

# Signaling via MYD88 in the pancreatic tumor microenvironment

## A double-edged sword

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We have recently shown that Toll-like receptor (TLR) signaling exacerbates pancreatic fibro-inflammation and promotes carcinogenesis in mice. Paradoxically, inhibition of the TLR-MYD88 signaling pathway is pro-tumorigenic owing to the dendritic cell-mediated  $T_H2$ -polarization of  $CD4^+$  T cells. TLR signaling appears to be central in pancreatic cancer-associated inflammation.

Pancreatic ductal adenocarcinoma (PDAC) is accompanied by a rich stromal component including both immune and mesenchymal cells. An intricate relationship between the epithelial and the stromal compartments exists in pancreatic cancer, which features bidirectional communication via cytokines, chemokines, growth factors and receptor-ligand interactions, and ultimately results in the growth of both compartments as well as in the resistance to most treatment modalities.

Since chronic inflammation is one of the established risk factors for the development of PDAC, we investigated the role of pattern-recognition receptors (PRRs) in pancreatic carcinogenesis. In two recent publications, we focused on the most well-known PRR family—Toll-like receptors (TLRs)—which recognize conserved microbial byproducts (pathogen-associated molecular patterns, PAMPs) as well as byproducts of sterile inflammation (damage-associated molecular patterns, DAMPs) and signal either via the TRIF (TLR3 and TLR4) or the MYD88 pathway (all TLRs except TLR3) to promote inflammation.<sup>1</sup> We found that TLR activation can accelerate pancreatic cancer development and TLR inhibition exerts

protective effect. However, directly blocking MYD88 signaling results in paradoxically pro-tumorigenic outcomes.<sup>2,3</sup>

We showed that TLR4 and TLR7 are overexpressed by pancreatic cancer cells in both mice and humans.<sup>2,3</sup> Continuous TLR activation using exogenous ligands augmented pancreatic inflammation in models of acute and chronic pancreatitis, and—as expected—accelerated pancreatic carcinogenesis in mice, while TLR blockade delayed tumor progression.<sup>2,3</sup> However, PRR signaling features a high degree of complexity, and the overall outcome of its manipulation may not be predictable. In an attempt to examine the role of the two main pathways ignited by TLR4 in the context of pancreatic cancer, we separately blocked TRIF and MYD88, using synthetic inhibitors. Although blocking the TRIF pathway protected from pancreatic tumorigenesis, inhibition of the MYD88 pathway surprisingly exacerbated pancreatic fibro-inflammation and accelerated carcinogenesis (Fig. 1).

The complexity of PRR/MYD88 signaling may be considered at two different levels. First, at the subcellular level, PRRs can have differential inflammatory outcomes, depending on the adaptor

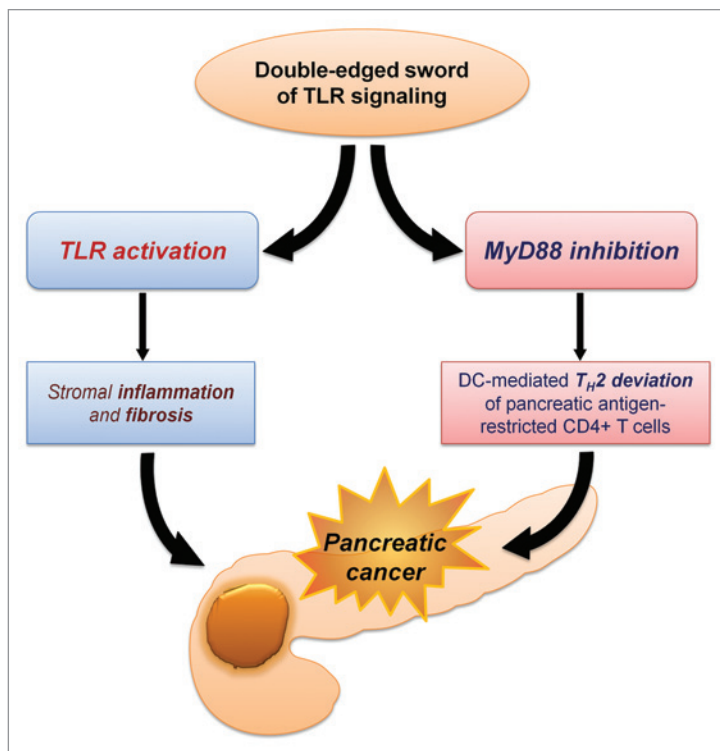
molecules recruited and the downstream signaling cascades initiated. Furthermore, the simultaneous activation of more than one PRR, as well as other immune receptors that have convergent or antagonistic signaling pathways, can influence the biological outcome.<sup>4,5</sup> Second, at the cellular level, PRR activation in different cell types can trigger different cellular behaviors, which can vary from cytotoxic and anti-carcinogenic to pro-inflammatory, tumor promoting effects. For example, TLR activation on dendritic cells (DCs) causes  $T_H1$  polarization of  $CD4^+$  T cells.<sup>6</sup> For this reason, TLR agonists are being employed as adjuvants in an attempt to boost the antitumor response elicited by adoptive immunotherapy.<sup>7</sup> However, TLR-induced DC activation also promotes the development of regulatory T cells (Tregs).<sup>6</sup> On the contrary, blocking MYD88 in DCs leads to the  $T_H2$  polarization of  $CD4^+$  T cells.<sup>6</sup> The latter phenomenon accounted for our intriguing findings in the setting of pancreatic cancer: MYD88 inhibition in DCs caused them to skew  $CD4^+$  T cells toward a  $T_H2$  profile, which in turn accelerated cancer progression by perpetuating inflammation.<sup>3</sup> Depletion of  $CD4^+$  T cells rescued from the pro-carcinogenic effects

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**Figure 1.** Model of the effects of Toll-like receptor (TLR) activation and MYD88 blockade on pancreatic carcinogenesis. DC, dendritic cell.

of MYD88 blockade in the pancreas, although it did not protect from pancreatic cancer in the absence of MYD88 inhibition.<sup>3</sup> Consistent with our findings, De Monte et al. have recently reported that DCs are found in high numbers in pancreatic cancer and in tumor draining lymph nodes, and that the amount of tumor-infiltrating  $T_H2$  cells inversely correlates with survival in human pancreatic cancer.<sup>8</sup>

In addition, TLRs and other immune receptors acting via the MYD88 pathway have the potential to influence signaling cascades within (pre)neoplastic cells that are important in tumorigenesis. In the case of pancreatic cancer, we showed that ligation of TLRs causes derangements in several tumor suppressor proteins (such as p16, p21, p27, p53 and pRb).<sup>2</sup> At least in part, this seems to constitute an indirect effect mediated by stromal cells, as mice that were made chimeric with *Tlr4*<sup>-/-</sup> or *Tlr7*<sup>-/-</sup> bone marrows were partially protected from pancreatic carcinogenesis.<sup>2,3</sup> Moreover, TLR agonists had no direct proliferative or mutagenic effects on pancreatic ductal epithelial cells.<sup>2,3</sup>

We found that TLR activation cross-talks with STAT3- and NOTCH-mediated signaling pathways, which have been recently demonstrated to transduce growth-promoting and apoptosis-resistance signals.<sup>2</sup> Specifically, we showed that the activation of STAT3 and the upregulation of NOTCH receptors and ligands occur in both the epithelial and the stromal component of pancreatic cancer, and—through NF $\kappa$ B and MAPK—contribute to the aggressive tumor phenotype induced by TLR7 activation. We speculate that all the aberrations induced by TLR ligation form a positive feedback loop between different components of the stroma as well as pancreatic epithelial cells, which includes both autocrine/paracrine communication (mediated by cytokines, chemokines and other soluble mediators) and contact-dependent signaling (e.g., NOTCH–NOTCH ligand interactions).

The importance of uncovering a pro-carcinogenic role of PRRs in pancreatic cancer is manifold. Since PRRs can promote pancreatic carcinogenesis, it is possible that their inhibition can serve as a

therapeutic target, while our findings support that direct MYD88 inhibition would be catastrophic. Notably, we believe that TLR agonists should be used with great caution as vaccine adjuvants in patients with pancreatic cancer. Further, it will be very interesting to explore the natural ligands of PRRs in the context of pancreatic carcinogenesis. It is known that DAMPs released subsequently to cell damage and necrosis can activate several TLRs.<sup>9</sup> We found that HMGB1 and S100A9—two DAMPs that bind TLR4—are present in the pancreas of PDAC patients.<sup>3</sup> It is also possible that PAMPs originating from endogenous intestinal microbiota can reach the pancreas and contribute to pancreatic carcinogenesis in individuals at risk. Finally, polymorphisms that affect the functionality of PRRs or their downstream signal transducers may modulate the risk for pancreatic cancer development, and may also have prognostic value in PDAC patients. Further investigation is warranted to address these unanswered questions.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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