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EDITORIAL

KRAS and Cancer Stem Cells in APC-Mutant Colorectal Cancer

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Akin to the situation for most human cancers, colorectal cancer (CRC) arises in part because of accumulated genetic and epigenetic defects in key signaling networks that regulate cell proliferation, differentiation, metabolism, and death. Among the most common recurrent somatic alterations in CRCs are inactivating mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene and activating mutations in the *KRAS* proto-oncogene (1–3). These gene lesions are often found to be present in all or nearly all of the neoplastic cells in primary CRCs and the metastatic progeny that might be present at diagnosis or recurrence. Nonetheless, a strong body of evidence has emerged over the past decade to indicate that the neoplastic cells in any given CRC, although sharing clonal somatic gene defects, likely manifest a range of malignant potentials as a result of differentiation lineages generated by the dominant malignant clones in the cancer (4,5). At the apex of these clones lies a population of cells referred to as "cancer initiating" or "cancer stem" cells that are capable of self-renewal as well as differentiation to generate the non-self-renewing cells constituting the bulk of cancer cells. Currently, the relationships between specific gene lesions, the resultant signaling pathway defects, and cancer stem cells (CSCs) populations in CRC are not well understood. The manuscript from Moon et al. in this issue of the Journal offers new findings highlighting how *APC* and *KRAS* mutations may collaborate in promoting CSC phenotypes (6).

Somatic inactivating mutations in the *APC* gene appear to be crucial initiating alterations in the genesis of most colon adenomas and CRCs. Although the APC protein has pleiotropic functions in the cell, the best understood function of APC is in regulation of the "free" or active pool of the β-catenin protein in the so-called "canonical" (or β-catenin–dependent) Wnt signaling pathway (1). In cells lacking APC function, coordinated phosphorylation, ubiquitination, and destruction of β-catenin is disrupted, mimicking the consequences when β-catenin is stabilized by Wnt ligands. As a result, β-catenin levels are constitutively elevated, and there is enhanced binding of β-catenin to T cell factor (TCF) DNA binding proteins and dysregulated β-catenin/TCF-dependent transcription of many genes (1). The TCF-dependent transcription program induced by β-catenin stabilization in CRC has been suggested to resemble that present in presumptive tissue stem cells at the base of normal intestinal and colon crypts (7). The potential role of Wnt signaling in CRC CSCs was previously examined by Vermeulen and colleagues, who reported that the commonly observed variability in β-catenin nuclear localization and β-catenin/TCF transcriptional activity among CRC cells were associated, such that CSC properties were manifest in the CRC cells with strong nuclear β-catenin and robust β-catenin/TCF transcriptional activity (8). Moreover, the findings of Vermeulen and colleagues suggested that differential exposure to factors such as hepatocyte growth factor in the tumor microenvironment might substantially modulate Wnt signaling and the CSC phenotype through a pathway involving c-Met, Akt, and β-catenin (8). Together, the findings suggest that the tumor microenvironment and mutation profile likely play complementary roles in regulating CSC fates.

Although approximately 90% of CRCs display mutations that alter APC or other canonical Wnt pathway factors (1–3), dysregulation of β-catenin levels is not sufficient for generating the CRC phenotype (1,7). Other somatic gene lesions, such as activation of the *KRAS*, *BRAF*, and *PIK3CA* proto-oncogenes or inactivation of the *TP53*, *SMAD4*, *ARIAD1A*, and *FBXW7* tumor suppressor genes, collaborate to promote progression of some *APC*-mutant adenomatous lesions to CRC (1–3). Interestingly, many of these gene lesions have been implicated in the regulation of CSCs in other systems (9). These observations raise the intriguing possibility that cooperation of multiple oncogene and tumor suppressor gene defects in promoting CSC phenotypes may be a more general phenomenon involved in carcinogenesis. The findings also serve to emphasize that the CSC and clonal evolution models of carcinogenesis are not mutually exclusive. Initiating events such as APC mutation may lead to clonal expansion of colonic stem cells, which results in adenoma formation. Secondary events, such as *KRAS* mutation, may then further enhance Wnt signaling, leading to further clonal expansion of this CSC population. We previously reported that expression of a mutant Kras allele in mouse colonic cells failed to expand the stem cell population but instead generated hyperplastic lesions reminiscent of human hyperplastic polyps (10). In contrast, inactivation of both Apc alleles resulted in expansion of the crypt stem cell population, generating adenomatous epithelium similar to human adenomas (10). These studies together with those of Moon and colleagues (6) emphasize that both the temporal sequence of oncogenic events and the differentiation state of the target cells involved may play pivotal roles in carcinogenesis.

Biochemical cross-talk between the Wnt/β-catenin and RAS signaling pathways in intestinal cells has been highlighted in some prior papers. Phelps and colleagues implicated KRAS mutational activation in increased nuclear localization of the free pool of β-catenin through a RAF1-dependent but MAPK-independent mechanism (11). More recently, Jeong and colleagues suggested that RAS protein levels are regulated by mechanisms similar to

those by which the APC-AXIN-GSK3β complex regulates the phosphorylation of β-catenin and its subsequent recognition by the β-TrCP-E3 ubiquitin ligase (12). As a result of APC defects, RAS proteins would be stabilized akin to β-catenin stabilization because of reduced GSK3β-dependent phosphoryation and reduced ubiquitination and degradation, with resultant increased activation of downstream signaling. In the article from Moon and colleagues in this issue of the Journal (6), the authors examine in depth, using human colon cancer–derived cell lines and genetically engineered mice, the ability of KRAS/Kras mutant alleles to modulate presumptive CSC populations as defined by cell surface marker expression patterns on the neoplastic cells and/or in assays of tumorigenicity (6). The authors found that oncogenic KRAS/Kras mutant proteins appeared to enhance nuclear localization of β-catenin in the setting of APC defects. In addition, the authors found that the oncogenic KRAS/Kras proteins were stabilized in cells with APC defects and β-catenin dysregulation

Further biochemical insights into the factors and mechanisms accounting for the observed collaboration between KRAS and Wnt/β-catenin signaling in enhancing CSCs in CRC are needed. However, the findings of Moon and colleagues (6) highlight some potentially important biological and clinical issues. The authors' findings of increased development of liver metastasis from KRAS- and APC-mutant CRCs are interesting in light of some clinical observations suggesting an increased frequency of poor prognosis and accelerated metastatic progression in the liver in CRC patients with KRAS-mutant cancers (13,14). Colon CSCs have often been suggested to express certain proteins, such as LGR5 and CD44, both of which are Wnt pathway target genes (7). The studies of Moon et al. (6) suggest that preclinical models might be used to determine whether simultaneous targeting of the KRAS and Wnt signaling pathways more effectively targets the colon CSC population than does targeting of a single pathway. Although KRAS itself has not been successfully targeted, inhibitors to downstream signaling factors, including MEK and PI3K, have been developed. In addition, inhibitors of key collaborating factors in Wnt signaling, such as the R-spondins, are now entering clinical trials. The study of these agents in preclinical models should inform the development of clinical trials using particular combinations of agents to more effectively target CRC CSCs.

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Notes

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