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Rectal cancer genomics

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A B S T R A C T

Cellular signaling abnormalities play an important role in the oncogenesis of rectal cancer. These signaling changes are frequently driven by genomic changes. This review describes five commonly altered cell signaling pathways in rectal cancer: WNT, RAS, TGF-β, p53, and PI3K. For these pathways, both physiologic (non-altered) function and common mutations that contribute to abnormal signaling are described. As rectal cancer is driven by genomic changes, a discussion of the prognostic value of pathway signaling mutations is included. Lastly, the use of genomic changes as predictive markers for response to preoperative radiotherapy is described.

1. Introduction

The initiation and progression of colorectal carcinoma (CRC) is driven by abnormalities that develop in cellular signaling pathways. Many details about the genomic basis of CRC have been clarified, although our understanding of this complex disease is certainly incomplete.

There are a wide variety of genomic alterations that occur in CRC. Individual gene mutations may be activating or inactivating, and even within a single gene, mutations at various loci may produce different biological effects. In addition to gene mutations, alterations in gene expression levels, chromosomal aberrations, and epigenetic changes such as hyper- or hypo-methylation contribute to the process of oncogenic transformation. Due to this complexity, the study of a single gene or even a single cell signaling pathway will yield a limited amount of information; a true genomic understanding of any given cancer requires a comprehensive analysis.

The literature on the genomics of CRC is massive, so we have intentionally been selective in reviewing topics that we think are of the greatest interest to this readership. In this paper we discuss details of genomic alterations in critical cell signaling pathways as they relate to CRC and we focus less on the downstream effects of these alterations. For example, mutations in the WNT–APC pathway lead to increased expression of C-MYC, which contributes to increased cellular proliferation.[1] Thus, increased levels of C-MYC in CRC represent a result of pathway dysregulation rather than a causative mechanism of oncogenesis. As our aim is to discuss the genomic alterations that drive the development of CRC rather than downstream effects of these pathways, the WNT pathway is discussed in greater detail below, while C-MYC is not further addressed.

Molecules involved in angiogenesis such as vascular endothelial growth factor and hypoxia-inducible factor also have an important role in CRC.[2] Angiogenesis pathway signaling has important implications for the development and use of targeted agents in the treatment of CRC. However, the activation of angiogenesis is most likely the result of altered cellular signaling rather than a cause, and therefore we will limit our discussion of this topic as well.

An extensive genome-scale analysis of colorectal tumors performed by The Cancer Genome Atlas (TCGA) project recently reported a detailed account of genomic changes in CRC, which is the most comprehensive analysis yet performed on colorectal cancers.[3] To accomplish this genomic analysis, TCGA investigated exome sequences, methylation patterns, chromosomal changes, and overall pathway alterations.

On a high-level evaluation, 16% of CRC tumors had very high mutation rates and were designated as hypermutated, while the remaining 84% of tumors were non-hypermutated. The vast majority of hypermutated tumors were located in the colon (predominantly in the right colon) and were rarely seen in the rectum. These tumors frequently had microsatellite instability and silencing or mutation of mismatch repair genes. Among the non-hypermutated tumors, there were no significant differences in patterns of mutations between tumors located in the rectum and in the colon. Despite the fact that anatomical differences between the colon and rectum have led to different treatment approaches for rectal cancer and colon cancer, rectal tumors do not appear to have a distinct genomic profile when compared to non-hypermutated tumors arising in the rest of the large bowel.

The role of various genomic alterations seen in CRC has been described in detail; WNT signaling abnormality due to mutant *APC* and mismatch repair abnormalities have been extensively

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reported, as these changes are associated with familial CRC syndromes.[4] Furthermore, alterations in P53, PI3K, and RAS signaling pathways have been shown to play an important role in CRC. The TCGA analysis further clarified details regarding mutations in these genes and pathways and also illustrated alterations in other pathways such as TGF-β. Details pertaining to the five most common signaling pathway alterations found in CRC are illustrated in a concise yet thorough manner in Figure 4 of the article published by TCGA. The purpose of this article is to review the genomic changes in CRC with a specific focus on pathways that contribute to oncogenesis as well as to discuss how alterations in these pathways relate to radiotherapy. To accomplish this, five of the most commonly altered signaling pathways in rectal cancer will be discussed in more detail: WNT, RAS, TGF-β, p53, and PI3K.

1.1. WNT

The WNT pathway plays an important part in the growth and differentiation of mucosal epithelial cells of the colon and rectum. This pathway is particularly important in CRC, as the vast majority of CRC have genomic aberrations that cause altered WNT signaling.[3]

A critical signaling molecule in the WNT pathway is β-catenin. This protein is an intracellular molecule that can enter the nucleus and bind to transcription factors in the TCF/LEF family that targets genes that lead to cellular proliferation. Under physiologic conditions β-catenin activity is regulated by GSK-3β, Axin, and APC. These proteins combine and phosphorylate β-catenin, which leads to the degradation of β-catenin in the proteasome. WNT is a signaling molecule that binds to plasma membrane-bound receptors such as Frizzled. Signaling from these receptors suppresses GSK-3β function. This leads to an increase in β-catenin levels, and thus, an increase in cellular proliferation (Fig. 1). As normal colonic cells grow and migrate away from the base of colonic crypts, there is a reduction in WNT signaling and a reduction in cell growth with a concomitant increase in cell differentiation.[5] The importance of the WNT pathway is seen most dramatically in patients with familial adenomatous polyposis, in which a mutated APC gene is unable to facilitate degradation of β-catenin, leading to uncontrolled cell growth and widespread polyp formation throughout the colon.

The most common alteration in the WNT pathway found in CRC patients is a mutation of APC. Approximately 93% of CRC tumors have altered WNT signaling, with 76% having mutations that lead to a loss of function in APC.[3] Because the WNT pathway is almost always altered in CRC, testing for mutations in this pathway is likely not useful as a predictive or prognostic marker. However, the high prevalence of mutations and changes in gene expression at various steps in the WNT pathway does suggest that the WNT pathway is a rich source of potential therapeutic targets in CRC.

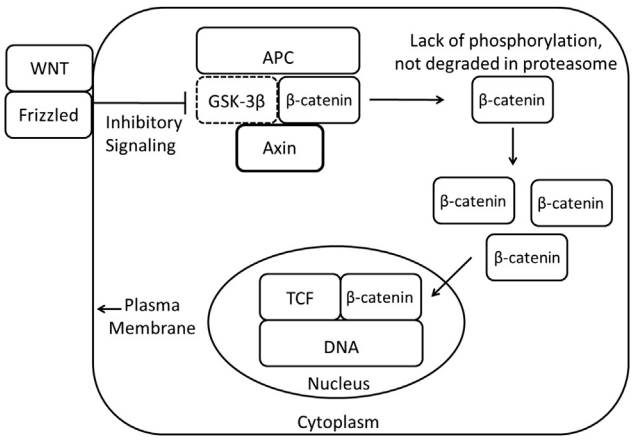

Fig. 1. WNT signaling causes increased β-catenin-mediated signaling via down-regulation of GSK-3β. When bound by APC, GSK-3β and axin phosphorylate β-catenin. This leads to degradation of β-catenin in the proteasome. Any loss-of-function mutation or signal-mediated down-regulation of APC, GSK-3β, or axin will lead to a decrease in phosphorylation and degradation of β-catenin. The resultant higher cytoplasmic concentration of β-catenin facilitates entry into the nucleus, where it can bind to transcription factors such as TCF which activate genes that lead to increased cellular proliferation.

Efforts to target individual components of the WNT pathway have met with limited success. *In vitro* and *in vivo* studies of CRC cell lines demonstrate that small interfering RNA (siRNA) directed against β-catenin inhibits the growth of cancer cells.[6] A variety of small-molecule β-catenin inhibitors have been identified, but more research is needed to evaluate toxicological and pharmacological properties of these drugs.[7] To date, none of these agents have seen widespread clinical use. Critical cell survival pathways appear to often have alternative means of activation, because they are so critical to cell survival. By targeting multiple portions of oncogenic pathways concurrently, the efficacy of targeted therapy may be further improved by removing the alternative means of activation. Silencing of both β-catenin and KRAS through the use of shRNA has been shown to cause induction of apoptosis *in vitro* and tumor growth suppression *in vivo*.[8] PKF115-584 is a specific inhibitor of the β-catenin/TCF4 interaction and pyrvinium pamoate is an anti-helminthic drug that induces degradation of β-catenin. When combined with salirasib, a specific RAS inhibitor, this three-drug combination was shown to be effective in inducing cell growth arrest and cell death *in vitro*. Unfortunately, preliminary *in vivo* results were negative due to poor drug absorption.[9] Furthermore, in a retrospective analysis, the synergistic effect of combined WNT and RAS targeting was not seen in cells with mutations in BRAF, which is observed in approximately 10% of CRC tumors.[3,9]

The WNT receptor Frizzled is overexpressed in approximately 17% of all CRC and is sometimes expressed at levels greater than 100-times normal.[3] Increased Frizzled expression activates the canonical WNT pathway independent of the presence of an APC mutation. Blocking Frizzled activity with siRNA decreased TCF transcriptional activity and cell viability.[10] A monoclonal antibody has been developed that targets Frizzled and blocks the canonical WNT signaling, resulting in tumor growth inhibition in animal models.[11]

The role of WNT signaling in sensitivity to chemoradiotherapy in rectal cancer is not clear. WNT signaling is aberrant in nearly all cases of CRC but response to neoadjuvant chemoradiotherapy in rectal cancer is variable, ranging from pathologic complete response (pCR) to no response. Although the mutations or changes in expression of genes involving the WNT pathway may be related to radioresistance, it seems virtually certain that other factors contribute to radioresistance of CRC tumors.

No data have established a direct causative mechanism for chemoradioresistance caused by aberrant WNT signaling. However, a study of the gene expression signature of 12 CRC cell lines found that cell lines with changes in expression of genes involved in the WNT pathway were significantly associated with increased radioresistance *in vitro*.[12] Furthermore, TCF4, a transcription factor that is a target of β-catenin, has been shown to be overexpressed in some rectal cancers resistant to chemoradiotherapy. The use of shRNA to silence TCF4 has been demonstrated to sensitize CRC cells to ionizing radiation *in vitro*.[13] These data suggest that genes in the WNT pathway are a potential target for the development of radiosensitizing drugs.

1.2. RTK–RAS

RAS is a membrane-bound nucleotide-binding protein that is normally activated in response to the binding of signal molecules.