

## **Macrophage Cytoplasmic Transfer in Melanoma Invasion**

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**Within tumors, macrophage infiltration can promote cancer cell invasiveness and consequently, metastatic dissemination. In this issue of *Developmental Cell*, Roh-Johnson et al. (2017) reveal that cytoplasmic transfer from macrophages to melanoma cells correlates with melanoma invasion and arises as a result of intimate cell-cell contact.**

In cancer the primary cause of death are metastases that arise as a consequence of cancer cells migrating away from the primary tumor and successfully colonizing new locations. Why cancer cells become invasive remains a subject of intense interest. Although acquisition of genetic lesions can contribute, current models suggest that a major driver of invasiveness is the intra-tumour microenvironment (Quail and Joyce, 2013). Oxygen and nutrient availability, as well as reciprocal signaling between multiple tumor-associated cell types, can all influence specific gene expression programs and as a consequence affect cell phenotype. Genetic lesions, including driver mutations, may sensitize cells to changes in the microenvironment, and are largely irreversible. By contrast, the impact of the microenvironment on cells is dynamic and potentially reversible. Understanding in detail the molecular mechanisms underpinning the phenotypic transition to invasion, which is frequently associated with drug-resistance, may reveal potential points for therapeutic intervention aimed towards either killing specific phenotypic sub-populations or by directing drug-resistant cells toward a phenotype sensitive to therapeutics. In this respect, the discovery by Roh-Johnson et al. (2017), reported in this issue of *Developmental Cell*, that macrophages donate cytoplasm to melanoma cells in vivo and in vitro, and that cytoplasm transfer correlates with invasion, has the potential to reveal a new aspect of the early steps in metastatic dissemination.

Macrophages are phagocytic cells that are a key part of the innate immune system. They have an important role in inflammation and tissue repair as well as in the response to infection (Aras and Zaidi, 2017). Significantly, it has become clear that macrophages can contribute to

many aspects of cancer progression including angiogenesis and proliferation, and importantly can stimulate invasion, epithelial-to-mesenchymal transition, and metastatic dissemination via paracrine signaling, especially at the leading edge of tumors (Aras and Zaidi, 2017; Quail and Joyce, 2013). However, while mouse models and human tissue samples have given some clues as to the role of macrophages in tumor cell dissemination, the conclusions that can be drawn are largely based on an end-point analysis. As such, there is a need for a system of metastatic dissemination that also allows the visualization in real time of cancer cell interactions with macrophages for better dissection of the interplay between cancer cells and the innate immune system.

The study from Roh-Johnson et al (2017) combines two key tools to address this need: melanoma, a highly aggressive skin cancer where the phenotypic transitions that occur to promote metastatic dissemination are relatively well characterized (Hoek and Goding, 2010) and zebrafish, a model system that permits high-resolution real-time intra-vital imaging of fluorescently-tagged cells and that has an innate immune system similar to that in humans. The authors first established that transplantation of 20-40 melanoma cells to the zebrafish larval hindbrain vesicle led to some cells adopting an altered morphology and migrating to the overlying skin as well as to distant sites without exhibiting any obvious tissue tropism. No significant migration was observed using control epithelial cells or fibroblasts.

To examine the interactions with cells from the innate immune system, the authors used transgenic zebrafish in which macrophages were fluorescently labeled. Real-time imaging revealed extensive intimate and dynamic contacts between infiltrating macrophages and the transplanted human melanoma cells or NRAS transformed zebrafish melanocytes. In mouse models of melanoma, TNF $\alpha$  secreted by infiltrating immune cells, including macrophages, can induce reversible melanoma cell de-differentiation (Landsberg et al., 2012) and melanoma cell invasion (Falletta et al., 2017). TNF $\alpha$ -mediated de-differentiation consequently drives resistance to adoptive T-cell therapy in which in vitro expanded cytotoxic T-cells that recognize differentiation-specific antigens are used to cause tumor regression in

vivo (Landsberg et al., 2012). In the zebrafish model, however, over time it is the TNF $\alpha$ -deficient macrophages that accumulate and predominate when melanoma cells adopt an invasive phenotype, suggesting that TNF $\alpha$  is less important in driving melanoma cell invasion.

It has previously been showed that in vivo highly metastatic breast cancer cells appear to induce invasive behavior in lowly metastatic breast cancer cells by the transfer of membrane bound vesicles (Zomer et al., 2015). Transfer in that study was detected by using highly invasive breast cancer cells expressing a CRE recombinase that activated a fluorescent reporter in the lowly metastatic breast cancer cells when delivered via vesicle transfer. Roh-Johnson et al. (2017) applied the same technique to their zebrafish system, in which melanoma cells receiving CRE-containing cytoplasm from the macrophages would undergo a red-to-green switch. The results were striking and indicated that close to 70% of invasive melanoma cells had received macrophage cytoplasm. Controls using melanocytes and keratin-expressing cells suggested that donation of cytoplasm was restricted to macrophages. The authors then went on to demonstrate that similar macrophage cytoplasm transfer could also occur and also correlate with metastatic dissemination in a B16 mouse model of melanoma. In contrast to the breast cancer model of previous work (Zomer et al, 2015), however, rather than relying on vesicle delivery of CRE, Roh-Johnson et al. (2017) showed in the melanoma system that cytoplasmic transfer from macrophages could occur via cell-cell contact, with interactions extending for almost 3 hours prior to melanoma cells undergoing the red-to-green switch indicative of cytoplasmic transfer. Importantly, in vivo cytoplasmic transfer occurred more frequently prior to metastatic spread and cells that received macrophage cytoplasm exhibited enhanced directional persistence during their migration.

Although it has previously been proposed that macrophage-melanoma fusion could potentially drive melanoma metastasis (Pawelek, 2007), it was expected that this would occur via cell-cell fusion, and to date the evidence for such a model has been limited. The new data from Roh-Johnson et al. (2017) certainly provide some support for a model in which

macrophages may contribute to melanoma invasion via cytoplasmic transfer arising from direct cell interactions, perhaps in addition to vesicle transfer as observed in the breast cancer model (Zomer et al., 2015). As always with such provocative and stimulating studies, the work raises several new questions that will need to be addressed in future work. As the authors point out, these questions include the nature of the molecules transferred and the mechanism by which they might influence melanoma behavior. In addition, the mechanism of transfer remains to be deciphered, especially since most melanoma-macrophage contacts do not appear to lead to cytoplasmic transfer. Finally, the results presented, elegant as they are, provide only a correlation between cytoplasmic transfer and melanoma cell invasion. As the authors acknowledge, cause and effect have not been established. It remains possible therefore that rather than cytoplasmic transfer inducing invasion, melanoma cells that adopt an invasive phenotype are instead predisposed to take up macrophage cytoplasm. Nevertheless, Roh-Johnson et al. (2017) provide some exciting observations that uncover a new twist in our understanding of the interactions between the innate immune system and cancer cells. The work presented will stimulate the field for the coming years.

## References:

- Aras, S., and Zaidi, M. R. (2017). TAMEless traitors: macrophages in cancer progression and metastasis. *British Journal of Cancer*. doi: 10.1038/bjc.2017.356
- Falletta, P., Sanchez-del-Campo, L., Chauhan, J., Effern, M., Kenyon, A., Kershaw, C. J., Siddaway, R., Lisle, R., Freter, R., Daniels, M., *et al.* (2017). Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes & Dev.* 31, 18-33.
- Hoek, K., and Goding, C. R. (2010). Cancer stem cells versus phenotype switching in melanoma. *Pigment Cell Melanoma Res* 23, 746-759.
- Landsberg, J., Kohlmeyer, J., Renn, M., Bald, T., Rogava, M., Cron, M., Fatho, M., Lennerz, V., Wolfel, T., Holzel, M., and Tuting, T. (2012). Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature* 490, 412-416.
- Pawelek, J. M. (2007). Viewing malignant melanoma cells as macrophage-tumor hybrids. *Cell Adh. Migr.* 1, 2-6.
- Quail, D. F., and Joyce, J. A. (2013). Microenvironmental regulation of tumor progression and metastasis. *Nature Medicine* 19, 1423-1437.

Roh-Johnson, M., Shah, A. N., Stonick, J. A., Poudel, J. A., Kargl, J., Yang, G. H., di Martino, J., Hernandez, R. E., Gast, C. E., Zarour, L. R., *et al.* (2017). Macrophage-dependent cytoplasmic transfer during melanoma invasion in vivo. *Dev Cell.* this issue

Zomer, A., Maynard, C., Verweij, F. J., Kamermans, A., Schafer, R., Beerling, E., Schiffelers, R. M., de Wit, E., Berenguer, J., Ellenbroek, S. I., *et al.* (2015). In Vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior. *Cell* *161*, 1046-1057.

**Figure Legend:**

**Figure 1. Donation of macrophage cytoplasm correlates with melanoma cell invasion.**

(Top) Injection of melanoma cells into zebrafish larvae hindbrain is accompanied over time by increased TNF $\alpha$ -negative M2 macrophage (M $\phi$ ) infiltration. (Bottom) Macrophages expressing Cre recombinase make intimate and prolonged contact with red fluorescent melanoma cells. Cytoplasmic transfer from the macrophages to the melanoma cells leads to donation of Cre recombinase, which confers a red-to-green switch in melanoma cell fluorescence that correlates with increased invasive behavior.

