Targeting Squalene Epoxidase in Breast-to-Brain Metastasis: A New Therapeutic Target for Brain Extravasation and Colonization Using Animal Models

Pharmacology Discovery Services

Abstract #1084

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Abstract

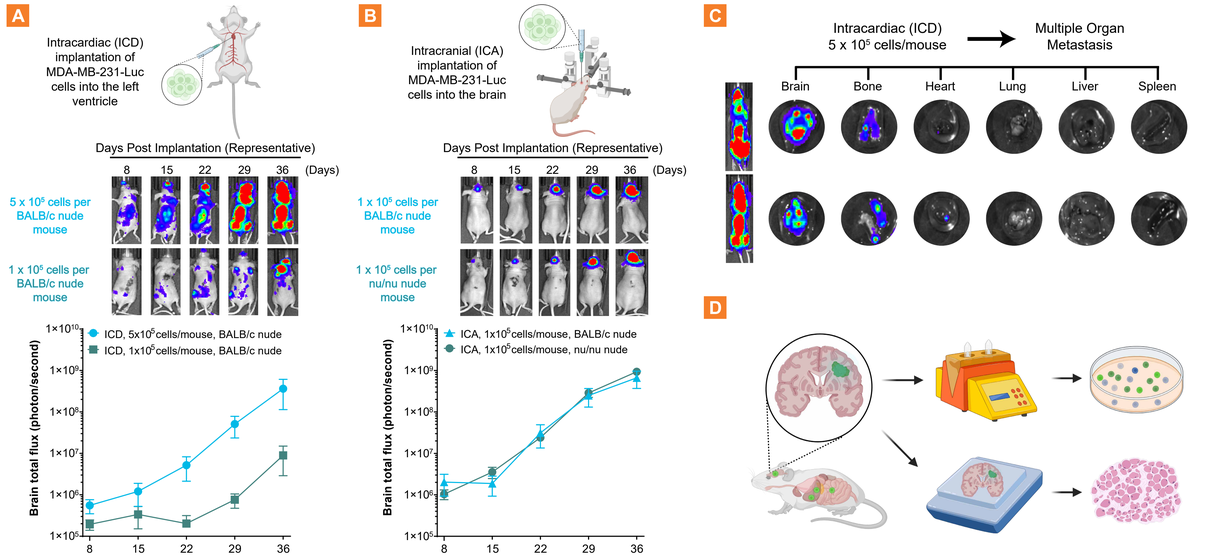
Background: Brain metastasis is the most common malignancy of the central nervous system which causes severe morbidity and mortality in multiple cancer types of patients and represents an unmet medical need. Several critical steps are required for a successful brain metastasis, including local invasion, intravasation, 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 (BCBM) 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 ex 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, 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.

Generation of *In Vivo* Breast Cancer-to-Brain Metastasis Mouse Models for Studying Breast Cancer Extravasation and Colonization into the Brain



Days post tumor cell implantation — Days post tumor cell implantation

Figure 2. *In vivo* breast-to-brain metastasis model with the highly metastatic human breast MDA-MB-231-luciferase (Luc) cell line in nude mice. A.-B. Intracardiac (ICD, A) and intracranial (ICA, B) injection of MDA-MB-231-Luc cells into nude mice followed by IVIS imaging. Tumor growth represented by bioluminescence signal in the brain. The graphical images were created with BioRender.com. C. Multiple organ metastasis with ICD implantation of tumor cells. D. Study scheme of ex vivo analysis: brain tumor cells were dissociated and cultured, or FFPE preserved for histopathology with H&E and IHC staining with anti-SQLE antibody. Created with BioRender.com.

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

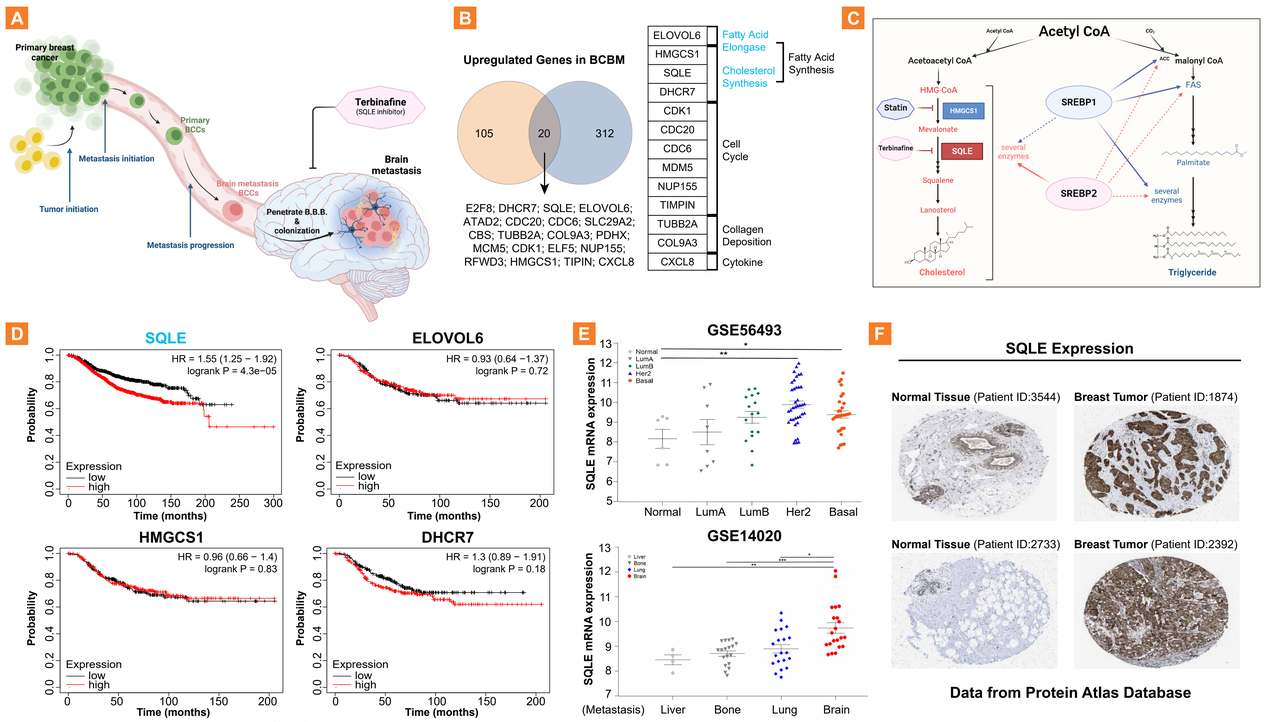


Figure 1. **Identification of squalene epoxidase (SQLE) as a novel therapeutic opportunity for breast cancer-to-brain metastasis.** A. Scheme of multiple steps involved in breast cancer-to-brain metastasis (BCBM) and the indication of targeting SQLE as a novel therapeutic opportunity. Created with BioRender.com. B. Left: Upregulated genes in BCBM with clinical breast carcinoma patients (GSE125989) and MDA-MB-231 cell line (GSE12237). Right: Among them, ELOVOL6, HMGCS1, SQLE and DHCR7 are involved in fatty acid synthesis. C. Scheme of key enzymes, including HMGCS1, SQLE and SREBP1/2, in the biosynthesis of cholesterol and triglyceride. D. Overall survival probability for patients with breast cancer with high and low mRNA expression of indicated genes. E. Differential expression of SQLE in various subtypes of breast carcinoma patients with higher expression in brain metastatic breast cancers. F. Overexpression of SQLE in breast tumor compared to normal breast tissues by IHC staining.

SQLE is Essential for Breast Cancer Cell Penetration and Transmigration Through the Blood–Brain Barrier (BBB) During Brain Metastasis

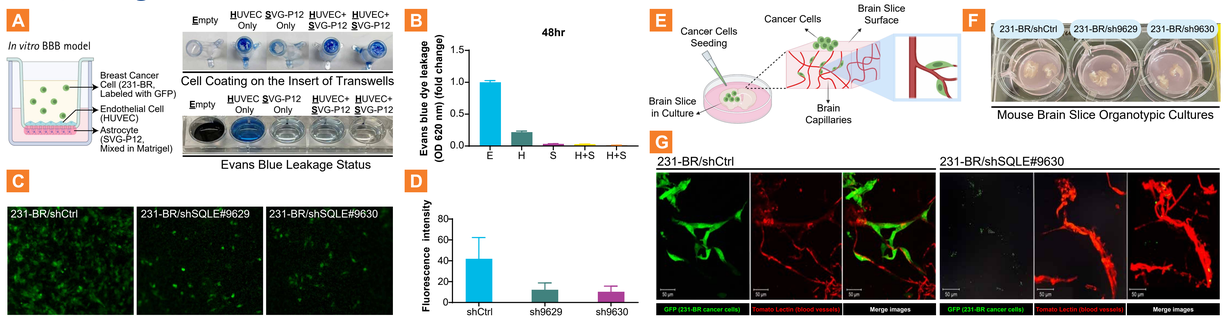


Figure 3. **SQLE may promote 231-BR cell penetration and transmigration through the blood–brain barrier (BBB).** A. Left: Scheme for the *in vitro* BBB model. Created with BioRender.com. Top right: Different cell coating on the insert of Transwells (representative examples). Bottom right: Evans blue dye leakage detection indicating the integrity of *in vitro* BBB models with different cell coating (representative examples). B. Quantitative results of Panel A study (mean ± SEM; n = 3). C. GFP fluorescence signals indicating the effects of shRNA-mediated knockdown (sh9629 or sh9630) of SQLE on 231-BR cell penetration through *in vitro* BBB models (representative examples). 231-BR cells were labeled with GFP. D. Quantitative results of Panel C study (mean ± SEM; n = 3). E. Scheme of brain slice organotypic cultures. Cancer cells placed on the surface of slices migrate into the tissue and seek microcapillaries. Created with BioRender.com. F. Control (shCtrl) and SQLE-KD (sh9629 or sh9630) 231-BR cells were seeded on mouse brain slides (representative examples). G. Representative confocal images of brain slices harboring infiltrated 231-BR/shCtrl cells, but not 231-BR/shSQLE cells, spreading over brain capillaries.

Summary

1. Squalene epoxidase (SQLE), one of the cholesterol biosynthesis enzymes, is highly expressed in brain metastatic breast cancers.
2. SQLE may promote breast cancer cell penetration and transmigration through the blood-brain barrier (BBB) during brain metastasis.
3. *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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