The human TLR4 variant D299G mediates inflammation-associated cancer progression in the intestinal epithelium

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Colon cancer is the second leading cause of cancer death in the Western world. Innate immunity influences tumor cell behavior, representing an important component of tumor development and progression in the intestine. Genetic, commensal and environmental factors may interact to modulate innate immune reactions, resulting in variable tumor phenotypes.

Toll-like receptors (TLR) play a key role in innate immunity of the intestinal mucosa, crucially involved in maintaining mucosal as well as commensal homeostasis through control of milieu influences. TLR4 is the major receptor for LPS activation which requires the presence of accessory molecules, CD14, LBP, and MD-2. Downstream, TLR4 signals via MyD88-dependent and MyD88-independent pathways. Basal TLR4 signaling exerts host-protective responses in the intestinal mucosa. TLR4 expression is low in healthy intestinal epithelium, but significantly upregulated in inflamed mucosa (e.g., in inflammatory bowel diseases (IBD)).

Evidence suggests that aberrant TLR4 signaling is linked to carcinogenesis. We have recently demonstrated that the common human TLR4 variant D299G exerts pro-inflammatory effects and drives malignant tumor progression in human colon cancer. Our findings were based upon 2 experimental models:

First, we generated IEC lines (Caco-2) that stably overexpress wild-type (WT) TLR4, or mutant TLR4-D299G or TLR4-T399I. Caco-2 cells (non-invasive enterocyte/adenoma cells) represent an accepted in vitro human IEC model to study differentiation, barrier integrity and tumorigenesis. Stable expression of TLR4-D299G in Caco-2 cells caused aberrant actin cytoskeletal disorganisation with nuclear atypia (multipolar spindles and misaligned chromosomes), suggestive of malignant transformation. These alterations were confirmed in several individual TLR4-D299G IEC clones, but were not present in IEC controls (TLR4-WT, TLR4-T399I or mock). We found that TLR4-D299G, but not TLR4-WT, activated the Wnt/β-catenin pathway, leading to IEC de-differentiation and a mesenchymal phenotype. TLR4-D299G IEC showed highly invasive behavior, whereas control clones did not. Mechanistically, TLR4-D299G induced IEC invasion via Wnt-dependent STAT3 activation. The major TLR4-D299G targets were dominated by pro-inflammatory and pro-tumorigenic genes. In addition,
TLR4-D299G constitutively secreted large protein amounts of pro-inflammatory mediators (involved in acute phase, coagulation and complement responses). Furthermore, xenografts in mice demonstrated TLR4-D299G-induced acceleration of intestinal tumor growth, which was blocked by STAT3 inhibition. By contrast, TLR4-WT, TLR4-T399I, or mock xenografts failed to grow.

Second, to assess the role of TLR4-D299G in the pathophysiology of human primary colon cancer, we examined human colonic specimens (214 cases) in a proof-of-concept approach. Primary human colon cancers endogenously carrying the TLR4-D299G mutation showed enhanced malignant progression, when compared with TLR4-WT colon cancer at the time of diagnosis. Advanced disease (UICC ≥ III with invasion and distant metastasis at initial diagnosis) was more frequent in TLR4-D299G colon cancer patients than TLR4-WT. This observation correlated with increased expression levels of STAT3 mRNA in TLR4-D299G colon cancers compared with TLR4-WT. However, TLR4 mRNA was significantly induced in most sporadic human colon cancers, regardless of genotype.

In conclusion, our results suggest that TLR4-D299G in established tumorigenic cells of the colon may accelerate the transition to invasion and metastasis (Fig. 1). Thus, we have identified a previously unknown role for the TLR4-D299G polymorphism as a gain-of-function mutation with enhanced oncogenic potential in the intestinal epithelium. TLR4-D299G promotes a pro-inflammatory microenvironment, which may be responsible for triggering cancer progression. Our data imply a novel mechanistic link between aberrant innate immune signaling and malignant progression via STAT3 in colon cancer.

Future studies will need to examine larger patient cohorts from different geographical origin and ethnicity. While TLR4-D299G may modify the intestinal tumor cell phenotype, it remains unclear whether carriers may actually have a significantly increased overall risk of developing colon cancer, or other types of cancer. Notably, patients with IBD are at greater risk of colon cancer than the general population. TLR4-D299G has been associated with IBD susceptibility. Whether IBD patients carrying the TLR4-D299G variant are at increased risk of developing a more aggressive phenotype of colitis-associated neoplasia remains to be determined.

In our in vitro cell culture model, cancer progression was due to mutation-driven and cell-autonomous, but TLR4 ligand-independent, activation. It will be essential to investigate in vivo whether environmental factors (e.g., commensal bacteria) may influence the IEC phenotype in TLR4-D299G mutation carriers. In addition, it must be examined whether abnormal signaling via TLR4-D299G in vivo skews the commensal composition, leading to changes in mucosal immune tolerance that may contribute to tumorigenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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