Genome-wide analysis of dendritic cell differentiation

Introduction and Objectives: Dendritic cells (DCs) comprise genetically and functionally distinct subsets, including the interferon-producing plasmacytoid DCs (pDCs) and the antigen-presenting classical DCs (cDCs). In the steady state, DC development from common progenitors in the bone marrow is driven primarily by the cytokine Flt3 ligand (Flt3L) and its receptor Flt3. Flt3 is expressed on uncommitted hematopoietic stem/progenitor cells as well as on DC progenitors and mature DCs. Moreover, Flt3L is sufficient to drive DC development and subset specification from murine primary bone marrow cells and immortalized progenitors. It remains unclear how the same signaling pathway supports both progenitor proliferation and DC lineage commitment, necessitating a systematic search for molecular regulators of the process.
Methods: The conditionally immortalized progenitor cell line HoxB8-FL can be induced to differentiate into functional pDCs and cDCs in a Flt3L-dependent manner, providing a unique tool to study Flt3L-driven DC development. We have retrofitted HoxB8-FL cells with Cas9 and used it to conduct a CRISPR-based knockout screen with a genome-wide sgRNA library. In a parallel approach, we used a targeted sgRNA library targeting more than 1,500 transcription factors. Results: Preliminary comparison of sgRNA content between undifferentiated HoxB8-FL progenitors and their DC progeny revealed highly significant differences, identifying candidate regulators of DC differentiation. Notably, sgRNAs targeting Pten were highly enriched in differentiated cells, suggesting that Pten deletion facilitates DC differentiation. These data are consistent with our previous identification of Pten as a negative regulator of cDC development that restricts the PI(3)K/mTOR signaling downstream of Flt3 (Sathaliyawala et al., Immunity 2010).
Conclusion: Our data provide a proof of principle for the bona fide genome-wide genetic analysis of DC differentiation, and suggest the utility of our system for the identification of known and novel pathways controlling DC development.

Keywords : DC development, CRISPR screen, Flt3L
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