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Expression and association of carbonic anhydrase IX and cyclooxygenase-2 in colorectal cancer

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ABSTRACT

Background: This work was designed to determine the relationship between hypoxia-inducible protein carbonic anhydrase IX (CA IX) and pro-inflammatory enzyme cyclooxygenase-2 (COX-2) in patients with colorectal cancer (CRC).

Methods: We examined CA IX and COX-2 expression in CRC tissues by immunohistochemical staining of 111 samples. We evaluated the correlation between the expression of these proteins and their correlation with the clinico-morphological parameters of CRC.

Results: CA IX was detected in 89 of 111 cases (80.2%). We predominantly observed membrane staining (70.3%) and a strong immunoreaction intensity (58.6%). The percentage of labeled cells in malignant lesions was less than 25% in 12.6% of cases, less than 50% in 15.3% of cases and more than 50% in 52.3% of CRC cases. The COX-2 protein was expressed in 94 of 111 cases (84.7%). We noticed only cytoplasmic localization, while immunoreaction intensity was predominantly strong (47.8%). The percentage of COX-2 positive cells was less than 25% only in 2.7% of the cases, less than 50% in 21.6% of the cases and more than 50% in 60.4% of the cases. No statistically significant correlations were observed between CA IX expression and clinico-morphological parameters. COX-2 expression was only significantly correlated with the tumor stage. Statistical analysis confirmed a significant correlation between the parameters of expression of the CA IX and COX-2 proteins.

Conclusion: CA IX/COX-2 interplay can promote hypoxia survival and the invasion of tumor cells. These proteins may represent independent prognostic factors of CRC.

1. Introduction

Colorectal cancer (CRC) is a significant global health, social and economic problem [1]. Worldwide, it is the third most common malignant cancer among males and the second most common malignant cancer among females [2]. In 2017, a total of 71,420 men and 64,010 women were diagnosed with CRC, while 27,150 men and 23,110 women died from this disease [3]. The incidence varies across countries and regions, diametrically fluctuating within age groups (increasing with higher age); it is more common in men than in women, and depending on ethnicity [4].

Many factors are considered important in the etiology and pathogenesis of CRC [5]. Among these factors, two environmental conditions – hypoxia and inflammation may also play a role in CRC growth and contribute to the aggressive phenotype of CRC cells [6].

Hypoxia is a condition that occurs when the oxygen supply to tumor cells is insufficient due to their rapid proliferation and a shift in their metabolism from oxidative phosphorylation to aerobic glycolysis [7,8]. Oncogenic metabolism leads to an extensive production of protons, lactate and carbon dioxide which promote the acidification of the tumor microenvironment [9]. From the clinical point of view, phenotypic changes caused by hypoxia are critical, because tumor hypoxia is associated with progression, resistance to therapy and increased mortality [10].

One of the tumor cells' adaptive mechanisms to hypoxia is increased expression and activation of carbonic anhydrase IX (CA IX), a cancer-related protein which plays a crucial role in tumor cell survival, proliferation, migration, adhesion and cell-signaling pathways. CA IX also catalyzes the reversible conversion of carbon dioxide to bicarbonate ions and protons. This catalytic activity contributes to the regulation of intracellular and extracellular pH. It results from hypoxia-induced changes in the cellular metabolism, thus producing an excess of acids

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[8,11,12].

CA IX expression may be regulated by HIF-1 (hypoxia inducible factor-1) transcription factor and also through the MAPK/ERK (Mitogen-activated protein kinase/Extracellular signal-regulated kinase) pathway activation [13,14]. HIF-1 is involved in regulation of multiple steps in tumorigenesis [15]. It consists of two subunits: O_2 labile HIF-1α and constitutively expressed HIF-1β [16]. In hypoxic conditions, the absence or low levels of O_2 cause HIF-1α stabilization and its dimerization with HIF-1β [17]. This HIF-1 dimer further binds to DNA at the HRE (hypoxia response element) and activates genes (including the *CA9* gene) involved in metabolic reprogramming, genetic instability and tumorigenesis [18]. The MAPK pathway is important in transduction of extracellular signals performed by several mitogenic and microenvironmental factors. Hypoxia activates ERKs by inducing their phosphorylation and nuclear translocation, thereby increasing the trans-activation capacity of HIF-1 and promoting the transcription of HIF-1-regulated genes (such as CA9) [19].

CA IX is also suggested to have a potential role as a prognostic indicator, diagnostic marker and tumor therapeutic target in several types of cancer [20,21]. This protein is not expressed in the majority of normal tissues (it is abundant only in the stomach and gallbladder). On the other hand, it is very often and strongly expressed in tumors (colon, breast, lung, ovaries, brain etc.), generally in their more aggressive variants. CA IX expression correlates with poor prognosis in many types of cancers (including CRC) [22,23].

Inflammation may also play a critical role in colon carcinogenesis [24,25]. Epidemiological observations cite the utility of CRC as a paradigm for inflammation associated with cancer [26]. Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in regulation of prostaglandin E_2 (PGE_2) synthesis, and is overexpressed at sites of inflammation and in several cancers [27]. COX-2 is also an important factor in colorectal tumorigenesis. It inhibits apoptosis by increasing Bcl_2 expression via the MAPK signaling pathways. PGE_2 activates β-catenin-dependent signaling, which promotes survival and proliferation. COX-2 also stimulates tumor angiogenesis by inducing the production of VEGF (Vascular endothelial growth factor) and basic fibroblast growth factor. It can increases tumor dissemination by altering the adhesive properties of cells and increasing matrix metalloproteinase activity [28].

COX-2 can be induced by aberrant growth factor signaling and oncogene activation during colorectal tumorigenesis. Kaidi et al [29] were the first to report a COX-2 protein level increase in CRC under hypoxic conditions. COX-2 up-regulation is transcriptional, associated with HIF-1α induction and during hypoxia it is accompanied by increased levels of PGE_2, thus promoting tumor cell survival. PGE_2 was shown to enhance intestinal adenoma growth via activation of the MAPK cascade [30]. HIF-1 can directly up-regulate COX-2 and PGE_2 levels. Afterwards PGE_2 activates MAPK pathway and enhance HIF-1 transcriptional activity. Therefore, PGE_2 up-regulation in colorectal tumor cells during hypoxia may modulate the expression of several other HIF-1-target genes (including *CA9*), which could have implications for tumor cell survival, angiogenesis, invasion and metastasis, and subsequently tumor progression [29]. Sansone et al [6], provided evidence that COX-2 up-regulates CA IX expression throughout the PGE_2 activation of MAPK/ERK pathway.

Due to there is only one article dealing with CA IX and COX-2 in CRC and clinical significance of these proteins, we have decided to review their relationship in our study. In the present work, we examined the correlation between the expression of these proteins and their correlation with the clinico-morphological parameters of CRC patients.

2. Materials and methods

The study was approved by the local Ethics Committee of the Jessenius Faculty of Medicine in Martin, and registered in the Office for Human Research Protection, U.S. Department of Health and Human Services under No: EK 1744/2015.

Table 1
Clinico-morphological characteristics of CRC patients.

Features	n	%
Age, years (n = 111)		
Mean	67.03	
Range	39 – 94	
Sex (n = 111)		
Female	39	35.1
Male	72	64.9
Histological grading (n = 106)		
G1	34	32.1
G2	60	56.6
G3	7	6.6
G4	5	4.7
Pathological staging (n = 61)		
Stage I	12	19.7
Stage II	23	37.7
Stage III	24	39.3
Stage IV	2	3.3
Lesion site (n = 111)		
Proximal colon	50	45.0
Distal colon	61	55.0
Lymph node (n = 65)		
Positive	24	36.9
Negative	41	63.1
Vascular invasion (n = 59)		
Positive	19	32.2
Negative	40	67.8

2.1. Patients group

Archival blocks of formalin-fixed paraffin-embedded tissue samples from 111 patients with colorectal cancer were included in this study. These tissue blocks were maximum four months old, because patient recruitment took from January 2016 to June 2016, while staining began in May 2016. In addition, the tissue blocks were stored in an air-conditioned room in the dark and cold. Colorectal carcinomas were histologically classified and graded according to WHO guidelines and pTNM (UICC) pathological staging criteria (Table 1) [31].

2.2. Immunohistochemical analysis

Each paraffin block was cut into 4 μm-thick sections and subjected to immunohistochemical staining, where 2 sections from each block were stained for detection of the CA IX and COX-2 proteins. TOMO® IHC Adhesive Glass Slides (Matsunami Glass Ind., Ltd., Japan), which had been in the thermostat for 3 h at 59 °C, were used to improve the adherence of the tissue sections to the glass surface.

The paraffin sections were automatically stained with Ventana BenchMark® ULTRA, according to the manufacturer's protocol. In order to detect the CA IX protein, the automated program for immunohistochemistry included deparaffinization at 72 °C; cell condition (CC1, pH 8.0) for 8 min at 97 °C; incubation with a 1:300 diluted mouse monoclonal CA IX antibody (Clone H-11, Zeta corporation) at 36 °C for 4 min; incubation with a UV Red Multimer for 12 min; incubation with a UV Red Enhancer for 4 min; incubation with a UV Fast Red A and UV Red Naphthol for 8 min; incubation with a UV Fast Red B for 8 min; incubation with Hematoxylin (for staining cellular nuclei) for 8 min and incubation with a Bluing reagent (for bluing the hematoxylin stained sections on the glass slides) for 8 min. In order to detect the COX-2 protein, the automated program for immunohistochemistry included deparaffinization at 72 °C; cell condition (CC1, pH 8.0) for 4 min at 95 °C; incubation with an OV Peroxidase Inhibitor for 4 min; incubation with a 1:300 diluted rabbit monoclonal COX-2 antibody (Clone SP21, Cell Marque) at 36 °C for 36 min; incubation with an OV UQ Universal

Linker for 8 min; incubation with an OV HRP Multimer for 8 min; incubation with an OV H_2O_2 and OV DAB for 8 min; incubation with an OV Copper for 4 min; and incubation with a Hematoxylin and Bluing reagent for 8 min each [32].

The staining was evaluated using light microscopy (Olympus BX41). All parameters were assessed semi-quantitatively by two independent observers (VT, MA) to achieve good reproducibility. Because of the rather straight criteria defined to categorize cases, disagreement among observers was rare, and when it happens, slides were reviewed and a consensus reached using a multi-headed microscope. We assessed the parameters such as subcellular localization, intensity of immunoreaction (weak, moderate, strong) and percentage of positive cells, while percentage ≤10% was considered negative. The subcellular localization of CA IX was the membrane (M) or the combined membrane and cytoplasmic (MC) and only cytoplasmic (C) in COX-2 localization.

2.3. Statistical analysis

The statistical evaluation was performed using the Chi-square ($\chi 2$) test in the R software (version 3.2.3) [33]. Results with a p-value below 0.05 were considered statistically significant.

3. Results

3.1. Evaluation of immunohistochemical staining

CA IX was expressed in 89 of the 111 colorectal carcinoma cases (80.2%).The positive cases showed membrane and combined (membrane and cytoplasmic) localization. Membrane staining was observed in 78 of 111 cases (70.3%), while combined CA IX staining was detected in 11 of 111 cases (9.9%). A weak immunoreaction intensity was detected in 5 of 111 cases (4.5%), moderate intensity was observed in 19 of 111 cases (17.1%) and strong intensity in 65 of 111 cases (58.6%) (Fig. 1). The percentage of labeled cells in malignant lesions was less than 25% in 14 of 111 carcinomas (12.6%), between 26˜50% in 17 of 111 cases (15.3%) and more than 50% in 58 of 111 cases (52.3%).

COX-2 was expressed in 94 of 111 colorectal carcinoma cases (84.7%). Cytoplasmic localization was observed in all positive cases. Immunoreaction intensity varied from weak to strong. Weak intensity was found in 19 of 111 cases (17.1%), moderate intensity was in 22 of 111 case (19.8%) and strong intensity was detected in 53 of 111 cases (47.8%) (Fig. 2). The percentage of COX-2 positive cells was less than 25% in 3 of 111 carcinomas (2.7%), between 26˜50% in 24 of 111 cases (21.6%) and more than 50% in 67 of 111 cases (60.4%).

3.2. Statistical analysis

The Chi-square (χ^2) confirmed no statistically significant association between CA IX expression and clinico-morphological characteristics (patient age, sex, histological grading, staging, lesion site, lymph node status, vascular invasion) (Table 2).The Chi-square (χ^2) only confirmed a statistically significant association between the percentage of COX-2 positive cells and the stage (p = 0.0001). Other clinico-morphological data did not associate with COX-2 expression (Table 3). Table 4 summarizes the relationship of CA IX and COX-2 proteins. Statistical analysis confirmed a significant correlation between CA IX and COX-2 expression (p = 2.12E–11), a significant difference in intensity of CA IX and COX-2 immunoreactivity (p = 2.88E–26), and a significant difference in the percentage of CA IX and COX-2 positive cells (p = 3.03E–24) (Fig. 3).

4. Discussion

In this investigation we observed hypoxia-inducible protein CA IX which is overexpressed in hypoxic tumors and is considered as an important biomarker for hypoxic tumor diagnosis [34]. In our group of 111 colorectal cancers we noticed overexpression of this protein. CA IX was expressed in 89 cases (80.2%) while 22 cases (19.8%) were negative. Saarnio et al [35], first observed CA IX expression in CRC tissue with the similar results (76% of CRC were positive for CA IX). Other investigators [36–38] also noticed high expression of this protein in CRC tissue. However, Saka et al [39] demonstrated that only 15.6% of CRC cases showed CA IX immunoreactivity. This discrepancy may be explained by the variability of the population size, antibodies and standardization of methods.

In the present study we observed CA IX membrane localization in 70.3% of the cases. CA IX is a transmembrane protein whose short part interferes with cytoplasm. Therefore we also assessed combined membrane and cytoplasmic localization. Only 9.9% of cases showed a combined localization of CA IX expression. Goethals et al [40] demonstrated CA IX membrane staining in CRC and tumors strongly positive for this protein had slightly cytoplasmic staining. Saka et al [39] also observed CA IX membranous staining in 89.3% of CRC with/without accompanying cytoplasmic reaction.

Most of our CRC cases showed strong CA IX immunoreaction intensity (58.6%) and the remaining cases indicated moderate (17.1%) or weak (4.5%) intensity. Saarnio et al [35] noticed weak immunoreaction intensity in 29% of cases and moderate to strong intensity in 47% of cases. Hong et al [37] observed that CA IX expression was weakly positive in 3.2% of CRC cases, moderately positive in 35.5% of cases and strong in 41.9% of cases. According to our results and the data of literature, the intensity of CA IX expression is mainly strong, which may be related to the high accumulation of this protein in CRC cells.

In our analysis, we found no significant differences between CA IX expression and clinico-morphological parameters (patient age, sex, histological grading, pathological staging, lesion site, lymph node status, vascular invasion). Our results are relatively consistent with other studies [39,41] that observed no significant correlation between CA IX expression and clinic-morphological characteristics of CRC patients (age, sex, tumor localization, stage, grade, lymph node status, angiolymphatic invasion). Cleven et al [36] found significant correlation between CA IX expression and TNM stage, with reduced expression in the more advanced tumors. Ho and Coomber [42] assumed that age was significantly associated with high CA IX expression (p = 0.012; ≥ 70 vs. < 70). On the other hand, they noticed no significant associations with CA IX expression and sex, histological grade, TNM stage, or primary tumor size in CRC patients. Rasheed et al [38] demonstrated a significant association between lymph node-negative tumors and CA IX positivity. In our investigation we showed only a trend of correlation between lymph node status and percentage of CA IX positive cells. In general, these results are considerably heterogeneous in literature that can be related to different methodology and grading systems used in the studies [43].

Further, we evaluated the expression of pro-inflammatory enzyme

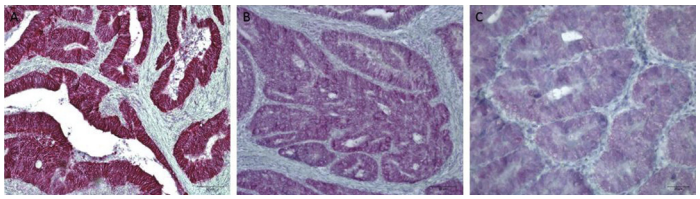

Fig. 1. CA IX immunohistochemical expression in CRC cells. The membrane positivity with strong (A), moderate (B) and weak (C) intensity.

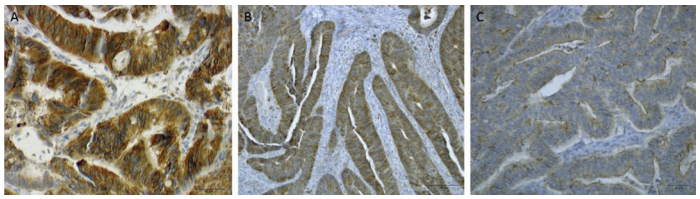

Fig. 2. Immunohistochemical demonstration of COX-2 expression in CRC cells. The cytoplasmic subcellular localization with strong (A), moderate (B) and weak (C) intensity.

COX-2 which is closely involved in the carcinogenesis process and is overexpressed in adenocarcinomas in contrast with non-cancerous mucosal regions [44]. In general, it has been reported that 80–90% of CRCs overexpress COX-2 [45]. In our series of 111 colorectal carcinoma, 94 cases (84.7%) were positive for COX-2 expression. Ogino et al [46] demonstrated that 83% of CRC cases were positive for COX-2 and 17% were negative. Mahmoud et al [44] revealed that COX-2 was positive in 77% of CRC cases and negative in normal colon tissue. Wu and Sun [47] showed that COX-2 is expressed in 77.97% of CRC.

We observed only the cytoplasmic localization of COX-2 protein. In most cases, immunoreaction intensity was strong (47.8%), but we also noticed moderate (19.8%) and weak intensity (17.1%) of COX-2 expression. Yamac et al [48] revealed that 37.4% of CRC patients had weak and moderate intensity of COX-2 expression and Wu et al [49] observed weak staining of COX-2 in 24.1% of CRC cases.

In our investigation, the tumor stage significantly associated with the percentage of COX-2 positive cells ($p \leq 0.05$). Other clinico-morphological data (patient age, sex, histological grading, pathological staging, lymph node status, vascular invasion) did not significantly associate with the parameters of COX-2 expression. Positive expression of COX-2 was more common among advanced-stage tumors than in early-stage tumors. This is in agreement with a study by Lim et al [50], who showed that COX2 expression was correlated with the depth of invasion and advanced tumor stage. Al-Maghrabi et al [51] also noticed significant correlation between COX-2 expression and tumor stage and observed no significant correlation between COX-2 expression and age, sex, tumor location and grade. Similarly, Elzagheid et al [52] confirmed significant association of COX-2 expression with tumor stage and they assumed COX-2 to be a prognostic factor in their cohort of patients with CRC. These findings support that COX2 plays an important role in advanced CRC.

Kaidi et al [29] concluded that under hypoxic conditions, COX-2 expression increases in CRC and is accompanied by increased levels of PGE_2. Previous studies linked this prostaglandin to the abnormal activation of the MAPK/ERK pathway [30]. Kopacek et al [19] showed the MAPK cascade to be involved in the regulation of CA IX expression. Sansone et al [6] demonstrated that upon hypoxia exposure, COX-2 upregulates the expression of the gene CA-IX, throughout the PGE_2-

Table 2
Association between CA IX expression and clinico-morphological parameters of CRC.

CA IX expression	A	Subcellular localization		Intensity of immunoreaction			Percentage of positive cells		
		M	MC	+	+ +	+ + +	11 – 25%	26 – 50%	> 50%
Age (n = 111)									
≤50	1	4	1	1	0	4	0	1	4
51-60	5	18	1	2	2	15	6	2	11
61-70	7	28	5	1	10	22	5	5	23
71-80	7	19	3	1	7	14	3	8	11
> 80	2	9	1	0	0	10	0	1	9
p-Value		0.978		0.395			0.371		
Sex (n = 111)									
Female	9	27	3	2	6	22	6	3	21
Male	13	51	8	3	13	43	8	14	37
p-Value		0.730		0.913			0.392		
Grade (n = 106)									
G1	10	23	1	1	5	18	6	4	14
G2	12	40	8	3	10	35	7	10	31
G3	0	6	1	0	2	5	1	1	5
G4	0	5	0	0	0	5	0	2	3
p-Value		0.247		0.551			0.482		
Stage (n = 61)									
I	2	8	2	1	1	8	3	1	6
II	5	16	2	1	5	12	1	4	13
III	1	20	3	0	5	18	3	7	13
IV	0	2	0	0	1	1	1	0	1
p-Value		0.620		0.551			0.329		
Lesion site (n = 111)									
Proximal site	9	33	8	1	11	29	5	6	30
Distal site	13	45	3	4	8	36	9	11	28
p-Value		0.150		0.442			0.504		
Lymph node(n = 65)									
Positive	1	20	3	0	6	17	4	7	12
Negative	10	27	4	2	6	23	4	5	22
p-Value		0.111		0.100			0.084		
Vascular invasion(n = 59)									
Positive	3	14	2	0	0	16	3	5	8
Negative	7	28	5	0	0	33	5	6	22
p-Value		0.957		0.688			0.698		

A, absent; M, membrane; MC, combined membrane and cytoplasmic; +, weak intensity; + +, moderate intensity; + + + strong intensity.

Table 3
Association between COX-2 expression and clinico-morphological parameters of CRC.

COX-2 expression	A	Intensity of immunoreaction			Percentage of positive cells		
		+	++	+++	11 – 25%	26 – 50%	> 50%
Age (n = 111)							
≤50	0	3	1	2	0	1	5
51-60	5	5	4	10	1	4	14
61-70	5	5	12	18	1	7	27
71-80	7	4	5	13	1	9	12
> 80	0	2	0	10	0	3	9
p-Value		0.114			0.564		
Sex (n = 111)							
Female	5	8	7	19	2	7	25
Male	12	11	15	34	1	17	42
p-Value		0.857			0.559		
Grade (n = 106)							
G1	10	3	5	16	0	4	20
G2	6	11	14	29	3	17	34
G3	1	1	1	4	0	2	4
G4	0	2	0	3	0	1	4
p-Value		0.234			0.245		
Stage (n = 61)							
I	2	2	3	5	0	2	8
II	1	4	7	11	0	9	13
III	2	6	3	13	0	7	15
IV	0	1	1	0	1	0	1
p-Value		0.693			**0.0001**		
Lesion site (n = 111)							
Proximal site	8	8	8	26	1	8	33
Distal site	9	11	14	27	2	16	34
p-Value		0.775			0.572		
Lymph node (n = 65)							
Positive	2	5	4	13	1	7	14
Negative	5	9	10	17	1	11	24
p-Value		0.761			0.943		
Vascular invasion (n = 59)							
Positive	2	4	5	8	1	6	10
Negative	4	9	9	18	1	11	24
p-Value		0.989			0.921		

A, absent; +, weak intensity; ++, moderate intensity; +++ strong intensity.

Table 4
Correlation between expression of CA IX and COX-2 proteins.

	CA IX	COX-2
Negative cases	22 (19.8%)	17 (15.3%)
Positive cases	89 (80.2%)	94 (84.7%)
p-Value	**2.12E–11**	
Intensity of immunoreaction		
+	5 (4.5%)	19 (17.1%)
++	19 (17.1%)	22 (19.8%)
+++	65 (58.6%)	53 (47.8%)
p-Value	**2.88E–26**	
Percentage of positive cells		
11 – 25%	14 (12.6%)	3 (2.7%)
26 – 50%	17 (15.3%)	24 (21.6%)
> 50%	58 (52.3%)	67 (60.4%)
p-Value	**3.03E–24**	

dependent activation of the MAPK/ERK pathway. In concordance with results of Sansone et al [6] we also confirmed a positive significant correlation between CA IX and COX-2 expression in CRC tissue using immunohistochemical analysis. Separately, we assessed intensity and percentage of CA IX and COX-2 positive cells, we found out statistically

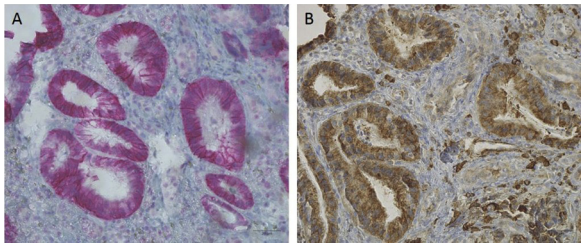

Fig. 3. Moderate membrane CA IX positivity (A) and moderate cytoplasmic COX-2 positivity (B) in CRC cells from identical case in the same site.

significant results. Sansone et al [6] also showed that expression of these proteins in CRC specimens is correlated and increases with tumor stage. In our study, stage was significantly correlated with COX-2 expression. In addition, our patient sample set contained more cases of CRC and we observed also lymph node status and vascular invasion compared with Sansone et al [6]. We did not confirm any significant associations but there was a tendency to correlation between lymph node status and percentage of CA IX positive cells.

Moreover, the potential of CA IX and COX-2 also plays an essential role as a therapeutic target for specific antibodies or inhibitors of their enzymatic activity. COX-2 inhibition with selective COX-2 inhibitors enhances tumor response to radiation and chemotherapeutic agents [53]. Selective inhibitors (celecoxib) and nonspecific inhibitors (aspirin) of COX-2 reduce CRC incidence [54]. COX-2 is also target of nonsteroidal anti-inflammatory drugs (NSAIDs) used for inflammation and pain [55]. Targeting CA IX is now considered as a relevant access for the development of new therapeutics against hypoxic tumors. Different approaches and new pharmacological agents for finding compounds that specifically target CA IX have been described in recent years [56]. Concurrently, the pharmacological down-regulation of CA IX and COX-2 may be a strategy for suppressing the aggressiveness of CRC cells [55].

5. Conclusion

In conclusion, in our study we detected overexpression of hypoxia-related protein CA IX and the pro-inflammatory enzyme COX-2 in CRC tissue. Our data are in line with the results of an only study, that there is a correlation between this proteins in CRC. These findings suggest that CA IX expression may be a marker of COX-2 activation as a result of the exposure of CRC cells to hypoxia. According to our results and review of the literature [6,9,57], CA IX/COX-2 interplay may generate aggressive CRC cell behaviour by promoting hypoxia survival and the invasion of tumor cells. Moreover, the understanding of the genes and pathways that control the earliest stages of CRC development as well as an understanding of the signals that indicate the metastatic phenotype can contribute to clinical management and provide information for developing drugs to control or prevent advanced disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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