

TUMOR CELL:MICROENVIRONMENTAL INTERACTION IN A XENOGRAFT MODEL OF BREAST CANCER BRAIN METASTASIS AND PIGMENT EPITHELIUM-DERIVED FACTOR AS A SUPPRESSOR OF BRAIN METASTASIS

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Brain metastases occur in 10%–20% of metastatic breast cancer patients. The 1-year survival rate is approximately 20%. We have developed a model system for the study of breast cancer brain metastasis using an EGFP-labeled “brain-seeking” derivative of the human MDA-MB-231 breast carcinoma cell line (231BR) in the mouse. One month after intracardiac injection of 231BR cells, metastases are observed in the brain of nude mice. An analysis of the pathology of our xenograft model, reported here, improves our understanding of metastatic progression in the brain. Tumors form in both the meninges and brain parenchyma. Metastases in all locations show high levels of proliferation (~53% by Ki67 staining); however, most parenchymal tumor growth manifests as clusters of neighboring “micrometastases.” A clonal analysis was performed by co-injecting a mixed population of tumor cells expressing one of several fluorescent proteins. The results proved that single tumor cells give rise to all the “micrometastases” in a given cluster. This results from preferential growth and migration along neighboring branches of the brain vasculature. Further analysis of the brain tissue surrounding metastases shows a massive inflammatory response with extensive reactive gliosis around metastases. Glial cells promote anchorage-independent growth of 231BR cells *in vitro*, suggesting that they secrete factors conducive to metastatic growth. We have chosen to pursue pigment epithelium- derived factor (PEDF, also known as SerpinF1) as a potential suppressor of brain metastasis. PEDF was downregulated in human brain metastases relative to the primary tumors (~2-fold), suggesting that it may have a negative impact on brain metastatic progression. PEDF, a secreted factor, has been shown to act as a tumor suppressor, has strong antiangiogenic activity, and affects the proliferation/viability/differentiation of neural tissue. Since metastases in our model system grow in close association with both the brain vasculature and with surrounding glial cells, we hypothesized that the multiple roles of PEDF might make it a potent agent that targets not only tumor cells but also the brain microenvironment. To investigate its effect on metastatic progression, PEDF was overexpressed (2- to 5-fold) in our 231-BR cells. *In vitro*, PEDF expression strongly inhibited (greater than 50-fold) anchorage-independent growth of 231-BR cells in soft agar. The *in vivo* effects of PEDF on angiogenesis and metastatic outgrowth of 231BR cells in the brain are currently under investigation.

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