Notch and TGFβ
Functional partners facilitating tumor progression

Hidetaka Ohnuki and Giovanna Tosato*

Laboratory of Cellular Oncology; Center for Cancer Research; National Cancer Institute; National Institutes of Health; Bethesda, MD USA

Keywords: Notch, TGFβ, tumor-infiltrating myeloid cells, head and neck tumors, tumor microenvironment, tumor progression

Transforming growth factor β (TGFβ), a key signaling molecule produced by tumor-infiltrating myeloid cells, plays dichotomous roles in oncogenesis. TGFβ is well-documented to accelerate the progression of established tumors, particularly in advanced disease, whereas it exhibits pro-apoptotic and cytostatic effects on normal cells and pre-malignant lesions.1 This functional spectrum is explained in selected cases by the emergence of inactivating mutations in the tumor cells affecting the receptor TGFβ receptor II (TGFBR2, best known as TGFβR2) and/or the signaling SMAD family member 3 (SMAD3) molecule. Such alterations could, conceptually, abrogate TGFβ anti-tumor biological activities by preventing TGFβ-mediated signaling cascades. In most cancers, however, TGFβ receptors and downstream signaling molecules are typically intact, and so it remains obscure how TGFβ acts as a tumor enhancer.

The Notch family of transmembrane receptors comprises four members, NOTCH 1–4.2 Notch signaling is induced by ligand binding through direct cell-to-cell contact. Notch ligands comprise 5 members, including 3 delta-like (DLL1, DLL3 and DLL4), Jagged 1 (JAG1) and Jagged 2 (JAG2), each of which displays selectivity for particular Notch receptors. For instance, DLL4 activates exclusively NOTCH1 and NOTCH4. Upon ligand binding, the γ-secretase protein complex becomes active and cleaves the Notch intracellular domain, which translocates to the nucleus where it transcriptionally activates multiple target genes, including v-Myc avian myelocytomatisis viral oncogene homolog (MYC, best known as c-Myc). In addition to its critical roles in regulation of cell fate and growth during development, aberrant Notch signaling is increasingly recognized as a contributor to cancer progression.3 Constitutive NOTCH activity from oncogenic breakpoints within the NOTCH1 locus have been found to contribute to the pathogenesis of human T cell acute lymphoblastic leukemia.4 Reduced levels of lunatic fringe (Lfng) N-acetylglucosamine transferase, an enzyme that reduces Notch activation in response to Jagged ligands, leads to sustained Notch signaling in mammary epithelium and mammary tumorigenesis.5 In many cases, however, the molecular architecture underlying aberrant Notch activity in cancer are poorly defined.

In our prior investigation of mouse Lewis lung carcinoma (LLC1) model, we identified mechanisms accounting for the pro-tumorigenic activities of TGFβ and Notch.6 We found that TGFβ activates Smad2/3 signaling in LLC1 and in a dose-dependent fashion, stimulates LLC1 cell proliferation. These effects of TGFβ are cell-density dependent, as they are observed only when the cells are at higher densities, suggesting a requirement for cell-to-cell contact. Although a myriad of cell surface molecules could mediate this requirement, we focused on Notch/Notch ligands because there is evidence for cross-talk between Notch and TGFβ signaling in embryonic cells in which physical interaction between the Notch intracellular domain and Smad3 was identified as the basis for this convergence.5,6 Given that Notch activation is induced through cell-to-cell contact, we examined whether Notch signaling contributes to TGFβ pro-tumorigenic function.

We found that LLC1 cells express Notch1 and Notch4 receptors as well as their activating ligands DLL1 and DLL4, and that Notch signaling is active in dense LLC1 cultures presumably via cell-cell interaction. Remarkably, inhibition of Notch signaling by γ-secretase inhibitors substantially attenuated both TGFβ signaling and TGFβ-associated growth in LLC1 cells. This demonstrated that cooperativity between Notch and TGFβ signaling supports TGFβ-mediated growth stimulation, raising the possibility that...
similar crosstalk could facilitate LLC1 tumor progression in mice.

In contrast to LLC1 cells in culture, LLC1-derived tumors in vivo are prominently infiltrated by pro-tumorigenic CD11b+Ly6C-Ly6G+ monocytes, recruited and activated by the tumor-derived cytokines, chemokine (C-C motif) ligand 2 (CCL2) and colony stimulating factor 1 (CSF1, best known as M-CSF). We found that these tumor-infiltrating monocytes secrete abundant TGFβ that, in turn, induces Smad signaling within the LLC1 tumor. Furthermore, these myeloid cells express Dil4 at high levels, even higher than those expressed by LLC1 cells. This monocyte-associated Dil4 is anticipated to activate Notch signaling within the tumor microenvironment in which monocytes and tumor cells engage. Indeed, treatment of LLC1 tumor-bearing mice with the γ-secretase inhibitor DAPT reduced Notch and TGFβ signaling within the tumor and reduced tumor growth. Since this antitumor effect was lacking in functionally monocyte-deficient mutant mice, collectively, these results show that tumor-infiltrating monocytes are critical inducers of Notch signaling within the tumor, and that TGFβ accelerates LLC1 progression by cooperating with Notch signaling. In this scenario, tumor-promoting monocytes play a dual role as both a source of TGFβ as well as inducers of Notch signaling among tumor cells (Fig. 1). This role would be most detrimental at the infiltrating edge of the tumor, a locale where monocyte cluster and tumor cell density is low, as monocytes would be essential inducers of Notch signaling in cells comprising the tumor mass.

By querying the Cancer Genome Atlas, we found that patient head and neck squamous cell carcinomas with significant monocyte infiltration, potentially signifying tumor aggressiveness, display a gene expression signature characterized by greater expression of DILL4, NOTCH4, the Notch signaling mediator hairy/ enhancer-of-split related with YRPW motif 1 (HEY1), TGFβ and the TGFβ receptor 1 (TGFβR1) relative to normal tissue. This expression profile is similar to the profile of experimental LLC1 tumors, suggesting that TGFβ/Notch signaling cooperation may contribute to progression of head and neck squamous cell carcinoma. Thus, these findings raise the possibility that dual targeting of TGFβ and Notch may represent a rational approach to the treatment of particular cancers. Fortuitously, many promising drugs targeting Notch1 and TGFβ4 are currently under development for clinical use.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.