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Therapeutic targets in the Wnt signaling pathway: Feasibility of targeting TNIK in colorectal cancer

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ABSTRACT

The genetic and epigenetic alterations occurring during the course of multistage colorectal carcinogenesis have been extensively studied in the last few decades. One of the most notable findings is that the great majority of colorectal cancers (>80%) have mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene. Loss of functional APC protein results in activation of canonical Wnt/β-catanin signaling and initiates intestinal carcinogenesis. Mutational inactivation of APC is the first genetic event, but colorectal cancer cells retain their dependency on constitutive Wnt signal activation even after accumulation of other genetic events. Accordingly, pharmacological blocking of Wnt signaling has been considered an attractive therapeutic approach for colorectal cancer. Several therapeutics targeting various molecular components of the Wnt signaling pathway, including porcupine, frizzled receptors and co-receptor, tankyrases, and cAMP response element binding protein (CREB)-binding protein (CBP), have been developed, and some of those are currently being evaluated in early-phase clinical trials. Traf2- and Nck-interacting protein kinase (TNIK) has been identified as a regulatory component of the T-cell factor-4 and β-catenin transcriptional complex independently by two research groups. TNIK regulates Wnt signaling in the most downstream part of the pathway, and its inhibition is expected to block the signal even in colorectal cancer cells with *APC* gene mutation. Here we discuss some of the TNIK inhibitors under preclinical development.

Contents

Abbreviations: ABC, ATP-binding cassette; APC, adenomatous polyposis coli; CBP, cAMP response element binding protein (CREB)-binding protein; CK, casein kinase; CSC, cancer stem cell; Dvl, disheveled; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; F-actin, filamentous-actin; FAP, familial adenomatous polyposis; FZD, frizzled; GCK, germinal center kinase; GSK3β, glycogen synthase kinase 3β; HTS, high-throughput screening; JNK, c-Jun N-terminal kinase; LEF, lymphoid enhancer factor; LRP, low-density lipoprotein receptor-related protein; MCR, mutation cluster region; MMTV, mouse mammary tumor virus; Stat3, signal transducer and activator of transcription-3; TCF, T-cell factor; TGF, transforming growth factor; TNIK, TRAF2- and NCK-interacting protein kinase; TNKS, tankyrase; VEGF, vascular endothelial growth factor.

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1. Introduction

Colorectal cancer is a major cause of cancer death, accounting for ~700,000 deaths annually worldwide. The majority of colorectal cancer patients without lymph node or distant organ metastasis can be readily cured by surgical resection, with 5-year survival rates of >80% (Okuno, 2007; Watanabe et al., 2015), but the outcome of patients with distant metastasis or postsurgical recurrence still remains unsatisfactory. Advances in combination chemotherapy [FOLFOX (folinic acid/fluorouracil/oxaliplatin), FOLFIRI (folinic acid/fluorouracil/irinotecan), XELOX/CAPOX (capecitabine/oxaliplatin), and their modifications] and supportive use of therapeutic antibodies against vascular endothelial growth factor (VEGF) (bevacizumab) and epidermal growth factor receptor (EGFR) (cetuximab and panitumumab) have significantly prolonged the survival of patients (Pasetto et al., 2005; Watanabe et al., 2015), and the median overall survival time of patients with metastatic colorectal cancer has now exceeded 30 months in the most recent phase 3 clinical trials (Cremolini et al., 2015). However, survival prolongation does not necessarily mean that the disease has been cured, and the 5-year survival rate of patients with stage-IV colorectal cancer is still around 15% (Watanabe et al., 2015).

In a recent phase 3 clinical trial, regorafenib (BAY 73–4506) significantly prolonged the survival of patients with metastatic colorectal cancer that had progressed after all approved standard therapies had failed (Grothey et al., 2013). However, this survival prolongation was still limited. The median overall survival period for patients treated with regorafenib was 6.4 months, and that of controls was 5.0 months. Therapy-related adverse events have been observed in 93% of patients receiving regorafenib, which is a multikinase inhibitor whose precise molecular target has not yet been established. It is therefore necessary to develop new therapeutics that target the fundamental cause of colorectal carcinogenesis, and currently accumulated knowledge of colorectal cancer genetics may provide some clues.

2. Wnt signaling and cancer

2.1. Frequent Wnt gene alterations in colorectal cancer

The great majority (>90%) of colorectal cancers carry mutations in at least one Wnt signaling pathway gene, such as the adenomatous polyposis coli (*APC*) and β-catenin (*CTNNB1*) genes. The *APC* gene was initially identified as being causative for the familial adenomatous polyposis (FAP) syndrome (Kinzler et al., 1991; Nishisho et al., 1991), but later found to be frequently (>80%) mutated also in sporadic colorectal cancers. *APC* mutations equally occur in replication error (RER)-positive and -negative colorectal cancers (Huang et al., 1996; Homfray et al., 1998) and are the earliest genetic event during the so-called adenoma–carcinoma sequence (Fig. 1) (Powell et al., 1992). *CTNNB1* mutation is mutually exclusive to *APC*. About half of colorectal cancers with wild-type *APC* have mutations in *CTNNB1* (Morin et al., 1997). Recent large-scale sequencing efforts by the Cancer Genome Atlas (TCGA) and other projects have revealed genetic alterations in other Wnt signaling molecules. Besides *APC* and *CTNNB1*, the genes encoding frizzled 10 (*FZD10*), T-cell factors-3 and -4 (TCF3/4) (*TCF7L1/2*), axis inhibitor 2 (*AXIN2*), and APC membrane recruitment protein 1 (*AMER1*, *WTX*, or *FAM123B*) have been found to be mutated in colorectal cancers (Bass et al., 2011; Cancer Genome Atlas, 2012).

2.2. Constitutive activation of Wnt signaling by genetic alterations

The APC protein forms a multiprotein complex with axin/axin2, casein kinase I α/ε (CKIα/ε), and glycogen synthase kinase 3β (GSK3β) (Clevers & Nusse, 2012), which plays a central role in the degradation of β-catenin and is thus referred to as the β-catenin destruction complex. Cytoplasmic β-catenin is recruited into the complex, phosphorylated with CKIα/ε and GSK3β, and subsequently ubiquitinated with β-TrCP-containing E3 ubiquitin ligase, which targets the β-catenin for proteosomal degradation (Fig. 2A). Mutations of *APC* are observed in the central part of the gene, called the mutation cluster region (MCR: amino acid 1263–1587), resulting in a truncated APC protein (Miyoshi et al., 1992) incapable of forming the destruction complex. Mutation of the *CTNNB1* gene has often been observed in the N-terminal phosphorylation sites at Ser45, Thr41, Ser37, and Ser33 (Polakis, 2000). Either loss-of-function *APC* mutations or gain-of-function *CTNNB1* mutations equally leads to inappropriate stabilization of β-catenin protein and mimics the activation of Wnt signaling (Polakis, 2000).

Surplus β-catenin translocates to the nucleus, where it acts as a co-activator for the T-cell factor (TCF)/lymphoid enhancer factor (LEF) family transcription factors. TCF/LEF transcription factors transactivate a large variety of target genes involved in the proliferation,

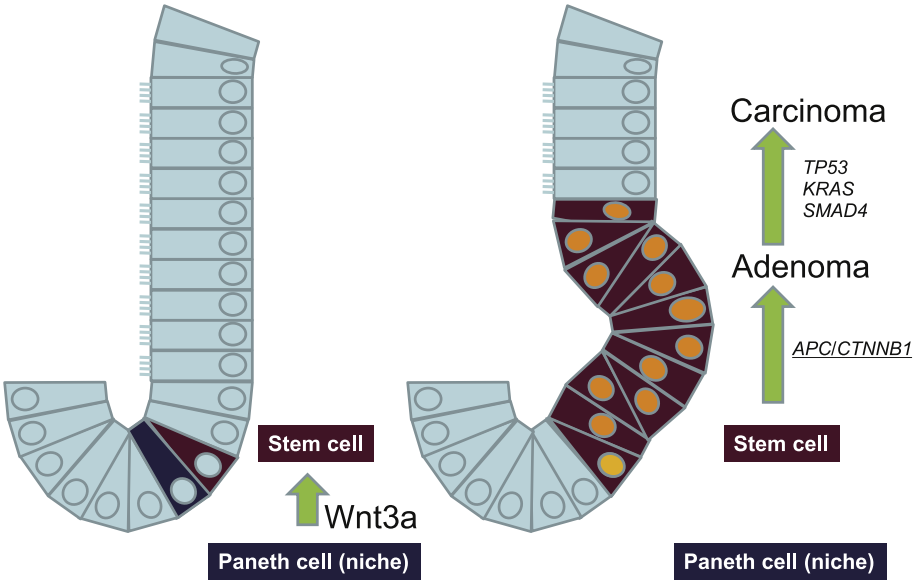

Fig. 1. Normal intestinal epithelial stem cells reside in the bottom of the crypt intermingled with Paneth cells. Paneth cells constitute the stem cell niche through supply of Wnt3a (left). Constitutive activation of Wnt signaling by *APC* or *CTNNB1* mutation transforms an intestinal epithelial stem cell into adenoma. Adenoma develops into carcinoma through accumulation of additional genetic alterations in the *SMAD4*, *KRAS*, and *TP53* genes (right).