

The macrophage: Switches from a passenger to a driver during anticancer therapy

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Abbreviations: CSF-1, colony stimulating factor 1; MAPK, mitogen-activated protein kinase; PD-L1, programmed death-ligand 1; PI3K/AKT, phosphatidylinositol 3'-kinase/ protein kinase B; TAMs, tumor-associated macrophages; VEGF, vascular endothelial growth factor.

We have recently discovered that BRAF inhibitors induce potent macrophage responses that confer melanoma resistance to therapy. Our studies lay a foundation for the hypothesis that macrophages switch their role from a passenger to a driver for tumor survival during therapeutic treatment, suggesting that agents that target macrophages can be an important component of "cocktail" anticancer therapy.

Small molecule inhibitor (SMI)-based anticancer targeted therapy for oncogene drivers, such as BRAF inhibitors (BRAFi) for mutant oncogenic BRAF in melanoma, achieves remarkable clinical efficacy. However, most tumors rapidly develop resistance to SMIs. Historically, much attention has been devoted to study genetic changes developed by tumor cells after SMIs treatment. SMIs are designed to specifically bind to the mutant oncogenic proteins with high affinity, which inhibits constitutive activation of kinase activity caused by mutant oncogenes. In BRAF mutant melanoma the main signaling pathway activated by mutant BRAF is the mitogen-activated protein kinase (MAPK) pathway, and its inhibition provides for the clinical efficacy of BRAF inhibition.¹ However, like most kinase inhibitors, BRAFi can weakly bind to wild-type BRAF in melanoma cells or oncogenic RAS transformed cells. This weak binding results in the paradoxical activation of the MAPK pathway in BRAF wild-type cells, leading to tumor cells non-responsive to BRAFi, or development of secondary tumors in patients receiving BRAFi.^{2,3} The biological significance of this paradoxical activation of the

MAPK pathway, especially in non-tumor cells, is not well defined.

Tumor-associated macrophages (TAMs) are among the most abundant stromal cells in many types of cancers, and are widely recognized as playing critical roles in tumor initiation, progression and metastasis. Emerging evidence also suggests that TAMs play an important role in tumor cell responses to treatment, especially to chemotherapy and radiotherapy, by modulating the immune response and angiogenesis among others.^{4,5} However, whether TAMs confer tumor cell resistance to targeted therapy and affect signaling pathways in tumor cells remains unclear. Because TAMs are abundant in melanomas and TAMs produce many pro-tumor growth factors,⁶ we reasoned that TAMs have an impact on melanoma resistance to BRAFi.

An *in vitro* co-culture system was used to study the effects of macrophages on melanoma cell response to BRAFi. In this model, differentiated macrophages induced by melanoma-conditioned media⁶ were loaded on collagen I pre-coated transwells and melanoma cells were seeded on the bottom of cell culture plates. This system allows cell-cell

communication without direct cell contact. We found that macrophages significantly promote cell growth and reduce cell death in the presence of BRAFi. Macrophages activate the MAPK pathway via macrophage-derived vascular endothelial growth factor (VEGF). Blockade of the MAPK pathway with MEK1/2 siRNA treatment or by preventing the VEGF/VEGFR interactions reversed macrophage-mediated BRAFi resistance.^{7,8} Smith et al. also report that macrophages confer melanoma resistance to BRAFi albeit through a TNF- α dependent mechanism.⁹ This discrepancy may be due to different cell culture systems and mouse models employed; or the complexity and plasticity of TAMs.

Paradoxical activation of the MAPK pathway occurs in BRAF wild-type cancer cells and fibroblasts transfected with oncogenic RAS. We found that BRAFi dramatically increases the activation of the MAPK pathway in macrophages, likely due to the already high levels of RAS activity. Activation of the MAPK pathway by BRAFi increases VEGF production, which results in a positive feedback loop for growth and survival of both tumor cells and macrophages.^{7,8} In addition, we

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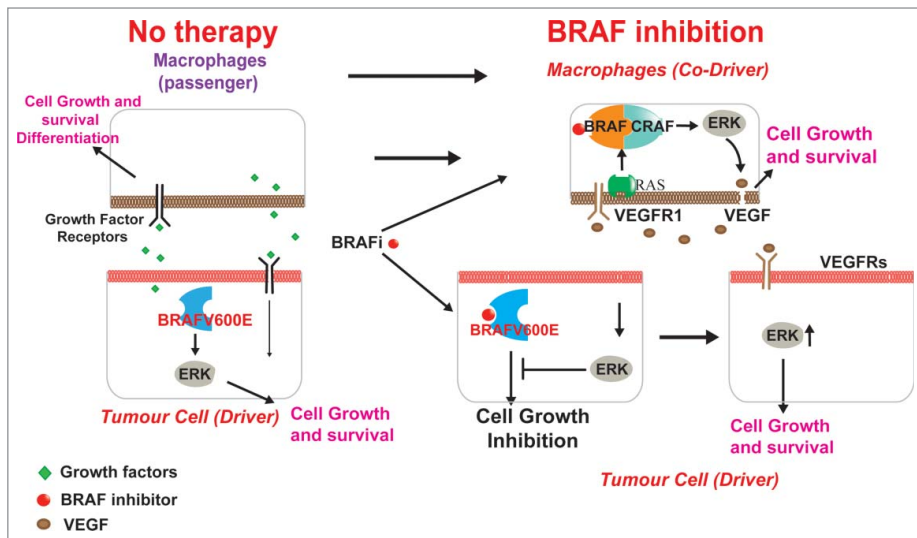


Figure 1. Macrophages switch their role from a passenger to a driver. Left panel – Without anticancer treatment: tumor cells produce cytokines and growth factors that recruit monocytes and differentiate monocytes to TAMs and provide growth and survival signaling for TAMs. TAMs in turn, produce cytokines and growth factors that promote tumor cell growth and survival, and induce invasion, among others. Although macrophages can provide growth and survival signals for tumor cells, tumor cells can play a role as a driver by inducing pro-tumor growth and survival signals provided by oncogenes. Right panel: with anticancer treatment, such as BRAFi in melanoma, the growth and survival signals for tumor cells are blocked, and TAMs are activated to produce more growth factors that provide growth and survival signals for tumor cells. Tumor cells may not survive without TAMs. Thus, both tumor cells and TAMs play a role as a driver for cancer cell growth and survival.

found that BRAFi also increase colony stimulating factor 1 (CSF-1) production⁸ and that there is a trend that BRAFi increase the number of Ki67-positive macrophages in patients' tumor tissues (unpublished data). Our data suggest that BRAFi induce potent macrophage responses leading to BRAFi resistance.

CSF-1 is the most potent factor for macrophage differentiation and survival. Blockade of CSF-1/CSF-1R signaling with monoclonal antibodies or SMIs has been shown to dramatically decrease the number of macrophages in tumors.⁵ We found that a CSF-1R inhibitor (CSF-1Ri) alone significantly inhibits tumor growth in a human xenograft model and increases the antitumor activity of BRAFi. CSF-1R inhibition also decreases the number of macrophages and inhibits activation of the MAPK pathway in mouse tumors. Similar results were obtained with a syngeneic mouse model.⁷

In combination with previous work by others, we propose a “Driver Switch” model for better understanding the role of TAMs in anticancer therapies. Without treatment, macrophages provide a survival signal for tumor cells. Because of the dominant effects of oncogenes, tumor cells only partially depend on macrophages or stromal cells. In these cases macrophages play the role of a passenger cell. When cancer cells are exposed to therapeutic stress, such as BRAFi treatment, their growth and survival pathways are interrupted. In this situation, tumor cells utilize all the resources they can rely on, including those from macrophages, to benefit their growth and survival. Under these circumstances macrophages switch their role from a passenger to a driver, or at least to a co-driver for tumor growth and survival. Supporting this model, our unpublished data indicate that macrophages have more profound effects on

tumor cells under stressful conditions of serum starvation. In this condition, macrophages activate both the MAPK and PI3K/AKT (phosphatidylinositol 3'-kinase/ protein kinase B) pathways and exert a profound effect on tumor cell survival. Our work indicates that the more stress cancer cells receive, the more they rely on macrophages (Fig. 1).

Many questions on how macrophages affect anticancer therapies remain to be answered. First, does activation of signaling pathways in macrophages by BRAFi also occur with other SMIs and if so, are the mechanisms of activation the same as BRAFi? It has been shown that AKT inhibitors similarly activate the PI3K signaling pathway (albeit via different mechanisms),¹⁰ but whether this also happens in macrophages or other stromal cells is not clear. If so, does this have an impact on anticancer therapies? Second, do TAMs also have an effect on antibody-based immune checkpoint inhibitors? This is important because numerous immune checkpoint inhibitors alone or in combination with targeted therapies are already used in the clinic or in clinical trials. The rationale for this hypothesis is based on the following: (1) macrophages produce many immune inhibitory molecules at high levels, such PD-L1 (programmed death-ligand 1) and PD-L2, and others; (2) therapeutic antibodies may also induce macrophage responses by binding to Fc receptors that are highly expressed on macrophages. Lastly, what is the best strategy to target TAMs, directly targeting TAMs or indirectly targeting macrophages by decreasing macrophage-derived factors?

In summary, our work reiterates the important roles of TAMs in anticancer therapy. We anticipate that targeting macrophages will be an important component in the next wave of anticancer therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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