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Role of c-Myc in tumor-associated macrophages and cancer progression

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**Abbreviations:** tumor associated macrophage (TAM)
Abstract

Transcription factors of the Myc family regulate several homeostatic cell functions, and their role as proto-oncogenes has been the focus of interest for decades. We recently demonstrated that c-Myc is expressed in tumor-associated macrophages (TAMs) and regulates their phenotype and pro-tumoral activities in vivo.

Main Text

Myc transcription factors have long been known to affect many cellular processes, and recent experiments in Drosophila melanogaster have revealed yet another new facet of these proto-oncogenes. In fly embryos, ‘winner’ cells with high dMyc levels actively eliminate neighboring ‘loser’ cells with lower dMyc activity to become the predominant cells.\(^1\) This so-called ‘cell competition’ phenomenon, which seems to be part of a developmental size and quality control program,\(^1\) has also been proposed to play an important role in mammalian cancer development, where an initial tumoral cell with higher expression/activity of proto-oncogenes, such as c-Myc, might have certain advantages over non-tumoral neighboring cells (eg, increased metabolic rate, ability to resist apoptosis and proliferative activity).\(^2, 3\) The establishment of this competitive niche by the tumor cell suggests the possibility that other cells in the tumor microenvironment might gain an advantage over neighbors from expressing Myc. Indeed, we recently detected c-Myc expression in tumor associated macrophages (TAMs) from human colon cancer.\(^4\)

TAMs produce factors that promote angiogenesis, remodel tissue and dampen the anti-tumor immune response. Macrophages acquire specialized phenotypes in response to signals from the local microenvironment that polarize them toward a specific activation state.\(^5\) The tumor microenvironment has been shown to skew TAMs towards a wound healing/regulatory state that resembles several aspects of the alternatively-activated macrophage phenotype.\(^6\) We have recently shown that c-MYC is induced in human macrophages during in vitro alternative activation and controls the expression of several alternative-specific markers,\(^4\) thus suggesting that targeting c-Myc function in TAMs may limit tumor growth. To test this possibility in a pre-clinical setting, we crossed c-Myc\(^{fl/fl}\) with LysM\(^{cre/+}\) mice to generate c-Myc\(^{fl/fl}-\)LysM\(^{cre/+}\) mice with myeloid cell-specific c-Myc inactivation (\(M\theta\)-c-Myc-KO).\(^7\) Compared with control
counterparts with intact c-Myc (c-Myc^{fl/fl}), bone-marrow derived macrophages and peritoneal macrophages from Mθ-c-Myc-KO mice showed lower c-Myc levels (70-100% lower across all analyzed mice), while expression of c-Myc was unaffected in other tissues such as liver, kidney and testis. Under steady-state conditions, Mθ-c-Myc-KO mice exhibited normal immune system parameters, including numbers of total bone marrow cells and bone marrow hematopoietic precursors (eg, multipotent, multiple erythroid, granulocyte-macrophage, common myeloid and macrophage progenitors, and long-term and short-term hematopoietic stem cells). Moreover, Mθ-c-Myc-KO and control c-Myc^{fl/fl} mice showed similar circulating cell counts, including total white blood cells, lymphocytes, neutrophils, classical- and non-classical monocytes, granulocytes, erythrocytes and platelets, as well as comparable numbers of similarly distributed infiltrated CD68+ macrophages in spleen, thymus and lymph nodes.

To investigate cancer development in mice with macrophage-specific deletion of c-Myc, we injected B16-F10 melanoma cells carrying the firefly luciferase gene into the flanks of Mθ-c-Myc-KO and control c-Myc^{fl/fl} mice and performed in vivo luciferase bioluminescent assays to assess tumor growth over time. These longitudinal studies together with post-mortem analysis performed 15 days after tumor cell inoculation revealed reduced tumor growth in Mθ-c-Myc-KO mice. Analysis of TAMs from Mθ-c-Myc-KO tumors showed reduced expression of c-Myc with no effect on their proliferation or apoptosis rates, which, as in controls, were very low. Interestingly, detailed phenotyping by flow cytometry showed a higher abundance of mature Ly6C^{low}MHCII^{high} TAMs in controls, whereas TAMs isolated from Mθ-c-Myc-KO mice mostly showed an immature Ly6C^{high}MHCII^{high} phenotype. This observation is consistent with previous reports indicating that different tumor types or tumor grades are infiltrated by different TAM subtypes. Our results provide evidence for a previously unrecognized role of c-Myc in the control of TAM maturation in vivo. It would be interesting to study if, over time, tumors from Mθ-c-Myc-KO mice eventually reach the size of tumors seen in controls and, at that point, if their TAMs have matured, somehow bypassing c-Myc inactivation. It should also be remembered that other transcription factors, such as NF-κB, are important drivers of cancer-related inflammation, therefore it would be interesting to analyze whether c-Myc and NF-κB act in parallel or sequentially and if they are expressed in the same macrophages or in distinct macrophage populations with different distributions within the tumor.
TAMs isolated from \( \mathcal{M}_\theta-\text{c-Myc-KO} \) mice also showed an attenuation of protumoral functions (such as reduced expression of VEGF, MMP9, and HIF1\( \alpha \)), and this was associated with impaired tissue remodeling and angiogenesis \textit{in vivo}, as assessed by molecular fluorescence tomography, and reduced development of new blood vessels, as revealed through the post-mortem confocal microscopy analysis of tumors.\(^7\) Consistent with these findings, TAMs lacking c-Myc obtained \textit{in vitro} by treating \( \mathcal{M}_\theta\text{-c-Myc-KO} \) bone-marrow derived macrophages with tumor-conditioned medium showed reduced expression of VEGF, MMP9, and HIF1\( \alpha \) than \textit{in vitro} control TAMs with intact c-Myc, and this was accompanied by decreased pro-tumoral activity, revealed by reduced metalloproteinase activity in zymogram assays, inhibition of endothelial cell proliferation and migration in wound healing assays, and inhibition of CD8+T lymphocyte proliferation.

Our work identified c-Myc as an important regulator of TAM biology and maturation \textit{in vivo} (Fig. 1). Compared with controls, \( \mathcal{M}_\theta\text{-c-Myc-KO} \) mice lacking c-Myc in myeloid cells exhibit defective tumor angiogenesis and reduced melanoma and fibrosarcoma development in xenograft models. These findings suggest c-Myc inactivation as an attractive strategy for cancer treatment. We believe that the new \( \mathcal{M}_\theta\text{-c-Myc-KO} \) mouse model will also be useful for analyzing the role of c-Myc in other inflammatory diseases associated with myeloid cell infiltration.
References

Figure 1. Role of c-Myc in tumor-associated macrophages and cancer progression. After recruitment from peripheral blood, monocytes differentiate within the tumor into immature TAMs which do not display yet fully pro-tumoral functions. In control c-Myc^{+/+} mice with intact c-Myc expression, TAMs mature and express high level of HIF1α, MMP9 and VEGF, which contribute to tissue remodeling, angiogenesis and tumor growth. Myeloid-specific deletion of c-Myc in M₀-c-Myc-KO mice impairs TAM maturation and expression of pro-tumoral factors, thus limiting cancer development.
Control mice (c-Myc^{fl/fl})

**Normal TAM maturation**

Inmature TAMs $<$ Mature TAMs

(Ly6C^{high}MHCII^{high}) (Ly6C^{low}MHCII^{high})

Higher tissue remodeling
Higher angiogenesis
Less T Lymphocyte infiltration

Mφ-c-Myc-KO mice (c-Myc^{fl/fl} LysM^{cre/+})

**Impaired TAM maturation**

Inmature TAMs $>$ Mature TAMs

(Ly6C^{high}MHCII^{high}) (Ly6C^{low}MHCII^{high})

Less tissue remodeling
Less angiogenesis
Higher T lymphocyte infiltration

**Peripheral blood**

Recruitment

Monocyte

Ly6C^{high}MHCII^{low}

**TAM differentiation**

Monocyte

Inmature TAM

Ly6C^{high}MHCII^{low}

Mature TAM

T Lymphocyte

Tumoral Cell

Neo-angiogenesis

Tissue remodeling

**Bigger tumor**

**Smaller tumor**

**Tumor Cell Neo-angiogenesis**

**Tissue remodeling**