Ubiquilin 1 Interaction with Ubiquilin 2 and Implications in Formation and Metastasis of Lung Adenocarcinoma
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Abstract

Introduction
Ubiquilin proteins are a family of adaptor proteins (UBQLN1-4 & UBQLN L) that mediate protein degradation by linking ubiquitinated proteins to the proteasome. UBQLN genes are lost or under-expressed in over 50% of lung cancers (Yadav et al. Neoplasia 2017). It has been shown that UBQLN1 can form heterodimers with UBQLN2 (Ford et al. Biochem J 2006). Also, loss of UBQLN1 and UBQLN2 expression has been previously shown to increase cell migration, cell invasion, actin skeleton reorganization, and epithelial mesenchymal transition in vitro, suggesting a role in cancer metastasis (Shah et al. Oncogene 2015).

Objective
We hypothesize that dimerization and localization of UBQLN1 and UBQLN2 are altered in lung adenocarcinoma. It is also hypothesized that overexpression of UBQLN1 in lung adenocarcinoma cell lines will attenuate clonogenicity, growth rate, and epithelial mesenchymal transition.

Methods
To investigate localization and dimerization, molecular cloning was used to construct plasmids with UBQLN1 and UBQLN2 genes attached to mClover2 and mRuby2 genes (fluorophores). To further investigate the role of UBQLN1 in cancer formation and metastasis, a stage 1 lung adenocarcinoma cell line (H2087, ATCC) was used with UBQLN1 over-expressed. Clonogenicity was evaluated by seeding 1000 cells/well in a 6 well plate and measuring the total area of clonal colonies 10 days later. Growth rate was evaluated with an alamarBlue assay. Epithelial mesenchymal transition (EMT) was evaluated by western blot analysis.

Results
UBQLN1 and UBQLN2 were successfully cloned into the N-terminus of mClover2 and mRuby2 (4 clones total) and confirmed by Sanger sequencing. These 4 clones were then transfected into A549 cells (lung adenocarcinoma cell line) in a 6 well plate using Lipofectamine 2000 transfection reagent. Stable expression of the clones was confirmed by fluorescence microscopy.

Results from the clonogenicity assay displayed a high degree of variability and could not be used to form any conclusions. Western blot analysis showed noticeable changes in phospho-SRC, a secondary messenger implicated in EMT, however this was only in one trial and the blots were not re-probed for total SRC. The alamarBlue assay showed no significant changes in cell viability.

Conclusions
The experiment investigating dimerization and localization of UBQLN1 and UBQLN2 in lung adenocarcinoma is still incomplete, and no conclusions can be drawn at this time.

No conclusions can be drawn from the H2087 experiments with overexpression of UBQLN 1. Results were too variable, and the experiments have not been repeated yet.

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