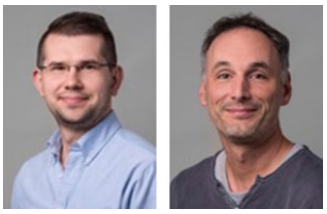
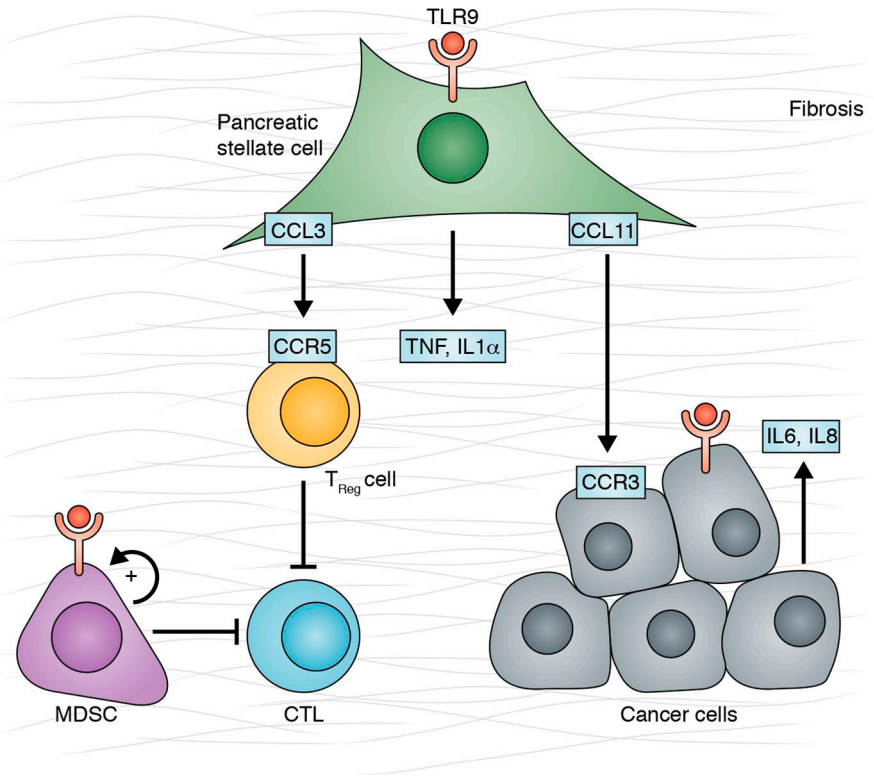
Pancreatic cancer takes its Toll



Insight from Pawel Mazur (left) and Julien Sage (right)

Development of pancreatic ductal adenocarcinoma (PDAC) is known to be driven by a persistent inflammatory state, as oncogenic mutations alone are not sufficient for tumorigenesis. Toll-like receptors (TLRs), the pillars of the innate immune system, are highly expressed in the tumor microenvironment and on circulating leukocytes in PDAC. Contrasting results have reported antitumorigenic effects or induction of intrapancreatic inflammation and tumor progression upon ligation of different members of this receptor family.

TLR9 in particular is expressed in both tumor and tumor-related cells, and its activation has been shown to impair tumor cell proliferation, suggesting that TLR9 agonists might be useful as adjuvant therapy. In this issue, Zambirinis et al. further examined the specific role of TLR9 signaling in PDAC. Gain- and loss-of-function experiments in a mouse model of PDAC showed that TLR9 activation is oncogenic in PDAC. Interestingly, these effects are only partially explained by activation of TLR9 signaling in the tumor cells themselves. Unexpectedly, the authors found expression of TLR9 on pancreatic stellate cells (PSCs; myofibroblast-like cells in the pancreas) and showed that TLR9 activation in these cells results in the production of the CCL3 and CCL11 chemokines. They show for the first time that CCL11 can promote the proliferation of pancreatic cancer cells in a dose-dependent manner. Activation of TLR9 in PSCs also leads to the recruitment of tumor suppressive regulatory T cells (T regs) that, together with TLR9-activated myeloid-derived suppressor cells (MDSCs), favor the generation of an immunosuppressive microenvironment.



Schematic depicts specific effects of TLR9 activation in epithelial, stromal, and inflammatory compartments in PDAC. A proposed model of tumor-promoting effects of TLR9 activation in pancreas tumorigenesis identifies PSCs as a key component of the inflammatory process. TLR9-activated PSCs mediate their protumorigenic effects on the epithelial compartment via CCL11 secretion. In addition, TLR9 activation may generate an immunosuppressive microenvironment by promoting MDSC proliferation and indirectly increasing T reg numbers via CCL3 secretion, both critical to disable cytotoxic T cells (CTL) in PDAC.

Perhaps one of the most important aspects of the crosstalk between tumor cells and their microenvironment is the inhibition of anticancer immune responses. This new study provides compelling evidence that, in addition to the adaptive immune system, specific molecules in the innate immune system, such as TLR9, can play crucial roles in cancer development. Importantly, the recent development of small molecule agonists and antagonists of TLRs may offer a new strategy to inhibit cancer growth. It will be interesting to determine how such manipulations may be combined with other strategies to activate antitumor T cells. Another exciting aspect of this work is the prominent role of PSCs, providing further support to the notion that desmoplasia from fibroblasts plays a critical role in the recruitment and (in)activation of immune infiltrates in pancreatic cancer. Unquestionably, the molecular complexity of tumor, stroma, and immune cells will continue to engage the efforts of pancreatic cancer researchers for many years to come.

Zambirinis, C.P., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142162

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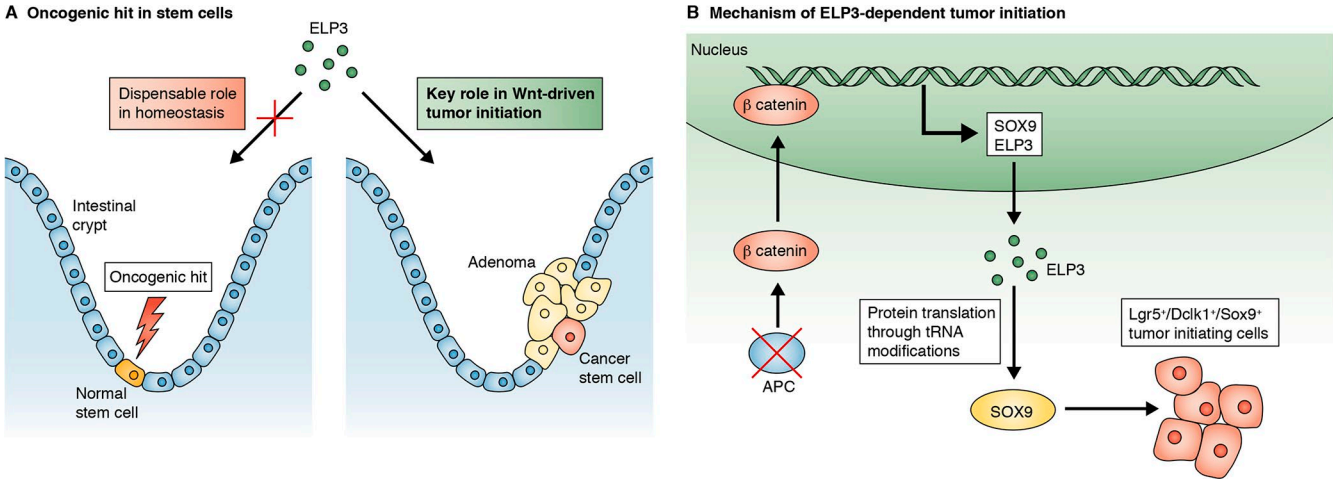
Lending an 'ELPing hand to tumor initiation

Insight from Jeremy Rich

Tumors are aberrant organ systems that develop through cooption of developmental and wound response programs. Wnt signaling represents a central organizing force in organ development and healing, but constitutive Wnt activation initiates and maintains tumor growth. Development of molecular targeted therapies against Wnt has proven challenging because of the diversity of ligands, receptors, and effectors.

In this issue, Ladang et al. discover that ELP3, an enzymatic component of Elongator, promotes Wnt-induced colon tumorigenesis and recovery from injury. As its name implies, the Elongator complex critically regulates transcriptional elongation through RNA polymerase II, but Elongator also functions in translation through tRNA modification, cytoplasmic kinase signaling, and exocytosis. Leveraging previous studies of oncogenic activity of Elongator, the authors found that Wnt induces ELP3 and that ELP3 levels are increased in colon cancers, a cancer commonly associated with Wnt dysregulation. Targeted disruption of ELP3 in the colon ablated Tuft cell generation, but no significant phenotype in colon organization or animal health was detectable at baseline. In contrast, loss of ELP3 severely attenuated tumor initiation and recovery from radiation injury, potentially through translation—not transcriptional—control of SOX9, a master organizer of the endodermal cellular hierarchy.

The colon undergoes continuous renewal with replacement of the colonic epithelium every seven days on average. Upon injury, like radiation, Wnt signaling is activated to accelerate self-renewal. The current study suggests that Elongator activity is necessary for injury responses in the colon, although future studies may define whether Elongator is sufficient for recovery of the colon and other tissues dependent on Wnt. Conceptually, activating Elongator could accelerate regeneration or improve the efficacy of cell-based therapies. As ELP3 is the catalytic subunit of the histone acetyltransferase elongator complex, its activity may be amenable to disruption through pharmacologic antagonists. Targeting ELP3 in the colon had minimal detrimental effects, suggesting that ELP3 may be an attractive target in Wnt-related cancers, especially in the colon where orally administered therapies could provide locoregional antagonism.



(A) A healthy intestinal crypt is illustrated in the left panel. Upon inactivation of the *Apc* gene suppressor ("Oncogenic hit"), Wnt signaling is constitutively activated and triggers adenoma development. Whereas the acetylase ELP3 is dispensable for the maintenance of normal stem cells and intestinal homeostasis, this factor is required for Wnt-dependent tumor initiation and induction of cancer stem cell self-renewal. (B) The inactivation of *Apc* triggers β-catenin stabilization and consequently drives the expression of Wnt target genes, such as SOX9 and ELP3. ELP3 chemically modifies tRNAs to promote SOX9 translation. As a result, a pool of $Lgr5^+/Dclk1^+/Sox9^+$ cells efficiently drives Wnt-dependent tumor initiation in the intestine.

Ladang, A., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142288

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Effective effector generation of CD8+ T cells and NK cells: A need for T-bet and ZEB-too

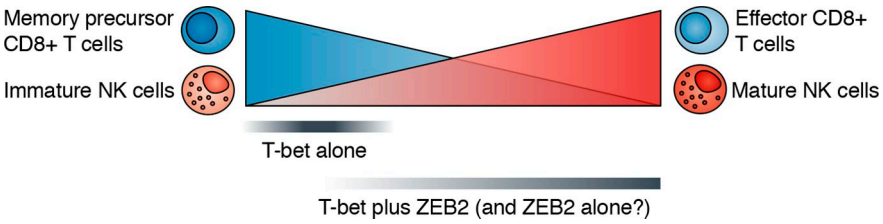
Insight from Sara Hamilton (left) and Stephen Jameson (right)

Both T cells and NK cells employ complex networks of transcriptional regulators to control their differentiation and functional prowess. In this issue, three studies report that the transcription factor ZEB2 is critical for generation and expansion of terminally differentiated effector cells, chiefly by working in partnership with T-bet.

Several transcription factors have been shown to regulate CD8+ T cell differentiation during an immune response. T-bet has emerged as a driving force in the development of KLRG1hi terminal effector cells. Both Dominguez et al. and Omilusik et al. show here that ZEB2, a molecule not previously associated with lymphocyte differentiation, is strongly up-regulated in early KLRG1hi effector cells and supports their progression to a short-lived effector status—yet appears dispensable for generation of long-lived memory cells. T-bet is required for ZEB2 expression, and the factors coregulate many of the same genes. However, the impact of ZEB2 deficiency could not be completely overcome by T-bet overexpression, and both cooperative and independent activities of these factors may be important.

Interestingly, van Helden et al. demonstrate a parallel role for ZEB2 in permitting NK cell maturation. *Zeb2* deficiency thwarted normal NK cell differentiation, migration into peripheral tissues, and control of melanoma growth. Again, many of these features echo the role of T-bet in NK cell development, and there was ample evidence of cooperation between the factors in controlling gene expression; however, ZEB2 was not completely subservient to T-bet because ZEB2 could partially restore NK development in T-bet gene–deficient mice.

These studies characterize ZEB2 as a novel player in the transcriptional control of lymphocyte differentiation, playing strikingly similar roles in CD8+ T cells and NK cells. Numerous phenotypic and functional changes accompany the differentiation of lymphocytes—a challenge has been to understand how these differentiation states relate to complex transcriptional networks within the cell and how generation of a stable subset is achieved. ZEB2 is downstream of T-bet, but cooperation between these factors is crucial for regulation of multiple genes—hence ZEB2 may serve to reinforce the differentiation program initiated by T-bet expression. However, whereas both ZEB2 and T-bet inhibit expression of memory precursor-associated genes in CD8+ T cells, Dominguez, et al. suggest that low level ZEB2 expression is required for generation of the CD8+ T cell effector-memory subset, indicating a more nuanced role. Also, whereas ZEB2 does not appear necessary for acquisition of key effector functions (such as cytolysis and IFN-γ production) by terminally differentiated CD8+ T cells or mature NK cells, it will be important to see how this factor impacts pathogen control in various contexts. Deciphering the pathways that promote effector cell generation may help direct strategies for better vaccine approaches, and understanding the cooperative and independent roles of T-bet and Zeb2 could provide new targets for therapeutic intervention.



It takes Zeb2 to tango: Cooperation between T-bet and Zeb2 is essential for CD8+ T cell effector differentiation and NK cell development. Although increasing T-bet expression alone can mediate some of the necessary gene expression changes, coordination with Zeb2 (which is itself under T-bet transcriptional control) is required for full commitment and expansion of terminally differentiated CD8+ T cells and mature NK cells.

Dominguez, C.X., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150186
Omilusik, K.D., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150194
van Helden, M.J., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150809

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Cutting to the chase: How pathogenic mutations cause Alzheimer's

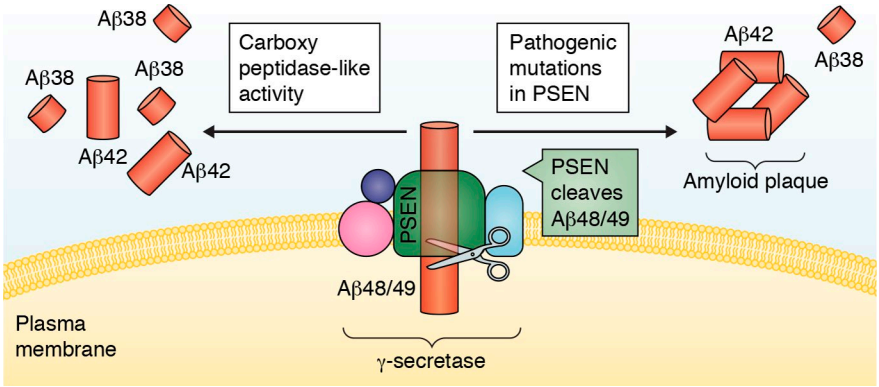
Insight from Michael Wolfe

How dominant mutations in presenilin (PSEN) cause early-onset familial Alzheimer's disease (FAD) has been debated since the discovery of such mutations 20 years ago. A study by Szaruga et al. in this issue of *JEM* now appears to provide a definitive answer.

Presenilin is the catalytic subunit of γ-secretase, a protease that cuts the transmembrane domain of the amyloid precursor protein (APP) to produce the C terminus of the amyloid β-peptide (Aβ) that notoriously deposits in the Alzheimer brain. Some argue that reduction of presenilin's proteolytic activity (i.e., a loss-of-function effect) is responsible for the neurodegeneration caused by FAD mutations. Others have shown that some mutations do not reduce proteolytic activity, but all increase the proportion of aggregation-prone 42-residue Aβ (Aβ42) to 40-residue Aβ (Aβ40; i.e., a gain of toxic function). Further complicating matters, γ-secretase initially cuts APP substrate via an endopeptidase activity to produce Aβ48 and Aβ49 and release the corresponding APP intracellular domain (AICD). These long Aβ peptides are then sequentially trimmed via a carboxypeptidase function of γ-secretase along two primary pathways: Aβ49 → Aβ46 → Aβ43 → Aβ40 and Aβ48 → Aβ45 → Aβ42 → Aβ38.

To address the loss- versus gain-of-function question, Szaruga et al. examined γ-secretase proteolytic activity in samples from post-mortem human brains from 24 FAD mutation carriers, covering nine different PSEN mutations. The samples contained endogenous human γ-secretase complexes and—importantly—both wild-type and PSEN mutant complexes. Under these natural conditions associated with the human disease state, the production of AICD—a measure of γ-secretase endoprotease activity—was not significantly different from that seen in control non-AD brains. Thus, the presence of the wild-type PSEN allele apparently compensates for any loss of endoproteolytic activity from the mutant allele. In contrast, clear reduction of carboxypeptidase activity—as measured by the ratio of Aβ38 from its precursor Aβ42—was seen for every mutation.

These findings have implications for the mechanism of Alzheimer pathogenesis and for drug discovery. In considering γ-secretase as a therapeutic target, one should first know what specific functional alterations in the enzyme lead to disease, and that appears to be decreased carboxypeptidase activity. Therefore, a search for stimulators of this activity would make sense. Such compounds have already been identified, although they appear to stimulate only the Aβ42 → Aβ38 step, insufficient if other long Aβ peptides are augmented in Alzheimer's and play pathogenic roles. As is so often the case, answering one key question leads to another.



Aβ is derived from its precursor protein APP by sequential proteolysis, first by β-secretase (not depicted) and then by γ-secretase, the latter hydrolyzing within the transmembrane (TM) domain. Initial cleavage occurs at the so-called ε site (indicated by the scissors), releasing the APP intracellular domain or AICD (red intracellular piece) and leaving Aβ49 or Aβ48 fragments in the membrane. Aβ49 or Aβ48 fragments are successively cut by the carboxypeptidase-like activity of γ-secretase, increasing the probability of release from the plasma membrane to the extracellular medium. Both ε cleavage and carboxypeptidase TM trimming depend on PSEN, the catalytic subunit of γ-secretase. Pathogenic mutations in PSEN cause a qualitative shift in Aβ profile production, increasing the proportion of released longer Aβ peptides, which are prone to aggregate and form the plaques observed FAD.

Szaruga, M., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150892

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