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REVIEW

Tumor microenvironment: becoming sick of Myc

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Abstract Several years ago, we described Myc as "the oncogene from hell", since evidence had just emerged that Myc, aside from being responsible for cell-cycle progression and tumor expansion, was also able to induce genomic instability in culture, wreaking havoc in tumor cells and accelerating tumor progression (Soucek and Evan, Cancer Cell 1:406–408, 2002; Vafa et al., Mol Cell 9:1031–1044, 2002). In this review, we discuss recent publications that expand Myc's evil armory to include coordination of the crosstalk between tumor and microenvironment. Indeed, endogenous Myc, acting as a client for upstream oncogenic lesions, instructs the tumor stroma, engages a complex inflammatory response and induces angiogenesis, thus allowing the tumor to thrive. This is highly topical in light of the fact that Hanahan and Weinberg have recently redefined the hallmarks of cancer and pointed out that genomic instability and inflammation are essential for both their acquisition and development (Hanahan and Weinberg, Cell 144:646–674, 2011). Myc, it seems, is behind it all.

Keywords Myc · Omomyc · Cancer · Microenvironment · Tumorigenesis · Inflammation · Angiogenesis

Abbreviations

VEGF	Vascular endothelial growth factor
IL-1β	Interleukin-1β
Hif1a	Hypoxia-inducible transcription factor 1 alpha
Bcl-xl	B-cell lymphoma-extra large
RIP	Rat insulin promoter 1
SV40	Simian virus 40

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Introduction

Myc is a highly pleiotropic transcription factor known to control proliferation, metabolism, differentiation, and apoptosis [4–6]. Normally its expression is tightly regulated. In human cancer, however, Myc's deregulated expression is often observed and is considered a poor prognostic factor [7–10]. Myc is evolutionarily conserved as an integrator of extracellular and intracellular signals leading to cell growth and division, tissue regeneration, and remodeling [11]. Indeed, during development, *myc* family gene expression is highest during embryonic stages and is downregulated in mature organs, due to cell growth arrest and differentiation [12].

Genetic knockout of the *c-myc* gene leads to embryonic lethality and, as elegantly shown by Baudino et al. [13], this is partially due to defects in vasculogenesis caused by the lack of proper Vascular Endothelial Growth Factor (VEGF) signaling [13]. In the same study, a role in murine embryonic stem cells and derived teratomas was attributed to Myc in the regulation of other proteins strictly involved in angiogenesis, such as thrombospondin-1 and angiopoietin-1 and -2, providing the first evidence to show Myc as a master regulator of vascular remodeling [13]. Myc's role in embryonic development has recently been defined more precisely by the use of cell lineage specific deletion [14]: embryos lacking *c-myc* in both endothelial and hematopoietic compartments phenocopied those lacking

c-myc in the entire embryo, as a consequence of defective hematopoiesis and vasculogenesis. Surprisingly though, *c-myc* deletion in endothelial cells alone did not prevent endothelial cell proliferation and vasculogenesis. Thus, it is *c-myc*-mediated hematopoiesis that is critical for blood vessel formation during mammalian development [14].

Further evidence for the regulation of VEGF and angiogenesis by Myc was provided by Mezquita and colleagues [15], who demonstrated that a human B-cell line increases VEGF production tenfold upon Myc overexpression, due to increased initiation of *VEGF* mRNA translation. Ngo et al. [16] confirmed in vivo Myc's down-modulation of thrombospondin-1 by making use of Myc-transformed Rat-1A fibroblasts that form vascular tumors in immunocompromised mice. Finally, Dews and colleagues [17] suggested that thrombospondin-1 is downregulated by Myc through induction of the *miR-17-92* microRNA cluster, using p53-null mouse colonocytes transformed in vitro by low-grade overexpression of activated K-Ras and Myc, and engrafted into the cecal wall of syngeneic mice. Collectively, these results show that Myc efficiently modulates in vivo various potent factors implicated in neoangiogenesis.

Another giant leap forward in understanding Myc's role as instructor of the tumor microenvironment was made using mouse models of Myc-induced tumorigenesis. In particular, the use of reversibly switchable models that allowed synchronous activation or deactivation of Myc also permitted deconvolution of the cause-and-effect processes consequent to activation of the oncogene in vivo. One of these models is the *pInsMycER^{Tam};RIP-BclXL* model of pancreatic β-cell tumorigenesis, in which expression of a MycER fusion protein is spatially controlled by the tissue-specific insulin promoter (*pIns*) and temporally regulated by the administration of 4-hydroxytamoxifen [18]. Apoptosis is prevented by co-expression of B-cell lymphoma-extra large (*Bcl-xl*) driven from the Rat insulin promoter 1 (*RIP*). In this model, Shchors et al. demonstrated that acute Myc activation in β-cells triggers the release of factors that induce proliferation of adjacent endothelial cells, and their subsequent formation of leaky and complex vessels [19]. This process is mediated by the release of interleukin-1β (IL-1β) by β-cells, which leads to mobilization of VEGF-A bound to the extracellular matrix, presumably through the action of extracellular proteases [19, 20].

In the same mouse model, we showed that Myc alone causes a complex inflammatory response, leading to the recruitment of various inflammatory cells. Among those, mast cells are absolutely required for tumor expansion and sustained proliferation of endothelial cells within the tumor [21]. We also showed that inhibitors of mast cell function rapidly triggered hypoxia and cell death in tumors and vessels, which suggests that there are Myc effectors in the tumor stroma, offering new therapeutic opportunities [21]. These data underscore the essential role of the activation of inflammatory pathways for Myc's oncogenic activity and, together with the previous work by Shchors et al. [19], demonstrate that Myc can directly instruct tissue remodeling, angiogenesis and inflammation.

Other studies have suggested the intriguing possibility of bi-directional crosstalk between Myc-driven tumors and their microenvironment. Giuriato et al. [22] and Rakhra et al. [23] showed that, in a conditional mouse model for Myc-induced tumorigenesis in hematopoietic cells, complete tumor regression, cellular senescence and shutdown of angiogenesis upon Myc inactivation can be achieved only through persistent expression of thrombospondin-1 [22] and in the presence of CD4($^{+}$) T cells [23].

Furthermore, hypoxia and other environmental stresses can affect c-Myc expression itself. For instance, low oxygen supply leads to stabilization of hypoxia-inducible transcription factor 1 alpha (Hif1a), whose transcriptional activity both antagonizes and cooperates with Myc [24], while low oxygen and glucose deficiency can destabilize Myc [25].

Yet, for a long time, Myc's interaction with the microenvironment was thought to be a prerogative of overexpressed and not physiological levels of Myc. More recently, we made use of a dominant-negative form of Myc, termed Omomyc, to assess the requirement for Myc activity in tumorigenesis. More specifically, we decided to study β-cell insulinomas arising in Rip-Tag2 mice that express Simian virus 40 (SV40) T/t antigens, the 'workhorse' for tumor microenvironment and angiogenic switch studies [26, 27]. We crossed these mice with the *TREOmomyc;CMVrtTA* strain, thus enabling controlled expression of Omomyc in most mouse organs [28]. This dominant-negative mutant interferes with Myc's transactivation activity [29], and we sought to establish the extent to which tumor angiogenesis is dependent upon endogenous Myc. Strikingly, given the enormous transforming potential of T/t antigens, tumor expansion was completely prevented by Myc inhibition, and furthermore, tumors collapsed after induced expression of Omomyc [30]. Notably, at no stage of tumor evolution was Myc overexpressed, suggesting it functions simply—but critically—as a client for upstream SV40 oncoproteins. Similarly, in most human cancers, Myc does not appear to be mutated itself, but more typically is induced by altered signal transduction [31]. Our results show that, even in cases where it is not upregulated, Myc still has a crucial part to play in tumorigenesis. Indeed, inhibition of *endogenous*—neither mutated nor overexpressed—Myc has a huge impact on the tumor stroma: it impairs VEGF signaling, causes disappearance of infiltrating inflammatory cells and leads to vasculature

collapse, all events that precede actual tumor regression [30]. Importantly, this holds true even when Myc is inhibited exclusively in tumor cells and not the microenvironment [30], showing that it is Myc in the tumor cells that directs changes in the tumor stroma.

This is particularly intriguing when considered together with recent data from Pello et al. [32], which define a clear role for Myc in controlling the activation of tumor-associated macrophages. This study indicates that Myc inhibition in the microenvironment also has therapeutic promise, being able to prevent alternative polarization of macrophages and their pro-tumorigenic behavior [32].

Various considerations follow:

First, since no emergence of resistance to Myc inhibition was observed in any tumor lesion, these results revealed a unique, non-adaptive link between tumor and microenvironment, which provides tremendous therapeutic opportunities.

Second, it remains to be established whether Myc's instruction of the microenvironment is conserved in different tumors and tissues, and downstream of different oncogenic lesions, or whether the degeneracy observed in signals upstream of Myc is also maintained in downstream pathways.

Third, Omomyc exerts an "edgetic" perturbation [33] of the Myc transcriptome [29, 34]—that is to say, Omomyc does not ablate *all* Myc activities but specifically Myc-dependent gene transactivation, rather than transrespression [29, 34]. This contrasts with approaches designed to totally ablate the gene product function, such as gene knockout or RNA interference techniques. Hence, the microenvironmental effects elicited by Omomyc are critically dependent on the inhibition of Myc's transactivated target genes.

Finally, it is well known that metastasis is a multistage process that requires cancer cells to escape from the primary tumor, survive in the circulation, seed at distant sites, and grow. Each of these processes is influenced by non-malignant cells of the tumor microenvironment [35]. It remains to be established whether Myc is again a critical node during such stages of tumorigenesis and whether inhibiting Myc has therapeutic activity against metastasis.

In summary, instruction of the microenvironment, previously considered a feature of aberrant, mutated or overexpressed Myc, is more likely just one of Myc's many and diverse physiological cellular activities. This should come as no surprise if we consider Myc as a nodal, central, and non-redundant integrator of intracellular and extracellular programs normally involved in organogenesis and tissue regeneration. It is this same physiological activity that is hijacked during tumorigenesis, turning Myc from healing and regenerating saint, to sinner.

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