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REVIEW ARTICLE

HIF-1α in colorectal carcinoma: review of the literature

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Summary

Colorectal cancer (CRC) is the third most common cancer worldwide and despite the abundance of molecular pathways and markers continually being reported, the mortality rates remain high. Hypoxia inducible factor 1alpha (HIF-1a) plays a major role in the response of tumors to hypoxia, and contributes to tumor aggressiveness, invasiveness and resistance to radiotherapy and chemotherapy. Targeting HIF-1a is an attractive strategy, with the potential for disrupting multiple pathways crucial for tumor growth.

In the current study, HIF-1a immunohistochemical expression in CRC is reviewed along with the relation to clinical outcome and prognosis. In addition, the significant correlation of HIF-1a to vascular endothelial growth factor (VEGF) expression is reported, as well as the possible role of HIF-1a in predicting the therapeutic response to anti-EGFR therapies.

Herein, an overview of the HIF-1a expression in CRC is presented. This review delineates the crucial role that HIF-1a plays in carcinogenesis, tumor angiogenesis and cancer progression. The evaluation of HIF-1a in patient biopsies could be useful as a prognostic and/or predictive biomarker in personalized cancer treatment.

Key words: *angiogenesis, colorectal carcinoma, HIF-1a, immunohistochemistry, prognosis*

Introduction

CRC is the third most common cancer, and is highly rated among factors resulting to cancer patient mortality [1]. CRC is the collective description of related diseases, emerging through the accumulation of aberrations leading to genomic - chromosomal instability, microsatellite instability (MSI), CpG island methylator phenotype, as well as genomic mutations of tumor suppressor genes and tumor oncogenes, microRNAs, and epigenetic changes [2,3]. As cancer progresses, invasion and metastases are established through the epithelial-mesenchymal transition with additional genetic alterations [2-4]. The subcellular molecular events which characterize these pathways have been exploited clinically in the diagnosis, screening and management of CRC and emerging biomarkers for early disease detection and risk stratification (diagnostic markers), prognosis (prognostic markers) and the prediction of treatment responses (predictive markers) have been developed [5].

This review outlines the current evidence on the role of Hypoxia-Inducible Factor 1 (HIF-1) and especially its subunit HIF-1α in CRC. Histopathological evidence of HIF-1α expression is presented along with the possible involvement in carcinogenesis, prognosis and tumor progression. In addition, the correlation of HIF-1α expression to the prediction of treatment response is also reviewed.

Tumor hypoxia

The fast proliferation of cancer cells in a solid tumor can outgrow the supply of nutrients and oxygen provided by the tumor poorly formed vas-

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culature. This results to the development of a hypoxic tumor microenvironment, which could be expected to curtail further tumor growth. However, hypoxic tumors have been shown to be more resistant to radiotherapy and chemotherapy and are associated with poorer prognosis [6]. Adaptation of cancer cells to hypoxia allows evasion of apoptosis or necrosis, sustained proliferation and increased migratory behavior. Moreover, hypoxia triggers metabolic reprogramming that causes tumor acidosis and stimulation of angiogenesis that allows sustained oxygenation and facilitates metastasis [7]. Direct detection of intratumoral hypoxia is possible by measuring pO2 with the help of oxygen electrodes but is invasive and limited by the clinical settings. An indirect but easier and increasingly used way is to monitor for the expression of hypoxia-inducible endogenous marker proteins. The most important of these are the α subunits of the hypoxia-inducible factors (HIFs). HIFs are heterodimeric transcription factors regulating the expression of hundreds of genes that respond to low oxygen levels [8,9]. The regulatory HIF-α subunit is often overexpressed in cancer cells either because of the ensuing tumor hypoxia or because of genetic changes occurring during malignant transformation. HIFs mediate the transcriptional program causing the aforementioned adaptive behavior of cells inside hypoxic tumors and, as expected, HIF-α overexpression is associated with increased tumor aggressiveness and higher patient mortality [10]. Both *in vitro* and *in vivo* studies have shown that targeting HIFs, especially in combination with traditional of antiangiogenic treatments, can be an effective way to restrict cancer cell and tumor growth [11,12].

HIF-1α

Three forms of HIFs are known: HIF-1, HIF-2 and HIF-3. They contain distinct HIF-α isoforms (HIF-1α, HIF-2α and HIF-3α, respectively) and a common beta subunit (HIF-1β or ARNT), which is constitutively expressed. In contrast, protein expression levels of the HIF-alpha subunits are largely regulated by oxygen levels. HIF-1α is expressed in all tissues under hypoxia and is the best-studied isoform and prototype of the family. Expression of HIF-2α is more tissue-specific and its function can overlap or be distinct from that of HIF-1α [13]. HIF-3α is poorly characterized.

According to the currently accepted model, HIF-1α is undetectable under physiological conditions because, although it is produced constantly, it is very rapidly and efficiently degraded by the proteasome. This process requires hydroxylation of two proline residues in the oxygen-dependent degradation domain (ODDD) of HIF-1α by a family of prolyl-hydroxylases (PHDs) that use molecular oxygen as substrate and also require 2-oxoglutarate, ascorbate (vitamin C) and iron for activity. These hydroxylations mediate interaction of HIF-1α with the von Hippel-Lindau (pVHL) tumor-suppressor protein, a component of an ubiquitin ligase complex [14,15]. As a result, HIF-1α becomes polyubiquitylated and is targeted to the proteasome for proteolysis. Under hypoxia, lack of oxygen causes inactivation of the PHDs and, consequently, HIF-1α is stabilized and accumulates inside the cell, translocates inside the nucleus and associates with HIF-1β to form an active HIF-1 complex that recognizes and binds to hypoxia-response DNA elements (HREs), thereby inducing transcription of hypoxia-target genes. The genes activated by HIF-1 include genes coding for proteins involved in the uptake and metabolism of glucose (such as GLUT-1 and glycolytic enzymes), erythropoiesis (e.g. erythropoietin), angiogenesis (e.g. VEGF), regulation of extracellular pH (e.g. CA IX) and many other processes that contribute to cancer cell proliferation, invasion and metastasis.

Apart of HIF-1α stabilization, oxygen also represses its transcriptional activity through an asparaginyl hydroxylase, called factor inhibiting HIF-1 (FIH), which modifies the C-terminal domain of HIF-1α [15]. Inhibitors of PHDs and FIH, such as 2-oxoglutarate analogs, oxidants that deplete ascorbate, heavy metals and iron chelators can stabilize HIF-1α and, therefore, imitate hypoxia by triggering a partially similar transcriptional response (called pseudohypoxia). Finally, HIF-1α expression and activity can be also upregulated by oxygen-independent mechanisms activated by oncogenes (such as EGFR, RAS and BRAF) or growth factors that stimulate the MAPK, mTOR and PI-3K/Akt pathways and by the lack of tumor suppressors such as VHL and PTEN [12,13].

HIF-1α in CRC

The literature for HIF-1α expression in CRC was systematically reviewed, using the terms "hypoxia inducible factor AND colorectal carcinoma" OR "HIF AND colorectal carcinoma" from 2003 to 2014. The inclusion criteria were: a) evaluation of colorectal cancer prognosis based on HIF-1α expression and b) application of immunohistochemistry. The main details of the studies focusing on HIF-1α in CRC are demonstrated in Table 1.

Table 1. Presentation of the studies regarding colorectal carcinoma that assessed HIF-1 alpha in relation to prognosis

First author (year) [Ref]	*Sample size/type*	*HIF-1alpha related remarks*	*Significant association with adverse outcomes*
Pez et al. 2001[52]	LS174Tr, Hct116, HT29/ human colon cancer cell lines BALB/c nude mice	LOX and HIF-1a act synergistically to promote tumorigenesis.	Yes
Jiang et al. 2003[18]	71 colorectal neoplasms (9 cases of colorectal adenoma and 62 CRC)	In CRC, decreased levels of PTEN are associated with increased expression of HIF-1a mRNA and VEGF protein.	Yes
Krishnamachary et al. 2003[23]	HCT-116 cells	HIF-1α overexpression is related to increased transcription of genes (Vimentin K14/k18/K19, Fibronectin 1, MMP2, uPAR, Cadhesin D, AMF), the protein products of which contribute to basement membrane invasion.	Yes
Koukourakis et al. 2005[62]	75/ CRCs	LDH5 expression is significantly associated with HIF-1 α and HIF-2α accumulation, as well as with high tumor grade and metastatic tumor behavior.	Yes
Kaidi et al. 2006[49]	HCT116 (human colon cancer cells), SW480, HEK293 (human embryonic kidney cells)	β-catenin interacts with HIF-1a and is significant contributor to colorectal tumorigenesis.	Yes
Wincewicz et al. 2007[50]	123/CRCs	Non-mucinous CRCs expressed HIF-1a and GLUT-1 with a greater frequency than mucinous CRCs.	Yes
Van der Bilt et al. 2007[54]	BALB*/c mice *Laboratory bred strain of albino mice.	Prolonged tissue hypoxia and thus stabilization of HIF-1a plays a crucial role in the altered behavior of micrometastases in the liver after ischemia/reperfusion.	Yes
Furlan et al. 2008[60]	78/CRCs	Significantly high levels of HIF-1α mRNA were observed in 33% of CRCs, while overexpression of HIF-1α protein was detected in 77% of the tumors.	Yes
Rajaganeshan et al. 2008[61]	52/CRCs	HIF-1α expression is significantly related to poor prognosis and overall survival. VEGF was also a crucial predictor of disease recurrence in primary CRCs.	Yes
Fan et al. 2008[21]	LS174T cell cultures	HIF-1α and survivin are significantly expressed in invasive CRCs.	Yes
Roberts et al. 2009[65]	HCT116 cells (DN)	HIF-1a has an important role in inhibiting oxaliplatin sensitivity in HCT116 cells. In monolayers, oxaliplatin was less effective in hypoxic relatively to aerobic cells.	Yes
Rajaganeshan et al. 2009[58]	55/ Metastatic CRCs	Positive connection is observed between HIF-1α and VEGF, and HIF-1α and VHL in primary CRC. No correlation is observed between HIF-1α and either Glut-1 or CA-9.	Yes
Sulkowska et al. 2009[59]	108/CRCs	HIF-1α is detected in 85% (92/108) of CRCs and is coexpressed with GLUT-1 and TGF-beta 1.	Yes
Chen et al. 2009[66]	2984/CRCs (metanalysis)	Overexpression of HIF-1α and HIF-2α are strongly associated with poor prognosis in CRC.	Yes
Baba et al. 2010[68]	731/CRCs (metaanalysis)	HIF-1α not only stimulates angiogenesis by upregulating multiple proangiogenic factors, including VEGF, but also promotes colon cancer cell invasion by regulating proteins such as MMP2, cathepsin D, vimentin, TGFA and PTGS2.	Yes
Zhao et al. 2010[53]	LS174T, RKO, SW1116, SW620/CRC cell lines	Galectin-1, a direct target of HIF-1a, mediates the hypoxia-induced migration and progression of CRC.	Yes
Shioya et al. 2011[64]	50/ Rectal adenocarcinoma	HIF-1α expression is associated with poor prognosis after hyperthermo-chemoradiotherapy (HCRT) for rectal cancer.	Yes
Rigopoulos et al. 2010 [47]	60 paraffin embedded primary CRC	Significant association between VEGF and HIF-1α . Deregulation of EGFR/VEGF/HIF-1α pathway in CRC	Yes
Murono et al. 2012[63]	HT-29, SW480/ Human colorectal cancer cells	SN-38 inhibits the expression of HIF-1α in cancer cells and can overcome chemoresistance under hypoxic conditions of colon cancer cells.	Yes
Shimomura et al. 2013[57]	64/CRLM (resection of colorectal liver metastasis)	Mutant PIK3CA induces the expression of HIF-1α. Significant expression of HIF-1α and VEGF is observed in liver metastasis as well as in the primary tumor. Overexpression of HIF-1a is an independent risk factor for recurrence.	Yes
Zhang et al. 2014[56]	Cells with/ without DFX treatment.	DFX induces HIF-1α expression. The latter induces epithelial-mesenchymal transition (EMT) in solid tumors.	Yes

The role of HIF-1α in colorectal carcinogenesis

The development of CRC is a complex and heterogeneous process arising from an interaction between multiple etiological factors, including genetic factors and environmental factors, such as diet and lifestyle. Over the last 25 years, remarkable progress has been made in understanding its biological and molecular features and in elucidating the steps involved in colon carcinogenesis. This, in turn, has led to improved treatment of CRC. While colorectal adenoma is the most frequent precancerous lesion, other potentially premalignant conditions, including chronic inflammatory bowel diseases and hereditary syndromes, such as familial adenomatous polyposis, Peutz-Jeghers syndrome and juvenile polyposis, are differentially localized along the gastrointestinal tract with an overall incidence of less than 5% [16]. Recently, significant progress has been made in the characterization of genetic and epigenetic alterations in CRC genomes in support of the genomic view of colorectal carcinogenesis. Like other types of human solid tumors, CRC exhibits a variety of genomic alterations ranging from small-scale changes (i.e., point mutations or small deletions) to large-scale chromosomal copy number changes or rearrangements [17]. Some of these alterations may contribute to the development of colorectal carcinogenesis, but the entity of causal genomic alterations in CRC genomes is still to be discovered.

Overexpression of HIF-1a in CRC

HIF-1α (mRNA and/or protein) is detected in both adenomas and colorectal adenocarcinomas, and is more frequently expressed in adenocarcinomas compared to adenomas, as shown in a number of immunohistochemical studies [18-20]. HIF-1α expression is also frequently correlated with disease stage, as shown in relative recent studies [19,21-23].

Accordingly, 66.7% of CRC in comparison to 12.25% of colorectal adenoma tissue microarrays were found positive for the expression of HIF-1α by immunohistochemistry. Moreover, the expression HIF-1α was significantly higher in patients with stage III than in patients with stage I – II CRC [21].

Moreover, Jiang et al. studied the expression of HIF-1α mRNA and vascular endothelial growth factor (VEGF) protein, by *in situ* hybridization and immunohistochemistry respectively, in 71 cases of colorectal neoplasms (9 cases of colorectal adenoma and 62 cases of CRC) [18]. They showed a significant increase of HIF-1α mRNA in adenocarcinomas and significant differences in HIF-1α mRNA and VEGF expression between adenomas and CRC. The levels of HIF-1 were positively correlated with VEGF expression. There was also a significant difference in the expression of both HIF-1α and VEGF in accordance with Dukes stage, and the level of HIF-1α and VEGF expression was significantly higher in Dukes stages C and D than that of Dukes stages A or B [18]. In the same study HIF-1α and VEGF expression was significantly associated with decreased levels of the tumor suppressor PTEN, indicating the involvement of the PI3K/Akt/FRAP (FKBP rapamycin-associated protein) pathway in the upregulation of HIF-1α [18]. In addition, Beltaziak et al. studied the immunohistochemical expression of HIF-1α in 125 CRCs and showed significant correlations regarding its expression with tumor grade and stage [18].

Finally, Simiantonaki et al. systematically investigated the expression of HIF-1α in CRC development, by analyzing HIF-1α protein expression in normal colonic mucosa, hyperplastic polyps (HPP), sessile serrated adenomas (SSA), low-grade (TA-LGD) and high-grade (TA-HGD) traditional adenomas as well as in non-metastatic and metastatic CRC, by immunohistochemistry and Western blot [19]. They found that HIF-1α was not expressed in normal mucosa, HPP and TA-LGD, but showed perinuclear and nuclear accumulation in half of the examined SSA and TA-HGD, and nuclear overexpression in all investigated CRCs. Although the overexpression of nuclear HIF-1α in CRCs was significant compared to the premalignant lesions, there was no significant correlation of HIF-1α with the metastatic status [19].

The finding that HIF-1α is present in adenomas along with its significantly higher expression in CRC and correlation with disease stage, point to the fact that the expression of HIF-1α occurs in early stages of CRC and escalates with tumor progression in the invasive stage. Thus, HIF-1α overexpression in adenomas probably represents an early stage of carcinogenesis, prior to angiogenesis or invasion. In a different line of thought, the detection of HIF-1α in the surface epithelium of the colorectal mucosa [20,24] is suggestive of a possible role of HIF-1α in the physiology of normal colon tissue, apart from its role in CRC.

HIF-1α is also suspected to be involved in inflammatory events related to CRC, as suggested

by its periinflammatory expression, which could contribute indirectly to the acquisition of a metastatic phenotype. In support of this notion, the expression of HIF-1α is linked to inflammation not only in CRC specimens but also in *in vitro* studies, where it was shown that the proinflammatory lipopolysaccharide (LPS) induced HIF-1α expression and nuclear translocation in CRC cell lines [19]. Moreover, HIF-1α overexpression in colon carcinoma cell lines has been shown to increase cell invasion in a Matrigel assay [23].

Von Hippel Lindau and HIF-1a

An alternative mechanism that can lead to HIF-1α overexpression, besides hypoxia or upregulation of signal transduction pathways, is loss of Von Hippel Lindau (VHL) expression. The reduction or loss of VHL in later stages of CRC is accompanied with upregulation of HIF-1α [24]. Moreover, Kuwai et al. examined paraffin-embedded tumors and identified 13 mutations in the coding region of the VHL gene in 11.4% of the specimens. Immunohistochemistry of the specimens showed that tumors bearing 7 of the 13 VHL mutations were also characterized by high HIF-1α protein expression, suggesting the possible role of VHL loss in the upregulation of HIF-1α in CRC [25]. This suggestion is supported by an earlier study showing frequent allelic loss of VHL in CRCs but not in adenomas [26].

APC, beta-catenin and HIF-1a

Adenomatous polyposis coli (APC), a tumor suppressor involved in the regulation of beta-catenin, has been recently found to mutually antagonize HIF-1α. HIF-1α directly regulates APC mRNA and protein levels, while APC represses HIF-1α through a beta-catenin 1 and nuclear factor-kappa B - dependent mechanism [27]. This has the implication that downregulation of APC by HIF-1α further enhances the increased survival of tumor cells at hypoxic conditions and vice versa, and loss of function APC mutations promotes survival by inducing HIF-1α and thus, a hypoxic response [28]. Since APC is mutated in most CRCs, it could be an important factor in the progression of colorectal tumors even at the early stages [27-29].

As mentioned above, beta-catenin is yet another important factor in CRC pathogenesis that interacts with HIF-1α. Interestingly, the interplay between beta-catenin and HIF-1α in CRC is not restricted to its regulation by APC. It has also been shown earlier that beta-catenin interacts with HIF-1 α and enhances the transcription of HIF-1 target genes, and thus, the hypoxic response and tumor cell survival [30].

HIF-1α and angiogenesis in CRC

The function of HIF-1 is multifaceted and can be oncogenic or tumor-suppressive in a tumor type-specific fashion [31]. In CRC, HIF1-α appears to promote oncogenesis via distinct mechanisms. Among them, HIF-1 mediates tumor angiogenesis, a critical factor for the development and progression of CRC.

Angiogenesis in CRC has been shown to be induced by HIF-1α through the activation of expression of the HIF-1 target gene VEGF [32-35]. Indeed, the activation of the HIF-1α/VEGF pathway in CRC tissue specimens has been shown in a number of studies using immunohistochemistry [19,36-38]. Moreover, VEGF mRNA and protein expression levels correlate with vascularity and disease progression in CRC [32,33,39-42].

Importantly, angiogenesis as well as HIFs' expression are poor prognostic factors and have been associated to worse survival rates in CRC patients [41,43]. HIF-1 mediated VEGF expression increases survival of colon cancer cells in culture under hypoxic conditions, dependent on the expression of a functional VEGFR-2 (KDR receptor). Calvani et al. showed that colon cancer cells differentially express a functional VEGF/KDR/HIF-1α autocrine loop that mediates survival under hypoxic conditions [44]. These results show that: (a) VEGF mediates survival of hypoxic HCT116 colon cancer cells in a HIF-1α–dependent fashion; (b) colon cancer cells can differentially express a functional KDR receptor; and (c) the presence of a functional response to VEGF may be associated with the outcome of anti-VEGF therapies in colon cancer patients [44].

Furthermore, a positive correlation between VEGF and VEGFR-2 expression has been identified in biopsies of patients with CRC [36], while agents such as bevacizumab, that target VEGF and its receptors, are included in the repertoire of CRC treatment [45].

However, there is more to the role of HIF in CRC angiogenesis, than the direct activation of well-established angiogenic genes like VEGF. In a recent study performed in a CRC cell line, HIF-1α appeared to act in synergy with epidermal growth factor (EGF), for the upregulation of a distinctive subgroup of genes, potentially promoting angiogenesis [46]. Rigopoulos et al. have also demonstrated significant association between VEGF

and HIF-1α expression, as well as deregulation of EGFR/VEGF/HIF-1α signalling pathway in colon adenocarcinoma tissue microarrays [47]. In addition, Thomaidis et al. investigated the role of multiple biomarkers of the epidermal growth factor receptor (EGFR-) and VEGFR pathways on the treatment outcome in patients with stage II/III CRC [48]. They showed that an adjuvant therapy containing irinotecan might be beneficial for AREG/EREG ligands of EGFR-negative, PTEN-positive and HIF-1α-negative patients. The authors suggested further prospective studies including a large number of patients with stage II/III CRC in order to evaluate which molecular patterns might serve as predictive markers for treatment outcome in these patients [48].

HIF-1α and CRC prognosis

In a number of studies, HIF-1α expression has been shown to promote tumor progression by modulating the expression of genes associated with tumor growth and survival as well invasion, metastases and resistance to chemotherapy [18,19,24,49-66]. However, there is no consensus regarding HIF-1α expression and CRC prognosis, as a number of studies show relatively heterogeneous results. From a pathological point of view, this could be attributed, at least in part, to differences in the mode of HIF-1α histopathological evaluation and differences in the type of antibody used, influence of fixation delay and perioperative ischemia, as there are currently no standardized methods to evaluate HIF expression by immunohistochemistry [67]. The inconsistency could also be to some extent attributed to the relatively small number of patients examined in many studies.

Most importantly, a large study by Baba et al., examining 731 CRC specimens, demonstrated that HIF-1α overexpression was independently associated with poor prognosis [68]. These results indicate that HIF-1α expression correlates with aggressive biological behavior of CRC. In the same study, HIF-2α expression was unrelated with clinical outcome and showed no significant prognostic role. Considering that HIF and related pathways are attractive therapeutic targets and that HIFs interact with many other pathways, these findings may have considerable clinical implications [68].

Valuable information on the association between HIF and CRC prognosis can be deduced from a meta-analysis by Chen et al. [66]. The authors combined the outcomes of 23 studies comprising 2984 CRC patients, in order to examine the association between HIF-1α and HIF-2α expression, CRC prognosis and clinicopathological features. The results indicated significant association of HIF overexpression with increased mortality risk, including overall and disease free survival. Importantly, sensitivity analysis showed that the association did not change after removing any of the 23 studies. Moreover, in subgroup analysis, overexpression of both HIF-1α and HIF-2α was associated with worse prognosis. Further subgroup analysis revealed an association of overexpressed HIF-1α with disease progression and unfavorable prognosis in Asian CRC patients. The analysis also indicated that the two HIF isoforms showed distinct clinicopathologic features; in contrast to HIF-1α, overexpression of HIF-2α did not correlate with disease progression and prognosis in these patients. However, HIF-2α was, unlike HIF-1α, significantly associated with grade of differentiation.

Large studies involving many patients are necessary in order to delineate the significance and the role of HIF-1α expression in immunohistochemistry specimens of CRC biopsies.

HIF-1α and targeted therapy

The molecular characteristics of CRC have been the target of recent research efforts, which have broadened our understanding of CRC pathobiology and facilitated the identification of CRC subtypes [2,4,67]. This categorization of CRC based also on clinical criteria has helped to outline new therapeutic options and improve CRC treatment outcome [5,70,71]. The new therapies include biological agents such as monoclonal antibodies against VEGF and EGFR inhibitors. HIF-1α expression has been studied in correlation to these factors in different groups of patients. Studies regarding anti-VEGF therapies have been discussed previously. In the following paragraphs we review the association between HIF-1α and anti-EGFR regimens in CRC.

EGFR has been identified as an oncogene in many different tumors, including CRC. Overexpression of EGFR is common; 65–70% of CRCs are EGFR-positive, and is more frequent in advanced-stage tumors [72]. In this context, EGFR is a front-line target in metastatic CRC treatment. Pharmacological inhibitors include monoclonal antibodies (cetuximab and panitumumab) and tyrosine-kinase inhibitors (gefitinib and erlotinib), which impede EGFR mediated signal transduction and have been proven valuable tools in CRC treatment [73]. There is evidence that the antitumor ef-

fects of the EGFR-blocking antibody cetuximab may be mediated through inhibition of the PI3K pathway, which in turn leads to downregulation of HIF-1α synthesis and activity [73-75]. Furthermore, the discovery of KRAS mutations and aberrations in related proteins, such as BRAF, PTEN, and PIK3-AKT, highlight a group of patients who may be resistant to anti-EGFR antibodies [5,77-79].

Indeed, in addition to the connection of HIF and EGFR, a unique interaction between KRAS and hypoxia has been also documented [80,81]. Recently, Kikuchi et al. demonstrated that mutually exclusive mutations of KRAS and BRAF have different effects on the induction of HIF-1α and HIF-2α in colon cancer cell lines. This study showed that oncogenic KRAS induced HIF-1α primarily at the level of translation in a PI3K-dependend manner. In contrast, oncogenic BRAF enhanced the mRNA expression of HIF-1α and HIF-2α, as well as HIF-2α protein synthesis. The distinct mode of HIF-1 and HIF-2 regulation by KRAS and BRAF mutants in colon cancer cells could account for the differences observed between colon tumors bearing KRAS and BRAF mutations [82].

The elucidation of the interactions between signaling molecules involved in CRC appears to be the strategy of choice for the treatment of patients with different CRC subtypes. In this context, further experimental studies are necessary to clarify the mechanisms that differentially regulate the hypoxia response system involving HIF-1 in CRC.

Conclusion

Hypoxia is involved in CRC and the role of HIF-1α is being thoroughly studied. Although the development of appropriate methods to accurately measure hypoxia in tumors, as well as the establishment of consensus for HIF-1α evaluation in tissue samples still remains a challenge for the future, a growing body of data supports the involvement of HIF-1α in various CRC aspects. HIF, also through interplay with cellular signal transduction pathways, is involved in carcinogenesis, tumor angiogenesis and cancer progression. These findings argue that the introduction of HIF-1α inhibitors in combination with existing treatments or other new-targeted therapies in the treatment of CRC patients may be very useful clinically.

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