HUMAN MACROPHAGES LACKING NF-κB P50 DISPLAY INCREASED PROINFLAMMATORY CYTOKINE EXPRESSION

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Background The NF-κB family of transcription factors plays a key role in inflammation. Proinflammatory signals induce IκB degradation to release cytoplasmic NF-κB p50:p65 heterodimers, which enter the nucleus and activate gene expression. NF-κB p50:p50 homodimers have 60-fold reduced affinity for IκB, and under basal conditions they repress proinflammatory genes. Multiple cancers grow slower in syngeneic p50(−/−) mice, or in p50(−/−);Lys-Cre mice lacking p50 in mature myeloid cells. Murine tumor macrophages lacking p50 display increased expression of a subset of proinflammatory (M1) genes, including IL-1β, IL-12β, and TNFα, and reduced expression of a subset of suppressive (M2) genes, which is associated with increased numbers of both total and activated tumor-infiltrating T cells.1–4 As a therapeutic approach, we find that adoptive transfer of immature myeloid cells lacking p50 (p50-IMC) slows the growth of syngeneic murine prostate cancer, pancreatic ductal carcinoma, and neuroblastoma tumors, when given after a dose of 5-fluorouracil.4–5 Immature rather than mature cells are utilized to favor tumor localization and retain dendritic cell potential. We have now sought to optimize development of human p50-IMC and determine whether macrophages that develop from these cells also manifest a more pro-inflammatory gene expression pattern.

Methods Human marrow CD34+ cells were nucleofected with Cas9:sgRNAs targeting the NFKB1 gene encoding p50, or with a non-targeting sgRNA. Cells were expanded in serum-free media that included SCF, TPO, FL, and IL-6, followed by transfer to serum-free media supplemented with M-CSF alone to produce monocytes, and finally to IMDM with human AB serum to generate macrophages. IFNγ or IL-4 were added for 24 hr to induce M1 or M2 polarization, followed by RNA isolation and qRT-PCR. Myeloid maturation in M-CSF was monitored by flow cytometry.

Results Highly efficient NFKB1 gene editing was confirmed by DNA analysis and Western blotting. Exome sequencing identified off-target edits affecting protein-coding regions of 27 genes, with 0.5–5.4% frequency; none are predicted to be transforming. Upon transfer of gene-edited cells to M-CSF, CD11b+CD14+ myeloid cells gradually accumulate and represent ~66% of the cell population after five days, with 2% granulocytes and rare erythroid cells and lymphocytes. Macrophages lacking human p50 manifested increased expression of a subset of M1 mRNAs, including IL-1β, IL-12β, and TNFα, and reduced expression of a subset of M2 mRNAs upon exposure to either IL-4 or IFNγ.

Conclusions Findings presented identify and validate a means to generate human p50-IMC for clinical application.

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