Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

Analysis of the expression and function of transcription factor Forkhead box Q1 (FoxQ1) in macrophages

Autor: Ilja Ovsiy
Institut / Klinik: Klinik für Dermatologie, Venerologie und Allergologie
Doktormutter: Prof. Dr. J. Kzhyshkowska

Monocyte polarization in the circulation during inflammation is a result of co-operative action of systemic and locally produced factors. However, the exact molecular mechanism of the specific activation of monocytes in blood stream is still poorly understood. Recent studies showed Th2-derived cytokine IL-4 as a key mediator of alternative activation of macrophages during chronic inflammation. In our laboratory novel transcription factor Forkhead box Q1 (FoxQ1) was identified as IL-4-induced gene in human primary macrophages. Upregulation of FoxQ1was also observed in monocytes of patients with acute atopic dermatitis. FoxQ1 is a member of forkhead transcription factors family that share specific DNA-binding motif known as the 'winged helix' domain. In human FoxQ1 is actively involved in epithelial mesenchymal transition and downregulates transcription of E-cadherin. However there is no evidence about the function of FoxQ1 in monocytes or macrophages. The main aim of the present study was to investigate FoxQ1 expression regulation in monocytes/macrophages, to identify the role of FoxQ1 in monocyte/macrophage functions, and the effects of FoxQ1 on the transcriptional changes in macrophages. Analysis of FoxQ1 expression in different stimulated human monocyte–derived macrophages by qRT-PCR revealed that IL-4 is primary factor needed for FoxQ1 expression. Human monocytes respond to IL-4 stimulation by FoxQ1 expression already after 3 hours, and this effect is increasing during monocyte to macrophage differentiation. The effect of IL-4 is amplified by TGF-β1 in macrophages that express TGF-βRII on their surface. Cell-based model system was generated in order to identify FoxQ1-induced genes and to analyse function of FoxQ1. Murine macrophage-like RAW 264.7 cells were stably transfected with pEF6/V5-HisB-mFoxQ1 construct or empty vector pEF6/V5-HisB. Comparison of three RAW-mFoxQ1 and three RAW-vector single cell-derived clones revealed, that overexpression of recombinant FoxQ1 does not affect basic macrophage functions endocytosis and phagocytosis, but suppresses both basic inflammatory response and inducible inflammatory response of macrophages to LPS by secretion of TNF-α. In order to identify possible target genes of transcription factor FoxQ1 expression profiles of five RAW-mFoxQ1 clones and five RAW-vector clones were analyzed and compared using Affymetrix microarray assay. Affymetrix chip assay revealed that FoxQ1 target genes can be involved in cell motility and cytoskeletal dynamics. Downregulation of cell migration suppressors Plexin C1, LSP1 and Claudin 11 in RAW-mFoxQ1 cells was confirmed by qRT-PCR. Semaphorin 7a receptor Plexin C1 was found to be suppressed in human-derived microphages after treatment with IL-4 and in monocytes of patients with atopic dermatitis, indicating that Plexin C1 is target gene of FoxQ1 in human monocytes. Migratory activity of five RAW-mFoxQ1 clones and five RAW-vector clones toward MCP-1 and FCS gradients was compared by *in vitro* transmigration assay. RAW-mFoxQ1 clones migrated toward nutritional gradient 15.5 times and toward MCP-1-gradient 19.5 times more efficient compared with RAW-vector cells. Scratch assay confirmed that FoxQ1 supports migration activity of monocytes/macrophages even in the absence of the gradient of chemoattractants. In addition, generated anti-FoxQ1 antibody (HFQC 1D5) were very specific in recognition of recombinant over expressed FoxQ1, however the sensitivity of HFQC 1D5 was insufficient for the quantitative analysis of endogenous FoxQ1. Therefore Real-time PCR remains to be the most reliable method for the FoxQ1 quantitative analysis in monocytes/macrophages. In conclusion, the results of the thesis project indicate that monocytes rapidly respond IL-4 by FoxQ1 upregulation that in turn mediate efficient migration of monocytes toward MCP-1 gradient. The FoxQ1-induced cell migration can be mechanistically explained by the suppression of Plexin C1, LSP1 and Claudin 11. Therefore FoxQ1 may support increased monocytes extravasation to the sites of chronic inflammation or into tumours.