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Inflaming Gastrointestinal Oncogenic Programming

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The etiology of gastrointestinal tumors implicates a role for chronic inflammation in response to pathogenic microflora as a promoting force for full neoplastic progression. Recently, Oguma and coworkers (2008) demonstrated that TNFα, derived from recruited macrophages, potentiates Wnt/β-catenin signaling and gastric carcinogenesis by activating Akt signaling and GSK3β phosphorylation independent of the NF-κB pathway in initiated epithelial cells. These observations provide a missing link in the mechanism whereby chronic inflammation, in response to *Helicobacter*, regulates the "penetrance" of initiating oncogenic mutations in the gastrointestinal tract leading to gastrointestinal tumorigenesis.

Epidemiologic studies have long supported a link between chronic inflammation and development of solid tumors (Coussens and Werb, 2002; Thun et al., 2004). More recently, through utilization of immunocompetent mouse models of multistage carcinogenesis, the molecular mechanisms whereby chronic engagement of the immune system (inflammation) potentiates development of epithelial cancers have begun to be elucidated (Balkwill et al., 2005; de Visser et al., 2006; Karin et al., 2006). Missing, however, has been insight into which soluble mediators derived from chronically activated immune cells are significant for regulating the penetrance of neoplastic cells harboring initiating mutations. For example, in the gastrointestinal tract, malignancy is frequently preceded by chronic inflammation sometimes associated with *Helicobacter pylori* infection (Blaser, 2000) or in individuals harboring activating mutations in the *APC* or *CTNNB1* genes or enhanced activation of Wnt/β-catenin signaling (Clements et al., 2002).

APC is a multifunctional cytoplasmic protein whose gene is frequently mutated in several types of gastrointestinal cancers. APC regulates both genomic instability and hyperactivation of the Wnt/β-catenin signaling pathway, and nuclear β-catenin accumulation is found in colon carcinoma cells at invasive fronts (Fodde and Brabletz, 2007). Recent experimental studies have revealed that malignant conversion of initiated *APC* mutant cells is potentiated by chronic activation of the Wnt/β-catenin signaling pathway (Fodde and Brabletz, 2007); however, links between these molecular mediators and chronic inflammation have not been previously identified.

Oguma and coworkers (2008) investigated this link and found that both enhanced Wnt expression and infection by gastric microflora induce submucosal infiltration by macrophages secreting high levels of tumor necrosis factor-alpha (TNFα). Binding of TNFα to TNF receptors on gastric epithelial cells enhances Akt phosphorylation that in turn induces glycogen synthase kinase 3β (GSK3β) phosphorylation, resulting in stabilization and nuclear accumulation of β-catenin that potentiates gastric carcinogenesis (Figure 1). To reveal this pathway, the authors utilized a mouse model of gastric tumorigenesis in which Wnt1 is expressed in gastrointestinal mucosal epithelia, K19-Wnt1 transgenic mice, which develop sporadic dysplastic lesions in the glandular stomach, mimicking hyperactivation of Wnt/β-catenin signaling commonly observed in patients with gastric carcinoma

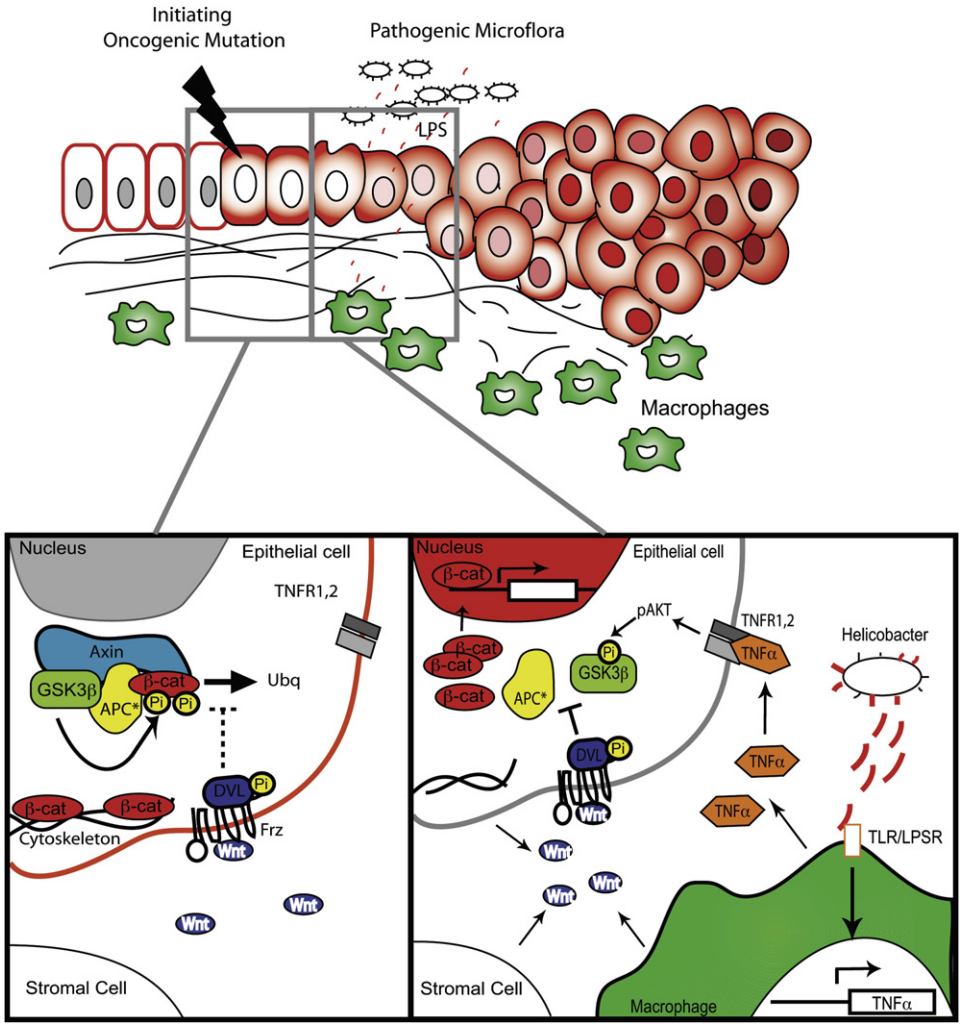

Figure 1. Macrophage-Derived TNFα Activates Wnt/β-Catenin during Gastrointestinal Cancer Development
Oncogenic mutations in tumor suppressors such as *APC* (APC*) are often associated with hyperactivation of Wnt/β-catenin pathways and stabilization of β-catenin. However, when such mutations occur in gastrointestinal cells, additional stimuli are required for translocation of β-catenin to the nucleus. Chronic inflammatory responses to pathogenic microflora such as *Helicobacter* can provide these stimuli via inducing macrophage infiltration. In response to bacterial components such as LPS or TLR ligands, macrophages produce TNFα, which in turn promotes nuclear accumulation of dephosphorylated β-catenin mediated by phosphoregulation of Akt and GSK3β in neoplastic epithelial cells in an NF-κB-independent manner. This process can be further exasperated by production of Wnt ligands by stromal cells that bind Frizzled receptors, leading to inhibition of the β-catenin degradation complex (APC, AXIN, GSK3β), thereby enabling nuclear translocation of stabilized β-catenin and transcriptional activation of Wnt target genes.

(Oshima et al., 2006). In K19-Wnt1 mice, macrophages accumulate in dysplastic areas of the glandular stomach adjacent to epithelia with nuclear accumulation of β-catenin, thus supporting a potential role for macrophages in regulating Wnt/β-catenin signaling. To functionally address this, the authors investigated a second model of gastrointestinal tumorigenesis, $Apc^{\Delta 716}$ mice, in which activation of Wnt/β-catenin signaling leads to development of intestinal polyps. By intercrossing $Apc^{\Delta 716}$ mice with *op/op* mice that have impaired colony-stimulating factor 1 (CSF1) signaling and decreased macrophage infiltration in tissues, the authors found a significant reduction in intestinal polyp formation, reduced levels of TNFα, and decreased Wnt/β-catenin activity that was largely due to inhibition of GSK3β and reduced Akt phosphorylation in gastric cancer cells.

To reveal whether this pathway is potentiated by infection, *Wnt1* transgenic mice were infected with *Helicobacter felis*—inflamed mucosa was histologically distinct from that found in dysplastic lesions of noninfected gut. Infected animals rapidly exhibited increased β-catenin accumulation in proliferating epithelial cells and decreased presence of H^+/K^+-ATPase-positive parietal cells, thus indicating that infection and inflammation promote Wnt/β-catenin signaling and suppress epithelial differentiation. Taken together, the results of the study by Oguma and coworkers (2008) reveal a new pathway whereby chronic infection of the gastrointestinal tract and the ensuing inflammatory microenvironment facilitate expansion of initiated cells by collaborating with existing oncogenic mutations in epithelia.

The tumor-promoting capability of macrophages has previously been demonstrated using a mouse model of

mammary carcinogenesis in which infiltration of macrophages into premalignant mammary tissue regulates mammary tumorigenesis (reviewed in Lin and Pollard, 2007). Failure to recruit macrophages into malignant tissue significantly delays development of late-stage carcinomas and attenuates pulmonary metastasis, likely via angiogenic mechanisms and altered paracrine interactions between epidermal growth factor (EGF) and CSF1 signaling pathways (Lin and Pollard, 2007). In breast and ovarian cancer, TNFα provided by tumor-infiltrating macrophages also promotes proinvasive phenotypes of neoplastic cells as well as potentiating metastatic spread in ovarian and hepatocellular cancer (Balkwill et al., 2005). The present study by Oguma et al. (2008) describes an additional tumor-promoting role for macrophages, independent of NF-κB-regulated pathways in epithelia, by providing a link between the proinflammatory cytokine TNFα and Wnt/β-catenin signaling.

In view of recent insights into mechanisms whereby microbial infections promote tumor development (Karin et al., 2006), it is intriguing that *Helicobacter*-associated inflammation promotes Wnt/β-catenin activity in initiated gastric epithelium since it raises the possibility that immune mediators directly influence stem cell niche expansion or "stemness" during gastric tumorigenesis. Cells of the gastrointestinal epithelium have a high turnover rate (3–6 days) whereby the balance between apoptosis, senescence, proliferation and differentiation is tightly regulated to maintain tissue homeostasis. Gastrointestinal epithelia are regenerated from stem cell populations residing in specific niches within the epithelium. Due to their relatively rapid turnover rate, differentiated gastrointestinal epithelia are not believed to persist long enough to acquire multiple genetic mutations that might be necessary for full malignancy before being shed into the lumen and/or undergoing apoptosis; thus, it has been postulated that gastrointestinal malignancies originate from stem cell populations. Wnt-regulated pathways are important for homeostatic stem cell renewal and are frequently upregulated during gastrointestinal carcinogenesis (Fodde and Brabletz, 2007). In addition to TNFα, macrophages have also been shown to directly produce Wnt ligands, including Wnt2 and Wnt5a in human colorectal tumors, further supporting a link between macrophages and activation of Wnt signaling pathways (Smith et al., 1999). It is unclear from the Oguma et al. (2008) study whether expression of Wnt ligands is also upregulated in stromal cells following *Helicobacter* infection, or whether Wnt ligands derived from macrophages act synergistically with TNFα to upregulate Wnt/β-catenin signaling. Considering the importance of Wnt signals during gastric carcinogenesis, it is tempting to speculate that chronically engaged immune cells may play a role in stem cell niche expansion during gastric tumorigenesis. In this manner, stem cell populations harboring initiating oncogenic or tumor suppressor gene mutations, such as *APC*, when present in a chronic inflammatory microenvironment might be subject to promotion-type events involving TNFα and/or Wnt ligands, leading to tissue expansion without differentiation, and in so doing might provide an expanded stem cell or stem cell-like population at risk for acquiring secondary tumor-promoting mutations. Thus, the findings of Oguma et al. (2008) provide a mechanism whereby *Helicobacter*-associated inflammation may facilitate expansion of initiated stem cells by promoting Wnt/β-catenin signaling and thereby promoting full neoplastic progression of initiated cells by nourishing the roots of cancer development.

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