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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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Would you like your abstract to be considered for an oral presentation (students and post docs only)?

Yes

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

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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.