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Poster Submissions

Poster Title
A KRAS dependency microRNA signature reveals a p62/Sqstm1-centered autophagy network regulated by mir-124

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Please describe the extent of your work in this research
Project design, experimental design, data analysis

Abstract Submission
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Yes

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Background Non-small cell lung cancer (NSCLC) is derived from broncho-alveolar epithelial cells. NSCLCs frequently harbor mutations in the KRAS oncogene, yet only a subset of NSCLC cell lines depend on KRAS for sustained survival signaling. Cells that are more epithelial are highly KRAS dependent, whereas mesenchymal-like cells are KRAS independent for cell survival signaling. KRAS mutations are commonly associated with resistance to anti-cancer therapeutics. An important drug resistance mechanism is altered cellular plasticity, via a process known as epithelial-to-mesenchymal transition (EMT). Here, we aimed to identify and elucidate a KRAS dependent microRNA (miRNA) signaling network in epithelial cancer cells that modulates malignant growth and proliferation as well as cellular plasticity in NSCLC.

Methods Differential miRNA expression in 6 KRAS mutant NSCLC cell lines (3 KRAS independent versus 3 KRAS dependent) was determined using Taqman low-density qPCR arrays (TLDA). To explore the functional relevance of differentially regulated miRNAs, gain-of-function studies by reconstitution experiments were performed using miRNA mimics in a panel of six KRAS independent cell lines. Cell viability analyses were performed in cell lines in medium throughput 96-well format, followed by functional validation of apoptotic, EMT and autophagy-related pathways by Western blotting, immunofluorescence microscopy and caspase-glo assays. The molecular targets of mir-124-2 were computationally identified by TargetScan or miRWalk and experimentally verified using 3’UTR luciferase-based assays. Functional rescue of cell viability defects was determined by forced expression of GFP-p62/Sqstm1 in KRAS independent lung cancer cell lines.

Results We identified a distinct differential miRNA expression profile in KRAS dependent versus KRAS independent NSCLC cells. This KRAS dependency miRNA signature included members of the mir-200 family, mir-34c and mir-205. Mir-200 and mir-205 reconstitution in KRAS independent cells modulated epithelial plasticity by down regulating Zeb1 protein expression and, thereby, increasing E-cadherin levels. Ectopic introduction of a subset of miRNAs in KRAS independent cells caused a consistent and pronounced loss of cell viability in all cell lines tested, including mir124, mir-625 and mir-518-3p. Ectopic expression of mir124 in KRAS independent cells caused cell viability defects by inducing apoptosis and autophagy, as assessed using mature mir-124 mimic and a lenti-viral vector encoding pri-mir-124-2. Using bioinformatics databases, we isolated p62/Sqstm1, TRAF6 and REL-A as key predicted targets of mir124-2. Mir-124-2
reconstitution in KRAS independent NSCLC cell lines caused decreased p62/Sqstm1, TRAF6 and REL-A protein expression levels. The effect of mir-124 on p62/Sqstm1 expression was verified using a p62-3’UTR-Luciferase construct. Overexpression of GFP-p62 in KRAS independent cells rescued the cell viability defects by mir124-2. Furthermore, mir124-2 re-expression down regulated the IL-6-IL-1β-NFκB inflammatory loop in KRAS independent cells, revealing a mechanism for the reduced cell viability induced by mir-124.

**Conclusion** MiR-124 expression is down regulated in KRAS independent NSCLC cells. Reintroduction of mir124-2 induces cell viability defects in part, by targeting p62/Sqstm1 and REL-A. We conclude that, mir-124 down regulates p62/Sqstm1 expression to activate autophagic flux. This leads to down regulation of the NFκB pathway and subsequent reduction in expression of pro-survival genes such as IL-6 and IL-1β. Taken together, mir124-2 acts as a tumor suppressor in KRAS mutant NSCLC cells and provides a mechanistic link between activation of autophagy and control of proinflammatory signaling in NSCLC cell survival.