Targeting Squalene Epoxidase for Breast Cancer Brain Metastasis: The Evaluation of a New Therapeutic Target for Brain Extravasation and Colonization Using Animal Models

Pharmacology Discovery Services

#1449

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Abstract

Breast cancer brain metastasis is one of the most common forms of breast cancer metastasis and a major cause of morbidity and mortality. Patients who develop brain metastases tend to be associated with progressive neurologic deficits and short overall survival; this represents an unmet medical need. Extensive research has been conducted to elucidate the mechanism of cancer metastasis with limited information gained about how cancer cells extravasate and colonize to the brain. Recently, we identified a novel mechanism by which squalene epoxidase (SQLE), the second rate-limiting enzyme in cholesterol biosynthesis, plays a critical role in the process of breast cancer metastasis to the brain. Thus, working toward a better understanding of the underlying molecular mechanisms and applications of molecular target(s) for brain metastasis therapy, we employed murine models for investigation of brain extravasation and colonization with intracardiac injection of GFP/FLuc-labeled human MDA-MB-231 metastatic breast carcinoma cells. The MDA-MB-231 cells were found to metastasize to the brain and other organs following intracardiac implantation into nude mice and were further isolated from each tissue. Interestingly, the isolated brain-metastasized tumor cells (MDA-MB-231-BrM) exhibited a significant upregulation of SQLE expression when compared with those that metastasized to other distal sites. The essential role of SQLE in the specific steps of the breast cancer-to-brain metastatic process was evaluated by *ex vivo* immunofluorescence analysis and immunohistochemistry staining of brain slices from the study subjects. Our data demonstrate that high SQLE expression is essential for MDA-MB-231-BrM to extravasate into the parenchyma, as well as the formation of micro- and macro-metastases in the brain. Interestingly, we found that blood vessel co-option and the surrounding neuronal cells play key roles in supporting MDA-MB-231-BrM to develop brain macro-metastases. *In vitro* blood-brain barrier (BBB) models further demonstrated the critical role of SQLE in promoting tumor cell invasion and penetration through the BBB. To verify SQLE as an oncogenic factor that can be selected as a potential therapeutic target in suppressing breast cancer brain metastasis, we evaluated the inhibitory effects of terbinafine (a SQLE inhibitor) in both the MDA-MB-231-BrM orthotopic and intracardiac mouse models. Pharmacologic inhibition of SQLE by terbinafine suppressed MDA-MB-231-BrM tumor growth at the mammary fat pad and distal metastases to the brain, suggesting that targeting SQLE represents a therapeutic opportunity for breast cancer brain metastasis.

Potential Role of Squalene Epoxidase (SQLE) in Breast Cancer-to-Brain Metastasis (BCBM)

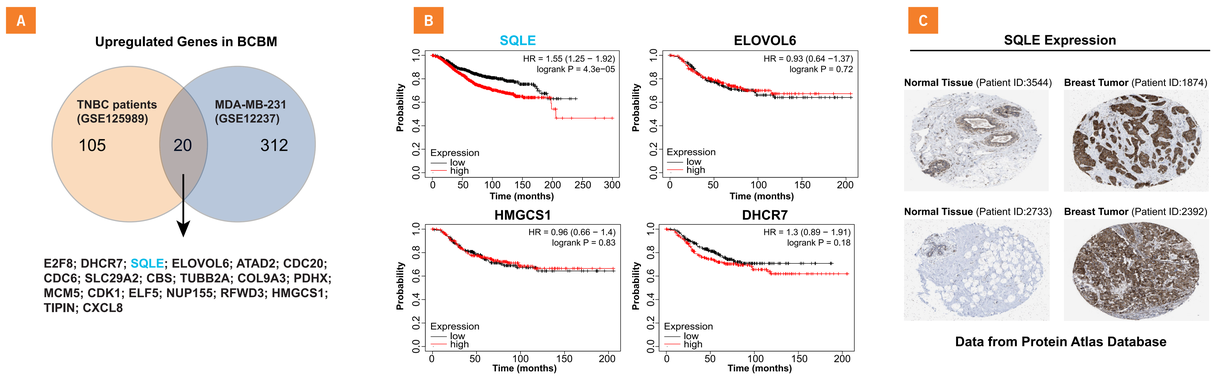

Figure 1. Identification of SQLE potential role in BCBM. A. Upregulated genes in BCBM with clinical triple-negative breast carcinoma (TNBC) patients (GSE125989) and MDA-MB-231 cell line (GSE12237). B. Overall survival probability for TNBC patients with the identified target genes showing patients with SQLE overexpression have a lower survival rate. C. Overexpression of SQLE in breast tumors compared to normal breast tissues by IHC staining.

Development of *In Vivo* Breast Cancer-to-Brain Metastasis Mouse Model with MDA-MB-231

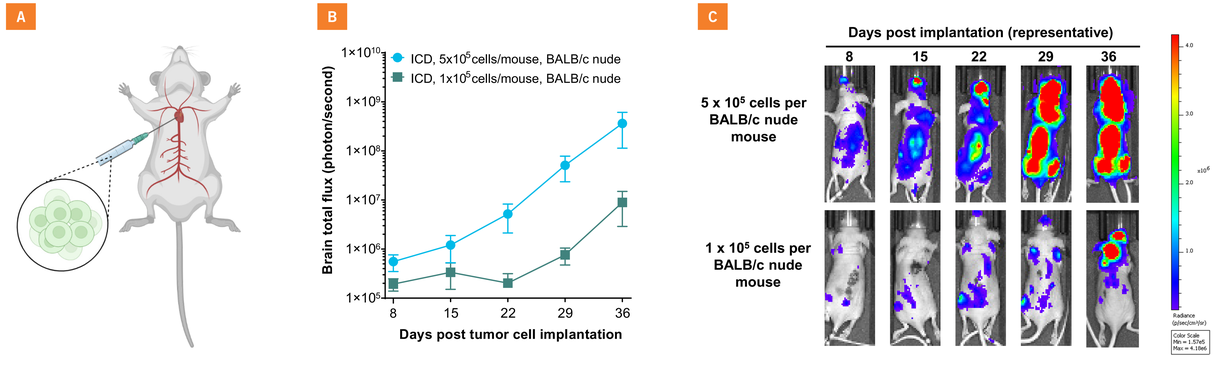

Figure 2. Development of *in vivo* BCBM model with the highly metastatic human TNBC MDA-MB-231-luciferase (Luc) cell line. A. Study scheme with intracardiac (ICD) injection of MDA-MB-231-Luc cells into nude mice for establishment of BCBM model. Created with Biorender.com. B. Establishment of tumor growth in the brain by bioluminescence imaging. C. In-life IVIS imaging demonstrates detectable bioluminescence signals in multiple organs upon ICD implantation of tumor cells with representative animals.

SQLE Is Essential for Breast Cancer Extravasation and Colonization into the Brain

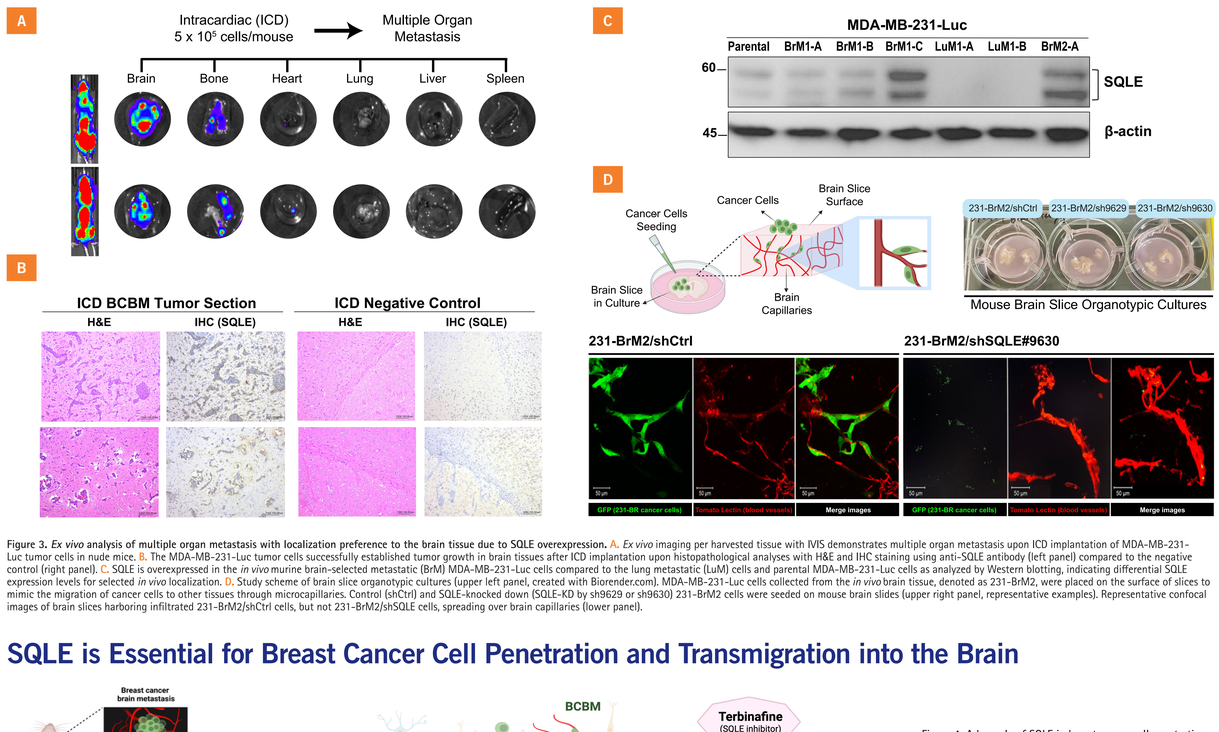

Figure 3. *Ex vivo* analysis of multiple organ metastasis with localization preference to the brain tissue due to SQLE overexpression. A. *Ex vivo* imaging per harvested tissue with IVIS demonstrates multiple organ metastasis upon ICD implantation of MDA-MB-231-Luc tumor cells in nude mice. B. The MDA-MB-231-Luc tumor cells successfully established tumor growth in brain tissues after ICD implantation upon histopathological analyses with H&E and IHC staining using anti-SQLE antibody (left panel) compared to the negative control (right panel). C. SQLE is overexpressed in the *in vivo* murine brain-selected metastatic (BrM) MDA-MB-231-Luc cells compared to the lung metastatic (LuM) cells and parental MDA-MB-231-Luc cells as analyzed by Western blotting, indicating differential SQLE expression levels for selected *in vivo* localization. D. Study scheme of brain slice organotypic cultures (upper left panel, created with Biorender.com). MDA-MB-231-Luc cells collected from the *in vivo* brain tissue, denoted as 231-BrM2, were placed on the surface of slices to mimic the migration of cancer cells to other tissues through microcapillaries. Control (shCtrl) and SQLE-knocked down (SQLE-KD by sh9629 or sh9630) 231-BrM2 cells were seeded on mouse brain slides (upper right panel, representative examples). Representative confocal images of brain slices harboring infiltrated 231-BrM2/shCtrl cells, but not 231-BrM2/shSQLE cells, spreading over brain capillaries (lower panel).

SQLE is Essential for Breast Cancer Cell Penetration and Transmigration into the Brain

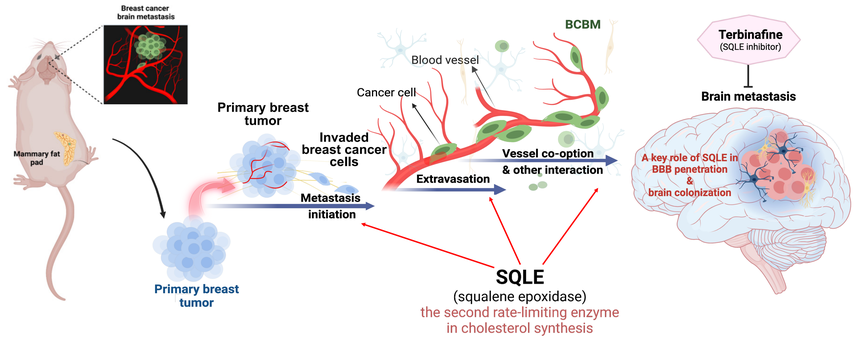

Figure 4. A key role of SQLE in breast cancer cell penetration through the blood–brain barrier (BBB) and brain colonization. SQLE plays a critical role in breast cancer stemness, intravasation, and invasion from the primary breast tumor to the surrounding tissues; In this study, a murine breast-to-brain metastasis model with the highly metastatic TNBC MDA-MB-231 cell line is employed to demonstrate the critical role of SQLE in the penetration of breast cancer cells through the blood-brain barrier (BBB) into the brain, followed by brain colonization. The overexpression of SQLE, specifically in brain-localized tumor cells, demonstrates that SQLE functions in the co-option of breast cancer cells with brain microcapillaries for tumor survival and growth in the brain environment. SQLE may serve as an essential biomarker for determination of breast-to-brain metastasis and further use as a target for therapeutic approach using terbinafine, a SQLE inhibitor. Terbinafine may suppress primary breast tumor growth at the mammary fat pad and distal metastases to the brain, suggesting a therapeutic opportunity for breast cancer brain metastasis (BCBM). Created with Biorender.com.

Summary

- Squalene epoxidase (SQLE), one of the cholesterol biosynthesis enzymes, is highly expressed in brain metastatic breast cancers.
- SQLE may promote breast cancer cell penetration and transmigration through the blood–brain barrier (BBB) during brain metastasis.
- *In vivo* BCBM mouse models provide a powerful assay tool for studying breast cancer extravasation and colonization into the brain.

References

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