References


To the editor:

TCF-1 mediates repression of Notch pathway in T lineage–committed early thymocytes

Notch-derived signals are essential for specification of hematopoietic progenitors to T-cell lineage and for promotion of β-selection at the CD4−CD8− double-negative 3 (DN3) stage. However, these signals are not required for further thymocyte maturation.1 Accordingly, the expression of Notch1 and its target genes, including Ptcra (encoding pre–T-cell receptor alpha [TCRα]), markedly decreases in late DN3 and DN4 cells.2,3 Notch1 downregulation has been attributed to pre-TCR–induced Id3, which antagonizes the
Notch1-positive regulator, E2A. A, Hes1, a Notch target gene, is negatively regulated by Ikaros in DN4 cells. However, there are 2 unresolved issues regarding attenuation of Notch signals: (1) although reduced in transcripts, surface expression of Notch1 protein remains equally high in DN3 and DN4 cells, suggesting that downregulation of Notch target genes requires other repressive mechanisms independent of Notch1; and (2) because pre-TCR signals are attenuated/terminated in DN4 cells due to Ptcra downregulation, sustained Notch1 repression may require additional factors.

We recently demonstrated that T-cell factor 1 (TCF-1; encoded by Tcf7) suppressed thymic malignancy. The neoplastic cells exhibited increased expression of Notch1 and its target genes and accumulated somatic Notch1 mutations, suggesting possible negative regulation of Notch signaling by TCF-1. Analysis of premalignant Tcf7+/− thymocytes revealed that TCF-1 deficiency caused increased expression of Notch1 and its known targets, Dxl1 and Ptcra, in DN3 and more markedly in DN4 cells (Figure 1A). TCF-1 interacts with Wnt-mediated β-catenin cofactor to regulate gene expression. To substantiate negative regulation of Notch pathway by TCF-1, we infected primary DN thymocytes with retrovirus expressing WT or constitutively active mutant β-catenin. As expected, forced expression of mutant β-catenin induced Axin2 and repressed Lef1 expression, with little effect on Tcf7 itself in DN3 thymocytes (Figure 1B). Interestingly, the mutant β-catenin greatly diminished expression of Notch1, Dxl1, and Ptcra in DN3 and DN4 thymocytes (Figure 1B), indicating direct involvement of the TCF-1–β-catenin complex in Notch repression. To exclude the possibility that β-catenin acts through factors other than TCF-1, we stabilized β-catenin in Tcf7−/− DN3 cells using BIO, a GSK3β inhibitor. Compared with its inactive analog MetBIO, BIO stimulation diminished the expression of Notch1, Dxl1, and Ptcra in control DN3 thymocytes (Figure 1C). In contrast, this effect was abolished in Tcf7−/− DN3 thymocytes but relatively unaffected in LEF-1-deficient DN3 cells (Figure 1C).

Our findings offer a unified answer to the 2 unresolved issues noted above. The answer being TCF-1 is responsible for early repression of Notch targets, including Ptcra, and hence attenuation of pre-TCR signaling, and is responsible for sustained repression of Notch1 after pre-TCR signals are diminished. Interestingly, TCF-1-mediated Notch1 downregulation is specific to thymocytes at the DN3 stage or beyond, where they are fully committed to the T-lineage, because Notch1 expression was not significantly affected by TCF-1 deficiency in DN1 cells and forced expression of the mutant β-catenin in DN1 did not repress Notch1 (supplemental Figure 1 on the BLOOD website). A requirement of β-catenin for normal thymopoiesis remains a contentious issue. It is therefore important to note that our gain-of-functional analysis demonstrates the sufficiency but not the necessity of activated β-catenin in repressing Notch signals in T-lineage–committed thymocytes. Nevertheless, our data revealed that the active Notch signaling is attenuated by a TCF-1-dependent mechanism during transition of DN1/early thymic progenitors to T-cell lineage-committed DN3 thymocytes. This model is consistent with the observations that Notch signaling is dispensable for late stages of thymocyte maturation and that ablation of both TCF-1 and LEF-1 arrests all thymocytes at the DN stage.

To the editor:

Low frequency of H3.3 mutations and upregulated DAXX expression in MDS

H3.3 represents a replication-independent histone 3 variant. H3.3 associates with alpha thalassemia/mental retardation syndrome X-linked (ATRX) and death domain-associated protein (DAXX) required for H3.3 chromatin assembly at pericentric heterochromatin and telomeres.1-3 Somatic mutations within H3F3A gene, which encodes the H3.3 histone variant, as well as within ATRX

References

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