Regulation of EGFR Expression and Localization by Cholesterol-Associated Genes: Insights from *C. elegans* Model

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Objectives: Epidermal growth factor receptor (EGFR) drives tumorigenesis in head and neck cancers (HNC) and therefore is an important protein target for development of novel and effective therapeutics. EGFR localization to the plasma membrane is regulated by cholesterol. Correlation analysis of EGFR expression in tumors in TCGA-HNC database with the expression of 115 endo-lysosomal genes involved in cholesterol trafficking identified 8 genes, expression of which either positively or negatively correlated with EGFR expression in HNC and survival duration of patients. Goal of this study was to determine if knock down of orthologs of those 8 genes in *C. elegans* could alter localization of LET-23 protein in worms or the *let-23* (EGFR ortholog)-driven multivulva phenotype of worms expressing constitutively active *let-23*.

Experimental Methods: *vha-8*, *vha-16*, *vha-17*, *tsp-7*, *gana-1*, *vps-26*, *vps-35*, *sulp-8* and *clh-6* were knocked down in *let-23* mutant or LET23::GFP expressing worms using RNAi. Thereafter, worms were imaged using a C2 confocal microscope to quantify the multivulva phenotype or determine localization of LET-23::GFP.

Results: Compared to vector control, knockdown of *vha-8* and *vps-26* whose human orthologs show positive correlation with EGFR expression, significantly caused mislocalization of LET-23::GFP. Knockdown of *gana-1*, *tsp-7* and *sulp-8* whose human orthologs show negative correlation with EGFR expression increased vulval localization of LET-23::GFP. Accordingly, a trend in decrease of multivulva phenotype was observed with *vps-26* knockdown, while an increase in multivulval phenotype was observed with knockdown of *gana-1*, *tsp-7* and *sulp-8*.

Conclusion: Our study provides novel insights into the role of cholesterol-associated genes in modulating LET-23 localization and function in *C. elegans*. These findings contribute to our understanding of the regulatory mechanisms governing EGFR function, with implications for human cancers. Further investigation is warranted to elucidate the precise molecular pathways underlying these effects and to explore potential therapeutic interventions targeting cholesterol-associated genes in HNC treatment.

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