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Research Progress of Carbonic Anhydrase in Colorectal Cancer

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Abstract: Carbonic anhydrase (CA) is an important group of proteins involved in the survival, invasion, and metastasis of solid cancers. It has been found that the CA family is closely related to the occurrence, diagnosis, treatment and prognosis of colorectal cancer, and CA research has become a potential direction for cancer treatment. Therefore, we reviewed the research progress of CA isoforms and their role in colorectal cancer.

Keywords: Carbonic anhydrase; Colorectal cancer; Occurrence; Diagnosis; Treatment; Prognosis

Colorectal cancer (CRC) is a malignant tumor of the digestive system that originates from the mucosal epithelium of the colorectum and ranks third in the global incidence of malignant tumors, and according to 2020 data, the death rate of colorectal cancer is the second highest among malignant tumors worldwide. In China, in recent years, the incidence rates of gastric, liver and esophageal cancers have gradually decreased, but the incidence rate of colorectal cancer in the population has increased [1]. As the world population increases, the incidence of colorectal cancer is expected to increase by 60%, with more than 2.2 million new cases and 1.1 million deaths by 2030 [2].

CA is a family of zinc-containing enzymes that exists in a variety of isomers and plays an important role in maintaining the stability of the internal environment. The primary function of CA is to regulate cellular pH by hydrating carbon dioxide (CO^2) to protons (H^+) and bicarbonate ions ($HCO3^-$). In addition, CA is involved in many physiological and pathological processes, including gluconeogenesis, lipogenesis, and iron metabolism [3]. Currently, several studies have found that CA families are closely related to colorectal carcinogenesis, diagnosis, treatment, and prognosis, and CA research has become a potential direction for tumor therapy. In this paper, the research results related to CA isoforms in colorectal cancer are analyzed and summarized.

1. Biological characteristics of the CA family

A characteristic feature of solid cancers is hypoxia, and the metabolic changes induced by hypoxia can promote activities associated with aggressive tumor cell behavior, including survival, invasion, and metastasis. The carbonic anhydrase (CA) family is a group of proteins important for these processes. Human CAs differ in their amino acid sequences, enzymatic properties, tissue distribution and expression sites, and there are 16 different human CA isoforms, of which only 13 are enzymatically active (CA1, CA2, CA3, CA4, CA5A, CA5B, CA6, CA7, CA9, CA12, CA13, CA14 and CA15). In contrast, the three carbonic anhydrase-related proteins CA8, CA10, and CA11 were not enzymatically active. Based on the subcellular localization of CAs, eight are cytoplasmic, including CA1, CA2, CA3, CA7, CA8, CA10, CA11, and CA13, CA5A and CA5B are mitochondrial, and four are membrane-bound, including CA4, CA9, CA12, and CA15, with CA6 being the only secreted isoform of a human CA family member. CA9 and CA12 have no enzyme activity, except for their roles in pH regulation, they also play important roles in chemotherapy resistance, tumor cell migration, cell adhesion, and tumorigenesis and growth. Some studies have found dysregulation of local immune responses and loss of effective anticancer mechanisms mediated by CA in CRC tissues. In this paper, we summarize the research results related to colorectal cancer and some of the CA mentioned above.

2. The CA family and colorectal carcinogenesis

2.1 CA1 and CA2 downregulation is associated with CRC development

Research has indicated a link between the downregulation of CA1 and CA2 and the genesis of colorectal cancer (CRC). Decreased expression levels of CA1, CA2, and CA13 have been documented in CRC cases [4-6]. Our earlier investigation noted a significant reduction in CA1 expression across various stages of CRC when contrasted with normal mucosa [4]. The diminished expression of CA2 may contribute to the enhancement of stem cell-like characteristics in adenomatous cells within CRC [7], and this downregulation is also correlated with colon

cancer progression [5]. Notably, among all cancer samples, the downregulation of CA13 was most markedly observed in CRC [6].

2.2 CA6 variants are associated with CRC development

CA6 is a regulator of innate immunity, and variant CA6 may lead to immune system disorders and associated oncogenic mechanisms, and therefore may be associated with the risk of CRC development, and researchers have hypothesized based on the current findings that variant CA6 plays a pathological role in CRC aetiology, and that CA6 regulation of innate immunity is critical for CRC development and progression [8]. Genetic variation in CA6 is a modifiers of CRC susceptibility, Jeong-Hwa Choi et al. found that the bitter taste receptors T2R38 and CA6 are involved in colorectal carcinogenesis and that their genetic variants are potential biomarkers of gastrointestinal tract function [8].

2.3 CA9 upregulation is associated with CRC development

CA9 is a hypoxia-inducible membrane protein that plays a key role in the regulation of pH in the tumor environment, and acidification of the tumor microenvironment promotes tumor progression through a variety of processes, including decreased intercellular adhesion, increased migration, and stromal invasion [9], and CA9 upregulation was associated with the development of CRC, and CA9 was positively correlated with the extent of colorectal cancer lesions. Several CA9 studies have shown that CA9 co-localizes with phosphorylated Ezrin (EZR) and activates the hypoxia-autophagy-EZR pathway in primary CRC tissues and that this pathway has been shown to be a novel regulatory mechanism for CRC progression [11]; in addition, part of the mechanism by which CA9 is involved in the carcinogenesis of CRC may be the CA9/COX-2 (cyclooxygenase 2) interaction: the interaction between cellular The hypoxic and mildly hypoxic environments generated by cell overgrowth trigger an increase in the expression of the COX-2/CA9 gene, which promotes hypoxic survival and invasive behavior of tumor cells and enhances the malignant invasive potential of CRC cells [12].

3. CA families and colorectal cancer diagnosis

As more research is conducted, additional members of the CA family have been found to be associated with the diagnosis of colorectal cancer (CRC), such as CA2 and CA9. CA2 has been identified as a metastasis-related gene in CRC. Proteomic analysis has identified CA2 as a potential biomarker [13, 14], which may be used for early diagnosis of CRC and could also serve as a marker for high-risk adenomas in CRC [7]. Additionally, research by Francis Yew Fu Tieng and colleagues has found that CA9 could serve as a potential serum marker for screening of both primary and metastatic colorectal cancer, acting as a potential early diagnostic test for CRC [15]. It is considered a specific marker for hypoxic CRC diagnosis [16]. Serum CA9 levels measured by ELISA may become a possible tool for diagnosing CA9 expression in CRC patients [16]. Xiao-Syun Guan's research suggests that a novel indium-111 labeled dual-CA9 targeted probe could serve as a potential single-photon emission computed tomography (SPECT) imaging radiotracer (nuclear imaging agent) for detecting hypoxic colorectal cancer cells. This would allow doctors to determine the degree of tumor hypoxia before treatment and monitor the progression of CRC in patients post-surgery or treatment. Moreover, the dual-CA9 targeted radiotracer has demonstrated high serum stability, high surface binding, and high affinity in vitro [16]. It may serve as a nuclear imaging probe for in vivo detection of CRC, adding more methods to the diagnosis of colorectal cancer.

4. The CA family is involved in colorectal cancer treatment

4.1 CA family inhibits CRC cell proliferation

Research indicates that CA2 exerts a significant inhibitory effect on the proliferation of CRC cells by inducing cell cycle arrest at the G0/G1 and G2 phases in the mature SW480 CRC cell line [7]. Researchers have recently discovered that CA2 inhibits the proliferation of Caco-2 colon cancer cells in a concentration-dependent manner, with a composite action pattern mediated by the apoptosis mechanism [2]. CA2 can also serve as a therapeutic target for metastasis [13]. The in vivo and in vitro research results by Rui Zhou and colleagues demonstrate that CA2 acts as a tumor suppressor gene and inhibits the growth and development of colorectal cancer, exhibiting significant anti-tumor activity against CRC. The inhibition mechanism is attributed to the cell cycle arrest of SW480 by CA2 at the G0/G1 and G2 phases, leading to a marked reduction in the S phase [14].

CA4 is a tumor suppressor in colon cancer, Jing wan zhang et al. demonstrated that CA4 is readily expressed in normal human colon tissues but is frequently silenced in CRC cell lines and primary CRC tumor tissues, and that high expression of CA4 inhibits cell proliferation in the G1 phase, induces apoptosis and cell cycle arrest, and inhibits colon cancer cells by modulating key EMT regulators migration and invasive ability [5].

CA9 is overexpressed in most hypoxic tumors, including CRC, and it has been found that potent CA9 inhibitors can block CA9 activation and thus inhibit tumor growth [16], and can be used as targeted drugs for the treatment of hypoxic, aggressive solid tumors. Novel synthetic sulfonamide derivatives (H-4i) and OX27 are potent CA9 inhibitors. H-4i can arrest the cell cycle in G2/M phase and induce apoptosis in colorectal cancer cells by activating oxidative stress and weakening antioxidant defenses, which can lead to high ROS production, cell cycle arrest, and cytotoxicity [17]; in addition, OX27 treatment can reduce CA9 expression, induce apoptosis and ROS production, and inhibits colo-

ny formation and migration of colon cancer cells [18].

4.2 CA8 downregulation inhibits CRC invasion and metastasis

CA8 is a carcinoembryonic antigen, and down-regulation of CA8 can inhibit CRC. Nishikata et al. found that overexpression of CA8 in human LOVO colorectal cancer cells led to an increase in proliferation and invasion of the cancer cells. Up-regulation of CA8 expression in the SW480 cell line increased vascular endothelial growth factor (VEGF) and decreased miRNA16-5p expression, which promotes the proliferation, metastasis and vasculogenesis of tumor cells. cell proliferation, metastasis and angiogenesis, and also attenuates the sensitivity of radiotherapy and chemotherapy. In addition, in a xenograft tumor angiogenesis model, decreased CA8 expression significantly reduced tumor growth and tumor-associated angiogenesis. Targeted modulation of the CA8/miRNA16-5p pathway may therefore provide a strategy to block colorectal cancer metastasis [19].

4.3 CA9 downregulation improves the efficacy of radiotherapy for CRC

Silencing of CA9 expression can improve the efficacy of radiotherapy, and down-regulation can also inhibit CRC. Jérome Doyen used shRNA silencing of CA9 expression in the human colorectal cell line LS174Tr in vitro in conjunction with radiotherapy and found that the cell death rate was increased by 50% to 70%, while in vivo silencing of CA9 expression in conjunction with radiotherapy significantly inhibited the growth of transplanted tumors in nude mice. Further studies found that silencing CA9 gene expression was found to improve the efficacy of radiotherapy in both in vivo and in vitro experiments by decreasing the number of cells in the S phase of cytokinesis and decreasing intracellular pH regulation [20].

5. The CA family and colorectal cancer survival prognosis

5.1 CA2 and CA7 downregulation is associated with poor prognosis in CRC

Some scholars have found that colorectal cancer tissues show down-regulation of CA2 protein expression and are associated with cancer aggressiveness [14], in addition, bioinformatics-based studies have shown that CA7 is an important repressor gene for the classification of normal and CRC tissues. Guang-Zhen Yang et al. found that the expression of CA7 was frequently down-regulated in CRC tissues at both the mRNA and protein levels, and the CA7 Decreased CA7 expression was significantly associated with poor differentiation, positive lymph node metastasis, advanced TNM staging, and poor clinical outcomes, and further staging-based survival analyses showed that decreased CA7 expression significantly predicted poor disease-specific survival (DSS) and was an independent poor prognostic indicator for patients with early-stage tumors [21].

5.2 CA9, CA8, and CA12 upregulation is associated with poor prognosis in CRC

CA9 may be an independent prognostic factor for CRC, and the higher the expression of CA9, the worse the prognosis. overexpression of CA8 leads to increased proliferation and invasion of colorectal cancer cells, and has a worse prognosis [22]. Some researchers even suggested that CA9 negativity may indicate negative colorectal cancer as an independent negative prognostic marker [23], and high CA9 expression has been shown to correlate with perineural infiltration, which is a marker of tumor metastasis and infiltration, and an indicator of poor prognosis in CRC [13, 24]. In addition, CA9/CA12 co-localization and their reduced expression or protein inhibition would hinder the survival of CRC cells [25], and we have made a reasonable guess that CA12 up-regulation is associated with poor prognosis of CRC in light of the current state of research.

6. Summary

In summary, the carbonic anhydrase family is closely related to the occurrence, diagnosis, treatment and prognosis of colorectal cancer. The study of carbonic anhydrase and colorectal cancer has important theoretical significance and clinical value for exploring the tumor pathogenesis, targeted therapy, and improving the anti-cancer effect, etc. It is expected that the relevant results will be further applied in the diagnosis and treatment of colorectal cancer, so as to benefit many colorectal cancer patients.

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