

## Sphingomyelin pathway modulation for epidermal growth factor receptor inhibition

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**Objective:** Overexpression of the epidermal growth factor receptor (EGFR) is associated with decreased survival, resistance to radiation, local treatment failure, and increased distant metastasis of oral squamous cell carcinoma (OSCC). In previous studies, we showed that pharmacological modulation of key enzymes in the sphingomyelin (SM) biosynthetic pathway leads to mislocalization of EGFR from the plasma membrane through depletion of cholesterol in CHO cells expressing GFP-tagged EGFR (CHO-EGFR). Goals of this study were to determine if SM pathway modulators inhibit proliferation and clonogenic survival of CHO-EGFR.

**Methods:** SM pathway modulators tested were myriocin (inhibitor of serine palmitoyl transferase), fumonisin B1 (inhibitor of ceramide synthase), GT11 (dihydroceramide desaturase inhibitor), 2-hydroxyoleic acid (2-OHOA; activator of SM synthase), D609 (inhibitor of SM synthase), SKI-II (sphingosine kinase I inhibitor), and L-threo-dihydrosphingosine (L-threo-DHS; a sphingosine kinase I and II inhibitor). In proliferation assays, cells seeded in 96-well plates were treated with a concentration range of drugs for 48 hours and quantified using CyQUANT (Thermofisher) assay. In clonogenic survival assays, cells were seeded in 6-well plates and drug treated every 72 hours for two weeks. Colonies were then fixed, stained with crystal violet, and imaged.

**Results:** Myriocin and fumonisin B1 poorly inhibited proliferation of the CHO-EGFR cells. GT11, K1, 2-OHOA, D609, SKI-II and L-threo-DHS dose-dependently and effectively inhibited proliferation of CHO-EGFR cells, with IC50s in the nanomolar to micromolar ranges. Efficacies of the drugs in clonogenic survival assays mirrored their effects in proliferation assays.

**Conclusions:** While myriocin and fumonisin B1 inhibit enzymes in the early stages of SM pathway biosynthesis, all other drugs target enzymes in latter stages. Our results show that pharmacological modulation of enzymes in latter stages of SM biosynthesis produces greater inhibitory effect on EGFR. Future studies should be conducted to further analyze their potential as chemotherapeutics for the treatment of OSCC.

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