A shortcut for early macrophage recruitment into tumors by activated oncogenes

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Macrophages play an important role in tumor promotion, usually acting as facilitators of cancer initiation and progression. However, it is not clear how macrophages impact early phases of tumorigenesis. Using genetically modified mouse models, Guo et al. (pp. 247–259) demonstrated that tumor-initiating cells with an activated Hippo pathway are able to recruit macrophages starting from the very early phases of cancer development, mainly through direct activation of genes encoding macrophage chemoattractants and survival factors. The recruited macrophages were of vital importance for protection of tumor-initiating cells against eradication by lymphocyte-mediated immune surveillance. Such a tight link between macrophages and a pathway controlling organ development and size may reflect the normal role of these cells in tissue morphogenesis.

Tumor development is invariably associated with the recruitment of leukocytes, among which macrophages represent a major and pathogenically relevant component (Ostuni et al. 2015). The role of macrophages is complex and not identical in different phases of tumorigenesis and different tumors, although, in most cases, they facilitate tumor development. In chronic inflammatory states, macrophages contribute to providing an environment that favors the occurrence of tumorigenic mutations in adjacent tissue cells as well as the positive selection and growth of cells accumulating mutations (Mantovani et al. 2008). In developing tumors, blood monocytes are recruited and induced to terminally differentiate into tumor-associated macrophages (TAMs) whose secretory products (such as interleukin 6 [IL-6] and vascular endothelial growth factor [VEGF]) directly support tumor cell growth or modify the local microenvironment, stimulating the development of new vessels and favoring tissue invasion (Noy and Pollard 2014). However, whether and how macrophages are recruited at very early stages of tumorigenesis are still unclear. Previous data indicate that, at the onset of chemically induced hepatic carcinogenesis, IL-6 production by macrophages and dendritic cells supports tumor growth. Conversely, macrophages are dispensable at later stages due to the acquired ability of cancer progenitor cells to autonomously produce IL-6 (He et al. 2013). In this well-characterized model, macrophages accumulate in proximity to small foci of altered hepatocytes (FAHs) (Pitot 1990), whose precise molecular and biological features are still unclear but likely represent early tumorigenic cells. FAHs were also shown to be associated with the activation of the Hippo pathway (He et al. 2013), in which stimulus-regulated nuclear translocation of the YAP coactivator protein enables transcriptional activation by the TEAD transcription factors (Pan 2010). YAP–TEAD control the expression of genes impacting cell proliferation and apoptosis and thus eventually organ size in normal development (Yu et al. 2015). It is therefore not surprising that this pathway is frequently deregulated in tumorigenesis. The study by Guo et al. (2017) investigated the possibility that the Hippo pathway may be directly involved in the recruitment of macrophages to promote liver carcinogenesis and used experimental strategies to track the interplay between macrophages and tumor-initiating cells from the very beginning of the process.

Specifically, Guo et al. (2017) used extensive genetic analyses, largely based on hydrodynamic tail vein injection (Chen and Calvisi 2014), which leads to plasmid uptake and integration in a fraction of hepatocytes. Stable expression of constitutively active (phosphorylation-resistant) YAP in ~1% of hepatocytes led to the formation of highly proliferative and poorly differentiated tumors in ~4 mo. Recruitment of macrophages in close proximity to YAP-expressing cells occurred as early as 2 d after liver transduction, when only scattered cells

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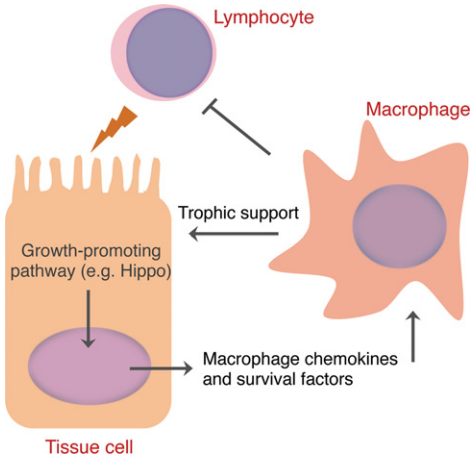

Figure 1. Activation of a growth-promoting oncogenic pathway such as the Hippo pathway in tissue cells results in direct and rapid macrophage recruitment, which can determine two groups of effects: direct trophic support to tissue cells and attenuation of lymphocyte-mediated responses. In principle, the same circuit may be operating in normal tissue development and oncogenesis.

expressing YAP were detected in the parenchyma. These data suggest that expression of active YAP may be sufficient to attract myeloid cells (Fig. 1). Indeed, the liver content of leukocytes (mainly composed of macrophages) rapidly increased about threefold upon YAP expression. Remarkably, expression of activated KRAS in the same system led to preferential recruitment of lymphocytes rather than macrophages (although myeloid cells also were abundantly recruited) (Kang et al. 2011), supporting the important notion that activation of specific oncogenes affects the type of immune infiltration and thus potentially the type of immune response to a given tumor. Similarly, mosaic deletion of *Lats1/2*, encoding the kinases that phosphorylate and inactivate YAP by promoting its cytoplasmic retention, induced the formation of tumors infiltrated by leukocytes, albeit with a longer latency (~8 mo) that may be explained by the lower level of YAP nuclear accumulation. A strong recruitment of macrophages was also obtained with a traditional genetic approach, leading to the deletion of the kinases upstream of Lats1/2; namely, Mst1/2.

Mechanistically, the ability of YAP to recruit macrophages depended on its interaction with TEAD, as inferred by the inability of a YAP mutant unable to interact with TEAD to promote leukocyte recruitment. Therefore, transduction of active YAP likely results in transcriptional changes mediated by TEAD that eventually result in macrophage recruitment. Indeed, the cross-talk between the Hippo pathway and macrophage recruitment appeared to depend on the main monocyte–macrophage chemoattractant the CCL2 chemokine, whose gene is a direct YAP–TEAD target. The delivery of a vector that simultaneously expressed activated YAP and depleted *Ccl2* and *Csf1* (the latter encoding the macrophage colony-stimulating factor [M-CSF]) abrogated macrophage recruitment and resulted in loss of tumor formation. By closely following the fate of YAP-expressing cells, it also became clear that CCL2 and M-CSF acted at an early stage of tumor development and thus that macrophages prevent the rapid removal of early tumorigenic cells. Importantly, elimination of early tumor cells was mediated by lymphocytes, since it was impaired in Rag-deficient mice (which lack mature B and T lymphocytes) (Fig. 1). However, how such elimination of early tumor cells is achieved and what specific antigens are recognized by lymphocytes remain unclear. Although it is still early to establish the relevance of these findings in human tumors (in which Hippo pathway mutations are uncommon, but the pathway is nonetheless commonly activated) (Zhao et al. 2007), half of the high-grade dysplastic nodules from human patients analyzed showed YAP activation and concomitant infiltration of macrophages, pointing toward a clinical relevance of these findings.

The notion that one of the mechanisms linking macrophages to enhanced tumorigenesis is represented by impaired anti-tumor immunity is supported by many studies (Ostuni et al. 2015). However, the remarkable conclusion that can be drawn from the study by Guo et al. (2017) is that even single tumor cells at the very beginning of tumorigenesis become able to evade immune clearance by recruiting macrophages, and this is due to the ability of specific activated oncogenes to directly attract these cells. Aside from its remarkable biological interest, this study is not devoid of potential medical implications, as it suggests that the interference with the activation of the Hippo pathway, in addition to its possible direct effects on parenchymal tumor cells, may unleash an efficient anti-tumor response.

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