Reprogramming multipotent tumor cells with embryonic microenvironments

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***Short Abstract* - The embryo regulates the invasion and differentiation programs of many different cell types, including the multipotent neural crest. What is largely unknown is whether adult neural crest-derived tumor cells reuse embryonic signaling networks or develop a unique program to metastasize. To analyze how tumor cells perceive and respond to microenvironmental signals, we transplant human multipotent tumor cells into the chick embryo neural crest microenvironment and study cell behaviors and gene expression changes using 2-photon in vivo time-lapse imaging and laser capture microdissection. We develop a quantitative framework to compare and identify convergence of the embryonic and tumorigenic signaling pathways.**

***Keywords* – cancer, reprogramming, microenvironment, embryonic, neural crest, 2-photon microscopy, laser capture microdissection.**

A dynamic, complex relationship exists between stem cells and their microenvironment, which plays a pivotal role in cell fate determination – critical to development, wound healing, tissue maintenance, and cancer progression. Key to identifying the molecular mechanisms underlying stem cell plasticity, is understanding the unique epigenetic role of the microenvironment on the emergence of cell phenotype. Previous studies from our laboratory have revealed the unexpected finding that metastatic human melanoma cells express genes that are associated with multiple cellular phenotypes and their respective precursor cells, the neural crest, suggesting a dedifferentiated, multipotent cancer cell with a plastic phenotype characteristic of stem cells [2,3]. Additional evidence supporting the concept of melanoma tumor cell plasticity from our laboratory includes recent findings that indicate the powerful influence of the chick neural crest-rich microenvironment with respect to reprogramming metastatic melanoma cells to a melanocyte-like phenotype – when exposed to this embryonic milieu [1,3]. Most interestingly, transplanting human metastatic melanoma cells into the neural crest-rich regions of chick embryos show the remarkable ability of the tumor cells to migrate along neural crest pathways resulting in the reprogramming of a subpopulation of melanoma cells induced to re-express melanocyte-like and neuronal-like phenotypes – both derivatives of neural crest cells. Therefore, based on these intriguing observations, we tested the central hypothesis that the embryonic microenvironment associated with chick neural crest-rich regions – contain informational cues with the potential to epigenetically reprogram the genotype and phenotype of human metastatic multipotent melanoma cells exposed to them.

Here, we present a quantitative experimental approach to elucidate the tissues and signals within the embryonic neural crest microenvironment capable of reprogramming metastatic melanoma cells. We use the chick embryo neural crest microenvironment, combined with 2-photon photoactivation cell labeling, time-lapse microscopy and laser tissue microdissection, and show how tumor cells respond to neural crest microenvironmental signals in more detail. Specifically, we will report tumor cell dynamics and gene expression changes as a function of position from the transplant site and within the neural crest microenvironment. Our results indicate that melanoma metastasis resembles the neural crest migratory pattern but is unpredictable in the spatio-temporal order of invasion. We propose that the chick embryo transplant model is a valid, *in vivo* model of melanoma metastastasis.

We develop a quantitative model to incorporate our cellular and molecular data, including changes in the expression of genes associated with the transplanted tumor cells. We show how we compare the embryonic neural crest and tumorigenic signaling pathways to identify components common to embryonic neural crest induction and invasion and tumorigenesis.

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