1:300 in 1% BSA-PBS, (4) incubation for 30 min with peroxidase-conjugated steptavidin (Dakoplatts) diluted 1:500 in PBS, and (5) incubation for 2 min in a solution containing 9 mg DAB in 15 ml PBS + 5 µl 30% H_2O_2. The sections were washed 3 times for 10 min in PBS after incubation steps 2 and 3, and 4 times for 5 min after step 4. All the incubations and washings were carried out at room temperature. After the immunostaining, the tumour sections were counterstained with haematoxylin. The stained sections were examined and photographed under a Zeiss Axioplan 2 microscope (Carl Zeiss).

The immunohistochemical results were semiquantitative, being based on the percentage of positive cells and the intensity of epithelial staining as evaluated in a total field of a single section. The extent of staining (EXT) was scored by four of the investigators (ML, JS, TJK, and SPar.) as 1 when 1-10% of the cells were stained, 2 when 11-50% of the cells were stained, and 3 when 51-100% of the cells were stained. A negative score (0) was given to tissue sections which had no evidence of specific immunostaining. The intensity of staining (INT) was scored on a scale of 0 to 3 as follows: 0, no reaction; 1, weak reaction; 2, moderate reaction; 3, strong reaction. In the normal and hyperplastic gastric mucosa, scores were first counted for the luminal surface, proliferative zone and glands and relative staining indices (on the scale 0-3) were calculated separately for each histological component using the formula $\sqrt{(EXT \times INT)}$. In the adenomas and metaplasias, EXT and INT scores were obtained separately for the deep and superficial parts of the lesions and relative staining indices calculated accordingly for both regions. Finally, the staining indices obtained in each region were used to calculate the mean values for each normal sample or lesion.

4.10 CA IX and CA II deficient mice (III, IV)

The generation of *Car9*$^{-/-}$ mice by targeted disruption of the *Car9* gene has been described earlier (Ortova Gut *et al.* 2002). These mice were introduced into the animal facility of Oulu University by embryo transfer, backcrossed ten generations (F10) to the C57BL/6 (papers III and IV) or Balb/c strain (paper III), and heterozygotes were then intercrossed to produce mice that were homozygous for the targeted gene. Generation of *Car2*$^{-/-}$ mice of the C57BL/6 strain by treating them with *N*-ethyl-*N*-nitrosourea has also been described earlier (Lewis *et al*. 1988). The breeding pairs of *Car2*$^{-/-}$ mice were obtained from Professor Gerolf Gros (Medizinische Hochschule Hannover, Germany). The C57BL/6 *Car2*$^{-/-}$ and *Car9*$^{-/-}$ mice were intercrossed to produce mice with different genotypic combinations of *Car2* and *Car9* mutant alleles (paper IV).

The mice were housed under pathogen-free conditions and their health status was monitored on a regular basis in accordance with the FELASA recommendations. The protocols were approved by the local Animal Care Committee.

4.11 High-salt diet (III)

To compare the effect of diet on *Car9*$^{-/-}$ and WT mice, two mouse strains were used, C57BL/6 and BALB/c. After four weeks on normal feed (R36 for mice and rats, Lactamin, Sweden), the mice were fed either standard or high salt food (normal feed + 7% NaCl, SD9S, Lactamin, Sweden). The protocol is described in Table 5.

Table 5. Characteristics of the mice analysed.

Strain	Genotype	Diet	n
BALB/c	WT	Standard	10
		High salt	9
	Car9$^{-/-}$	Standard	10
		High salt	10
C57/BL6	WT	Standard	9
		High salt	9
	Car9$^{-/-}$	Standard	10
		High salt	10

4.12 Histological evaluation of mouse tissues (III, IV)

The mouse tissue samples from the gastrointestinal tract were fixed with 10% formalin overnight (III) or for 8-27 days (IV) and then processed for paraffin embedding. 5 μm sections were placed on microscope slides and stained with haematoxylin and eosin, and the stained sections were then photographed using a Zeiss Axioskop 40 microscope (Carl Zeiss). Foveolar hyperplasia and glandular atrophy in the body mucosa were scored as absent, mild, moderate or severe according to the length of the foveolae and thickness of the glandular layer. The presence of mucosal and submucosal inflammation, intestinal metaplasia and dysplasia was evaluated.

4.13 *CA9* genotyping (III, IV)

Mouse DNA was isolated from ear marks with a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany). The *CA9* gene was amplified with a polymerase chain reaction (PCR) using Reddy Mix PCR Master Mix (ABgene, Epsom, UK). Each reaction was performed using 150 ng of DNA as a template. The primers, chosen from the targeted disruption area in exon I (Ortova Gut *et al.* 2002), were 5'CCAGTCAGCTGCATGGCC3' and 3'AGGAGCCTCGGGAGTCGA5' for the WT mice and 5'AGGAGCAAAGCTGCTATTGG3' and 3'AGGAGCCTCGGGAGTCGA5' for the knockout mice. The PCR program was: +96°C for 5 min and 35 cycles of +96°C for 30 s, 56°C for 60 s and 72°C for 60 s. The PCR products were characterized in 1.2%

agarose gel (LE, analytical grade, Promega Madison, WI, USA) containing 0.005% nucleic acid gel stain GelStar (BMA, Rockland, ME, USA) and visualized in UV light.

4.14 Measurement of CA activity (IV)

CA activity in mouse blood samples (treated with EDTA, diluted 1:5000) was determined by the imidazole-tris technique (Brion *et al*. 1988). As CA II constitutes the major fraction of CA activity in the blood, the test is considered reliable for monitoring CA II activity.

4.15 Duodenal cannulation (IV)

Duodenal bicarbonate secretion in the mice was analysed by Dr. Markus Sjöblom at Uppsala University (Sweden). The mice were anaesthetized with spontaneous inhalation of isoflurane (Forene®; Abbott Scandinavia, Kista, Sweden), the inhalation gas, containing a mixture of ~40% oxygen, ~60% nitrogen and 2.2 ± 0.2% isoflurane, being administered continuously through a breathing mask. Body temperature was maintained at ~37.5°C by means of a heating pad controlled by a rectal thermistor probe. A catheter containing heparin (20 IU/ml) dissolved in isotonic saline was placed in the left carotid artery to monitor blood pressure and for continuous infusion of an isotonic sodium carbonate solution ([in mM] 200 Na^+ and 100 CO_3^{2-}) at a rate of 0.35 ml/h.

he bile and pancreatic ducts were ligated very closely to the entrance into the duodenum to prevent pancreaticobiliary juice from entering the duodenum. Silicone tubing was introduced through a hole made in the forestomach, guided through the stomach and pylorus and secured with a ligature 2–3 mm distal to the pylorus. A PE-200 cannula (Becton-Dickinson, Parsippany, NJ) was inserted into the duodenum ~1.5 cm distal to the pylorus and secured with ligatures. The proximal duodenal tubing was connected to a peristaltic pump and the segment was perfused with isotonic saline (150 mM NaCl) at a rate of 0.25 ml/min. To complete the surgery, the abdominal cavity was closed with sutures. After surgery, ~30 min was allowed for cardiovascular, respiratory and gastrointestinal functions to stabilize before the experiments were commenced.

The systemic blood acid/base balance was checked (AVL Compact 3, Graz, Austria) in 40 µl arterial blood samples taken at the end of the experiments.

4.16 Measurement of luminal alkalinization (IV)

The rate of luminal alkalinization was determined by back titration of the perfusate to pH 4.90 with 10 mM HCl under continuous gassing (100% N_2) using pH-stat equipment (Schott, TitroLine-easy, Mainz, Germany). The amount of HCl titrated was considered equivalent to the duodenal HCO_3^- secretion. The pH electrode was routinely calibrated with standard buffers before commencing the titration. The rates of luminal alkalinization

are expressed as micromoles of base secreted per centimetre of intestine per hour ($\mu mol \cdot cm^{-1} \cdot h^{-1}$).

The experiments were run in the following way. Basal HCO_3^- secretion in the C57BL/6J mice was monitored continuously for 90 min and recorded at 10-min intervals when perfused luminally with saline alone. In the test groups, 47 µM of PGE_2 was present in the duodenal perfusate for 20 min (between time points t=30 and 50 min).

Duodenal bicarbonate secretion, mean arterial blood pressure and body temperature were monitored continuously in all the groups and recorded at 10-min intervals. Arterial blood samples for acid/base analysis were taken at the end of the experiment (t=90 min).

4.17 Statistical analysis

The results in papers II and III were analysed statistically using SPSS for Windows software (SPSS Inc.). Statistically significant differences were always accepted at the $p<0.05$ level. A one-way analysis of variance was used in paper II to compare staining for CA isoenzymes between the types of lesion. Pairwise comparisons between group means were performed using multiple comparison tests: Bonferroni, Tukey's honestly significant difference test, Sidak, Gabriel, Hochberg and the least significant difference. The Mann-Whitney U–test was used to analyse differences between the histological findings in the groups formed by genotype, diet or strain (paper III). Significance levels for the statistics were calculated using the 2-tailed exact method.

The descriptive statistics in paper IV were expressed as means ± SEM, with the number of experiments given in parentheses. Statistical significance was tested with a repeated measures analysis of variance. To test differences within a group, a one-factor repeated measures ANOVA was used, followed by Fisher's PLSD post hoc test. Between groups, a two-factor repeated measures ANOVA followed by a one-way ANOVA at each time point was used. If the ANOVA was significant at a given time point, Fisher's PLSD post hoc analysis was used. All the statistical analyses were performed on an IBM-compatible computer using StatView 5.0 software.

5 Results

5.1 CA expression in leukaemia (I)

5.1.1 CA expression in malignant haematopoietic cell lines

Out of the five previously characterized leukaemia cell lines and one lymphoma cell line that were subjected to CA inhibitor affinity chromatography and analysed by SDS-PAGE, the RPMI 8226 and OCI/AML-2 cell lines showed 30-kDa polypeptides, corresponding to the molecular mass of cytosolic CA I and II. The positive polypeptides were further characterized by Western blotting, which revealed strong CA II polypeptides in these cell lines and a weak positive signal in the HL-60 cells. Other blots using anti-CA I, anti-CA IX and anti-CA XII antibodies were negative.

5.1.2 CA expression in leukaemia cells

As CA II was expressed in some malignant haematopoietic cell lines, it was felt important to study its expression in bone marrow samples collected from leukaemia patients. 62% of the AML samples, 73% of the ALL samples and one out of the two CMML samples showed positive reactions for CA II. Both the mean staining intensity and the percentage of positive cells varied markedly in each patient category, and neither the extent nor the intensity of staining correlated with the mortality rate (data not shown). Positive signals showed a typical cytoplasmic location in blast cells. Double immunofluorescence staining using anti-CA II and anti-CD34 antibodies further confirmed that most CA II-positive cells expressed CD34, a marker of immature blast cells. Control staining using normal rabbit serum instead of the anti-CA II serum showed no specific reaction.

5.2 Human CA IX and XII in gastric adenomas and carcinomas (II)

5.2.1 Expression of CA IX

CA IX was localized in all the major cell types of the normal mucosa, including parietal cells, chief cells and mucus producing surface epithelial cells, as described previously by Pastoreková *et al*. (1997). The positive staining covered all regions of the gastric mucosa, including the glands, proliferative zone and superficial epithelium. The slightly weaker staining observed in the proliferative zone (data not shown) contrasts with previous results in the intestine, where CA IX is mainly located in the proliferative enterocytes (Saarnio *et al*. 1998). It is notable that positive staining was observed in the crypts of the metaplastic epithelium, which is in line with the high expression found in the Lieberkühn crypts of the normal gut. The average staining reactions in the normal and hyperplastic gastric mucosa were quite similar, but they were significantly weaker in the dysplastic and malignant lesions. CA IX staining indices in the adenomas declined from lesions with slight or moderate dysplasia towards those with severe dysplasia, but the staining index in the adenocarcinomas remained at the same level as in the normal or hyperplastic mucosa in grade 1, declining towards the higher malignancy grades.

5.2.2 Expression of CA XII

CA XII showed little or no immunoreaction in the non-neoplastic gastric mucosa, but the mean staining indices increased significantly in the hyperplastic and adenomatous lesions and in the grade 1 and 2 adenocarcinomas and metastases, although they did not reach the values observed for CA IX. The indices were lower in the grade 3 adenocarcinomas and diffuse carcinomas, where they did not differ significantly from those in the non-neoplastic mucosa.

5.3 CA IX deficient mice with a standard or high-salt diet (III)

Among the total of 77 mice, grouped according to strain, diet and *Car9* genotype, that were analysed (Table 5), those that were homozygous for the targeted *Car9* allele were born at the expected Mendelian frequency, developed normally and were fertile. Samples of the stomach, which is known to express the highest levels of the CA IX enzyme in normal mice (Hilvo *et al.* 2004), were dissected for histological analysis.

The results implied that the *Car9*$^{-/-}$ genotype and the C57/BL6 strain were the main factors which increased susceptibility to histological abnormalities in the stomach (Fig. 4). The gastric morphology of all the wild-type BALB/c mice (n=19) was completely normal regardless of their diet, and the majority of the BALB/c *Car9*$^{-/-}$ mice fed on a standard diet (n=10) showed either moderate (50%) or mild (40%) hyperplasia of the gastric surface epithelium ($p<0.001$), with 30% having moderate and 50% mild glandular

atrophy (p=0.001). 10% of the mice that had received high-salt feed (n=10) showed moderate and 90% mild gastric hyperplasia, while 40% had mild and 10% moderate atrophy. The salt content of the diet had no statistically significant effect on the gastric histology of the BALB/c *Car9*$^{-/-}$ mice.

The wild-type C57/BL6 mice showed a slight susceptibility to gastric hyperplasia: 33% of those fed on a standard diet (3 out of 9) had mild hyperplasia of the epithelial cells, whereas all those with a high-salt diet (n=9) had a normal gastric epithelium. Most importantly, all the C57/BL6 *Car9*$^{-/-}$ mice fed on a standard diet showed strong (40%), moderate (20%) or mild (40%) hyperplasia of the gastric epithelium, whereas the high-salt diet did not bring about any significant change in the degree of pit cell hyperplasia, the proportions being 30%, 50% and 20%, respectively. Statistical comparison of the *Car9*$^{-/-}$ BALB/c and C57/BL6 groups fed on a standard diet indicated no significant difference in susceptibility to pit cell hyperplasia between them.

All the wild-type C57/BL6 mice showed normal glandular histology, whereas 90% of the C57/BL6 *Car9*$^{-/-}$ mice in both diet groups had abnormal gastric glands. Among the mice fed a standard diet, 20% had moderate and 70% mild glandular atrophy, whereas 80% of the mice fed on a high-salt diet showed moderate and 10% mild atrophy.

There was no evidence of mucosal inflammation in any of the groups, and no cases of intestinal metaplasia or dysplasia were seen. Gastric submucosal inflammation was detectable in the majority of the C57/BL6 *Car9*$^{-/-}$ mice. The degree of inflammation was moderate in one mouse and mild in five mice in both the standard and the high-salt diet groups. It is notable that the WT C57/BL6 mice and all the BALB/c mice had normal submucosal histology.

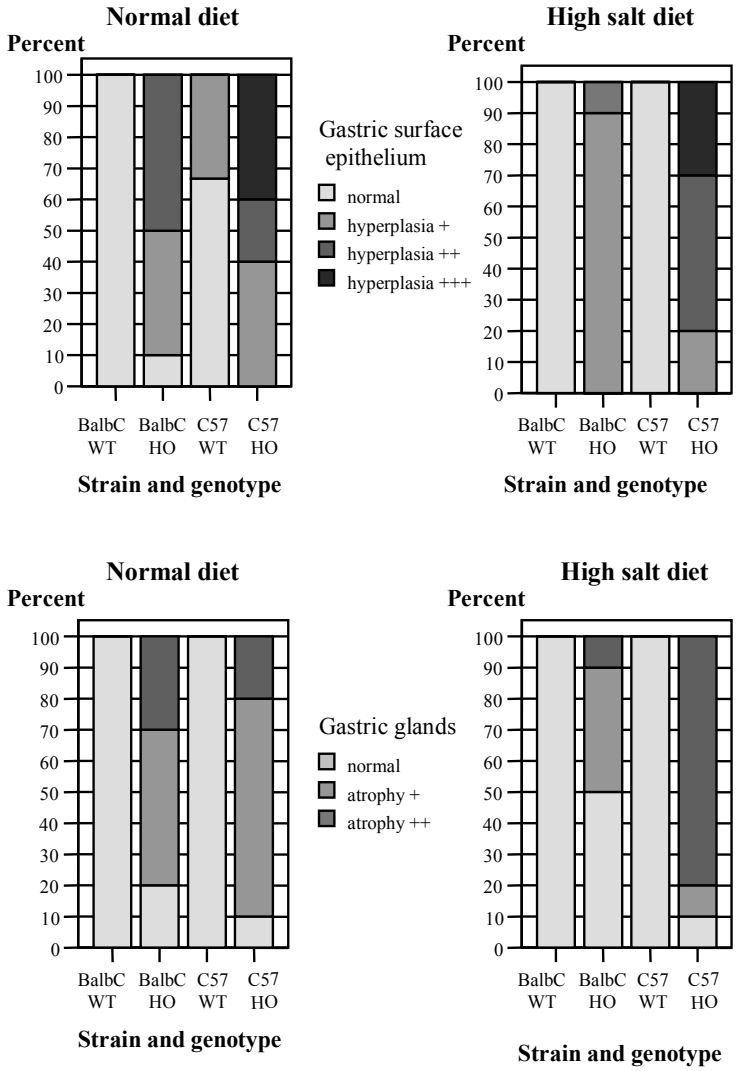

Fig. 4. A graphic summary of gastric pathology detected in different mice. *Car9*$^{-/-}$ genotype clearly predisposes mice to gastric pit cell hyperplasia and glandular atrophy in both C57/BL6 and BALB/c strains. The salt content in the diet has no statistical significant effect on the gastric histology in BALB/c *Car9*$^{-/-}$ mice, whereas the glandular atrophy becomes more severe in the C57/BL6 *Car9*$^{-/-}$ mice fed with high-salt diet.

5.4 Bicarbonate secretion in CA II and/or IX deficient mice (IV)

The matings between proven heterozygous *Car2*$^{+/-}$ / *Car9*$^{+/-}$ mice yielded all the expected genotypic variations, but the genotypes deviated from the expected Mendelian ratios in that only one double knockout mouse (*Car2*$^{-/-}$ / *Car9*$^{-/-}$) was born although ten such mice could have been expected from the total number of pups.

The duodenal segment spontaneously secreted HCO_3^- at a steady basal rate in all the groups tested. The secretory rate was not influenced by the systemic isotonic saline infusion or the duodenal luminal vehicle (isotonic saline) perfusion. Similarly, neither the systemic infusion nor the duodenal vehicle perfusion affected the mean arterial blood pressure (MAP).

The basal rate of bicarbonate secretion was measured in control (WT C57BL/6J) mice originating from two breeding centres over a period of 90 min. The duodenal lumen was perfused with saline alone and the secretory rate was recorded at 10-min intervals. The steady basal secretory rates of the mice originating from the Swedish and Finnish animal centres were not significantly different, being 5.3 ± 0.55 μmol·cm^{-1}·h^{-1} and 5.6 ± 0.65 μmol·cm^{-1}·h^{-1}, respectively.

Perfusing of the duodenal lumen with 47 μM of prostaglandin E_2 (PGE_2) for 20 min caused a significant increase in duodenal mucosal HCO_3^- secretion in the WT mice, from 5.6 ± 0.65 μmol·cm^{-1}·h^{-1} to 13.0 ± 2.9 μmol·cm^{-1}·h^{-1}, n=5, P<0.0001. This secretory increase in response occurred promptly, and also returned to the baseline level immediately after the removal of PGE_2 from the luminal perfusate. Duodenal luminal PGE_2 had no effect on MAP.

5.4.1 Bicarbonate secretory response to PGE_2

To evaluate the role of CAs in the PGE_2 regulation of duodenal alkalinisation, a number of mice of various *Car2* and *Car9* genotypes were investigated. Stimulation of the duodenal lumen for 20 min with 47 μM of PGE_2 caused a significant increase in duodenal mucosal HCO_3^- secretion in the double heterozygous *Car2*$^{+/-}$ / *Car9*$^{+/-}$ mice (n=3), from 4.97 ± 0.67 μmol·cm^{-1}·h^{-1} to 11.6 ± 4.5 μmol·cm^{-1}·h^{-1}, P<0.01, but neither the basal nor the stimulated secretions were significantly different from those in the control group. The genetic manipulation or PGE_2 did not affect the MAP.

The stimulatory effect of PGE_2 on DBS was completely absent in the mice lacking both alleles coding CA II enzyme. Two variants of CA II deficient mice were tested, *Car2*$^{-/-}$ / *Car9*$^{+/+}$ mice (n=3, P>0.05) or *Car2*$^{-/-}$ / *Car9*$^{+/-}$ mice (n=5, P>0.05), and PGE_2 did not change the DBS in either. The basal DBS in the *Car2*$^{-/-}$ mice was not significantly different from that in the control group or in the *Car2*$^{+/-}$ group, although previous studies have reported that the CA inhibitors reduce basal bicarbonate secretion (Knutson *et al*. 1995, Furukawa *et al*. 2004, Hirokawa *et al*. 2004). The *Car2*$^{-/-}$ mice had a basal secretory rate of ~4.5 ± 0.4 μmol·cm^{-1}·h^{-1} during the 90-min experimental period. MAP was not affected by either the genetic manipulation or PGE_2.

The *Car2*$^{+/-}$ / *Car9*$^{-/-}$ mice (n=5) significantly increased their duodenal alkaline secretion in response to luminal PGE_2, from 4.92 ± 0.55 μmol·cm^{-1}·h^{-1} to 13.8 ± 6.4

µmol·cm^{-1}·h^{-1} (P<0.0001), an effect close to that observed in the control and double heterozygous mice. In the *Car2*$^{+/+}$ / *Car9*$^{-/-}$ mice (n=4), both the net basal bicarbonate secretion and the net bicarbonate output during PGE_2 stimulation were significantly increased compared with both the control and the *Car2* $^{+/-}$, *Car9* $^{+/-}$ mice, the basal secretion being 6.55 ± 0.95 µmol·cm^{-1}·h^{-1}, P<0.001 and the peak secretory rate during PGE_2 exposure 17.0 ± 3.9 µmol·cm^{-1}·h^{-1}, P<0.0001. Again neither the genetic manipulation nor PGE_2 affected the MAP.

The mouse lacking both CA II and CA IX showed no stimulation in its secretory response to PGE_2. Although it is not possible to reach any firm conclusions on the basis of one experiment, the basal secretory rate was lower than in the controls and the secretion did not increase when PGE_2 was applied to the duodenal lumen. The MAP was the same as in the controls.

The main results of PGE_2-mediated stimulation of DBS are shown graphically in Fig. 5.

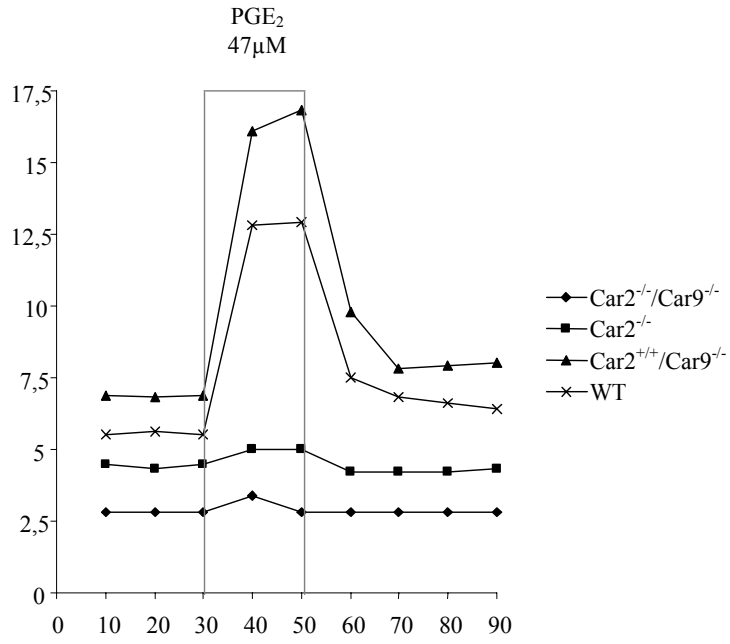

Fig. 5. PGE_2-mediated stimulation of DBS. Perfusing the duodenal lumen with PGE_2 (47 µM) caused a marked increase in duodenal mucosal HCO_3^- secretion in the WT group (n=6). The *Car2*$^{+/+}$*/Car9*$^{-/-}$ group (n=4) had significantly increased response to PGE_2 compared to the WT group. Also the basal levels of DBS were elevated in the *Car2*$^{+/+}$*/Car9*$^{-/-}$ group. No secretory increase in response to PGE_2 was observed in CA II deficient mice (n=8). In the *Car2*$^{-/-}$*/Car9*$^{-/-}$ mouse (n=1), the basal secretion was low and no secretory increase in response to PGE_2 was observed.

5.4.2 Acid/base balance

All the samples for blood gas analysis were taken at the end of the experiments (t=90). The mice lacking CA II showed respiratory acidosis, as was to be expected, as this plays an important role in CO_2 metabolism in the erythrocytes, as reported previously (Wistrand 1981). Otherwise, no significant differences in pH, pCO_2, base excess, final plasma HCO_3^-, or pO_2 were observed between the animals.

5.4.3 Morphological changes

Apart from the physiological findings, there were also several morphological changes in the stomach and intestine associated with the CA deficiencies. The *Car2*$^{-/-}$ / *Car9*$^{-/-}$ mouse had mild pit cell hyperplasia in the stomach and enlarged crypts in the small intestine.

Pit cell hyperplasia was in fact seen in all the *Car9*$^{-/-}$ mice regardless of the *Car2* genotype, being moderate in 40% and mild in 60%. In addition, 40% of the *Car9*$^{-/-}$ mice had mild glandular atrophy. Two *Car9*$^{-/-}$ mice in addition to the double knockout mouse had enlarged small intestinal crypts.

Interestingly, 50% of the *Car2*$^{-/-}$ / *Car9*$^{+/+}$ and *Car2*$^{-/-}$ / *Car9*$^{+/-}$ mice had mild pit cell hyperplasia, 13% had mild glandular atrophy and 50% had gastric cysts. In addition, six mice had increased numbers of intraepithelial lymphocytes in the duodenum, of which five had the genotype *Car2*$^{-/-}$ / *Car9*$^{+/+}$ or *Car2*$^{-/-}$ / *Car9*$^{+/-}$ and one was the double heterozygote.

The morphological changes noted in the CA deficient mice are listed in Table 6.

Table 6. Histological abnormalities in the mice, by CA genotype

Genotype	Histological abnormalities
Car2$^{-/-}$	Mild pit cell hyperplasia (50%, 4 out of 8)
	Mild glandular atrophy (13%, 1 out of 8)
	Gastric cysts (50%, 4 out of 8)
Car9$^{-/-}$	Moderate pit cell hyperplasia (44%, 4 out of 9)
	Mild pit cell hyperplasia (56%, 5 out of 9)
	Mild glandular atrophy (44%, 4 out of 9)
	Enlarged crypts in the small intestine (22%, 2 out of 9)
Car2$^{-/-}$ / *Car9*$^{-/-}$	Mild pit cell hyperplasia (100%, 1)
	Enlarged crypts in the small intestine (100%, 1)

6 Discussion

The principal aim of this work was to clarify the function and expression of CA IX, especially in the alimentary tract. The recently produced CA IX deficient mice offered useful tools for assessing the role of this protein in the normal structure and function of organs. Since CA II is another important CA in the alimentary tract, this isoenzyme was also investigated in some parallel experiments. Emphasis was placed on the phenotypic changes that the lack of these enzymes alone or together might cause in mice.

6.1 CA IX in gastric neoplasms and leukaemias

CA IX is a unique isoenzyme among the CA gene family. In contrast to the others, it is thought to be involved in cell proliferation, adhesion and malignant cell invasion in addition to its role in pH regulation. It is often referred to as a cancer-associated isoenzyme, as it is present in several human carcinomas, although it is usually absent or only weakly expressed in the normal tissues from which these tumours have originated, e.g. in the kidney and cervix (Liao *et al*. 1994, Mc Kiernan *et al*. 1997). A divergent expression pattern has been reported in the stomach, intestine and gallbladder, however, where CA IX is normally expressed, but as it is absent or diminished in malignant gallbladder and stomach specimens (Pastoreková *et al*. 1997, Saarnio *et al.* 2001). As tumours arising from the gastric mucosa had not been systematically studied earlier, a series of specimens from the normal gastric mucosa and from various dysplastic and neoplastic gastric lesions were analysed here. Interestingly, CA IX expression was sustained in most cases of gastric neoplasias, even though diminished relative to the normal and hyperplastic benign gastric mucosa. CA IX expression levels were very low in adenomas with severe dysplasia, but normal in grade I adenocarcinomas, declining again towards the carcinomas with higher malignancy grades. These findings support the hypothesis that these tumours do not follow the typical adenoma-carcinoma sequence that has been described in colorectal cancer (Maesawa *et al*. 1995, Tamura *et al*. 1995, Tamura 1996, Tsukashita *et al*. 2001). The recent results obtained by Chen *et al*. (2005) regarding CA IX in gastric tumours confirm our findings that CA IX expression is indeed lower in malignant gastric tumours than in the normal epithelium. A subgroup of gastric

tumours in their material retained CA IX expression at the invasion front, and interestingly, they also found that CA IX positivity predicted poor survival, which agrees well with some previous observations on tumours of various types. Based on our present understanding, it seems plausible that CA IX expression could represent a significant prognostic factor for certain cancers, and that the M75 monoclonal antibody could serve as an excellent reagent for such prognostic studies.

The extracellular proteoglycan-like region causes CA IX to stand out from the other isoenzymes, as the CA domain is probably involved in the regulation of acid-base homeostasis on the basolateral surfaces of the gastrointestinal epithelial cells. CA IX is a very active enzyme which may acidify the extracellular microenvironment of tumour cells in response to hypoxia, and its expression usually promotes tumour growth and invasion, and thus impairs prognosis (Wingo *et al*. 2001, Ivanov *et al*. 1998, Helmlinger *et al*. 2002, Svastova *et al.* 2004). Even though the results presented in paper II indicate that CA IX is not a specific biomarker for gastric tumours, so that its clinical significance may be limited in this tumour type, it is a promising target for cancer therapy in the case of several other tumours (Ivanov *et al*. 1998, Uemura *et al*. 1999). As shown in paper I, haematological malignancies are among the cancers which do not generally express CA IX, and thus cannot be considered potential applications for CA IX-targeted cancer therapy. Nonetheless, reasonably rapid progress has been made in developing novel anti-cancer drugs, and a German biotechnology company, Wilex AG, has announced that a chimeric anti-CA IX monoclonal antibody for the treatment of RCC is already undergoing phase II and III trials (http://www.wilex.com). In addition, many CAIs which seem to be promising anti-cancer therapeutic agents, have been developed. Further studies and evaluation will still be needed to clarify the possible clinical use of CA inhibitors in cancer therapy (Supuran & Scozzafava 2001, 2002, Supuran *et al*. 2001). One target for future research should be to develop inhibitors with high isoenzyme specificity. A CA IX-specific inhibitor, for example, would indeed be a very promising candidate drug for therapeutic and diagnostic purposes. There are many such compounds now available, with nanomolar affinity and high specificity ratios for inhibiting CA IX over other isoenzymes (Garaj *et al*. 2004).

6.2 CA IX deficiency and the development of gastric structural abnormalities

The PG region of CA IX is probably responsible for the cell adhesion properties of this interesting enzyme (Zàvada *et al*. 2000). This unique feature of CA IX may be involved in the maintenance of mucosal integrity, contributing to proper intercellular contacts and communication (Zàvada *et al*. 1997, 2000). Studies on *Car9*$^{-/-}$ mice have indicated that CA IX is an important factor for gastric morphogenesis and for homeostasis in the gastric epithelium (Ortova Gut *et al*. 2002). The *Car9*$^{-/-}$ mice had abnormalities in gastric morphology, the most prominent of which was epithelial hyperplasia caused by an increased number and proportion of mucus-producing cells. In addition, the number and proportion of pepsinogen-producing chief cells was lower than in normal mice, while the number of parietal cells remained unchanged. These observations suggest that CA IX

plays a role in gastric morphogenesis, possibly via cell differentiation and proliferation, but the exact mechanism and the relationship to other morphogenic factors are still unknown. Svastova *et al*. (2003) elucidated the adhesion role of CA IX by investigating its subcellular localization and its relationship to E-cadherin, which is a key adhesion molecule whose loss or destabilization is linked with tumour invasion. They generated MDCK cells with constitutive expression of human CA IX protein. CA IX was located at the cell–cell contacts in the same areas of the lateral membranes that contain E-cadherin molecules. In addition to this overlapping localization, both proteins responded to equal external stimuli by internalization, and their removal restored the plasma membrane position. Hypoxic treatment of these cells similarly resulted in a disruption of the cell–cell contacts and internalization of E-cadherin. Overexpression of CA IX in MDCK cells reduced the binding of E-cadherin to β-catenin, which destabilizes intercellular adhesion, because the formation of complexes between E-cadherin and β-catenin is critical for cell adhesive function. This finding clearly supported a functional relationship between CA IX and E-cadherin. Thus, CA IX may have a capacity to modulate E-cadherin-mediated cell adhesion through interaction with β-catenin, which could be an important factor in tumour progression induced by hypoxia. These results suggest that CA IX can be classified as an important regulatory molecule, like EGFR, ErbB2, MUC1, and IQGAP, which have similar modes of action via different but interconnected signal transduction pathways (Hoschuetzky *et al*. 1994, Ochiai *et al*. 1994, Yamamoto *et al*. 1997, Kuroda *et al*. 1998, Svastova *et al*. 2003). It is possible that CA IX may perform its function by combining its enzyme activity and adhesion capacity in response to stimuli coming from adjacent cells. Very little is known, however, about whether these functions could be interconnected, i.e. what kind of effect pH has on cell adhesion. There are just a few pieces of information to indicate that the intracellular pH of human fibroblasts may be dependent on cell density (Galkina *et al.* 1995). Furthermore, the adhesion of melanoma cells seems to be regulated by extracellular pH (Stock *et al.* 2005) and was found to be strongest at pH 6.6. Migration of the malignant cells was hindered when the interaction was too strong (at an acidic pH) or too weak (at an alkaline pH). Even though CA IX probably contributes to the pH balance in close proximity to the intercellular junctions, it has not been established whether pH regulation by CA IX plays any role in ensuring proper cell adhesion.

It will be interesting in the future to examine other tissues in CA IX deficient mice. Because CA IX has a role in adhesion and morphogenesis, it is possible that there may be histological abnormalities in some other tissues, especially in the colon, brain, thymus and spleen, where CA IX is expressed (Hilvo *et al*. 2004).

It is common that the targeted mutants of mice are initially on a mixed genetic background and gradually moved to a pure background, and the phenotype of such mutants seems to shift with time during this back-crossing (Tominaga *et al*. 2004). Since previous analyses of *Car9*$^{-/-}$ mice had been performed on a mixed genetic background involving the C57/BL6 and 129/Ola strains (Ortova Gut *et al*. 2002), the present *Car9*$^{-/-}$ mice were back-crossed into the pure genetic backgrounds of the commonly used strains C57/BL6 or BALB/c. The morphological results confirmed that the phenotype of the mice with a pure genetic background was slightly different from the mixed one. In addition, there were clearly detectable phenotypic differences between the C57/BL6 and BALB/c strains, suggesting that the natural genetic heterogeneity associated with

different mouse strains can significantly alter the phenotype of CA IX knockout mice. These results again emphasise that it is important to pay attention to the animal strain when designing a study in which genetically manipulated animals are to be used. Our results obtained with C57/BL6 and BALB/c *Car9*$^{-/-}$ mice possessing a pure genetic background showed that CA IX deficiency induces gastric pit cell hyperplasia and glandular atrophy in the gastric body mucosa in both strains, but the changes were slightly more prominent in the C57/BL6 mice. On the other hand, the morphological changes in both strains were milder than those reported previously (Ortova Gut *et al.* 2002), in that no large pathological cysts were observed. The absence of actual neoplastic lesions such as dysplasias or carcinomas agrees well with the previously documented findings.

6.3 Gastric effects of CA IX deficiency and a high-salt diet

In order to investigate the morphological changes brought about by CA IX deficiency together with a high-salt diet, a known co-factor of *H. pylori*-induced carcinogenesis (Lee *et al*. 2003, Correa *et al*. 2004), an experiment was conducted on mice that were free of significant gastric infections, including *H. pylori* infection, and were housed under pathogen-free conditions. The findings indicated that the high-salt diet had no additive effect on the histological abnormalities observed in *Car9*$^{-/-}$ BALB/c mice, but that the glandular atrophy in the gastric body mucosa of the C57/BL6 mice was slightly increased. This suggests that the mouse strain again influenced the results, and that CA IX deficiency alone or together with a high-salt diet does not cause gastric cancer to develop, although it may initiate a carcinogenic process by affecting cell proliferation, intercellular adhesion or communication, thereby inducing atrophic changes in the gastric body mucosa. It is generally accepted that gastric atrophy is an important step in gastric carcinogenesis (Lauwers 2003). Considering the results presented in papers II and III together, it is notable that the gastric pathologies mentioned, i.e. pit cell hyperplasia and glandular atrophy in mice and gastric dysplasias and carcinomas in humans, were all associated with loss or diminished levels of CA IX expression. Based on that observation, one could speculate that CA IX may play an important role in the cascade of events that finally lead to a carcinogenic process in the gastric mucosa.

6.4 Gastric submucosal inflammation in CA IX deficient mice

Gastric submucosal inflammation, which consisted of T and B lymphocytes, plasma cells and occasional neutrophilic granulocytes, was seen in most of C57/BL6 *Car9*$^{-/-}$ mice fed on either a normal or a high-salt diet, whereas no evidence of this was seen in the C57/BL6 WT or BALB/c mice. The mechanisms of the reaction are unknown, because the affected mice had neither a systemic infection nor pathogenic microbial agents, but these results suggest that CA IX may have a role in the regulation of inflammatory or immunological responses in some mouse strains.

The difference between the C57/BL6 and BALB/c mouse strains is known to apply to *H. pylori* infection, since BALB/c mice do not respond to this, whereas C57/BL6 mice show a strong immune response (Fox *et al*. 1999). It is conceivable that these strains react to *H. pylori* infection by different mechanisms, C57/BL6 mice showing a balanced Th1 response, while BALB/c mice display a mixed Th cytokine profile (Mohammadi *et al*. 1996, Smythies *et al*. 2000).

CA IX mRNA has been detected in the mouse thymus and spleen (Hilvo *et al*. 2004), and the protein has been found in a human subpopulation of germinal centre cells in reactive lymph nodes (Stewart *et al*. 2002), suggesting that CA IX may be expressed in all the major organs of the immunological system. Thymic expression of CA IX (Hilvo *et al*. 2004) may be significant in the development of tolerance to this protein, as with other potential autoantigens (Anderson *et al*. 2002). All these results together indicate that CA IX may have a role in regulation of the immunological Th1 response and in the development of autoimmunity. Inflammation was restricted to the submucosa, however, and not to the glandular layer, which does not support this hypothesis. Further studies are definitely needed in order to clarify the meaning of these observations. An examination of phenotypic changes and inflammation in *Car9*$^{-/-}$ mice infected with *H. pylori,* for example, could help elucidate the role of CA IX in regulation of the immune response. It is important to recognize that aberrations of the immunological system are considered important pathogenic factors in the development of gastric cancer (Zheng *et al*. 2004), and CA IX deficient mice may provide a useful animal model for studying the pathogenic components of *H. pylori*-induced carcinogenesis.

6.5 CA IX and CA II deficiency and duodenal bicarbonate secretion

Although our examination of the role of CA IX deficiency in the DBS response to PGE_2 revealed only mild physiological changes, the *Car2*$^{+/+}$ / *Car9*$^{-/-}$ mice showed higher basal and PGE_2-induced DBS rates than the WT mice. These results indicate for the first time that CA IX is indeed involved in the regulation of bicarbonate homeostasis in the normal gastrointestinal tract, but unfortunately the physiological mechanism remains unknown. These mice also had enlarged crypts in the small intestine, which supports a role for CA IX in cell adhesion (Svastova *et al*. 2003).

It can be concluded from previous studies and the present results that CA IX has a major role in the regulation of gastroduodenal morphogenesis and that its influence on bicarbonate homeostasis represents only a minor function. It will be attractive in the future to study further the pathways by which CA IX influences cell adhesion. Microarray analysis of WT and *Car9*$^{-/-}$ mice could provide a valuable device for this purpose. CA II is expressed in all organs of the alimentary tract, which makes it a unique isoenzyme within the CA family (Lönnerholm *et al*. 1985, Parkkila & Parkkila 1996, Parkkila 2000). The present study made use of the previously developed CA II deficient mice as a tool for evaluating the role of CA II in gastroduodenal function and morphology, and interestingly, mild pit cell hyperplasia, mild glandular atrophy and gastric cysts were found in some of the *Car2*$^{-/-}$ mice. These results indicate for the first time that CA II may also participate in normal gastric morphogenesis and raise the question of whether all the

observed morphological abnormalities result from defective pH homeostasis caused by a gastric CA deficiency. To our knowledge this is not likely, because CA IX seems to play only a minor role in DBS, but it would be very interesting to analyse gastric acid secretion in both CA II and CA IX deficient mice. Such an analysis was planned when the work for paper IV was being designed, but it appeared to be technically extremely difficult. The normal serum gastrin levels observed in the CA IX deficient mice nevertheless, suggested that acid secretion is close to normal in these mice (Ortova Gut *et al.* 2002).

Epithelial bicarbonate secretion is important for protecting the duodenal mucosa against luminal acid (Flemström & Kivilaakso 1983, Kivilaakso & Flemström 1984), and low doses of PGE_2 induce secretory responses that are closely similar to those elicited by acid (Hirokawa *et al*. 2004). The bicarbonate stimulatory action of PGE_2 in the duodenum is thought to include both 3',5'-cyclic adenosine monophosphate (cAMP) and intracellular Ca^{2+} (Flemström 1994, Takeuchi *et al*. 1997, Jacob *et al*. 2000), both second messengers that are thought to excite CA activity (Knutson *et al*. 1995). Furthermore, DBS deficiency has been suggested as a contributor to the pathogenesis of duodenal ulcer disease (Isenberg *et al*. 1987). It was therefore reasonable to study the role of CA II deficiency in the DBS response to PGE_2.

It was demonstrated here for the first time that CA II deficiency induces a clear abnormal gastrointestinal phenotype, and that the stimulatory effect of the duodenal secretagogue PGE_2 is completely dependent on CA II. Other studies have shown lower basal and PGE_2-stimulated secretory rates than those recorded here, and also a smaller DBS in response to luminal PGE_2 (Hirokawa *et al*. 2004a,b, Takeuchi *et al*. 1999). The difference could be explained by minor biological or experimental differences. Previous studies have also shown reduced basal secretion of bicarbonate after CA inhibition (Knutson *et al*. 1995), whereas the present results suggest that basal HCO_3^- secretion is not significantly affected in CA II deficient mice relative to WT animals. In the previous studies the CA inhibitors probably suppressed more or less all the major CA isoenzymes expressed in the gastrointestinal epithelia, while only a single isoenzyme was shut down in the genetically manipulated mice used here. Genetic approaches clearly provide valuable material for physiological analyses, and similar specificity could be achieved only if CA isoenzyme-specific inhibitors were available.

After several matings we obtained only one double knockout ($Car2^{-/-}$ / $Car9^{-/-}$) mouse, which makes interpretation of the result somewhat uncertain. Basal bicarbonate secretion was nevertheless lower in this case than in any other group, and did not increase when PGE_2 was applied to the duodenal lumen. One possible explanation for this is that CA IX may slightly compensate for the missing CA II activity in $Car2^{-/-}$ / $Car9^{+/+}$ mice, and that the absence of both isoenzymes led to lower basal bicarbonate secretion.

7 Conclusions

1. Since haematological and gastric malignancies show only occasional expression of CA IX or none at all, these cancers cannot be considered promising for CA IX-targeted therapy. The variations in CA IX levels observed in gastric tumours support the notion that gastric adenomas and carcinomas are distinct entities and do not represent progressive steps in a single pathway.
2. CA IX deficiency alone is not a significant carcinogenic factor in mice, but it may initiate a carcinogenic process by affecting cell proliferation, intercellular communication and/or adhesion and thereby inducing atrophic changes in the gastric body mucosa.
3. Since CA IX deficiency is associated with submucosal inflammation in some mice, CA IX may play a role in the regulation of immunological responses.
4. CA II is essential for PGE_2-mediated stimulation of duodenal HCO_3^- secretion in the mouse *in vivo*. It may also play a minor role in gastric morphogenesis.
5. The major role of CA IX is linked to gastric morphogenesis. It may also have a minor role in the regulation of gastroduodenal bicarbonate homeostasis.

References

Akama Y, Yasui W, Yokozaki H, Kuniyasu H, Kitahara K, Ishikawa T & Tahara E (1995) Frequent amplification of the cyclin E gene in human gastric carcinomas. Jpn J Cancer Res 86: 617-621.

Akisawa Y, Nishimori I, Taniuchi K, Okamoto N, Takeuchi T, Sonobe H, Ohtsuki Y & Onishi S (2003) Expression of carbonic anhydrase-related protein CA-RP VIII in non-small cell lung cancer. Virchows Arch 442: 66-70.

Aldred P, Fu P, Barrett G, Penschow JD, Wright RD, Coghlan JP & Fernley RT (1991) Human secreted carbonic anhydrase: cDNA cloning, nucleotide sequence, and hybridization histochemistry. Biochemistry 30: 569-575.

Allen A & Carroll NJ (1985) Adherent and soluble mucus in the stomach and duodenum. Dig Dis Sci 30: 55S-62S.

Allen A & Flemström G (2005) Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am J Physiol Cell Physiol 288: C1-C19.

Allen A, Leonard AJ, Pearson JP, Sellers LAA & Bennet MA (1991) Pepsin induced mucosal damage compared with that with ethanol or hypertonic saline: the role of the adherent mucus barrier. In: Garner A & O'Brien P (eds) Mechanisms of injury and mucosal repair in the upper gastrointestinal tract. Wiley, New York, 7-18.

Allen A, Pain RH & Robson TR (1976) Model for the structure of the gastric mucous gel. Nature 264: 88-89.

Amemiya H, Kono K, Mori Y, Takahashi A, Ichihara F, Iizuka H, Sekikawa T & Matsumoto Y (2000) High frequency of c-Met expression in gastric cancers producing alpha- fetoprotein. Oncology 59: 145-151.

Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C & Mathis D (2002) Projection of an immunological self shadow within the thymus by the aire protein. Science 298: 1395-1401.

Ashida S, Nishimori I, Tanimura M, Onishi S & Shuin T (2002) Effects of von Hippel-Lindau gene mutation and methylation status on expression of transmembrane carbonic anhydrases in renal cell carcinoma. J Cancer Res Clin Oncol 28: 561-568.

Awad M, Al-Ashwal AA, Sakati N, Al-Abbad AA & Bin-Abbas BS (2002) Long-term follow up of carbonic anhydrase II deficiency syndrome. Saudi Med J 23: 25-29.

Bahari HM, Ross IN & Turnberg LA (1982) Demonstration of a pH gradient across the mucus layer on the surface of human gastric mucosa in vitro. Gut 23: 513-516.

Ballesteros MA, Wolosin JD, Hogan DL, Koss MA & Isenberg JI (1991) Cholinergic regulation of human proximal duodenal mucosal bicarbonate secretion. Am J Physiol 261: G327-G331.

Barnea G, Grumet M, Milev P, Silvennoinen O, Levy JB, Sap J & Schlessinger J (1994a) Receptor tyrosine phosphatase beta is expressed in the form of proteoglycan and binds to the extracellular matrix protein tenascin. J Biol Chem 269: 14349-14352.

Barnea G, Grumet M, Sap J, Margolis RU & Schlessinger J (1994b) Close similarity between receptor-linked tyrosine phosphatase and rat brain proteoglycan. Cell 76: 205.

Barnea G, Silvennoinen O, Shaanan B, Honegger AM, Canoll PD, D'Eustachio P, Morse B, Levy JB, Laforgia S, Huebner K, Musacchio JM, Sap J & Schlessinger J (1993) Identification of a carbonic anhydrase-like domain in the extracellular region of RPTP gamma defines a new subfamily of receptor tyrosine phosphatases. Mol Cell Biol 13: 1497-1506.

Bartošová M, Parkkila S, Pohlodek K, Karttunen TJ, Galbavý S, Mucha V, Harris AL, Pastorek J & Pastoreková S (2002) Expression of carbonic anhydrase IX in breast is associated with malignant tissues and is related to overexpression of c-erbB2. J Pathol 197: 314-321.

Basu A, Dong B, Krainer AR & Howe CC (1997) The intracisternal A-particle proximal enhancer-binding protein activates transcription and is identical to the RNA- and DNA-binding protein p54nrb/NonO. Mol Cell Biol 17: 677-686.

Bataller L, Sabater L, Saiz A, Serra C, Claramonte B & Graus F (2004) Carbonic anhydrase-related protein VIII: autoantigen in paraneoplastic cerebellar degeneration. Ann Neurol 56: 575-579.

Beasley NJ, Wykoff CC, Watson PH, Leek R, Turley H, Gatter K, Pastorek J, Cox GJ, Ratcliffe P & Harris AL (2001) Carbonic anhydrase IX, an endogenous hypoxia marker, expression in head and neck squamous cell carcinoma and its relationship to hypoxia, necrosis, and microvessel density. Cancer Res 61: 5262-5267.

Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR & Hofler H (1994) E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res 54: 3845-3852.

Behrens J, Mareel MM, Van Roy FM & Birchmeier W (1989) Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. J Cell Biol 108: 2435-2447.

Bell AE, Sellers LA, Allen A, Cunliffe WJ, Morris ER & Ross-Murphy SB (1985) operties of gastric and duodenal mucus: effect of proteolysis, disulfide reduction, bile, acid, ethanol, and hypertonicity on mucus gel structure. Gastroenterology 88: 269-280.

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR & Sultan C (1976) Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 33: 451-458.

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR & Sultan C (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51: 189-199.

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR & Sultan C (1985) Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 103: 620-625.

Ben-Ze'ev A & Geiger B (1998) Differential molecular interactions of beta-catenin and plakoglobin in adhesion, signaling and cancer. Curr Opin Cell Biol 10: 629-639.

Bergenhem NC, Hallberg M & Wisen S (1998) Molecular characterization of human carbonic anhydrase-related protein (HCA-RP VIII). Biochim Biophys Acta 1384: 294-298.

Bertini I, Luchinat C & Scozzafava A (1982) Carbonic anhydrase: An insight into the zinc binding site and into the active cavity through metal substitution. Struct Bonding 48: 45–92.

Briganti F, Pierattelli A, Scozzafava A & Supuran CT (1996) Carbonic anhydrase inhibitors. Part 37. Novel classes of carbonic anhydrase inhibitors and their interaction with the native and cobalt-substituted enzyme: Kinetic and spectroscopic investigations. Eur J Med Chem 31: 1001–1010.

Brion LP, Schwartz JH, Zavilowitz BJ & Schwartz GJ (1988) Micro-method for the measurement of carbonic anhydrase activity in cellular homogenates. Anal Biochem 175: 289-297.

Brion LP, Suarez C & Saenger P (2001) Postnatal disappearance of type A intercalated cells in carbonic anhydrase II-deficient mice. Pediatr Nephrol 16: 477-481.

Brizel DM, Scully SP, Harrelson JM, Layfield LJ, Bean JM Prosnitz LR & Dewhirst MW (1996) Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma Cancer Res 56: 941-943.

Brown D, Zhu XL & Sly WS (1990) Localization of membrane-associated carbonic anhydrase type IV in kidney epithelial cells. Proc Natl Acad Sci U S A 87: 7457-7461.

Bui MH, Seligson D, Han KR, Pantuck AJ, Dorey FJ, Huang Y, Horvath S, Leibovich BC, Chopra S, Liao SY, Stanbridge E, Lerman MI, Palotie A, Figlin RA & Belldegrun AS (2003) Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res 9: 802-811.

Bundy HF (1977) Carbonic anhydrase. Comp Biochem Physiol 57B: 1-7.

Butterworth PHW, Barlow JH, Brady HJM, Edwards M, Lowe N & Sowden JC (1991) The structure and regulation of the human carbonic anhydrase I gene. In: Dodgson SJ, Tashian RE, Gros G & Carter ND (eds) The Carbonic Anhydrases. Cellular Physiology and Molecular Genetics. Plenum Press, New York, 197-207.

Cabiscol E & Levine RL (1995) Carbonic anhydrase III. Oxidative modification in vivo and loss of phosphatase activity during aging. J Biol Chem 270: 14742-14747.

Carneiro F & Sobrinho-Simoes M (2000) The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. Cancer 88: 238-240.

Carter N, Wistrand PJ & Lonnerholm G (1989) Carbonic anhydrase localization to perivenous hepatocytes. Acta Physiol Scand 135: 163-167.

Chan AO, Luk JM, Hui WM & Lam SK (1999) Molecular biology of gastric carcinoma: from laboratory to bedside. J Gastroenterol Hepatol 14: 1150-1160.

Chen JC & Chesler M (1992) pH transients evoked by excitatory synaptic transmission are increased by inhibition of extracellular carbonic anhydrase. Proc Natl Acad Sci U S A 89: 7786-7790.

Chen J, Rocken C, Hoffmann J, Kruger S, Lendeckel U, Rocco A, Pastorekova S, Malfertheiner P & Ebert MP (2005) Expression of carbonic anhydrase 9 at the invasion front of gastric cancers. Gut 54: 920-927.

Chia SK, Wykoff CC, Watson PH, Han C, Leek RD, Pastorek J, Gatter KC, Ratcliffe P & Harris AL (2001) Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma J Clin Oncol 19: 3660-3668.

Cho M, Grabmaier K, Kitahori Y, Hiasa Y, Nakagawa Y, Uemura H, Hirao Y, Ohnishi T, Yoshikawa K & Ooesterwijk E (2000) Activation of the MN/CA9 gene is associated with hypomethylation in human renal cell carcinoma cell lines. Mol Carcinog. 27: 184-189.

Christie KN, Thomson C, Xue L, Lucocq JM & Hopwood D (1997) Carbonic anhydrase isoenzymes I, II, III, and IV are present in human esophageal epithelium. J Histochem Cytochem 45: 35-40.

Correa P, Piazuelo MB & Camargo MC (2004) The future of gastric cancer prevention. Gastric Cancer 7: 9-16.

Cowin P & Burke B (1996) Cytoskeleton-membrane interactions. Curr Opin Cell Biol 8: 56-65.

Crampton JR, Gibbons LC & Rees WD (1987) Effect of luminal pH on the output of bicarbonate and PGE2 by the normal human stomach. Gut 28: 1291-1295.

Danjo Y & Gipson IK (1998) Actin 'purse string' filaments are anchored by E-cadherin-mediated adherens junctions at the leading edge of the epithelial wound, providing coordinated cell movement. J Cell Sci 111: 3323-3332.

Davenport HW (1939) Gastric carbonic anhydrase. J Physiol (Lond.) 97: 32-43.

Davenport HW & Fisher RB (1938) Carbonic anhydrase in the gastrointestinal mucosa. J Physiol (Lond.) 94: 16P-17P.

De Raeve HR, Vermeulen PB, Vanderkerken K, Harris AL & Van Marck E (2004) Microvessel density, endothelial-cell proliferation and carbonic anhydrase IX expression in haematological malignancies, bone-marrow metastases and monoclonal gammopathy of undetermined significance. Virchows Arch 445: 27-35.

Dodgson SJ (1991) Liver mitochondrial carbonic anhydrase (CA V), gluconeogenesis, and ureagenesis in the hepatocytes. In: Dodgson SJ, Tashian RE, Gros G, Carter ND, editors. The Carbonic Anhydrases. Cellular Physiology and Molecular Genetics. Plenum Press, New York, 297-306.

Dodgson SJ, Forster RE & Storey BT (1984) The role of carbonic anhydrase in hepatocyte metabolism. Ann NY Acad Sci USA 429: 516-524.

Dong B, Horowitz DS, Kobayashi R & Krainer AR (1993) Purification and cDNA cloning of HeLa cell p54nrb, a nuclear protein with two RNA recognition motifs and extensive homology to human splicing factor PSF and Drosophila NONA/BJ6. Nucleic Acids Res 21: 4085-4092.

Drubin DG & Nelson WJ (1996) Origins of cell polarity. Cell 84: 335-344.

Earnhardt JN, Qian M, Tu C, Lakkis MM, Bergenhem NC, Laipis PJ, Tashian RE & Silverman DN (1998) The catalytic properties of murine carbonic anhydrase VII. Biochemistry 37: 10837-10845.

Ebert MP, Fei G, Kahmann S, Muller O, Yu J, Sung JJ, & Malfertheiner P (2002a) Increased beta-catenin mRNA levels and mutational alterations of the APC and beta-catenin gene are present in intestinal-type gastric cancer. Carcinogenesis 23: 87-91.

Ebert MP, Schandl L & Malfertheiner P (2002b) Helicobacter pylori infection and molecular changes in gastric carcinogenesis. J Gastroenterol 37: 45-49.

Edwards YH (1991) Structure and expression of the carbonic anhydrase III gene. In: Dodgson SJ, Tashian RE, Gros G & Carter ND (eds) The Carbonic Anhydrases. Cellular Physiology and Molecular Genetics, Plenum Press, New York, 215-224.

El-Rifai W & Powell SM (2002) Molecular biology of gastric cancer. Semin Radiat Oncol 12: 128-140.

Engberg P, Millqvist E, Pohl G & Lindskog S (1985) Purification and some properties of carbonic anhydrase from bovine skeletal muscle. Arch Biochem Biophys 241: 628-638.

Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ & Ratcliffe PJ (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107: 43-54.

Eriksson & Liljas (1993) Refined structure of bovine carbonic anhydrase III at 2.0 A resolution Proteins 16: 29-42.

Feldstein JB & Silverman DN (1984) Purification and characterization of carbonic anhydrase from the saliva of the rat. J Biol Chem 259: 5447-5453.

Fenoglio-Preiser CM, Wang J, Stemmermann GN & Noffsinger A (2003) TP53 and gastric carcinoma: a review. Hum Mutat 21: 258-270.

Fernley RT (1991) Secreted carbonic anhydrases. In: Botré F, Gros G & Storey BT (eds) Carbonic anhydrase. From Biochemistry and Genetics to Physiology and Clinical Medicine. VCH Verlagsgesellschaft, Weinheim, 178-185.

Fernley RT, Wright RD & Coghlan JP (1979) A novel carbonic anhydrase from the ovine parotid gland. FEBS Lett 105: 299-302.

Fernley RT, Wright RD & Coghlan, JP (1988) Complete amino acid sequence of ovine salivary carbonic anhydrase. Biochemistry 27: 2815-2820.

Fiocca R, Luinetti O, Villani L, Mastracci L, Quilici P, Grillo F & Ranzani GN (2001) Molecular mechanisms involved in the pathogenesis of gastric carcinoma: interactions between genetic alterations, cellular phenotype and cancer histotype. Hepatogastroenterology 48: 1523-1530.

Fleming RE, Moxley MA, Waheed A, Crouch EC, Sly WS & Longmore WJ (1994) Carbonic anhydrase II expression in rat type II pneumocytes. Am J Respir Cell Mol Biol 10: 499-505.

Fleming RE, Parkkila S, Parkkila A-K, Rajaniemi H, Waheed & Sly WS (1995) Carbonic anhydrase IV expression in rat and human gastrointestinal tract: Regional, cellular and subcellular localization. J Clin Invest 96: 2907-2913.

Flemström G (1977) Active alkalinization by amphibian gastric fundic mucosa in vitro. Am J Physiol 233: E1-E12.

Flemström G (1980) Stimulation of HCO3- transport in isolated proximal bullfrog duodenum by prostaglandins. Am J Physiol 239: G198-G203.

Flemström G (1986) Gastroduodenal mucosal secretion of bicarbonate and mucus. Physiologic control and stimulation by prostaglandins. Am J Medicine 81: 18-22.

Flemström G (1994) Gastric and duodenal mucosal bicarbonate secretion. In: Johnson LR (ed) Physiology of the gastrointestinal tract. 3rd ed. Raven, New York, 1285–1309.

Flemström G & Garner A (1982) Gastroduodenal HCO3(-) transport: characteristics and proposed role in acidity regulation and mucosal protection. Am J Physiol 242: G183-G193.

Flemström G, Garner A, Nylander O, Hurst BC & Heylings JR (1982) Surface epithelial HCO3(-) transport by mammalian duodenum in vivo. Am J Physiol 243: G348-G358.

Flemström G & Kivilaakso E (1983) Demonstration of a pH gradient at the luminal surface of rat duodenum in vivo and its dependence on mucosal alkaline secretion. Gastroenterology 84: 787-794.

Florey H (1955) Mucin and the protection of the body. Proc R Soc Lond B Biol Sci 143: 147-158.

Fox JG, Dangler CA, Taylor NS, King A, Koh TJ & Wang TC (1999) High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances Helicobacter pylori colonization in C57BL/6 mice. Cancer Res 59: 4823-4828.

Fujikawa-Adachi K, Nishimori I, Sakamoto S, Morita M, Onishi S, Yonezawa S & Hollingsworth MA (1999a) Identification of carbonic anhydrase IV and VI mRNA expression in human pancreas and salivary glands. Pancreas 18: 329-353.

Fujikawa-Adachi K, Nishimori I, Tagychi T & Onishi S (1999b) Human mitochondrial carbonic anhydrase VB. cDNA cloning, mRNA expression, subcellular localization, and mapping to chromosome X. J Biol Chem 274: 21228-21233.

Fujikawa-Adachi K, Nishimori I, Taguchi T & Onishi S (1999c) Human carbonic anhydrase XIV (CA14): cDNA cloning, mRNA expression, and mapping to chromosome 1. Genomics 61: 74-81.

Fujikawa-Adachi K, Nishimori I, Taguchi T, Yuri K & Onishi S (1999d) cDNA sequence, mRNA expression, and chromosomal localization of human carbonic anhydrase-related protein, CA-RP XI. Biochim Biophys Acta 1431: 518-524.

Furukawa O, Bi LC, Guth PH, Engel E, Hirokawa M & Kaunitz JD (2004) NHE3 inhibition activates duodenal bicarbonate secretion in the rat. Am J Physiol Gastrointest Liver Physiol 286: G102-G109.

Galkina SI, Sud'ina GF, Dergacheva GB & Margolis LB (1995) Regulation of intracellular pH by cell-cell adhesive interactions. FEBS Lett 374: 17-20.

Garaj V, Puccetti L, Fasolis G, Winum JY, Montero JL, Scozzafava A, Vullo D, Innocenti A & Supuran CT (2004) Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides incorporating 1,2,4-triazine moieties. Bioorg Med Chem Lett 14: 5427-5433.

Ghandour MS, Langley OK, Zhu XL, Waheed A & Sly WS (1992) Carbonic anhydrase IV on brain capillary endothelial cells: a marker associated with the blood-brain barrier. Proc Natl Acad Sci U S A 89: 6823-6827.

Ghandour MS, Parkkila A-K, Parkkila S, Waheed A & Sly WS (2000) Mitochondrial carbonic anhydrase in the nervous system: expression in neuronal and glial cells. J Neurochem 75: 2212-2220.

Ghandour MS, Skoff RP, Venta PJ & Tashian RE (1989) Oligodendrocytes express a normal phenotype in carbonic anhydrase II-deficient mice. J Neurosci Res 23: 180-190.

Giatromanolaki A, Koukourakis MI, Sivridis E, Pastorek J, Wykoff CC Gatter KC & Harris AL (2001) Expression of hypoxia-inducible carbonic anhydrase-9 relates to angiogenic pathways and independently to poor outcome in non-small cell lung cancer. Cancer Res 61: 7992-7998.

González CA, Sala N & Capella G (2002) Genetic susceptibility and gastric cancer risk. Int J Cancer 100: 249-260.

Grabmaier K, Vissers JL, De Weijert MC, Oosterwijk-Wakka JC, Van Bokhoven A, Brakenhoff RH, Noessner E, Mulders PA, Merkx G, Figdor CG, Adema GJ & Oosterwijk E (2000) Molecular cloning and immunogenicity of renal cell carcinoma-associated antigen G250. Int J Cancer 85: 865-870.

Gumbiner BM (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84: 345-357.

Gumbiner BM (2000) Regulation of cadherin adhesive activity. J Cell Biol 148: 399-404.

Haapasalo JS, Nordfors KM, Hilvo M, Rantala IJ, Soini Y, Parkkila A-K, Pastorekovà S, Pastorek J, Parkkila SM & Haapasalo HK (2005) Expression of carbonic anhydrase IX in astrocytic tumors predicts poor prognosis. Clin Cancer Res (in press).

Hageman GS, Zhu XL, Waheed A & Sly WS (1991) Localization of carbonic anhydrase IV in a specific capillary bed of the human eye. Proc Natl Acad Sci U S A 88: 2716-2720.

Hallier M, Tavitian A & Moreau-Gachelin F (1996) The transcription factor spi-1/PU.1 binds RNA and interferes with the RNA-binding protein p54nrb. J Biol Chem 271: 11177-11181.

Halsted CH, Villanueva J, Chandler CJ, Ruebner B, Munn RJ, Parkkila S & Niemelä O (1993) Centrilobular distribution of acetaldehyde and collagen in the ethanol-fed micropig. Hepatology 18: 954-960.

Handschuh G, Candidus S, Luber B, Reich U, Schott C, Oswald S, Becke H, Hutzler P, Birchmeier W, Hofler H & Becker KF (1999) Tumour-associated E-cadherin mutations alter cellular morphology, decrease cellular adhesion and increase cellular motility. Oncogene 18: 4301-4312.

Hara T, Ooi A, Kobayashi M, Mai M, Yanagihara K & Nakanishi I (1998) Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization. Lab Invest 78: 1143-1153.

Hayashi N, Miyata S, Yamada M, Kamei K & Oohira A (2005) Neuronal expression of the chondroitin sulfate proteoglycans receptor-type protein-tyrosine phosphatase beta and phosphacan. Neuroscience 131: 331-348.

Hazen SA, Waheed A, Sly WS, LaNoue KF & Lynch CJ (1996) Differentiation-dependent expression of CA V and the role of carbonic anhydrase isozymes in pyruvate carboxylation in adipocytes. FASEB J 10: 481-490.

Heck RW, Tanhauser SM, Manda R, Tu C, Laipis PJ & Silverman DN (1994) Catalytic properties of mouse carbonic anhydrase V. J Biol Chem 269: 24742-24746.

Helmlinger G, Sckell A, Dellian M, Forbes NS & Jain RK (2002) Acid production in glycolysis-impaired tumors provides new insights into tumor metabolism. Clin Cancer Res 8: 1284-1291.

Henkin RI, Martin BM & Agarwal RP (1999a) Decreased parotid saliva gustin/carbonic anhydrase VI secretion: an enzyme disorder manifested by gustatory and olfactory dysfunction. Am J Med Sci 318: 380-391.

Henkin RI, Martin BM & Agarwal RP (1999b) Efficacy of exogenous oral zinc in treatment of patients with carbonic anhydrase VI deficiency. Am J Med Sci 318: 392-405.

Hewett-Emmett D (2000) Evolution and distribution of the carbonic anhydrase gene families. In: Chegwidden WR, Carter ND & Edwards YH (eds.) The Carbonic Anhydrases. New Horizons. Birkhäuser Verlag, Basel, 29-76.

Hewett-Emmett D & Tashian RE (1996) Functional diversity, conversation, and convergence in the evolution of the α-, β-, and γ-carbonic anhydrase gene families. Mol Phylogenet Evol 5: 50-77.

Hilvo M, Rafajova M, Pastorekovà S, Pastorek J & Parkkila S (2004) Expression of carbonic anhydrase IX in mouse tissues. J Histochem Cytochem 52: 1313-1322.

Hilvo M, Tolvanen M, Clark A, Shen B, Shah GN, Waheed A, Halmi P, Hanninen M, Hamalainen JM, Vihinen M, Sly WS & Parkkila S (2005) Characterization of CA XV, a new GPI-anchored form of carbonic anhydrase. Biochem J 392: 83-92.

Hirokawa M, Furukawa O, Guth PH, Engel E & Kaunitz JD (2004a) Low-dose PGE2 mimics the duodenal secretory response to luminal acid in mice. Am J Physiol Gastrointest Liver Physiol 286: G891-G898.

Hirokawa M, Takeuchi T, Chu S, Akiba Y, Wu V, Guth PH, Engel E, Montrose MH & Kaunitz JD (2004b) Cystic fibrosis gene mutation reduces epithelial cell acidification and injury in acid-perfused mouse duodenum. Gastroenterology 127: 1162-1173.

Höckel M, Schlenger K, Aral B, Mitze M, Schaffer U & Vaupel P (1996) Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 56: 4509-4515.

Hofler H & Becker KF (2003) Molecular mechanisms of carcinogenesis in gastric cancer. Recent Results Cancer Res 162: 65-72.

Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R & Harris CC (1996) Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. Nucleic Acids Res 24: 141-146.

Holmes RS (1976) Mammalian carbonic anhydrase isozymes: Evidence for a third locus. J Exp Zool 197: 289-295.

Hoogsteen IJ, Marres HA, Wijffels KI, Rijken PF, Peters JP, van den Hoogen FJ, Oosterwijk E, van der Kogel AJ & Kaanders JH (2005) Colocalization of carbonic anhydrase 9 expression and cell proliferation in human head and neck squamous cell carcinoma. Clin Cancer Res 11: 97-106.

Horii A, Nakatsuru S, Miyoshi Y, Ichii S, Nagase H, Kato Y, Yanagisawa A & Nakamura Y (1992) The APC gene, responsible for familial adenomatous polyposis, is mutated in human gastric cancer. Cancer Res 52: 3231-3233.

Hoschuetzky H, Aberle H & Kemler R (1994) Beta-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. J Cell Biol 127: 1375-1380.

Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR & Wang TC (2004) Gastric cancer originating from bone marrow-derived cells. Science 306: 1568-1571.

Houghton & Wang (2005) Helicobacter pylori and gastric cancer: a new paradigm for inflammation-associated epithelial cancers. Gastroenterology 128: 1567-1578.

Hu PY, Ernst AR, Sly WS, Venta PJ, Skaggs LA & Tashian RE (1994) Carbonic anhydrase II deficiency: single-base deletion in exon 7 is the predominant mutation in Caribbean Hispanic patients. Am J Hum Genet 54: 602-608.

Hu PY, Lim EJ, Ciccolella J, Strisciuglio P & Sly WS (1997) Seven novel mutations in carbonic anhydrase II deficiency syndrome identified by SSCP and direct sequencing analysis. Hum Mutat 9: 383-387.

Hu PY, Roth DE, Skaggs LA, Venta PJ, Tashian RE, Guibaud P & Sly WS (1992) A splice junction mutation in intron 2 of the carbonic anhydrase II gene of osteopetrosis patients from Arabic countries. Hum Mutat 1: 288-292.

Hyafil F, Morello D, Babinet C & Jacob F (1980) A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. Cell 21: 927-934.

Hynes RO (1999) Cell adhesion: old and new questions. Trends Cell Biol 9: M33-M37.

Ichihara N, Sasaki K, Iseri S, Kimura H, Miura K, Nishita T & Asari M (1997) Immunohistolocalization of carbonic anhydrase isozymes (CA-I, CA-II and CA-III) in bovine male reproductive tracts. Okajimas Folia Anat Jpn 74: 193-198.

Ilyas M (2005) Wnt signalling and the mechanistic basis of tumour development. J Pathol 205: 130-144.

Imazeki F, Omata M, Nose H, Ohto M & Isono K (1992) p53 gene mutations in gastric and esophageal cancers. Gastroenterology 103: 892-896.

Isenberg JI, Hogan DL, Koss MA & Selling JA (1986) Human duodenal mucosal bicarbonate secretion. Evidence for basal secretion and stimulation by hydrochloric acid and a synthetic prostaglandin E1 analogue. Gastroenterology 91: 370-378.

Isenberg JI, Selling JA, Hogan DL & Koss MA (1987) Impaired proximal duodenal mucosal bicarbonate secretion in patients with duodenal ulcer. N Engl J Med 316: 374-379.

Ivanov SV, Kuzmin I, Wei MH, Pack S, Geil L, Johnson BE, Stanbridge EJ & Lerman MI (1998) Down regulation of transmembrane carbonic anhydrases in renal cell carcinoma cell lines by wild-type von Hippel–Lindau transgenes. Proc Natl Acad Sci USA 95: 12596–12601.

Ivanov S, Liao SY, Ivanova A, Danilkovitch-Miagkova A, Tarasova N, Weirich G, Merrill MJ, Proescholdt MA, Oldfield EH, Lee J, Zàvada J, Waheed A, Sly W, Lerman MI & Stanbridge EJ (2001) Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am J Pathol 158: 905-919.

Jacob P, Christiani S, Rossmann H, Lamprecht G, Vieillard-Baron D, Muller R, Gregor M & Seidler U (2000) Role of Na(+)HCO(3)(-) cotransporter NBC1, Na(+)/H(+) exchanger NHE1, and carbonic anhydrase in rabbit duodenal bicarbonate secretion. Gastroenterology 119: 406-419.

Jeffery S, Edwards Y & Carter N (1980) Distribution of CA III in fetal and adult human tissue. Biochem Genet 18: 843-849.

Jiao Y, Yan J, Zhao Y, Donahue LR, Beamer WG, Li X, Roe BA, Ledoux MS & Gu W (2005) Carbonic Anhydrase-Related Protein VIII Deficiency Is Associated With a Distinctive Lifelong Gait Disorder in Waddles Mice. Genetics 171: 1239-1246.

Jones NL, Shannon PT, Cutz E, Yeger H & Sherman PM (1997) Increase in proliferation and apoptosis of gastric epithelial cells early in the natural history of Helicobacter pylori infection. Am J Pathol 151: 1695-1703.

Joo YE, Rew JS, Choi SK, Bom HS, Park CS & Kim SJ (2002) Expression of e-cadherin and catenins in early gastric cancer. J Clin Gastroenterol 35: 35-42.

Kadoya Y, Kuwahara H, Shimazaki M, Ogawa Y & Yagi T (1987) Isolation of a novel carbonic anhydrase from human saliva and immunohistochemical demonstration of its related isoenzymes in salivary gland. Osaka City Med J 33: 99-109.

Kaelin Jr WG (2005) Proline Hydroxylation and Gene Expression. Annu Rev Biochem 74: 115-128.

Kaluz S, Kaluzova M, Opavský R, Pastoreková S, Gibadulinova A, Dequiedt F, Kettmann R &, Pastorek J (1999) Transcriptional regulation of the MN/CA 9 gene coding for the tumor-associated carbonic anhydrase IX. Identification and characterization of a proximal silencer element. J Biol Chem 274: 32588-32595.

Kannan KK, Petef M, Fridborg K, Cid-Dresdner H & Lovgren S (1977) Structure and function of carbonic anhydrases. Imidazole binding to human carbonic anhydrase B and the mechanism of action of carbonic anhydrases. FEBS Lett 73: 115-119.

Karhumaa P, Kaunisto K, Parkkila S, Waheed A, Pastoreková S, Pastorek J, Sly WS & Rajaniemi H (2001a) Expression of the transmembrane carbonic anhydrases, CA IX and CA XII, in the human male excurrent ducts. Mol Hum Reprod. 7: 611-616.

Karhumaa P, Leinonen J, Parkkila S, Kaunisto K, Tapanainen J & Rajaniemi H (2001b) The identification of secreted carbonic anhydrase VI as a constitutive glycoprotein of human and rat milk. Proc Natl Acad Sci U S A 98: 11604-11608.

Karhumaa P, Parkkila S, Tureci O, Waheed A, Grubb JH, Shah G, Parkkila A, Kaunisto K, Tapanainen J, Sly WS & Rajaniemi H (2000a) Identification of carbonic anhydrase XII as the membrane isozyme expressed in the normal human endometrial epithelium. Mol Hum Reprod 6: 68-74.

Karhumaa P, Parkkila S, Waheed A, Parkkila A-K, Kaunisto K, Tucker PW, Huang CJ, Sly WS & Rajaniemi H (2000b) Nuclear NonO/p54(nrb) protein is a nonclassical carbonic anhydrase. J Biol Chem 275: 16044-16049.

Kato K (1990) Sequence of a novel carbonic anhydrase-related polypeptide and its exclusive presence in Purkinje cells. FEBS Lett 271: 137-140.

Katoh Y & Katoh M (2005) Hedgehog signaling pathway and gastric cancer. Cancer Biol Ther 4: 1050-1054.

Kaunisto K, Parkkila S, Parkkila A-K, Waheed A, Sly WS & Rajaniemi H (1995) Expression of carbonic anhydrase isoenzymes IV and II in rat epididymal duct. Biol Reprod 52: 1350-1357.

Kaunisto K, Parkkila S, Rajaniemi H, Waheed A, Grubb J & Sly WS (2002) Carbonic anhydrase XIV: luminal expression suggests key role in renal acidification. Kidney Int 61: 2111-2118.

Kaunisto K, Parkkila S, Tammela T, Ronnberg L & Rajaniemi H (1990) Immunohistochemical localization of carbonic anhydrase isoenzymes in the human male reproductive tract. Histochemistry 94: 381-386.

Kelly CD, Carter ND, de Boer P, Jeffery S, Moorman AF & Smith A (1991) Detection of CAIII mRNA in rat skeletal muscle and liver by in situ hybridization. J Histochem Cytochem 39: 1243-1247.

Khalifah RG (1971) The carbon dioxide hydration activity of carbonic anhydrase. I. Stop-flow kinetic studies on the native human isoenzymes B and C. J Biol Chem 246: 2561-2573.

Kim G, Lee TH, Wetzel P, Geers C, Robinson MA, Myers TG, Owens JW, Wehr NB, Eckhaus MW, Gros G, Wynshaw-Boris A & Levine RL (2004) Carbonic anhydrase III is not required in the mouse for normal growth, development, and life span. Mol Cell Biol 24: 9942-9947.

Kim G, Selengut J & Levine RL (2000) Carbonic anhydrase III: The phosphatase activity is extrinsic. Arch Biochem Biophys 377: 334–340.

Kim JH, Takahashi T, Chiba I, Park JG, Birrer MJ, Roh JK, De Lee H, Kim JP, Minna JD & Gazdar AF (1991) Occurrence of p53 gene abnormalities in gastric carcinoma tumors and cell lines. J Natl Cancer Inst 83: 938-943.

Kim SJ, Rabbani ZN, Dewhirst MW, Vujaskovic Z, Vollmer RT, Schreiber EG, Oosterwijk E & Kelley MJ (2005) Expression of HIF-1alpha, CA IX, VEGF, and MMP-9 in surgically resected non-small cell lung cancer. Lung Cancer 49: 325-335.

Kim WY & Kaelin WG (2004) Role of VHL gene mutation in human cancer. J Clin Oncol 22: 4991-5004.

Kivelä AJ, Parkkila S, Saarnio J, Karttunen TJ, Kivelä J, Parkkila A-K, Pastoreková S, Pastorek J, Waheed A, Sly WS & Rajaniemi H. (2000b) Expression of transmembrane carbonic anhydrase isoenzymes IX and XII in normal human pancreas and pancreatic tumours Histochem Cell Biol 114: 197-204.

Kivelä A, Parkkila S, Saarnio J, Karttunen TJ, Kivelä J, Parkkila A-K, Waheed A, Sly WS, Grubb JH, Shah G, Tureci O & Rajaniemi H (2000a) Expression of a novel transmembrane carbonic anhydrase isozyme XII in normal human gut and colorectal tumors. Am J Pathol 156: 577-584.

Kivelä J, Parkkila S, Metteri J, Parkkila A-K, Toivanen A & Rajaniemi H (1997a) Salivary carbonic anhydrase VI concentration and its relation to basic characteristics of saliva in young men. Acta Physiol Scand 161: 221-225.

Kivelä J, Parkkila S, Parkkila A-K & Rajaniemi H (1999) Low concentration of carbonic anhydrase isoenzyme VI in saliva predisposes to caries. Caries Res 33: 178-184.

Kivelä J, Parkkila S, Waheed A, Parkkila A-K, Sly WS & Rajaniemi H (1997b) Secretory carbonic anhydrase isoenzyme (CA VI) in human serum. Clin Chem 43: 2318-2322.

Kivilaakso E & Flemström G (1984) Surface pH gradient in gastroduodenal mucosa. Scand J Gastroenterol Suppl 105: 50-52.

Kivilaakso E & Silen W (1981) The role of mucosal carbonic anhydrase in the protection of gastric mucosa against luminal H+. Scand J Gastroenterol 67: 219-221.

Kleinke T, Wagner S, John H, Hewett-Emmett D, Parkkila S, Forssmann WG & Gros G (2005) A distinct carbonic anhydrase in the mucus of the colon of humans and other mammals. J Exp Biol 208: 487-496.

Knutson TW, Koss MA, Hogan DL, Isenberg JI & Knutson L (1995) Acetazolamide inhibits basal and stimulated HCO3- secretion in the human proximal duodenum. Gastroenterology 108: 102-107.

Kolega J (1986) The cellular basis of epithelial morphogenesis. Dev Biol 2: 103-143.

Konturek SJ, Bilski J, Tasler J & Laskiewicz J (1984) Gastroduodenal alkaline response to acid and taurocholate in conscious dogs. Am J Physiol 247: G149-G154.

Krueger NX & Saito H (1992) A human transmembrane protein-tyrosine-phosphatase, PTP zeta, is expressed in brain and has an N-terminal receptor domain homologous to carbonic anhydrases. Proc Natl Acad Sci U S A 89: 7417-7421.

Kumpulainen T (1984) Immunohistochemical localization of human carbonic anhydrase isozymes. Ann N Y Acad Sci 429: 359-368.

Kumpulainen T & Jalovaara P (1981) Immunohistochemical localization of carbonic anhydrase isoenzymes in the human pancreas. Gastroenterology 80: 796-799.

Kumpulainen T, Rajaniemi H, Myllylä VV & Korhonen LK (1985) A single-step solid phase radioimmunoassay for quantifying human carbonic anhydrase I and II in cerebrospinal fluid. Clin Chim Acta 150: 205-212.

Kuroda S, Fukata M, Nakagawa M, Fujii K, Nakamura T, Ookubo T, Izawa I, Nagase T, Nomura N, Tani H, Shoji I, Matsuura Y, Yonehara S & Kaibuchi K (1998) Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin- mediated cell-cell adhesion. Science 281: 832-835.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lai LW, Erickson RP, Venta PJ, Tashian RE & Lien YH (1998) Promoter activity of carbonic anhydrase II regulatory regions in cultured renal proximal tubular cells. Life Sci 63: 121-126.

Laitala-Leinonen T, Lowik C, Papapoulos S & Väänänen HK (1999) Inhibition of intravacuolar acidification by antisense RNA decreases osteoclast differentiation and bone resorption in vitro. J Cell Sci 112: 3657-3666.

Lakkis MM, Bergenhem NC & Tashian RE (1996) Expression of mouse carbonic anhydrase VII in E. Coli and demostration of its CO2 hydrase activity. Biochem Biophys Res Commun 226: 268-272.

Lakkis MM, O'Shea KS & Tashian RE (1997) Differential expression of the carbonic anhydrase genes for CA VII (Car7) and CA-RP VIII (Car8) in mouse brain. J Histochem Cytochem 45: 657-662.

Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou F, Li H, Wei MH, Chen F, Glenn G, Choyke P, Walther MM, Weng Y, Duan D-SR, Dean M, Glavaè D, Richards FM, Crossey PA, Ferguson-Smith MA, Le Paslier D, Chumakov I, Cohen D, Chinault AC, Maher ER, Linehan WM, Zbar B & Lerman MI (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260: 1317-13120.

Laurèn P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Path Microbiol Scand 64: 31-49.

Lauwers GY (2003) Defining the pathologic diagnosis of metaplasia, atrophy, dysplasia, and gastric adenocarcinoma. J Clin Gastroenterol 36: S37-S43.

Law JS, Nelson N, Watanabe K & Henkin RI (1987) Human salivary gustin is a potent activator of calmodulin-dependent brain phosphodiesterase. Proc Natl Acad Sci U S A 84: 1674-1678.

Lee JH, Abraham SC, Kim HS, Nam JH, Choi C, Lee MC, Park CS, Juhng SW, Rashid A, Hamilton SR & Wu TT (2002) Inverse relationship between APC gene mutation in gastric adenomas and development of adenocarcinoma. Am J Pathol 161: 611-618.

Lee SA, Kang D, Shim KN, Choe JW, Hong WS & Choi H. (2003) Effect of diet and Helicobacter pylori infection to the risk of early gastric cancer J Epidemiol 13: 162-168.

Lehenkari P, Hentunen TA, Laitala-Leinonen T, Tuukkanen J & Väänänen HK (1998) Carbonic anhydrase II plays a major role in osteoclast differentiation and bone resorption by effecting the steady state intracellular pH and Ca2+. Exp Cell Res 242: 128-137.

Lehtonen JM, Parkkila S, Vullo D, Casini A, Scozzafava A & Supuran CT (2004b) Carbonic anhydrase inhibitors. Inhibition of cytosolic isozyme XIII with aromatic and heterocyclic sulfonamides: a novel target for the drug design. Bioorg Med Chem Lett 14: 3757-3762.

Lehtonen J, Shen B, Vihinen M, Casini A, Scozzafava A, Supuran CT, Parkkila A-K, Saarnio J, Kivelä AJ, Waheed A, Sly WS & Parkkila S (2004) Characterization of CA XIII, a novel member of the carbonic anhydrase isozyme family. J Biol Chem 279: 2719-2727.

Leinonen J, Kivelä J, Parkkila S, Parkkila A-K & Rajaniemi H (1999) Salivary carbonic anhydrase isoenzyme VI is located in the human enamel pellicle. Caries Res 33: 185-190.

Leinonen J, Parkkila S, Kaunisto K, Koivunen P & Rajaniemi H (2001) Secretion of carbonic anhydrase isoenzyme VI (CA VI) from human and rat lingual serous von Ebner's glands. J Histochem Cytochem 49: 657-662.

Levy JB, Canoll PD, Silvennoinen O, Barnea G, Morse B, Honegger AM, Huang JT, Cannizzaro LA, Park SH, Druck T, Huebner K, Sap J, Ehrlich M, Masacchio JM & Schlessinger J (1993) The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. J Biol Chem 268: 10573-10581.

Lewis SE, Erickson RP, Barnett LB, Venta PJ & Tashian RE (1988) N-ethyl-N-nitrosourea-induced null mutation at the mouse Car-2 locus: an animal model for human carbonic anhydrase II deficiency syndrome. Proc Natl Acad Sci U S A 85: 1962-1966.

Liao SY, Aurelio ON, Jan K, Zàvada J & Stanbridge EJ (1997) Identification of the MN/CA9 protein as a reliable diagnostic biomarker of clear cell carcinoma of the kidney. Cancer Res 57: 2827-2831.

Liao SY, Brewer C, Zàvada J, Pastorek J, Pastoreková S Manetta A, Berman ML, DiSaia PJ & Stanbridge EJ (1994) Identification of the MN antigen as a diagnostic biomarker of cervical intraepithelial squamous and glandular neoplasia and cervical carcinomas Am J Pathol 145: 598-609.

Liao SY & Stanbridge EJ (1996) Expression of the MN antigen in cervical papanicolaou smears is an early diagnostic biomarker of cervical dysplasia. Cancer Epidemiol Biomarkers Prev 5: 549-557.

Lieskovska J, Opavský R, Zacikova L, Glasova M, Pastorek J & Pastoreková S (1999) Study of in vitro conditions modulating expression of MN/CA IX protein in human cell lines derived from cervical carcinoma. Neoplasma 46: 17-24.

Lindahl M, Vidgren J, Eriksson E, Habash J, Harrop S, Helliwell J, Liljas A, Lindeskog M & Walker N (1991). Crystallographic studies of carbonic anhydrase inhibition. In: Botre` F, Gros G, StoreyBT (eds) Carbonic anhydrase. VCH, Weinheim, 111–118.

Lindskog S, Engberg P, Forsman C, Ibrahim SA, Jonsson B-H, Simonsson I & Tibell L (1984) Kinetics and mechanism of carbonic anhydrase isoenzymes. Ann NY Acad Sci 429: 61-75.

Ling B, Bergenhem NCH, Dogson SJ, Forster RE & Tashian RE (1994) Determination of mRNA levels for six carbonic anhydrase isozymes in rat lung. Isozyme Bull 28: 32.

Lönnerholm G, Selking O & Wistrand PJ (1985) Amount and distribution of carbonic anhydrases CA I and CA II in the gastrointestinal tract. Gastroenterology 88: 1151-1161.

Lovejoy DA, Hewett-Emmett D, Porter CA, Cepoi D, Sheffield A, Vale WW & Tashian RE (1998) Evolutionarily conserved, "acatalytic" carbonic anhydrase-related protein XI contains a sequence motif present in the neuropeptide sauvagine: the human CA RP XI gene (CA11) is embedded between the secretor gene cluster and the DBP gene at 19q13.3. Genomics 54: 484-493.

Lowe N, Brady HJ, Barlow JH, Sowden JC, Edwards M & Butterworth PH (1990) Structure and methylation patterns of the gene encoding human carbonic anhydrase I. Gene 93: 277-283.

Lu SH, Takeuchi T, Fujita J, Ishida T, Akisawa Y, Nishimori I, Kohsaki T, Onishi S, Sonobe H & Ohtsuki Y (2004) Effect of carbonic anhydrase-related protein VIII expression on lung adenocarcinoma cell growth. Lung Cancer 44: 273-280.

Lynch CJ, Hazen SA, Horetsky RL, Carter ND & Dodgson SJ (1993) Differentiation-dependent expression of carbonic anhydrase II and III in 3T3 adipocytes. Am J Physiol 265: C234-C243.

Maesawa C, Tamura G, Suzuki Y, Ogasawara S, Sakata K, Kashiwaba M & Satodate R (1995) The sequential accumulation of genetic alterations characteristic of the colorectal adenoma-carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. J Pathol 176: 249-258.

Maseide K, Kandel RA, Bell RS, Catton CN, O'Sullivan B Wunder JS, Pintilie M, Hedley D & Hill RP (2004) Carbonic anhydrase IX as a marker for poor prognosis in soft tissue sarcoma. Clin Cancer Res 10: 4464-4471.

Maurel P, Rauch U, Flad M, Margolis RK & Margolis RU (1994) Phosphacan, a chondroitin sulfate proteoglycan of brain that interacts with neurons and neural cell-adhesion molecules, is an extracellular variant of a receptor-type protein tyrosine phosphatase. Proc Natl Acad Sci U S A 91: 2512-2516.

McKiernan JM, Buttyan R, Bander NH, Stifelman MD, Katz AE, Chen MW, Olsson CA & Sawczuk IS (1997) Expression of the tumor-associated gene MN: a potential biomarker for human renal cell carcinoma. Cancer Res 57: 2362-2365.

McKiernan JM, Buttyan R, Bander NH, de la Taille A, Stifelman MD, Emanuel ER, Bagiella E, Rubin MA, Katz AE, Olsson CA & Sawczuk IS (1999) The detection of renal carcinoma cells in the peripheral blood with an enhanced reverse transcriptase-polymerase chain reaction assay for MN/CA9. Cancer 86: 492-497.

McNeill H, Ozawa M, Kemler R & Nelson WJ (1990) Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. Cell 62: 309-316.

Meldrum NU & Roughton FJW (1932) Some properties of carbonic anhydrase, the carbon dioxide enzyme present in blood. J Physiol (London) 75: 15-16.

Meldrum, NU & Roughton FJW (1933) Carbonic anhydrase. Its preparation and properties. J. Physiol (London) 80: 113-142.

Meyers & Orlando (1992) In vivo bicarbonate secretion by human esophagus. Gastroenterology 103: 1174-1178.

Miller JR & Moon RT (1996) Signal transduction through beta-catenin and specification of cell fate during embryogenesis. Genes Dev 10: 2527-2539.

Miyaji E, Nishimori I, Taniuchi K, Takeuchi T, Ohtsuki Y & Onishi S (2003) Overexpression of carbonic anhydrase-related protein VIII in human colorectal cancer. J Pathol 201: 37-45.

Mohammadi M, Czinn S, Redline R & Nedrud J (1996) Helicobacter-specific cell-mediated immune responses display a predominant Th1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice. J Immunol 156: 4729-4738.

Montgomery JC, Venta PJ, Eddy RL, Fukushima YS, Shows TB & Tashian RE (1991) Caharacterization of human gene for newly discovered carbonic anhydrase, CA VII, and its localization to chromosome 16. Genomics 11: 835-848.

Mori K, Ogawa Y, Ebihara K, Tamura N, Tashiro K, Kuwahara T, Mukoyama M, Sugawara A, Ozaki S, Tanaka I & Nakao K (1999) Isolation and Characterization of CA XIV, a Novel Membranebound Carbonic Anhydrase from Mouse Kidney. J Biol Chem 274: 15701-15705.

Moss SF, Calam J, Agarwal B, Wang S & Holt PR (1996) Induction of gastric epithelial apoptosis by Helicobacter pylori. Gut 38: 498-501.

Mühlhauser J, Crescimanno C, Rajaniemi H, Parkkila S, Milovanov AP, Castellucci M & Kaufmann P (1994) Immunohistochemistry of carbonic anhydrase in human placenta and fetal membranes. Histochemistry 101: 91-98.

Munemitsu S, Albert I, Souza B, Rubinfeld B & Polakis P (1995) Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci U S A 92: 3046-3050.

Murakami H, Marelich GP, Grubb JH, Kyle JW & Sly WS (1987) Cloning, expression, and sequence homologies of cDNA for human carbonic anhydrase II. Genomics 1: 159-166.

Murakami H & Sly WS (1987) Purification and characterization of human salivary carbonic anhydrase. J Biol Chem 262: 1382-1388.

Nagao Y, Platero JS, Waheed A & Sly WS (1993) Human mitochondrial carbonic anhydrase: cDNA cloning, expression, subcellular localization, and mapping to chromosome 16. Proc Natl Acad Sci U S A 90: 7623-7627.

Nagelhus EA, Mathiisen TM, Bateman AC, Haug FM, Ottersen OP, Grubb JH, Waheed A & Sly WS (2005) Carbonic anhydrase XIV is enriched in specific membrane domains of retinal pigment epithelium, Müller cells, and astrocytes. Proc Natl Acad Sci U S A 102: 8030-8035.

Nakai H, Byers MG, Venta PJ, Tashian RE & Shows TB (1987) The gene for human carbonic anhydrase II (CA2) is located at chromosome 8q22. Cytogenet Cell Genet 44: 234-235.

Niemelä O (1993) Acetaldehyde adducts of proteins: Diagnostic and pathogenic implications in diseases caused by excessive alcohol consumption. Scand J Clin Lab Invest 53: 45-54.

Niemelä O, Juvonen T & Parkkila S (1991) Immunohistochemical demonstration of acetaldehydemodified epitopes in human liver after alcohol consumption. J Clin Invest 87: 1367-1374.

Niemelä O, Parkkila S, Ylä-Herttuala S, Halsted C, Witztum JL, Lanca A & Israel Y (1994) Covalent protein adducts in the liver as a result of ethanol metabolism and lipid peroxidation. Lab Invest 70: 537-546.

Nishimori I, Miyaji E, Morimoto K, Nagao K, Kamada M & Onishi S (2005a) Serum antibodies to carbonic anhydrase IV in patients with autoimmune pancreatitis. Gut 54: 274-281.

Nishimori I, Vullo D, Innocenti A, Scozzafava A, Mastrolorenzo A & Supuran CT (2005b) Carbonic anhydrase inhibitors: inhibition of the transmembrane isozyme XIV with sulfonamides. Biorg Med Chem Lett 15: 3828-3833.

Nishimori I, Vullo D, Innocenti A, Scozzafava A, Mastrolorenzo A & Supuran CT (2005c) The mitochondrial isozyme VB as a new target for sulfonamide and sulfamate inhibitors. J Med Chem 48: 7860-7866.

Nishizaki Y, Guth PH, Quintero E, Bover J, Del Rivero M & Kaunitz JD (1994) Prostaglandin E2 enhances gastric defense mechanisms against acid injury in uremic rats. Gastroenterology 107: 1382-1389.

O'Brien P, Rosen S, Trencis-Buck L & Silen W (1977) Distribution of carbonic anhydrase within the gastric mucosa. Gastroenterology 72: 870-874.

Ochiai A, Akimoto S, Kanai Y, Shibata T, Oyama T & Hirohashi S (1994) c-erbB-2 gene product associates with catenins in human cancer cells. Biochem Biophys Res Commun 205: 73-78.

Oda T, Kanai Y, Oyama T, Yoshiura K, Shimoyama Y, Birchmeier W, Sugimura T & Hirohashi S (1994) E-cadherin gene mutations in human gastric carcinoma cell lines. Proc Natl Acad Sci U S A 91: 1858-1862.

Ogawa Y, Chang CK, Kuwahara H, Hong SS, Toyosawa S & Yagi T (1992) Immunoelectron microscopy of carbonic anhydrase isozyme VI in rat submandibular gland: comparison with isozymes I and II. J Histochem Cytochem 40: 807-817.

Ogawa Y, Hong SS, Toyosawa S, Kuwahara H, Shimazaki M & Yagi T (1993) Immunoelectron microscopy of carbonic anhydrase isozyme VI in human submandibular gland: comparison with isozymes I and II. J Histochem Cytochem 41: 343-351.

Ogawa Y, Toyosawa S, Inagaki T, Hong SS & Ijuhin N (1995) Carbonic anhydrase isozyme VI in rat lacrimal gland. Histochem Cell Biol 103: 387-394.

Okamoto N, Fujikawa-Adachi K, Nishimori I, Taniuchi K & Onishi S (2001) cDNA sequence of human carbonic anhydrase-related protein, CA-RP X: mRNA expressions of CA-RP X and XI in human brain. Biochim Biophys Acta 1518: 311-316.

Okuyama T, Batanian JR & Sly WS (1993) Genomic organization and localization of gene for human carbonic anhydrase IV to chromosome 17q. Genomics 16: 678-684.

Okuyama T, Sato S, Zhu XL, Waheed A & Sly WS (1992) Human carbonic anhydrase IV: cDNAcloning, sequence comparison, and expression in COS cell membranes. Proc Natl Acad Sci USA 89: 1315-1319.

Okuyama T, Waheed A, Kusumoto W, Zhu XL & Sly WS (1995) Carbonic anhydrase IV: role of removal of C-terminal domain in glycosylphosphatidylinositol anchoring and realization of enzyme activity. Arch Biochem Biophys 320: 315-322.

Oosterwijk E, Ruiter DJ, Hoedemaeker PJ, Pauwels EK, Jonas U, Zwartendijk J & Warnaar SO (1986) Monoclonal antibody G 250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney. Int J Cancer 38: 489-494.

Opavský R, Pastoreková S, Zelnik V, Gibadulinova A, Stanbridge EJ, Zàvada J, Kettmann R & Pastorek J (1996) Human MN/CA9 gene, a novel member of the carbonic anhydrase family: structure and exon to protein domain relationships. Genomics 33: 480-487.

Ortova Gut MO, Parkkila S, Vernerova Z, Rohde E, Zàvada J, Hocker M, Pastorek J, Karttunen T, Gibadulinova A, Zàvadovà Z, Knobeloch KP, Wiedenmann B, Svoboda J, Horak I & Pastorekovà S (2002) Gastric hyperplasia in mice with targeted disruption of the carbonic anhydrase gene Car9. Gastroenterology 123: 1889-1903.

Özensoy O, Nishimori I, Vullo D, Puccetti L, Scozzafava A & Supuran CT (2005). Carbonic anhydrase inhibitors: inhibition of the human transmembrane isozyme XIV with a library of aromatic/heterocyclic sulfonamides. Bioorg Med Chem 13: 6089-6093.

Park WS, Oh RR, Park JY, Lee SH, Shin MS, Kim YS, Kim SY, Lee HK, Kim PJ, Oh ST, Yoo NJ & Lee JY (1999) Frequent somatic mutations of the beta-catenin gene in intestinal-type gastric cancer. Cancer Res 59: 4257-4260.

Parkkila A-K, Herva R, Parkkila S & Rajaniemi H (1995) Immunohistochemical demonstration of human carbonic anhydrase isoenzyme II in brain tumours. Histochem J 27: 974-982.

Parkkila A-K, Parkkila S, Juvonen T & Rajaniemi H (1993) Carbonic anhydrase isoenzymes II and I are present in the zona glomerulosa cells of the human adrenal gland. Histochemistry 99: 37-41.

Parkkila A-K, Parkkila S & Rajaniemi H (1996) Carbonic anhydrase isoenzyme II is located in corticotrophs of the human pituitary gland. J Histochem Cytochem 44: 245-250.

Parkkila A-K, Parkkila S, Reunanen M, Niemelä O, Tuisku S, Rautakorpi I & Rajaniemi H (1997) Carbonic anhydrase II in the cerebrospinal fluid: its value as a disease marker. Eur J Clin Invest 27: 392-397.

Parkkila A-K, Parkkila S, Serlo W, Reunanen M, Vierjoki T & Rajaniemi H (1994) A competitive dual-label time-resolved immunofluorometric assay for simultaneous detection of carbonic anhydrase I and II in cerebrospinal fluid. Clin Chim Acta 230: 81-89.

Parkkila A-K, Scarim AL, Parkkila S, Waheed A, Corbett JA & Sly WS (1998) Expression of carbonic anhydrase V in pancreatic B-cells suggests role for mitochondrial carbonic anhydrase in insulin secretion. J Biol Chem 273: 24620-24623.

Parkkila S (2000) Roles of carbonic anhydrases in the alimentary tract. In: Chegwidden WR, Carter ND & Edwards YH (eds) The Carbonic Anhydrases New Horizons. Birkhäuser Verlag, Basel, 461-474.

Parkkila S, Halsted CH, Villanueva JA, Väänänen HK & Niemelä O (1999) Expression of testosterone-dependent enzyme, carbonic anhydrase III, and oxidative stress in experimental alcoholic liver disease. Dig Dis Sci 44: 2205-2213.

Parkkila S, Kaunisto K, Kellokumpu S & Rajaniemi H (1991a) A high activity carbonic anhydrase isoenzyme (CA II) is present in mammalian spermatozoa. Histochemistry 95: 477-482.

Parkkila S, Kaunisto K & Rajaniemi H (1991b) Location of the carbonic anhydrase isoenzymes VI and II in human salivary glands by immunohistochemistry, In: Botre F, Gros G & Storey BT (eds) Carbonic Anhydrase. From Biochemistry and Genetics to Physiology and Clinical Medicine. VCH, Weinheim, 254-257.

Parkkila S, Kaunisto K, Rajaniemi L, Kumpulainen T, Jokinen K & Rajaniemi H (1990) Immunohistochemical localization of carbonic anhydrase isoenzymes VI, II, and I in human parotid and submandibular glands. J Histochem Cytochem 38: 941-947.

Parkkila S, Kivelä AJ, Kaunisto K, Parkkila A-K, Hakkola J, Rajaniemi H, Waheed A & Sly WS (2002) The plasma membrane carbonic anhydrase in murine hepatocytes identified as isozyme XIV. BMC Gastroenterol 2: 13.

Parkkila S & Parkkila A-K (1996) Gastrointestinal carbonic anhydrases. Duodecim 112: 2383-2388.

Parkkila S, Parkkila A-K, Juvonen T & Rajaniemi H (1994) Distribution of carbonic anhydrase isoenzymes I, II and VI in the human alimentary tract. Gut 35: 646-650.

Parkkila S, Parkkila A-K, Juvonen T, Waheed A, Sly WS, Saarnio J, Kaunisto K, Kellokumpu S & Rajaniemi H (1996) Membrane-bound carbonic anhydrase IV is expressed in the luminal plasma membrane of the human gallbladder epithelium. Hepatology 24: 1104-1108.

Parkkila S, Parkkila A-K, Kaunisto K, Waheed A, Sly WS & Rajaniemi H (1993) Location of a membrane-bound carbonic anhydrase isoenzyme (CA IV) in the human male reproductive tract. J Histochem Cytochem 41: 751-757.

Parkkila S, Parkkila A-K, Lehtola J, Reinilä A, Södervik H-J, Rannisto M & Rajaniemi H (1997) Salivary carbonic anhydrase protects gastroesophageal mucosa from acid injury, Digest Dis Sci 42: 1013-1019.

Parkkila S, Parkkila A-K & Rajaniemi H (1995) Circadian periodicity in salivary carbonic anhydrase VI concentration. Acta Physiol Scand 154: 205-211.

Parkkila S, Parkkila A-K, Rajaniemi H, Shah GN, Grubb JH, Waheed A & Sly WS (2001) Expression of membrane-associated carbonic anhydrase XIV on neurons and axons in mouse and human brain. Proc Natl Acad Sci U S A 98: 1918-1923.

Parkkila S, Parkkila A-K, Saarnio J, Kivelä J, Karttunen TJ, Kaunisto K, Waheed A, Sly WS, Türeci O, Virtanen I & Rajaniemi H (2000a) Expression of the membrane-associated carbonic anhydrase isozyme XII in the human kidney and renal tumors. J Histochem Cytochem 48: 1601-1608.

Parkkila S, Rajaniemi H, Parkkila A-K, Kivelä J, Waheed A, Pastoreková S, Pastorek J & Sly WS (2000b) Carbonic anhydrase inhibitor suppresses invasion of renal cancer cells in vitro. Proc Natl Acad Sci U S A 97: 2220-2224.

Pastorek J, Pastoreková S, Callebaut I, Mornon JP, Zelnik V, Opavský R, Zat'ovicova M, Liao S, Portetelle D, Stanbridge EJ, Závada J, Burny A & Kettmann R (1994) Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment Oncogene 9: 2877-2888.

Pastoreková S, Parkkila S, Parkkila A-K, Opavský R, Zelnik V, Saarnio J & Pastorek J (1997) Carbonic anhydrase IX, MN/CA IX: analysis of stomach complementary DNA sequence and expression in human and rat alimentary tracts. Gastroenterology 112: 398-408.

Pastoreková S, Parkkila S, Pastorek J & Supuran CT (2004) Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib Med Chem 19: 199-229.

Pastoreková S & Závada J (2004) Carbonic anhydrase IX (CA IX) as a potential target for cancer therapy. Cancer Therapy 2: 245-262.

Pastoreková S, Zàvadovà Z, Kostal M, Babusikova O & Zàvada J (1992) A novel quasi-viral agent, MaTu, is a two-component system Virology 187: 620-626.

Quigley EM & Turnberg LA (1987) pH of the microclimate lining human gastric and duodenal mucosa in vivo. Studies in control subjects and in duodenal ulcer patients. Gastroenterology 92: 1876-1884.

Räisänen SR, Lehenkari P, Tasanen M, Rahkila P, Härkönen PL & Väänänen HK (1999) Carbonic anhydrase III protects cells from hydrogen peroxide-induced apoptosis. FASEB J 13: 513-522.

Ranzani GN, Luinetti O, Padovan LS, Calistri D, Renault B, Burrel M, Amadori D, Fiocca R & Solcia E (1995) p53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type. Cancer Epidemiol Biomarkers Prev 4: 223-231.

Rebello G, Ramesar R, Vorster A, Roberts L, Ehrenreich L, Oppon E, Gama D, Bardien S, Greenberg J, Bonapace G, Waheed A, Shah GN & Sly WS (2004) Apoptosis-inducing signal sequence mutation in carbonic anhydrase IV identified in patients with the RP17 form of retinitis pigmentosa. Proc Natl Acad Sci U S A 101: 6617-6622.

Register AM, Koester MK & Noltmann EA (1978) Discovery of carbonic anhydrase in rabbit skeletal muscle and evidence for its identity with "basic muscle protein". J Biol Chem 253: 4143-4152.

Riethmacher D, Brinkmann V & Birchmeier C (1995) A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. Proc Natl Acad Sci USA 92: 855-859.

Ross JS & McKenna BJ (2001) The HER-2/neu oncogene in tumors of the gastrointestinal tract. Cancer Invest 19: 554-568.

Roth DE, Venta PJ, Tashian RE & Sly WS (1992) Molecular basis of human carbonic anhydrase II deficiency. Proc Natl Acad Sci U S A 89: 1804-1808.

Saarnio J, Parkkila S, Parkkila A-K, Haukipuro K, Pastoreková S, Pastorek J, Kairaluoma MI & Karttunen TJ (1998a) Immunohistochemical study of colorectal tumors for expression of a novel transmembrane carbonic anhydrase, MN/CA IX, with potential value as a marker of cell proliferation. Am J Pathol 153: 279-285.

Saarnio J, Parkkila S, Parkkila A-K, Pastoreková S, Haukipuro K, Pastorek J, Juvonen T & Karttunen TJ (2001) Transmembrane carbonic anhydrase, MN/CA IX, is a potential biomarker for biliary tumours J Hepatol 35: 643-649.

Saarnio J, Parkkila S, Parkkila A-K, Waheed A, Casey MC, Zhou ZY, Pastoreková S, Pastorek J, Karttunen T, Haukipuro K, Kairaluoma MI & Sly WS (1998b) Immunohistochemistry of carbonic anhydrase isozyme IX (MN/CA IX) in human gut reveals polarized expression in the epithelial cells with the highest proliferative capacity. J Histochem Cytochem 46: 497-504.

Sababi M, Nilsson E & Holm L (1995) Mucus and alkali secretion in the rat duodenum: effects of indomethacin, N omega-nitro-L-arginine, and luminal acid. Gastroenterology 109: 1526-1534.

Saldanha G (2001) The Hedgehog signalling pathway and cancer. J Pathol 193: 427-432.

Sanyal G & Maren TH (1981) Thermodynamics of carbonic anhydrase catalysis. A comparison between human isoenzymes B and C. J Biol Chem 256: 608-612.

Sasaki K, Igarashi S-I, Amasaki T, Amasaki H, Nishita T, Kano Y & Asari M (1993) Comparative immunohistolocalization of carbonic anhydrase isozymes I, II and III in the equine and bovine digestive tract. Histochem J 25: 304-311.

Sato A, Spicer SS & Tashian RE (1980) Ultrastructural localization of carbonic anhydrase in gastric parietal cells with the immunoglobulin-enzyme bridge method. Histochem J 12: 651-659.

Scozzafava A, Mastrolorenzo A & Supuran CT (2004) Expert Opin Ther Pat 14, 667-702.

Sefton M, Johnson MH & Clayton L (1992) Synthesis and phosphorylation of uvomorulin during mouse early development. Development 115: 313-318.

Sellers LA, Allen A, Morris ER & Ross-Murphy SB (1988) Mucus glycoprotein gels. Role of glycoprotein polymeric structure and carbohydrate side-chains in gel-formation. Carbohydr Res 178: 93-110.

Semenza GL (2000) Oxygen-regulated transcription factors and their role in pulmonary disease. Respir Res 1: 159-162.

Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 10: 721-732.

Sender S, Gros G, Waheed A, Hageman GS & Sly WS (1994) Immunohistochemical localization of carbonic anhydrase IV in capillaries of rat and human skeletal muscle. J Histochem Cytochem 42: 1229-1236.

Shah GN, Bonapace G, Hu PY, Strisciuglio P & Sly WS (2004) Carbonic anhydrase II deficiency syndrome (osteopetrosis with renal tubular acidosis and brain calcification): novel mutations in CA2 identified by direct sequencing expand the opportunity for genotype-phenotype correlation. Hum Mutat 24: 272.

Shah GN, Hewett-Emmett D, Grubb JH, Migas MC, Fleming RE, Waheed A & Sly WS (2000) Mitochondrial carbonic anhydrase CA VB: differences in tissue distribution and pattern of evolution from those of CA VA suggest distinct physiological roles. Proc Natl Acad Sci U S A 97: 1677-1682.

Shitara K, Yamada H, Watanabe K, Shimonaka M & Yamaguchi Y (1994) Brain-specific receptor-type protein-tyrosine phosphatase RPTP beta is a chondroitin sulfate proteoglycan in vivo. J Biol Chem 269: 20189-20193.

Skaggs LA, Bergenhem NC, Venta PJ & Tashian RE (1993) The deduced amino acid sequence of human carbonic anhydrase-related protein (CARP) is 98% identical to the mouse homologue. Gene 126: 291-292.

Sly WS, Hewett-Emmett D, Whyte MP, Yu Y-S L & Tashian RE (1983) Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc Natl Acad Sci USA 80: 2752-2756.

Sly WS & Hu PY (1995) Human carbonic anhydrases and carbonic anhydrase deficiencies. Annu Rev Biochem 64: 375-401.

Smythies LE, Waites KB, Lindsey JR, Harris PR, Ghiara P & Smith PD (2000) Helicobacter pylori-induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFN-gamma, gene-deficient mice J Immunol. 165: 1022-1029.

Soda H, Yukizane S, Yoshida I, Aramaki S & Kato H (1995) Carbonic anhydrase II deficiency in a Japanese patient produced by a nonsense mutation (TAT-->TAG) at Tyr-40 in exon 2, (Y40X). Hum Mutat 5: 348- 350.

Soda H, Yukizane S, Yoshida I, Koga Y, Aramaki S & Kato H (1996) A point mutation in exon 3 (His 107-->Tyr) in two unrelated Japanese patients with carbonic anhydrase II deficiency with central nervous system involvement. Hum Genet 97: 435-437.

Sok J, Wang XZ, Batchvarova N, Kuroda M, Harding H & Ron D (1999) CHOP-Dependent stress-inducible expression of a novel form of carbonic anhydrase VI. Mol Cell Biol 19: 495-504.

Spicer SS, Ge ZH, Tashian RE, Hazen-Martin DJ & Schulte BA (1990) Comparative distribution of carbonic anhydrase isozymes III and II in rodent tissues. Am J Anat 187: 55-64.

Spicer SS, Lewis SE, Tashian RE & Schulte BA (1989) Mice carrying a CAR-2 null allele lack carbonic anhydrase II immunohistochemically and show vascular calcification. Am J Pathol 134: 947-954.

Spicer SS, Sens MA & Tashian RE (1982) Immunocytochemical demonstration of carbonic anhydrase in human epithelial cells. J Histochem Cytochem 30: 864-873.

Stadtman ER & Berlett BS (1991) Fenton chemistry. Amino acid oxidation. J Biol Chem 266: 17201-17211.

Stadtman ER, Berlett BS & Chock PB (1990) Manganese-dependent disproportionation of hydrogen peroxide in bicarbonate buffer. Proc Natl Acad Sci U S A 87: 384-388.

Sterling D, Alvarez BV & Casey JR (2002) The extracellular component of a transport metabolon. Extracellular loop 4 of the human AE1 Cl-/HCO3- exchanger binds carbonic anhydrase IV. J Biol Chem 277: 25239-25246.

Stewart M, Talks K, Leek R, Turley H, Pezzella F, Harris A & Gatter K (2002) Expression of angiogenic factors and hypoxia inducible factors HIF 1, HIF 2 and CA IX in non-Hodgkin's lymphoma. Histopathology 40: 253-260.

Stock C, Gassner B, Hauck CR, Arnold H, Mally S, Eble JA, Dieterich P & Schwab A (2005) Migration of human melanoma cells depends on extracellular pH and Na+/H+ exchange. J Physiol 567: 225-238.

Strisciuglio P, Hu PY, Lim EJ, Ciccolella J & Sly WS (1998) Clinical and molecular heterogeneity in carbonic anhydrase II deficiency and prenatal diagnosis in an Italian family. J Pediatr 132: 717-720.

Strisciuglio P, Sartorio R, Pecoraro C, Lotito F & Sly WS (1990) Variable clinical presentation of carbonic anhydrase deficiency: evidence for heterogeneity? Eur J Pediatr 149: 337-340.

Supuran CT, Briganti F, Tilli S, Chegwidden WR & Scozzafava A (2001) Carbonic anhydrase inhibitors: Sulfonamides as antitumor agents? Bioorg Med Chem 9: 703-714.

Supuran CT & Scozzafava A (2000) Carbonic anhydrase inhibitors and their therapeutic potential. Exp Opin TherPatents 10: 575–600.

Supuran CT & Scozzafava A (2001) Carbonic anhydrase inhibitors. Curr Med Chem 1: 61-97

Supuran CT & Scozzafava A (2002) Applications of carbonic anhydrase inhibitors and activators in therapy. Exp Opin Ther Patents 12: 217–242.

Supuran CT, Scozzafava A & Casini A (2003) Carbonic Anhydrase Inhibitors. Medicinal Research Reviews 23: 146-189.

Sutherland GR, Baker E, Fernandez KE, Callen DF, Aldred P, Coghlan JP, Wright RD & Fernley RT (1989) The gene for human carbonic anhydrase VI (CA6) is on the tip of the short arm of chromosome 1. Cytogenet Cell Genet 50: 149-150.

Svastova E, Hulikova A, Rafajova M, Zat'ovicova M, Gibadulinova A, Casini A, Cecchi A, Scozzafava A, Supuran CT, Pastorek J & Pastoreková S (2004) Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. FEBS Lett 577: 439-445.

Svastova E, Zilka N, Zat'ovicova M, Gibadulinova A, Ciampor F, Pastorek J & Pastoreková S (2003) Carbonic anhydrase IX reduces E-cadherin-mediated adhesion of MDCK cells via interaction with beta-catenin. Exp Cell Res 290: 332-345.

Tahara E (1995) Molecular biology of gastric cancer. World J Surg 19: 484-490.

Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251: 1451-1455.

Takeuchi K, Ukawa H, Kato S, Furukawa O, Araki H, Sugimoto Y, Ichikawa A, Ushikubi F & Narumiya S (1999) Impaired duodenal bicarbonate secretion and mucosal integrity in mice lacking prostaglandin E-receptor subtype EP(3). Gastroenterology 117: 1128-1135.

Takeuchi K, Yagi K, Kato S & Ukawa H (1997) Roles of prostaglandin E-receptor subtypes in gastric and duodenal bicarbonate secretion in rats. Gastroenterology 113: 1553-1559.

Tamura G (1996) Molecular pathogenesis of adenoma and differentiated adenocarcinoma of the stomach. Pathol Int 46: 834-841.

Tamura G, Sakata K, Maesawa C, Suzuki Y, Terashima M, Satoh K, Sekiyama S, Suzuki A, Eda Y & Satodate R (1995) Microsatellite alterations in adenoma and differentiated adenocarcinoma of the stomach. Cancer Res 55: 1933-1936.

Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, Kong D, Smolinski KN, Wilson KT, James SP, Silverberg SG, Nishizuka S, Terashima M, Motoyama T & Meltzer SJ (2000) E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. J Natl Cancer Inst 92: 569-573.

Tanaka M, Kitajima Y, Edakuni G, Sato S & Miyazaki K (2002) Abnormal expression of E-cadherin and beta-catenin may be a molecular marker of submucosal invasion and lymph node metastasis in early gastric cancer. Br J Surg 89: 236-244.

Tanaka M, Maeda N, Noda M & Marunouchi T (2003) A chondroitin sulfate proteoglycan PTPzeta /RPTPbeta regulates the morphogenesis of Purkinje cell dendrites in the developing cerebellum. J Neurosci 23: 2804-2814.

Taniuchi K, Nishimori I, Takeuchi T, Fujikawa-Adachi K, Ohtsuki Y & Onishi S (2002a) Developmental expression of carbonic anhydrase-related proteins VIII, X, and XI in the human brain. Neuroscience 112: 93-99.

Taniuchi K, Nishimori I, Takeuchi T, Ohtsuki Y & Onishi S (2002b) cDNA cloning and developmental expression of murine carbonic anhydrase-related proteins VIII, X, and XI. Brain Res Mol Brain Res 109: 207-215.

Tashian RE (1989) The carbonic anhydrases: widening perspectives on their evolution, expression and function. Bioessays 10: 186-192.

Tashian RE (1992) Genetics of the mammalian carbonic anhydrases. Adv Genet 30: 321-356.

Tashian RE, Hewett-Emmett D, Carter ND & Bergenhem NCH (2000) Carbonic anhydrase (CA)-related proteins (CA-RPs), and transmembrane proteins with CA or CA-RP domains. In: Chegwidden WR, Carter ND & Edwards YH (eds) The Carbonic Anhydrases: New Horizons. Birkhäuser Verlag Basel, Switzerland, 105-120.

Terry MB, Gaudet MM & Gammon MD (2002) The epidemiology of gastric cancer. Semin Radiat Oncol 12: 111-127.

Thatcher BJ, Doherty AE, Orvisky E, Martin BM & Henkin RI (1998) Gustin from human parotid saliva is carbonic anhydrase VI. Biochem Biophys Res Commun 250: 635-641.

Tominaga K, Matsuda J, Kido M, Naito E, Yokota I Toida K, Ishimura K, Suzuki K & Kuroda Y (2004) Genetic background markedly influences vulnerability of the hippocampal neuronal organization in the "twitcher" mouse model of globoid cell leukodystrophy J Neurosci Res 77: 507-516.

Tsukashita S, Kushima R, Bamba M, Sugihara H & Hattori T (2001) MUC gene expression and histogenesis of adenocarcinoma of the stomach. Int J Cancer 94: 166-170.

Türeci O, Sahin U, Vollmar E, Siemer S, Gottert E, Seitz G, Parkkila A-K, Shah GN, Grubb JH, Pfreundschuh M & Sly WS (1998) Human carbonic anhydrase XII: cDNA cloning, expression, and chromosomal localization of a carbonic anhydrase gene that is overexpressed in some renal cell cancers. Proc Natl Acad Sci U S A 95: 7608-7613.

Turner JR, Odze RD, Crum CP & Resnick MB (1997) MN antigen expression in normal, preneoplastic, and neoplastic esophagus: a clinicopathological study of a new cancer-associated biomarker. Hum Pathol 28: 740-744.

Uchino S, Noguchi M, Ochiai A, Saito T, Kobayashi M & Hirohashi S (1993) p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. Int J Cancer 54: 759-764.

Uemura H, Nakagawa Y, Yoshida K, Saga S, Yoshikawa K, Hirao Y & Oosterwijk E (1999) MN/CA IX/G250 as a potential target for immunotherapy of renal cell carcinomas. Br J Cancer 81: 741-746.

Ulmasov B, Waheed A, Shah GN, Grubb JH, Sly WS, Tu C & Silverman DN (2000) Purification and kinetic analysis of recombinant CA XII, a membrane carbonic anhydrase overexpressed in certain cancers. Proc Natl Acad Sci U S A 97: 14212-14217.

Uusitalo M, Heikkilä M & Vainio S (1999) Molecular genetic studies of Wnt signaling in the mouse. Exp Cell Res 253: 336-348.

Väänänen HK & Autio-Harmainen H (1987) Carbonic anhydrase III: a new histochemical marker for myoepithelial cells. Histochem Cytochem 35: 683-686.

Väänänen HK, Carter ND & Dodgson SJ (1991) Immunocytochemical localization of mitochondrial carbonic anhydrase in rat tissues. J Histochem Cytochem 39: 451-459.

Väänänen HK, Paloniemi M & Vuori J (1985) Purification and localization of human carbonic anhydrase. III. Typing of skeletal muscle fibers in paraffin embedded sections. Histochemistry 83: 231-235.

Venta PJ, Montgomery JC & Tashian RE (1987) Molecular genetics of carbonic anhydrase isozymes. Isozymes Curr Top Biol Med Res 14: 59-72.

Venta PJ, Shows TB, Curtis PJ & Tashian RE (1983) Polymorphic gene for human carbonic anhydrase II: a molecular disease marker located on chromosome 8. Proc Natl Acad Sci U S A 80: 4437-4440.

Venta PJ, Welty RJ, Johnson TM, Sly WS & Tashian RE (1991) Carbonic anhydrase II deficiency syndrome in a Belgian family is caused by a point mutation at an invariant histidine residue (107 His----Tyr): complete structure of the normal human CA II gene. Am J Hum Genet 49: 1082-1090.

Vermylen P, Roufosse C, Burny A, Verhest A, Bosschaerts T, Pastoreková S, Ninane V & Sculier JP (1999) Carbonic anhydrase IX antigen differentiates between preneoplastic malignant lesions in non-small cell lung carcinoma. Eur Respir J 14: 806-811.

Vestweber D & Kemler R (1984) Rabbit antiserum against a purified surface glycoprotein decompacts mouse preimplantation embryos and reacts with specific adult tissues. Exp Cell Res 152: 169-178.

Vollmers HP, Dammrich J, Hensel F, Ribbert H, Meyer-Bahlburg A, Ufken-Gaul T, von Korff M & Muller-Hermelink HK (1997) Differential expression of apoptosis receptors on diffuse and intestinal type stomach carcinoma. Cancer 79: 433-440.

Vullo D, Voipio J, Innocenti A, Rivera C, Ranki H, Scozzafava A, Kaila K & Supuran CT (2005) Carbonic anhydrase inhibitors. Inhibition of the human cytosolic isozyme VII with aromatic and heterocyclic sulfonamides. Biorg Med Chem Lett 15: 971-976.

Vuotikka P, Uusimaa P, Niemelä M, Väänänen K, Vuori J & Peuhkurinen K (2003) Serum myoglobin/carbonic anhydrase III ratio as a marker of reperfusion after myocardial infarction. Int J Cardiol 91: 137-144.

Waheed A, Zhu XL & Sly WS (1992) Membrane-associated carbonic anhydrase from rat lung. Purification, characterization, tissue distribution, and comparison with carbonic anhydrase IVs of other mammals. J Biol Chem 267: 3308-3311.

Wåhlstrand T & Wistrand PJ (1980) Carbonic anhydrase C in the human renal medulla. Ups J Med Sci 851: 7-17.

Wang C, Curtis JE, Minden MD & McCulloch EA (1989) Expression of a retinoic acid receptor gene in myeloid leukemia cells. Leukemia 3: 264-269.

Wang JY, Lin SR, Hsieh JS, Hsu CH, Huang YS & Huang TJ (2001) Mutations of p53 gene in gastric carcinoma in Taiwan. Anticancer Res 21: 513-520.

Watson PH, Chia SK, Wykoff CC, Han C, Leek RD, Sly WS, Gatter KC, Ratcliffe P & Harris AL (2003) Carbonic anhydrase XII is a marker of good prognosis in invasive breast carcinoma. Br J Cancer 88: 1065-1070.

Wenger RH (2000) Mammalian oxygen sensing, signalling and gene regulation. J Exp Biol. 203: 1253-1263.

Werner M, Becker KF, Keller G & Höfler H (2001) Gastric adenocarcinoma: pathomorphology and molecular pathology. J Cancer Res Clin Oncol 127: 207-216.

White PS, Jensen SJ, Rajalingam V, Stairs D, Sulman EP, Maris JM, Biegel JA, Wooster R & Brodeur GM (1998) Physical mapping of the CA6, ENO1, and SLC2A5 (GLUT5) genes and reassignment of SLC2A5 to 1p36.2. Cytogenet Cell Genet 81: 60-64.

Whitney PL & Briggle TV (1982) Membrane-associated carbonic anhydrase purified from bovine lung. J Biol Chem 257: 12056-12059.

Whittington DA, Grubb JH, Waheed A, Shah GN, Sly WS & Christianson DW (2004) Expression, assay, and structure of the extracellular domain of murine carbonic anhydrase XIV: implications for selective inhibition of membrane-associated isozymes. J Biol Chem 279: 7223-7228.

Whittington DA, Waheed A, Ulmasov B, Shah GN, Grubb JH, Sly WS & Christianson DW (2001) Crystal structure of the dimeric extracellular domain of human carbonic anhydrase XII, a bitopic membrane protein overexpressed in certain cancer tumor cells. Proc Natl Acad Sci U S A 98: 9545-9550.

Williams SE & Turnberg LA (1981) Demonstration of a pH gradient across mucus adherent to rabbit gastric mucosa: evidence for a 'mucus-bicarbonate' barrier. Gut 22: 94-96.

Wingo T, Tu C, Laipis PJ & Silverman DN (2001) The catalytic properties of human carbonic anhydrase IX. Biochem Biophys Res Commun 288: 666-669.

Wistrand PJ (1980) Human renal cytoplasmic carbonic anhydrase. Tissue levels and kinetic properties under near physiological conditions. Acta Physiol Scand 109: 239-248.

Wistrand PJ (1981) The importance of carbonic anhydrase B and C for the unloading of CO2 by the human erythrocyte. Acta Physiol Scand 113: 417-426.

Wistrand PJ & Knuuttila KG (1989) Renal membrane-bound carbonic anhydrase. Purification and properties. Kidney Int 35: 851-859.

Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, Pugh CW, Ratcliffe PJ & Harris AL (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res 60: 7075-7083.

Yamada Y, Yoshida T, Hayashi K, Sekiya T, Yokota J, Hirohashi S, Nakatani K, Nakano H, Sugimura T & Terada M (1991) p53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases. Cancer Res 51: 5800-5805.

Yamamoto M, Bharti A, Li Y & Kufe D (1997) Interaction of the DF3/MUC1 breast carcinoma-associated antigen and beta-catenin in cell adhesion. J Biol Chem 272: 12492-12494.

Yang Y-S, Hanke JH, Carayannopoulos L, Craft CM, Capra JD & Tucker PW (1993) NonO, a non-POU-domain-containing, octamer-binding protein, is the mammalian homolog of Drosophila nonAdiss. Mol Cell Biol 13: 5593-5603.

Yang Y-S, Yang M-CW, Tucker PW & Capra JD (1997) NonO enhances the association of many DNA-binding proteins to their targets. Nucleic Acids Res 25: 2284-2292.

Yao B, Hogan DL, Bukhave K, Koss MA & Isenberg JI (1993) Bicarbonate transport by rabbit duodenum in vitro: effect of vasoactive intestinal polypeptide, prostaglandin E2, and cyclic adenosine monophosphate. Gastroenterology 104: 732-740.

Yasui W, Oue N, Kuniyasu H, Ito R, Tahara E & Yokozaki H (2001) Molecular diagnosis of gastric cancer: present and future. Gastric Cancer 4: 113-121.

Yokozaki H, Yasui W & Tahara E (2001) Genetic and epigenetic changes in stomach cancer. Int Rev Cytol 204: 49-95.

Závada J, Závadová Z, Machon O, Kutinova L, Nemeckova S, Opavský R & Pastorek J (1997) Transient transformation of mammalian cells by MN protein, a tumor-associated cell adhesion molecule with carbonic anhydrase activity. Int J Oncol 10: 857-863.

Zàvada J, Zàvadovà Z, Pastorek J, Biesova Z, Jezek J & Velek J (2000) Human tumour-associated cell adhesion protein MN/CA IX: identification of M75 epitope and of the region mediating cell adhesion. Br J Cancer 82: 1808-1813.

Zàvada J, Zàvadovà Z, Pastorekovà S, Chiampor F, Pastorek J & Zelník V (1993) Expression of MaTu-MN protein in human tumor cultures and in clinical specimens. Int J Cancer 54: 268-274.

Zàvada J, Zàvadovà Z, Zat'ovicova M, Hyrsl L & Kawaciuk I (2003) Soluble form of carbonic anhydrase IX (CA IX) in the serum and urine of renal carcinoma patients. Br J Cancer 89: 1067-1071.

Zheng L, Wang L, Ajani J & Xie K (2004) Molecular basis of gastric cancer development and progression. Gastric Cancer 7: 61-77.

Zhu XL & Sly WS (1990) Carbonic anhydrase IV from human lung. Purification, characterization, and comparison with membrane carbonic anhydrase from human kidney. J Biol Chem 265: 8795-8801.

Book orders:
OULU UNIVERSITY PRESS
P.O. Box 8200, FI-90014
University of Oulu, Finland

Distributed by
OULU UNIVERSITY LIBRARY
P.O. Box 7500, FI-90014
University of Oulu, Finland

UNIVERSITY OF OULU P.O. Box 7500 FI-90014 UNIVERSITY OF OULU FINLAND

ACTA UNIVERSITATIS OULUENSIS

ISBN 951-42-7993-X (nid.)
ISBN 951-42-7994-8 (PDF)
ISSN 0355-3221