

The role of CUX1 in antagonizing NF- κ B signaling in TAMs

Benjamin Kühnemuth and Patrick Michl*

Department of Gastroenterology, Endocrinology and Metabolism; Philipps-University Marburg; Marburg, Germany

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Macrophages represent a major component of the tumor microenvironment and contribute to neoplasia initiation and cancer progression. However, the molecular mechanisms underlying these phenomena are only partially understood. Manipulating the transcriptional activity of the macrophage functional specification factor NF- κ B by virtue of a novel regulatory factor cut-like homeobox 1 (CUX1) may provide a potential target for therapeutic intervention.

Macrophages in Cancer

Chronic inflammatory conditions are well known to promote the development of cancer. Infiltrating inflammatory cells are present in preneoplastic lesions and in established tumors. Tumor-associated macrophages (TAM) exhibit high cellular plasticity linking innate and adaptive immunity but also executing fundamental functions in wound healing and tissue repair.

Investigations of cancer patient specimens and studies in genetic engineered mouse models have revealed that macrophages frequently promote tumor development. Nevertheless, in some tumor entities such as colorectal carcinoma, high macrophage infiltration has been found to be a positive prognostic marker. These contradictory findings are indicative of functionally distinct phenotypes of macrophages dependent upon the cellular context and the tumor type.^{1,2}

Macrophage Phenotypes

Mimicking endogenous specialization, macrophages can be polarized by exogenous application of immunogenic agents *in vitro* toward differing functional states described as classically (M1) or

alternatively (M2) polarized phenotypes. M1 macrophages can be generated by exposure to pro-inflammatory cytokines or microbial products. They are integrally involved in fostering immune responses and are potentially tumor inhibiting. M2 macrophages and their corresponding inducing agents, on the other hand, are highly heterogeneous. For example, these potentially tumor-promoting M2 macrophages may be induced by the interleukins IL-10 and IL-6, the chemokines CCL2 and CXCL4, or hypoxia. Functions described for M2 macrophages range from tissue remodelling to immune regulation. M2-like phenotypes predominate in most human and murine tumors.²

NF- κ B Signaling in TAMs

The phenotype of macrophages is shaped by a complex network of signaling pathways and a multitude of transcription factors. Nuclear factor κ B (NF- κ B) has emerged as a central regulator of macrophage functional specification and as such is crucial for the response of macrophages toward M1-polarizing stimuli including Toll-like receptor (TLR) ligands or tumor-necrosis factor- α (TNF- α).³ In the canonical pathway, 5 distinct but structurally analogous NF- κ B family members (RelA, RelB, c-Rel, p50, and p52)

form hetero- and homodimers that are functionally regulated by sequestration via binding to the Inhibitor of κ B (I- κ B) in the cytoplasm. Upon stimulation of the NF- κ B signal transduction cascade, I κ B gets phosphorylated by activated I κ B-kinase complex (IKK) inducing the proteasomal degradation of I κ B and the subsequent release and translocation of NF- κ B into the nucleus for transcriptional regulation of target genes.

Several studies have highlighted the significance of myeloid cell NF- κ B activity in the initiation of neoplastic lesions by fostering tumor-promoting inflammation.⁴ Interestingly, macrophages isolated from established tumors exhibit defects in NF- κ B signaling and impaired expression of inflammatory mediators, phenotypes consistent with a M2-like specification. Mechanistically the inactivation of NF- κ B pathways has been attributed to delayed phosphorylation of the I κ B and increased nuclear localization of p50 homodimers, known to compete with p65/p50 heterodimers for binding site occupancy.⁵

Our group has observed an additional mechanism by which NF- κ B signaling is repressed in TAMs.⁶ Cut-like homeobox 1 (CUX1), a homeodomain transcription factor known to be highly expressed in several tumor types (e.g., pancreatic cancer), was found to displace RelA from the promoters of M1-associated genes, such as

*Correspondence to: Patrick Michl; Email: michlp@med.uni-marburg.de

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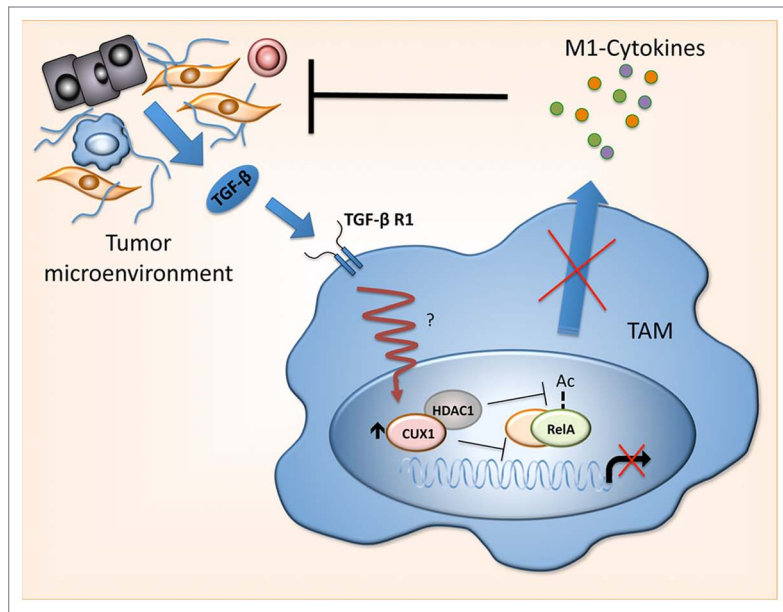


Figure 1. Role of CUX1 in antagonizing NF-κB signaling in tumor-associated macrophages. Transforming growth factor-β (TGFβ) secreted by various cells in the tumor microenvironment induces expression of cut-like homeobox 1 (CUX1) in tumor-associated macrophages (TAMs). CUX1 both directly represses nuclear factor-κB (NF-κB) DNA binding and recruits histone-deacetylase-1 (HDAC1) to NF-κB promoters where it modulates the acetylation status of the RelA component, thereby leading to the reduced transactivation of NF-κB regulated cytokines. This inactivation of NF-κB signaling contributes to the impaired cytokine secretion of TAMs found in established tumors.

chemokine (C-X-C) ligand 10 (CXCL10) or chemokine (C-C) ligand 5 (CCL5). Furthermore, we found evidence that CUX1 impedes NF-κB's transcriptional activity by interfering with regulatory acetylation status. De-acetylation of RelA is mediated by CUX1-mediated recruitment of histone deacetylase 1 (HDAC1) to the promoters of NF-κB target genes.⁶ Hence, CUX1 is involved in modulating NF-κB signaling in TAMs, thereby inhibiting the secretion of pro-inflammatory factors that are capable of impeding tumor progression. Furthermore, CUX1 is a transcriptional target of the immunosuppressive cytokine transforming growth factor-β (TGFβ) produced by a broad spectrum of cells in established tumors. However, the pathways involved in the transcriptional upregulation of CUX1 by TGFβ remain to be fully elucidated. In summary, as shown in **Figure 1**, based on our data CUX1 represents a novel downstream mediator of immunosuppressive, stroma-modulating signaling cascades such as TGFβ in macrophages, linking TGFβ signaling with NF-κB-mediated tumor antagonism.

Targeting NF-κB in TAMs

Given the role of macrophages in malignant disease progression and the central function of NF-κB in manifesting the phenotype of these cells, intervention in the pathways involved in this process might be worthwhile targets for cancer therapy. Beside strategies such as inhibition of macrophage recruitment or depletion of myeloid cells with drugs such as liposomal clodronate, one goal is the “re-education” of M2-like phenotypes to a cell state akin to M1 phenotypes.

To date, attempts to reactivate canonical NF-κB signaling have included the evaluation of toll-like receptor (TLR) agonists or antibodies (Ab) against CD40. TLR-agonists such as Poly(I:C), a TLR3 ligand, or monophosphoryl lipid A, a TLR4 ligand, are well known inducers of NF-κB activity and have proven their ability to redirect M2 polarized macrophages to an M1 phenotype. Some of these agents have shown antitumor effects in vivo and are currently under investigation in clinical trials.⁷

The exact role of CD40 in macrophage polarization is not fully understood. Ligand binding to CD40 is known to activate the non-canonical NF-κB signaling pathway previously reported to increase the expression of M2-associated genes.³ However, treatment of murine macrophages with agonistic anti-CD40 Ab activated these cells to secrete interferon-γ (IFNγ) and mediate destruction of tumor cells in vitro.⁸ Furthermore, Beatty and colleagues reported that activation of macrophages with an agonistic CD40 antibody increased their tumoricidal activity and facilitated depletion of tumor stroma in a genetic mouse model of pancreatic cancer, a functional alteration accompanied by increased expression of M1 associated genes.⁹ In support, cotreatment of human pancreatic ductal adenocarcinoma patients with CD40 Ab and gemcitabine lead to marked tumor regression in some cases.⁹

It should be mentioned that activation of NF-κB pathways does not uniformly trigger M1-phenotype conversion of TAMs and the effects of modulating NF-κB signaling on tumor progression remain controversial. In this context, Hagemann et al. reported that inhibition of NF-κB signals specifically in TAMs rendered them cytotoxic to tumor cells concurrently with the acquisition of an M1 phenotype.¹⁰

Conclusion

Due to its crucial role in the specification of macrophage phenotypes toward tumor-promoting or tumor-inhibitory TAMs, the NF-κB signaling nexus represents a tantalizing target for cancer treatment. However, the complexity and context dependency of NF-κB in TAMs warrants further investigations of this signaling network to enhance therapeutic benefit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010; 11:889-96; PMID:20856220; <http://dx.doi.org/10.1038/ni.1937>
2. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* 2012; 122:787-95; PMID:22378047; <http://dx.doi.org/10.1172/JCI59643>
3. Biswas SK, Lewis CE. NF- κ B as a central regulator of macrophage function in tumors. *J Leukoc Biol* 2010; 88:877-84; PMID:20573802; <http://dx.doi.org/10.1189/jlb.0310153>
4. Arkan MC, Greten FR. IKK- and NF- κ B-mediated functions in carcinogenesis. *Curr Top Microbiol Immunol* 2011; 349:159-69; PMID:20845109; http://dx.doi.org/10.1007/82_2010_97
5. Saccani A, Schioppa T, Porta C, Biswas SK, Nebuloni M, Vago L, Bottazzi B, Colombo MP, Mantovani A, Sica A. p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. *Cancer Res* 2006; 66:11432-40; PMID:17145890; <http://dx.doi.org/10.1158/0008-5472.CAN-06-1867>
6. Kühnemuth B, Mühlberg L, Schipper M, Griesmann H, Neesse A, Milosevic N, Wisniewski T, Buchholz M, Gress TM, Michl P. CUX1 modulates polarization of tumor-associated macrophages by antagonizing NF- κ B signaling. *Oncogene* 2013; (Forthcoming); PMID:24336331; <http://dx.doi.org/10.1038/onc.2013.530>
7. Tang X, Mo C, Wang Y, Wei D, Xiao H. Anti-tumour strategies aiming to target tumour-associated macrophages. *Immunology* 2013; 138:93-104; PMID:23113570; <http://dx.doi.org/10.1111/imm.12023>
8. Buhtoiarov IN, Lum H, Berke G, Paulnock DM, Sondel PM, Rakhmilevich AL. CD40 ligation activates murine macrophages via an IFN-gamma-dependent mechanism resulting in tumor cell destruction in vitro. *J Immunol* 2005; 174:6013-22; PMID:15879094
9. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, Sharp LL, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 2011; 331:1612-6; PMID:21436454; <http://dx.doi.org/10.1126/science.1198443>
10. Hagemann T, Lawrence T, McNeish I, Charles KA, Kulbe H, Thompson RG, Robinson SC, Balkwill FR. "Re-educating" tumor-associated macrophages by targeting NF-kappaB. *J Exp Med* 2008; 205:1261-8; PMID:18490490; <http://dx.doi.org/10.1084/jem.20080108>