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Chapter 19

Carbonic Anhydrase IX in Adult and Pediatric Brain Tumors

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1. Introduction

Carbonic anhydrases (CAs) are zinc-containing metalloenzymes present in prokaryotes and eukaryotes (Sly and Hu 1995). CAs have been investigated since 1930’s (Meldrum and Roughton 1933). They are important in normal human physiology, e.g., in gluconeogenesis, lipogenesis, ureagenesis, bone resorption, and formation of gastric juice and cerebrospinal fluid (Sly and Hu 1995, Pastoreková et al. 2004). There are at least 15 members in human alpha-CA family: Five active family members are cytosolic (CA I-III, VII, and XIII), four are membrane associated (CA IV, IX, XII, and XIV), two are mitochondrial (CA VA and VB), and one is a secretory form (CA VI). In addition, there are three acatalytic forms, which are called CA-related protein (CARPs). CAs can be categorized to catalytically active or inactive, intracellular or extracellular, and wide-spread or restricted to few tissues.

Their main physiological function is to catalyze the conversion of CO2 to bicarbonate ion and proton, as described by the following reaction:

\[
\text{CA} \quad \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ 
\]

In addition to their functions in normal physiology, the roles of CAs in different diseases have been extensively investigated during the last decades. They are involved in certain neurological and hereditary disorders, edema, and most importantly, in cancer. There are at least three tumor associated isoforms; CA II, IX and XII. Especially carbonic anhydrase IX (CA IX) has been associated to neoplastic growth and cancer. The following chapter will discuss the role of CA IX in brain tumors.
2. Carbonic anhydrase IX

Carbonic anhydrase IX was first found by Pastroreková et al. (1992) and the CA9 gene was cloned by the Pastrorek et al. (1994). Previously, a research group from Netherlands described a monoclonal antibody, named G250, which stained cell membranes of renal cell carcinoma, but did not stain normal epithelium (Oosterwijk et al. 1986). They continued their studies to establish G250 as a tool in cancer diagnostics and treatment. Afterwards, the protein recognized by the G250 antibody was characterized to be CA IX (Grabmaier et al. 2000).

CA 9 gene was originally located to the chromosome 17q21.2 by fluorescence in situ hybridization (Opavský et al. 1996), but it was later localized to the chromosome 9p13-p12 by radiation hybrid mapping (http://www.ncbi.nlm.nih.gov/gene/768). CA9 gene consists of eleven exons and ten introns (Opavský et al. 1996), and encodes a protein containing 466 amino acids. It has a proteoglycan (PG) domain, central catalytic (CA) domain, transmembrane anchor, and short COOH-terminal cytoplasmic tail. CA IX was initially called MN, found from human carcinoma cell line, and later associated to neoplastic growth in carcinomas of ovary, uterine cervix and endometrium (Závada et al. 1993). A detailed characterisation of human CA IX protein has shown that the recombinant CA IX protein exhibits the highest catalytic activity ever measured for any CA isozyme (Hilvo et al. 2008).

3. Carbonic anhydrase IX in normal tissue

The expression of CA IX in normal tissues has been thoroughly investigated. In mouse tissues, the highest expression has been detected in gastric mucosa, moderate expression in colon and brain, whereas low expression has been reported in pancreas and small intestine (Hilvo et al. 2004). The similar distribution pattern has been detected in human tissues; high CA IX staining has been discovered in GI-tract, especially in the epithelia of the gallbladder and gastric mucosa (Pastorek et al. 1994, Pastoreková et al. 1997, Saarnio et al. 1998a). Furthermore, Saarnio et al. (1998b) have reported the most intensive signals of CA IX in the epithelium of the duodenum and jejunum, whereas the expression diminishes towards the large intestine. Mesothelium, epithelial cells of the esophagus, and pancreatic and biliary ducts express CA IX (Turner et al. 1997, Pastoreková et al. 1997, Kivelä et al. 2000, Ivanov et al. 2001). CA IX has been detected in the male reproductive organs, whereas the female reproductive tract express only low amounts of CA IX (Liao et al. 1994; Karhumaa et al. 2001). Generally, expression of CA IX is generally low in the human brain, although positive signal has been reported in the epithelial cells of the choroid plexus (Ivanov et al. 2001, Proescholdt et al. 2005). Similarly, lower levels of CA IX mRNA have been reported in the normal brain as compared to brain neoplasms (Said et al. 2007a).
4. Carbonic anhydrase IX in neoplastic tissue

The von Hippel–Lindau (VHL) tumor suppressor gene was the first link to the major pathway controlling CA IX expression (Wykoff et al. 2000). Importantly, CA IX turned out to be one of the enzymes regulated by the hypoxia pathway, in which hypoxia inducible factor 1 (HIF-1) plays a role as a key transcription factor, especially in hypoxic tumors. Under normoxia, the encoded VHL protein (pVHL) binds to hydroxylated hypoxia inducible factor 1 – alpha and causes degradation by the ubiquitin-mediated proteasome system, inactivating the downstream target genes. (Ivanov et al. 1998). HIF-1 is stabilized under hypoxic conditions and binds to hypoxia-responsive elements in many genes, e.g. VEGF, erythropoietin, and glucose transporter. This leads to the induction of erythropoiesis, angiogenesis, and glycolysis (Carmeliet et al. 1998). HIF-1 also activates CA9 gene and CA IX expression level increases dramatically in hypoxic conditions. In line with this, high CA IX expression is often found in perinecrotic regions of tumors (Wykoff et al. 2000). The similar induction of CA IX has been proposed to take place in brain tumors. CA IX mRNA has been studied in human malignant glioma cell lines and distinct patterns of hypoxic expression of CA IX have been observed (Said et al. 2007b). The finding indicated that low oxygen concentration is probably the driving force for the increased CA IX expression due to the presence of a hypoxia responsive element (HRE) in the CA9 promoter (Wykoff et al. 2000). The activation of hypoxia-inducible genes is illustrated in Figure 1.

**Figure 1.** Activation of hypoxia-inducible genes. Under normoxia, HIFα is degraded by ubiquitin-proteasome system. Prolyl-4-hydroxylases (PHD) hydroxylate two conserved proline residues of HIF-1α, then von Hippel-Lindau protein (VHL) binds to the hydroxylated HIF-1α. Under hypoxia, PHDs are inactive in the absence of dioxygen, and HIF-1α is not recognized by VHL protein. HIF-1α accumulates and is translocated to the nucleus. HIF-1β constitutive subunit dimerizes with HIF-1α, resulting in the active transcription factor, which binds to hypoxia response element (HRE). Therefore the transcription of target genes, such as CA9 and GLUT1, is induced. Adapted from Pastoreková et al. (2008) and Haapasalo (2011).
The overexpression of CAIX in majority of renal cell carcinomas (RCCs) is due to the loss of functional VHL protein, which causes the stabilization of HIF-1. (Gnarra et al. 1994, Wykoff et al. 2000). In other words, the CA IX is no longer regulated by hypoxia. On the contrary, the majority of tumors do not contain VHL mutations, and in these, CA IX is usually found in focal perinecrotic areas, supporting the role of hypoxia in CA IX regulation (Wykoff et al. 2000). Furthermore, it has been suggested that HIF activates genes that change the expression profile of tumor cells suffering from hypoxia; thus, either leading to adaptation to the hypoxic stress or resulting in cell death. If this adaptation is successful, the surviving tumor cell population is associated with increasingly aggressive behaviour involving invasion and metastases, resistance to anti-cancer treatment, and finally, worse patient prognosis. (Harris 2002). This mechanism is supported by various immunohistochemical studies in which the CA IX expression is located in in the perinecrotic regions of solid tumors.

The mechanisms behind the role of CA IX in cancer have been studied widely. The hypoxia, measured by needle electrores, has been shown to correlate with CA IX expression in cervical cancer (Loncaster et al. 2001). This finding has been further clarified in genetic analysis, showing that CA9 was the most induced gene among the 32 identified hypoxia responsive genes, which included VEGF, in human solid tumors (Lal et al. 2002). In vitro, CA9 has been shown to be hypoxia-regulated in glioblastoma cells (Said et al. 2008).

The pivotal feature of the malignant tumor cells is their capability to maintain the normal intracellular pH, whereas the extracellular pH is significantly acidic. CA IX increases the extracellular acidification by shifting the site of CO\(_2\) hydration from intra- to extracellular (Svastová et al. 2004). This in turn increases the capability of tumor cells to survive and invade, and the selective sulfonamide inhibitors disturb this process. Acetazolamide, a potent CA inhibitor, has been shown to suppress the invasion of renal cancer cells in vitro (Parkkila et al. 2000b). Interestingly, CA IX has an optimal catalytic activity for CO\(_2\) hydration to bicarbonate and proton in acidic pH (Innocenti et al. 2009). Furthermore, when studied in cancer-derived cell lines, CA IX diminishes the intracellular pH gradient in the hypoxic core of three-dimensional tumor spheroids (Swietach et al. 2008). These findings support the theory that CA IX is an essential factor for tumor cells in adaptation to hypoxia and their survival, and is illustared in Figure 2.

Even though the expression of CA IX is mainly regulated by hypoxia, it has been shown that acidosis increases CA IX expression via a hypoxia-independent mechanism (Ihnatko et al. 2006). CA IX has been proposed to be regulated by low oxygen concentrations or constitutive, oncogene-related mechanisms (Said et al. 2007a). Furthermore, CA IX modulates E-cadherin mediated cell adhesion by decreasing the binding of this cell adhesion molecule to beta-catenin (Svastová et al. 2003). This, in turn, could possibly benefit the cancer cells by an increase in cell motility and invasion. As mentioned before, acetazolamide can suppress the invasion of renal cancer cells in vitro (Parkkila et al. 2000b). However, the inhibition of CA IX in RNAi-treated breast cancer cells reduced the invasion capacity only slightly (Robertson et al. 2004). There is also evidence that CA IX expression is a negative predictive factor when evaluating the treatment efficacy in oestrogen receptor-positive tumors treated with adjuvant tamoxifen after
primary chemo-endocrine therapy (Generali et al. 2006). The role of CA IX in resistance to chemotherapy could be explained by the effects of pH on tamoxifen uptake.

Ectopic expression of CA IX is induced in various tumors. These include the malignancies of breast, cervix uteri, esophagus, kidney, and lung (Liao et al. 1994, McKiernan et al. 1997, Liao et al. 1997, Turner et al. 1997, Vermylen et al. 1999, Bartosová et al. 2002). When these tumors are considered, CA IX is absent in the corresponding normal tissue. Conversely, CA IX expression is usually absent or reduced in tumors which have originated from CA IX-positive tissues. These include carcinomas of stomach and gallbladder (Saarnio et al. 2001, Leppilampi et al. 2003). This makes the CA IX a promising molecule as a prognostic factor as well as a potential target for therapeutic methods.

In cervical cancer, the CA IX expression correlates to tumor hypoxia and poor patient prognosis, and could be used in the selection of suitable patients for hypoxia-modification therapies (Loncaster et al. 2001). In lung cancer CA IX is a marker of poor prognosis (Swinson et al. 2003, Kim et al. 2004), and it has been associated to proteins linked to angiogenesis, disruption of cell-cell adhesion and inhibition of apoptosis (Giatromanolaki et al. 2001). CA IX could also been used as a differentiation tool between preneoplastic lesions and lung cancer (Vermeylen et al. 1998) and a high concentration of CA IX in plasma serves as an independent prognostic
biomarker in patients with non-small cell lung cancer (Ilie et al. 2010). CA IX expression is sensitive for diagnostics of mesothelioma and metastatic clear cell renal cell carcinoma of the lung (Ramsey et al. 2012). Furthermore, CA IX expression has been associated with poor prognosis for patients with head and neck cancer (Beasley et al. 2001, Koukourakis et al. 2001), esophageal cancer (Birner et al. 2011), ovarian cancer (Hynninen et al. 2006, Choschzick et al. 2011), soft tissue sarcoma (Måseide et al. 2004), and bladder carcinoma (Hoskin et al. 2003). It has been confirmed in several studies that CA IX correlates with poor prognosis in breast cancer (Chia et al. 2001, Brennan et al. 2006, Hussain et al. 2007) and is related to overexpression of c-erbB2 (Bartosová et al. 2002).

The most widely investigated tumor type, considering CA IX, is renal cell carcinoma (RCC) in which CA IX represents a useful marker (Liao et al. 1997, McKiernan et al. 1997, Parkkila et al. 2000a). In its most common subtype, clear cell carcinoma, CA IX expression is higher than in other renal cell cancer types (Sandlund et al. 2007). In addition, patients with both conventional renal cell cancer and low CA IX expression had a less favourable prognosis. In renal cancer CA IX is a promising therapeutic target for novel oncological applications, including immunotherapy and radioisotopic methods (Pastoreková et al. 2006, Bleumer et al. 2006). CA IX and CA XII are functionally involved in tumor growth (Chiche et al. 2009). Renal cell cancer in vivo experiments showed that CA9 gene silencing alone led to a 40% reduction in xenograft tumor volume, and silencing of both CA9 and CA12 resulted in an 85% reduction in tumor volume.

5. Carbonic anhydrases in adult brain tumors

The incidence of brain tumors is similar in different countries and rather stable over the past two decades (Pollack et al. 2011). There are about 50 new pediatric and 1000 adult brain tumors every year in Finland with five million inhabitants (Statistics Finland 2011). The etiology of brain tumors has been under intense investigation but no clear evidence between different environmental, nutritional or lifestyle and carcinogenesis have been found (Baldwin et al. 2004). Most common primary CNS tumors of the adult are gliomas and meningiomas. Glioblastoma is a highly malignant and unfortunately common, glial tumor and the 5-year survival of the patients is less than 10% (Stupp et al. 2009). On the other hand, almost all (90-98%) patients with meningioma are alive after five years (Statistics Finland 2011).

The expression of carbonic anhydrases in brain tumors has been previously reported (Parkkila AK et al. 1995). The first findings assessed CA II, which was stained positively by immunohistochemistry in astrocytic tumors, oligodendrogliomas and medulloblastomas. The expression of CA IX was first reported by Ivanov et. al (2001). In this first study, they screened tumors of different genetic background as well as several malignant cell lines for the expression of CA IX. mRNA analysis revealed high-to-moderate levels of expression of CA9 and CA12 in glioma cell lines. Immunolocalization of CA IX was further studied in 11 gliomas; low-grade gliomas were not stained for CA IX, whereas grade II-IV gliomas were all CA IX positive. In addition, 3 oligodendrogliomas were included in the analysis and they failed to express CA IX. Fur-
thermore, all hemangioblastomas (3 tumors), meningiomas (5 tumors), and two out of three choroid plexus tumors were positive for CA IX.

This overview of different tumors was followed by a study of Proescholdt et al. (2005) on CA IX and CA XII, which combined brain tumors of different histology and grade of malignancy. The material consisted of total of 112 tumor samples (grade I-IV astrocytomas, meningiomas, metastases, primitive neuroectodermal tumors (PNETs), and hemangioblastomas). Generally, low-grade astrocytomas did not show any positive staining for CA IX and the expression increased with increasing WHO grade. The strongest staining of all glioma samples was observed in the glioblastomas, and almost all of the samples (97%) were positive. In these, the staining was detected around necrotic areas. However, more diffuse staining pattern without any association to necrosis was detected and CA IX expression was found in almost all tumor cells, including those near blood vessels, suggesting the induction also without the hypoxia-inducible mechanism. In the meningiomas, increased CA IX staining, with diffuse and evenly distributed pattern, was found in comparison to the normal brain. The authors found the most widespread CA IX and XII staining of all tumors in hemangioblastoma samples.

As to brain tumors, the first large study to describe the expression of CA IX in human diffusely infiltrating astrocytomas was published year after (Haapasalo et al. 2006). The study material consisted of 362 diffusely infiltrating astrocytoma samples (grades II-IV), which were obtained from surgically operated patients. Cellular CA IX immunopositivity was observed in 78% of diffusely infiltrating astrocytomas and the percentages according the WHO grade were as follows; 65% of grade II astrocytomas, 73% of grade 3 astrocytomas, and 82% of grade 4 astrocytomas. The immunohistochemical results were verified by mRNA analysis. The statistical comparison of cytoplasmic CA IX intensity and tumor grade revealed significantly higher CA IX intensity in tumors of higher malignancy grade. Again, CA IX was expressed in areas close to the necrotic regions and cytoplasmic staining was seen in the neoplastic cells of the infiltrative zone. When important clinicopathological features were assessed, CA IX showed no association with p53 expression nor did it correlate with epidermal growth factor receptor–amplification, apoptosis, or cell proliferation by Ki-67/MIB-1. There was a significant correlation between increasing CA IX intensity and increasing patient age. For the first time, CA IX positivity was associated to shortened patient survival in univariate analysis: CA IX intensity divided the tumors into four significantly differing prognostic subsets (Figure 3). The survival difference was even significant within grade II and grade IV tumors separately. Most importantly, statistical analysis of the data revealed that the patient age, tumor grade, and CA IX intensity all had independent prognostic value when evaluated by Cox multivariate analysis.

The finding that CA IX predicts poor prognosis has been confirmed by others. Korkolopoulou et al. (2007) showed that increasing CA IX immunopositivity was associated with a shortened survival in univariate analysis. Furthermore, they reported similar independent prognosticators in multivariate analysis including CA IX, tumor grade and patient age. The perinecrotic distribution of CA IX immunostaining was detected and intensity increased in parallel with the extent of necrosis and histological grade. In concordance, Sathornsumetee et al. (2008) conducted a trial, in which patients with recurrent malignant astrocytomas treated with a combination of VEGF -neutralizing antibodies were retrospectively evaluated. Survival
analysis revealed that high CA IX expression was associated with poor survival outcome. VEGF was associated with radiographic response but not with survival. Interestingly, they tested both CA IX and HIF simultaneously in a Cox model as separate factors, and only CA IX remained as a statistically significant factor. One opposite result has been published in glioma patient cohort (Flynn et al. 2008). In this study, no significant correlations between the CA IX expression and patient survival or tumor grade were found, although the patients with CA IX-positive tumors seemed to have a trend towards a worse prognosis. This might be due to different immunostaining method used. The most reliable method for CA IX immunostaining having no cross-reactivity with other CAs (Saarnio et al. 1998b), is based on M75 antibodies used by us and e.g. Korkolopoulou et al. (2007). Flynn et al. (2008) had also smaller number of patients. Yoo et. al (2010) assessed the issue again, and showed that CA IX expression was a predictive factor for poor survival and correlated positively with increasing WHO grade.

The expression of CA IX has been studied in other gliomas as well. Järvelä et al. (2008) showed by immunohistochemistry that 80% of studied 86 oligodendroglial tumors stained positively for CA IX. In addition, CA IX predicted poor prognosis in univariate analysis and in multivariate analysis CA IX expression, patient age and histological component (pure oligodendroglioma vs. mixed oligoastrocytoma) showed independent prognostic values. Abraham et
al. (2012) have recently assessed the expression of all hypoxia related molecules HIF-1, VEGF, Glut-1, and CA IX, in oligodendrogliomas. They found that all these proteins were statistically significantly different between grade II and III oligodendrogliomas, anaplastic oligodendrogliomas having stronger expression. Interestingly, low CA IX expression predicted better prognosis in anaplastic oligodendrogliomas but not in grade II oligodendrogliomas, whereas the prognostic significance was not reported in the whole tumor material.

As for mostly benign tumors, Yoo et al. (2007) showed that 50% of all meningiomas contained regions of hypoxia as judged by expression of CA IX. CA IX expression was significantly associated with higher-grade histology and tended to be more common in recurrent tumors. Furthermore, Korhonen et al. (2009) reported 11.6% cytosolic CA IX expression in meningiomas. CA IX positivity was neither associated with the studied clinicopathological factors nor survival.

Recently, Jensen et al. (2012) assessed the molecular markers of hypoxia, vascularity, and proliferation in meningiomas, including CA IX. As expected, VEGF, HIF, CA IX, and Glut-1 were positively correlated. There was an association between higher-grade tumors with higher scores for CA IX, VEGF, and HIF-1alpha, but CA IX was not associated to overall survival.

CA IX is also significantly upregulated in craniopharyngiomas and is associated with increased cyst size (Proescholdt et al. 2011). The mechanisms of CA IX regulation remain unknown, since neither hypoxia nor p53 appear to play a role in these tumors. The authors state, that inhibition of CA IX may be a potential target for the adjuvant treatment in patients with cystic craniopharyngiomas.

6. CA IX in pediatric brain tumors

Pediatric cancers are still the main cause of death in children aged 1-14 years in the UK and Finland (Gatta et al. 2005, Statistics Finland 2011). After leukemia brain tumors are the second most common tumor group. Approximately 60-70% of the patients with brain tumors are alive five years from the diagnosis (Pokhrel and Hakulinen 2009). The most common pediatric brain tumors are pilocytic astrocytoma, medulloblastoma and ependymoma. Neurosurgical operation is the most important treatment modality. Inoperable or highly malignant tumors are treated also with radio- and chemotherapy. CNS is vulnerable, especially when evolving. Most of the patients do survive but the tumor and the different treatment modalities can cause side effects that reduce the quality of life. Supratentorial tumors, tumor reoperations, shunt revisions and chemotherapy increase the risk of these problems (Reimers et al. 2003, Pietilä et al. 2012). There is only a limited scope for improvement with conventional chemotherapy and thus, there is an urgent need of therapeutic agents for these patients. CA IX is one novel molecule that might serve as a prognostic/diagnostic tool, and perhaps, a target for various therapeutic methods.

Some publications have assessed the CA IX expression in pediatric brain tumors. Ivanov et al. (2001) screened a small amount of brain tumors and found the following immunohistochem-
istry results: most of the 7 central/peripheral PNETs expressed the CA IX, all of 6 studied ependymomas were positive. Preusser et al. (2005) assessed the CA IX in intracranial ependymomas: 84 out of 100 tumors expressed CA IX, and it was associated with a bizarre angiogenesis and necrosis. However, CA IX failed to reach a prognostic significance in univariate analysis. The most common, solid, extracranial pediatric nervous tumor is neuroblastoma. Dungwa et al. (2012) found positive membranous/cytoplasmic CA IX expression in 21 (23%) of 91 neuroblastomas but was absent in ganglioneuromas. Neuroblastomas with 1p deletion and MYCN amplification had even stronger membranous expression. 18% of the neuroblastomas showed nuclear CA IX expression in 10% or more tumoral cells. Nuclear CA IX expression associated with worse overall—and event-free survival.

We have previously studied CA IX in 39 medulloblastomas and PNETs (Nordfors et al. 2010). CA IX positivity was found in 23% of tumors and the expression was linked to necrosis. CA IX expression was analysed in concordance with various clinical features and molecular markers. Proliferation (Ki-67/MIB-1), apoptosis or expression of Bcl-2, p53 or c-erbB-2 were not associated with CA IX in any of the groups except for the correlation between positive c-erbB-2 and positive CA IX expression in PNETs. CA IX was also positively associated with female gender. There was no significant difference in the expression of CA IX between primary and recurrent tumors in any of the groups. Moreover, there was no correlation between the tumor type (MBs/PNETs) and CA IX intensity. Interestingly, CA IX-positivity was a marker of worse outcome in patients with MB/PNET in univariate and multivariate analyses (Figure 4). Generally, CA IX is associated with higher grade, necrosis, and worse CA IX seems to have several inductors. CA IX is often found in perinecrotic areas. Because necrosis is an uncommon feature and is not considered to be a significant prognostic factor in MBs, the induction of CA IX in MBs/PNETs may also involve hypoxia-independent mechanisms. In addition, there is the evidence that CA IX is expressed in grade I pilocytic astrocytomas, and the immunopositivity for CA IX is associated to histopathological features of degeneration and increased proliferation (our unpublished results).

In children, possible side-effects of therapeutic interventions may be more severe and the exact biology of hypoxia and its clinical relevance in childhood tumors is still unclear. Thus, further studies will be needed before novel agents concerning hypoxia can be introduced into pediatric oncology.

7. Future aspects

The tumor tissue specific CA IX expression has led researchers to propose several novel treatment strategies. A promising treatment strategy is to use CA selective inhibitors (Pastoreková et al. 2004). Tumor cells probably use CAs as key enzymes to adapt to the hostile environment caused by metabolic stress of cancer cells, and thus, acidification facilitates the spread and invasion of cancer cells (Svastová et al. 2004). High CA IX expression might increase the capability of cells to infiltrate the neighboring tissue. The inhibition of this process would potentially disturb the invasion processes of cancer cells.
Another possible treatment option is to use cancer-specific antibodies. The CA and proteoglycan domains give the molecule a unique extracellular structure. CA IX expression is high in renal cell carcinoma (Liao et al. 1997) and this has enabled therapeutic trials with high-dose radiolabeled CA IX antibody (cG250) and CA IX-loaded dendritic cells. Unfortunately, a significant breakthrough in clinical trials remains to be achieved and in RCC trials are still in phase II (Stillebroer et al. 2010).

CA IX could also be used as a potential target for immune therapy. Greiner et al. (2006) found a significant correlation between high mRNA levels of CA IX and a longer overall survival in acute myeloid leukemia. This might be due to the induction of a strong antileukemic immune response by CA IX. Similar findings have been found in metastatic RCC patients (Uemura et al. 2006). Vaccination with tumor-RNA pulsed dendritic cells led to increased numbers of CA IX peptide-specific cytotoxic T76 lymphocytes and IgG levels without any major adverse event. Metastasis of three patients shrank or even disappeared and the overall survival was longer for six patients. However, further studies are required to confirm these findings in larger study cohorts.

Bevacizumab, an anti-VEGF antibody, inhibits the developing vasculature of tumors, but resistance is common. Antiangiogenic therapy induces hypoxia and thus, CA IX. Curiously, McIntyre et al. (2012) knocked down CAIX expression in a colon cancer and a glioblastoma cell lines and combined the results with bevacizumab. They found that CAIX expression was associated with increased necrosis and apoptosis in vivo and in vitro. Added
to this, acidity seemed to inhibit CAIX activity, and this may be the mechanism whereby excess acid self-limits the build-up of extracellular acid. It seems that inhibition of the hypoxic adaptation to antiangiogenic therapy enhances bevacizumab treatment and highlights the value of developing small molecules or antibodies which inhibit CAIX for combination therapy.

8. Conclusions

CA IX has been linked to several cancer tissues, whereas the normal tissue is mostly negative. This seems to be the case also when different brain tumors are concerned. Malignant astrocytomas and oligodendrogliomas express CA IX and it has been shown as a useful prognosticator. Being a hypoxia/necrosis marker, CA IX can be used as a diagnostic tool in the grading of astrocytomas and oligodendrogliomas. Tumor biopsies containing a small amount of tissue for diagnosis, are especially good targets. In addition, CA IX is associated to more malignant phenotype in meningiomas and some other mostly benign brain tumors.

CA IX is present in the most common malignant brain tumor of children, medulloblastoma, as well as in PNETs. Interestingly, in these tumors CA IX predicts poor prognosis and could be used as a marker when planning the cancer therapy. Added to this, CA IX has been shown to be expressed in other pediatric brain tumors, such as ependymomas and pilocytic astrocytomas, where it is linked to degenerative histopathological features.

CA IX is associated with hypoxia, necrosis, and angiogenesis – features traditionally linked to the tumorigenesis of brain tumors. Several studies show that CA IX could be used as a target molecule in adult and pediatric brain tumors. Further clinical trials for cancer treatment are needed aiming to either eradicate the tumor cell population or turn the tumor into a more chronic and stable disease.

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