Targeting Squalene Epoxidase (SQLE) as a Novel Therapeutic Opportunity for Breast Cancer-to-Brain Metastases

Abstract

Background: Brain metastasis is the most common malignancy of the central nervous system which causes severe morbidity and mortality in multiple cancer types of patients and represents an unmet medical need. Several critical steps are required for a successful brain metastasis, including local invasion, extravasation, dissemination, extravasation, and colonization. Extensive research has been conducted to elucidate the mechanism of cancer metastasis with limited information toward how cancer cells extravasate and colonize.

Methods: To understand the underlying molecular mechanisms and applications of molecular targets for brain metastasis therapy, murine models are employed for investigation of brain extravasation and colonization. We generated in vivo breast cancer-to-brain metastasis (RCBM) mouse models by intracardiac or direct intracranial injections of MDA-MB-231-luciferase (Luc) cell line into immune deficient nude mice. In vivo brain extravasation and colonization were monitored by IVIS imaging. To investigate the role of SQLE in breast cancer-to-brain extravasation and colonization, we silenced SQLE expression directly with lentiviral shRNA in the brain metastatic MDA-MB-231-BR cell line (231-BR/shSQLE) and used 231-BR cells expressing a control shRNA (231-BR/shCtrl) as the control. The essential roles of SQLE in the specific steps of breast-to-brain metastatic process were evaluated by in vitro blood-brain barrier (BBB) models as well as in vivo immunofluorescence analysis of brain slices from the animals.

Results: Here, we identified a novel mechanism by which squalene epoxidase (SQLE), the second rate-limiting enzyme in the cholesterol biosynthesis pathway, plays a critical role in the metastatic processes of breast cancer to the brain, especially in brain extravasation and colonization. Our data demonstrated that SQLE is essential for 231-BR cells to extravasate into the parenchyma as well as the formation of micro- and macro-metastases in the brain. In vitro blood-brain barrier (BBB) models further demonstrated the critical roles of SQLE in promoting 231-BR cell invasion and penetration through BBB.

Conclusions: Recently, the pharmacologic inhibition of SQLE has been widely used against fungal infections, and the next-generation SQLE inhibitors have been shown to exert an anticancer effect. Our findings suggest that pharmacologic inhibition of SQLE by SQLE inhibitors (e.g., Terbinafine or NB-598) may represent a therapeutic opportunity for breast cancer-to-brain metastases.

References:


Figure 1. Identification of squalene epoxidase (SQLE) as a novel therapeutic opportunity for breast cancer-to-brain metastasis. A. Scheme of multiple steps involved in breast cancer-to-brain metastasis (BCBM) and the inactivation of targeting SQLE as a novel therapeutic opportunity. Cancer cells (blue) and blood vessels (red). B. Upregulated genes in EBCM with breast tumors and lung metastatic patients (GSE13928) and MDA-MB-231 and 231-BR (GSE12217). Right: Visualization of the volcano plot. C. Heatmap. D. Most enriched biological processes for patients with breast cancer with low and high mRNA expression of indicated genes. E. Differential expression of SQLE in various subtypes of breast cancers patients with higher expression in hereditary breast cancer. F. Expression of SQLE in breast tumor compared to normal breast tissue by IHC staining.

Figure 2. In vivo RCBM model with the highly metastatic human breast MDA-MB-231-HAoma (Luc) cell line in nude mice. A. Intracardiac (ICD) and intracranial (ICA) injection of MDA-MB-231-Luc cells into nude mice followed by IVIS imaging. B. In vivo brain metastasis model with luciferase-expressing cells. C. Multiple organ metastasis with ICD implantation of tumor cells. D. Right: Immunohistochemical analysis of brain metastasis. E. Immunofluorescence analysis of brain slices from the animals. F. Expression profile of SQLE in breast cancer cell lines.

Figure 3. SQLE may promote 231-BR cell penetration and transmigration through the blood–brain barrier (BBB) during brain metastasis. A. Left: Scheme for in vivo RCBM model. Create a blood–brain barrier (BBB) using Evans blue dye leakage. Right: In vivo blood–brain barrier (BBB) model. Create BBB using naïve mouse brain barrier. B. SQLE is essential for breast cancer cell penetration and transmigration through the BBB during brain metastasis. C. SQLE is effective in breast cancer cell penetration and transmigration through the BBB during brain metastasis. D. SQLE may promote 231-BR cell penetration and transmigration through the BBB during brain metastasis. E. SQLE may promote 231-BR cell penetration and transmigration through the BBB during brain metastasis. F. SQLE may promote 231-BR cell penetration and transmigration through the BBB during brain metastasis. G. SQLE may promote 231-BR cell penetration and transmigration through the BBB during brain metastasis. H. SQLE may promote 231-BR cell penetration and transmigration through the BBB during brain metastasis. I. SQLE may promote 231-BR cell penetration and transmigration through the BBB during brain metastasis.