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Caught in the Akt: Regulation of Wnt signaling in the intestine

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There is a strong association between inflammation and initiation of cancer in a number of organ systems. This correlation has been observed in patients with chronic inflammatory conditions and is highlighted in patients with inflammatory bowel disease (IBD), who have an increased risk of cancer. One major hurdle in definitively linking these two pathologic states is the lack of clear underlying and implicating molecular mechanisms. One intriguing link may be in regulatory crosstalk between cellular signaling pathways that are modulated by inflammatory cytokines and those that are implicated in tumor initiation. The Wnt and PI3K/Akt signaling pathways are two likely players. While β -catenin-mediated Wnt signaling is well-known for its role in stimulation of epithelial proliferation, it has been shown recently to be modulated by the PI3K/Akt signaling pathway on at least two levels, one directly within the nucleus through inhibition of β -catenin nuclear localization, and another through regulation of GSK3 β phosphorylation. Furthermore, inflammatory cytokines have been implicated in activation of the Akt signaling pathway¹, supporting a molecular link between inflammation and epithelial proliferation. In this issue of *Gastroenterology*, Lee et al, demonstrate that inhibition of the PI3K/AKT signaling pathway in a chronic intestinal inflammatory microenvironment resulted in decreased nuclear localization of β -catenin and a subsequent decrease in epithelial proliferation. These data provide a potential molecular basis for linking inflammation and cancer. Significantly, however, it also provides evidence that inhibition of the PI3K/AKT signaling pathway might offer a viable therapeutic target for treatment of chronic intestinal inflammatory disease.

Modulation of Wnt Signaling in an inflammatory setting

While it is well-known that activation of numerous signaling pathways are key to initiation and propagation of tumor growth², there is no clear molecular link between inflammation-mediated modulation of cell signaling pathways and tumorigenesis. The logic that links inflammation to cancer presumes that a reduction in inflammation will result in a reduction of dysregulated epithelial proliferation, a precursor to cancer initiation. Dysregulation of the canonical Wnt signaling pathway has been well-established as a proliferative carcinogenic precursor. Therefore, direct activation of the Wnt pathway from other inflammation-stimulated pathways would establish the basis for a causal molecular connection. Not surprisingly, regulation of the canonical Wnt signaling pathway is complex and occurs at multiple levels within the pathway (i.e. the ligand/receptor, cytoplasmic regulation of phosphorylation of β -catenin, nuclear localization of β -catenin and transcriptional activation). As such, the Wnt signaling pathway has been shown to be regulated by multiple

signaling pathways³. One interesting cell signaling pathway that has been shown to be activated in an inflammatory microenvironment, the PI3K/AKT pathway, has also been shown to interface with the canonical Wnt signaling pathway⁴.

Previous studies have supported a molecular intersection between components of the Wnt and PI3K/Akt signaling pathways, thereby suggesting potential for cross-regulation of transcriptional activity. At the transcriptional level, the cellular localization of the downstream Wnt signaling modulator, β -catenin, has been shown to be regulated by a novel nuclear shuttle protein, Chibby, that functions upon activation of AKT and subsequent phosphorylation of 14-3-3 proteins⁵ (Figure 1A). Functional partitioning of β -catenin into the cytoplasm antagonizes its transcriptional function, ultimately translating Wnt transcriptional regulation from the PI3K/Akt pathway and demonstrating one direct regulatory link between the two signaling pathways.

The most commonly investigated and controversial molecular link between the two signaling pathways lies at the Gsk3 β node. Multiple published studies suggest that the crosstalk between the Akt and Wnt signaling pathways converge at a common pool of Gsk3 β (Figure 1A; reviewed in 6). Supporting this viewpoint, Fukumoto et al showed that AKT binds the AXIN/GSK3 β complex in the presence of the Wnt pathway member, Dishevelled, and increased the levels of cytoplasmic β -catenin and subsequent transcriptional Wnt activity as measured with a TOPFLASH Wnt reporter assay in a rat pheochromocytoma cell line⁴. However, equally convincing data using breast and prostate cancer cell lines and identical experimental assays suggested that modulation of the Akt signaling pathway does not alter Wnt transcriptional activation⁶. Taken together, these data highlight the potential for tissue specific differences in pathway crosstalk and ultimately, differences in their regulation. While previous studies have been performed in a number of cancer cell lines, a thorough examination of the regulatory relationship between WNT and PI3K/AKT signaling pathways within the intestine is needed in order to define molecular influences of intestinal inflammation on intestinal cancer.

PI3K/Akt signaling pathway in cancer and inflammation

It is well-accepted that dysregulated activation of AKT kinases is a common molecular alteration and driving force in human cancer progression⁷. In some cases, such as breast and non-small cell lung cancer, hyperactivation of the PI3K/AKT pathway is correlated to advanced or aggressive disease. Similarly, activation of the AKT pathway is implicated in the pathogenesis of colorectal cancer⁸ and in chemoresistance⁹. Recently, Rychahou et al have shown that both AKT1 and AKT2 are upregulated in colorectal cancer and that AKT2 activation is critical for establishing colorectal cancer derived liver metastases¹⁰. Consistent with this, Ericson et al showed that deletion of both AKT1 and AKT2 disrupted clonal growth *in vitro*, as well as impeded the ability for xenografted cells to “metastasize” *in vivo*¹¹. Furthermore, in agreement with previous data from Hans Clevers’ group⁶, they found that PI3K/AKT signaling in colorectal cancer cell lines does not act through the Wnt pathway, but rather through downstream FOXO proteins (See Figure 1A). These data, along with other observations, suggest that the PI3K/Akt pathway signals differently in cancer cells compared to other cells, and that there may be subtle tissue specific differences in pathway regulation. The notion that normal and transformed cells may convey a differential response to modulation of a discrete signaling pathway provides an exciting potential focus for development of therapeutic targets, as the ultimate goal is to ablate malignant cells and spare surrounding normal tissue. In addition, these differences also point to the importance of investigating whether the PI3K/Akt pathway can be beneficially modulated therapeutically for other intestinal diseases.

In contrast to its function in cancer, the role of the PI3K/Akt pathway in inflammation is less well-established. While it has been implicated in upregulating inflammation in the lung^{12, 13} and vascular endothelium¹⁴, little is known about the role of the PI3K/Akt pathway in colonic inflammation and IBD. Work by Dahan and colleagues showed that intestinal lymphocytes could induce activation of the PI3K/Akt signaling pathway in intestinal epithelial cells isolated from patients with IBD. This activation, which was specific to inflamed epithelium when compared to normal colonic epithelial cells, resulted in induction of cellular differentiation and cell death¹. In this issue of *Gastroenterology*, Lee and colleagues utilized a mouse model of IBD to show that Akt activity is increased in inflamed intestinal epithelial cells. Next, when they ablated the PI3KR1 gene within the intestinal epithelium this led to effective inhibition of the PI3K/Akt signaling pathway as well as suppression of the canonical Wnt signaling pathway, as measured by nuclear localization of β -catenin. Strengthening their observation, they also showed that pharmacologic inhibition of the PI3K/Akt pathway reduced activation of the Wnt signaling pathway and, most intriguingly, reduced intestinal inflammation and dysplasia when compared to controls. The significance of their findings is that modulation of the PI3K/Akt signaling pathway may represent an important regulatory mechanism for controlling cellular proliferation in the intestinal epithelium. Although these data might provide a potential molecular link between inflammation and cellular proliferation, the most important impact of the work may be in revealing that therapeutic targeting the PI3K/Akt signaling pathway represents a novel and potentially exciting treatment for IBD. Currently, a number of compounds derived to inhibit the PI3K/Akt signaling pathway are in early stage clinical trials for a variety of epithelial malignancies.

Current Therapeutic Treatments for Inflammatory Bowel Disease

At present, pharmacologic therapy for IBD is primarily aimed at reduction of inflammation, both to reduce clinical symptoms, and to prevent disease progression. The cumulative incidence of colorectal cancer in IBD patients is 5–10% over 20 years, and the rate of synchronous colorectal cancer in IBD patients with dysplasia ranges from 20–40%¹⁵. Although chronic inflammation is associated with an increased risk of progression to epithelial dysplasia and colorectal cancer in patients with IBD, the complicating factor for treatment and disease management is that there lacks a clear identifier or marker for predicting progression to cancer.

Agents currently in clinical use for IBD therapy include steroids and non-steroidal anti-inflammatory compounds such as mesalamine and sulfasalazine; immunosuppressive agents such as azathioprine and methotrexate; and biologic agents, primarily the anti-TNF β monoclonal antibody Infliximab (Figure 1B). Other, direct immune modulating therapies such as anti-IL-6 antibodies¹⁶ and recombinant IL-10¹⁷ have been evaluated in small, early-phase clinical trials. Recent studies have shown that early and aggressive multi-agent treatment of IBD improves symptoms and can even induce remission¹⁸. While these agents are clearly effective, they have significant side effects including global immune suppression rather than specifically targeting molecular pathways responsible for inducing and maintaining the inflammatory state.

Conclusions and Future Directions

The increased risk of carcinoma development in patients with chronic intestinal inflammation emphasizes the importance of elucidating a molecular link between these two pathophysiologic states. Lee et al, present compelling evidence that the inflammation-mediated activation of the PI3K/Akt signaling pathways can modulate the downstream target gene transcriptional activation of the Wnt signaling pathway, providing insight into a

potential molecular mechanism for inflammation-induced carcinogenesis. Both of these signaling pathways are subject to regulatory feedback loops^{19, 20} that can result in perpetual stimulation and activation of their respective downstream targets. Drugs targeted to inhibit the PI3K/Akt pathway could break the cycle of Wnt and PI3K/Akt pathway induced inflammation and dysplasia, potentially treating not just the inflammatory component of IBD, but the transition to dysplasia and subsequent initiation of carcinoma. Based upon the work presented by Lee et al, the PI3K/Akt pathway represents novel and eminently druggable targets for the treatment of IBD. A variety of compounds which inhibit PI3K, Akt or the downstream target mTOR are currently undergoing early phase clinical trials in a variety of malignancies (Figure 1B, reviewed in Liu et al²¹). In general, these compounds appear to be well-tolerated and have the advantage – especially compared to current IBD therapies – of not inducing global immune suppression but, are apparently specific to the aerodigestive and gastrointestinal tracts as well as the endothelium. Because of the complex and incompletely understood network of intersecting regulatory signaling pathways, it is likely that additional downstream actors in these pathways (such as FOXO1 or the mTOR pathway) will need to be targeted in order to obtain maximal clinical benefit.

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Figure 1.

Interaction between PI3K/Akt and Wnt signaling pathways mediates diverse cellular effects through downstream messengers. (A) PI3K/Akt pathway (left) is activated by binding of insulin or growth factors to the PI3K receptor. Activation of PI3K in turn activates AKT, eliciting a broad range of downstream signaling events. The canonical WNT pathway (right) is activated by the binding of WNT ligand to the Frizzled receptor, activating Disheveled and disrupting the GSK3 β /Axin complex, stabilizing β -catenin which is then able to translocate to the nucleus and activate WNT target genes. These pathways converge both at the GSK3 β node and at the level of β -catenin nuclear translocation. Additional points of interaction are possible as well. (B) In inflammatory bowel disease, the progression from inflammation to carcinoma progresses through a number of steps. Immune modulators (red) suppress inflammation and proliferation directly. PI3K and AKT inhibitors inhibit the growth of carcinomas. In this study, the PI3K inhibitor LY294002 was shown to inhibit inflammation and progression to dysplasia. Other PI3K and AKT inhibitors may also be useful in preventing inflammation and progression to dysplasia and carcinoma.