DUOX1 silencing in lung cancer promotes epithelial-to-mesenchymal transition, enhances invasive properties, and increases resistance to erlotinib

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Rationale and Objective: Dual oxidase 1 (DUOX1) is an H₂O₂-generating enzyme within the airway epithelium is suppressed in lung cancers by epigenetic silencing, although the importance of DUOX1 silencing in lung cancer is unknown. We used RNAi approaches to investigate the impact of DUOX1 silencing in lung cancer.

Results: Stable transfection of DUOX1-targeted shRNA in H292 cells (H292-shDUOX1) was found to result in loss of epithelial characteristics and increased mesenchymal features suggesting the occurrence of epithelial-to-mesenchymal transition (EMT). This was confirmed by molecular analysis of EMT phenotypes. EMT in lung cancer has also been associated with enhanced resistance to EGFR tyrosine kinase inhibitors (TKI) as well as an enrichment of cancer stem cell (CSC) populations. Interestingly, H292-shDUOX1 cells are indeed resistant to the EGFR TKI erlotinib and display elevated expression of the following CSC markers: CD24<sup>high</sup>/CD44<sup>low</sup>, CD133 and intracellular ALDH1 activity. Furthermore, cell sorting of EMT subpopulations indicated dramatically reduced DUOX1 expression. Congruently, DUOX1 silencing also lead to enhanced invasiveness/metastatic potential in vitro and in vivo. Finally, overexpression of DUOX1 in A549 cells lead to the recovery of epithelial signatures. Collectively, these data prove the close association between DUOX1 and EMT.

Conclusions: Our findings indicate that DUOX1 silencing in lung epithelial cells promotes features of EMT, and may therefore be strongly associated with invasive and metastatic lung cancer. Furthermore, DUOX1 silencing is associated with CSC phenotypes and TKI resistance. Finally, DUOX1 silencing may also be relevant to other EMT-related lung pathologies such as chronic obstructive pulmonary disease (COPD).

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