Characterization of RIT1 signaling by proteome, phosphoproteome, and transcriptome profiling

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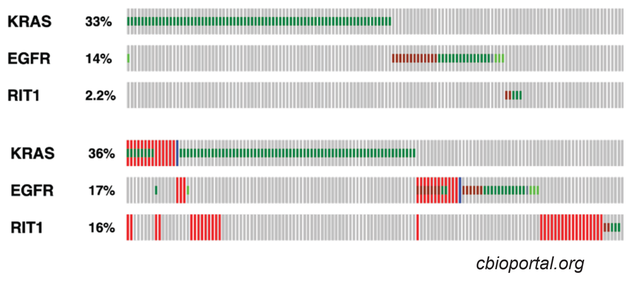
Background

Ras-like-in-all-tissues (*RIT1*) is a small GTPase of the Ras family that shares 50% homology with KRAS.

RIT1 is mutated in 2% of lung adenocarcinomas and amplified in a further 7-14%[1].

However, while RIT1's role in some Ras-related pathways has been investigated, **there has been no unbiased mapping of downstream RIT1 regulation and signaling pathways in human cells.**

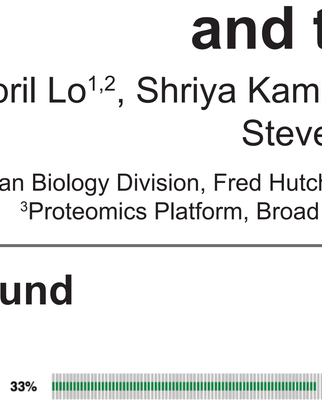


Questions

- Does RIT1 regulate the same pathways and processes as KRAS?
- How does increased expression of wild-type RIT1 affect signaling, compared to RIT1 and KRAS oncogenic variants?

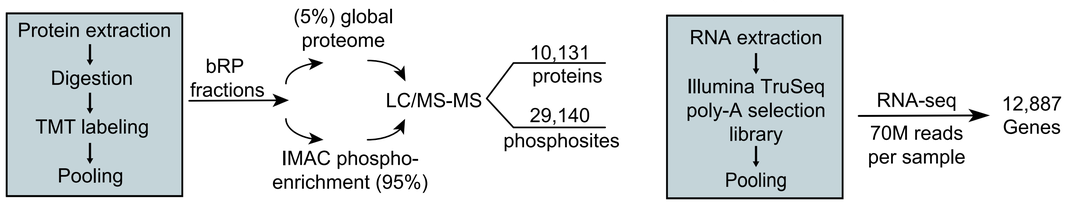
Methods

We transduced wild-type and oncogenic variant KRAS and RIT1 into AALE cells, a non-transformed, immortalized, human lung epithelial



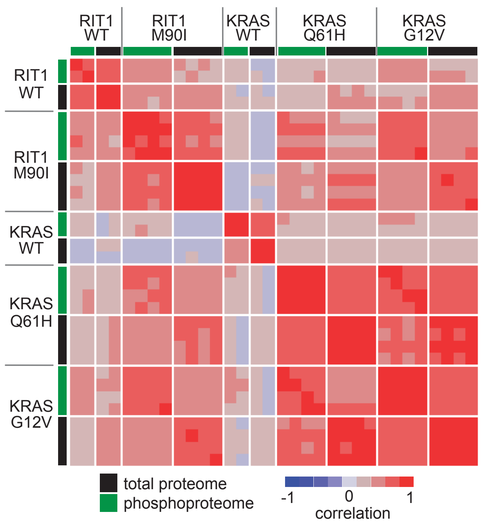
Expression of wild-type or oncogenic variants of RIT1 and KRAS promotes growth in soft agar.

We then performed proteomics and phosproteomics profiling by LC/MS-MS and transcriptomic profiling by RNA-seq.

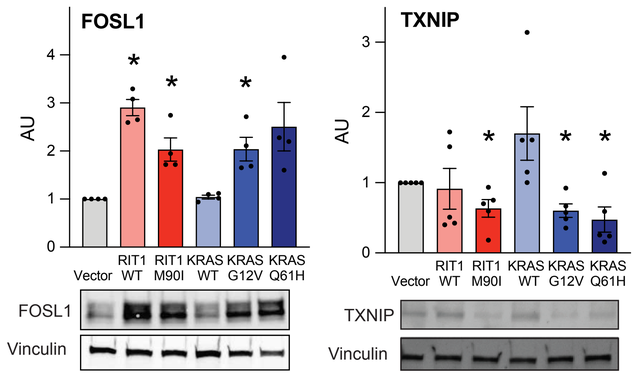


Results

RIT1^{WT} and RIT1^{M90I} induce changes in the proteome and phosproteome similar to KRAS variants



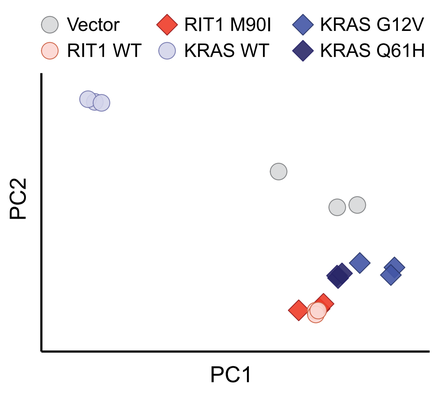
The proteome and phosphoproteome profiles of KRAS^{G12V} and KRAS^{Q61H} were highly correlated with each other, and with the proteome and phosphoproteomes of RIT1^{WT} and RIT1^{M90I} (r = 0.7-0.85).

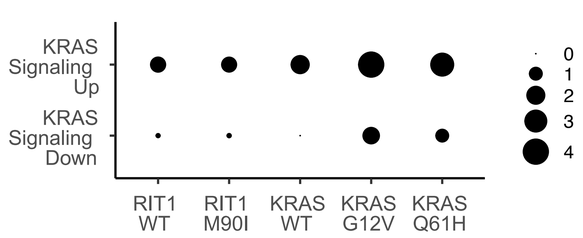


Among the top proteins with increased abundance in KRAS variant and RIT1 cells was FOSL1, a transcription factor known to be upregulated by RAS.

Similarly, TXNIP, a protein shown to be suppressed by HRASG12V, was among the top proteins with decreased abundance.

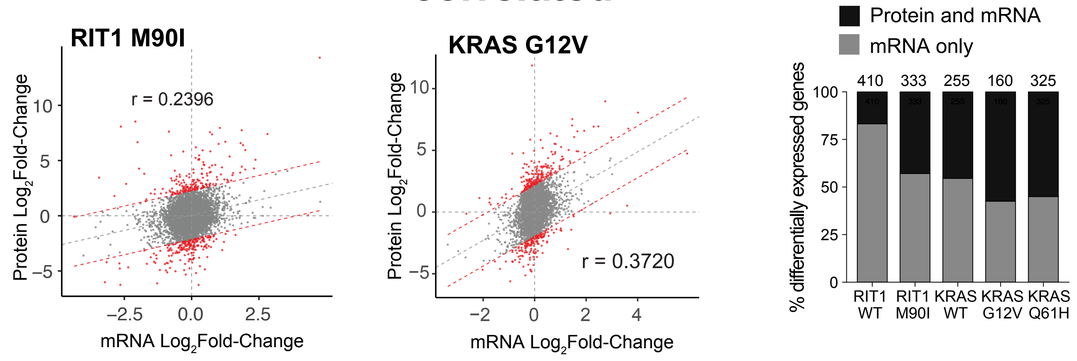
RIT1^{WT} and RIT1^{M90I} also induce changes in the transcriptome similar to KRAS variants



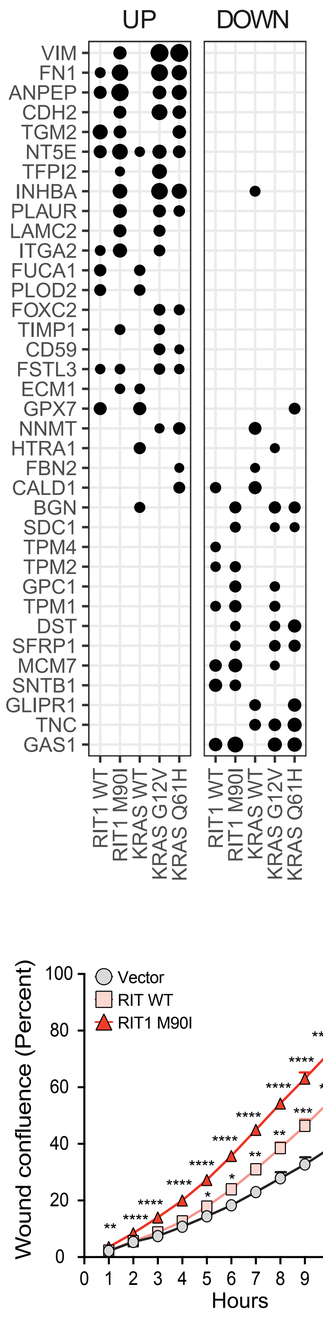


Gene set enrichment analysis of upregulated transcripts show **expected increase in activity of KRAS signaling pathways**.

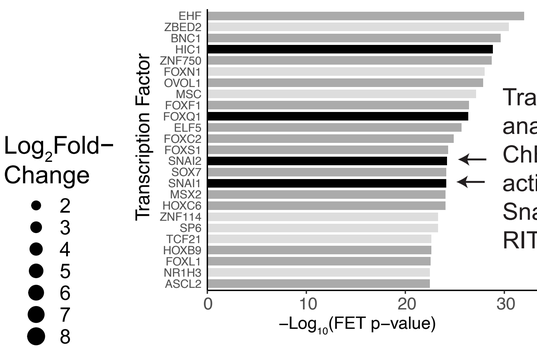
Proteome and transcriptome profiles are moderately correlated



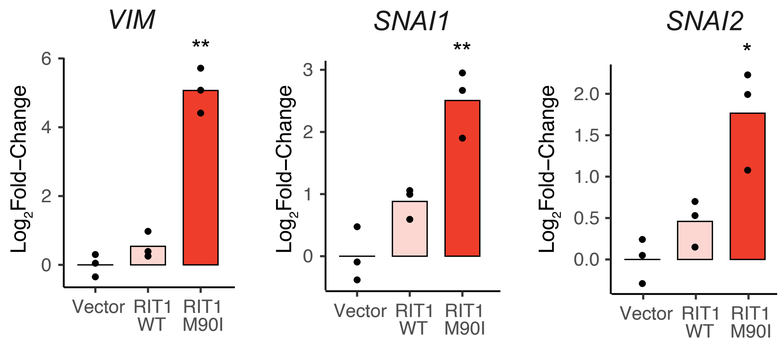
RIT1 promotes an epithelial-to-mesenchymal transition



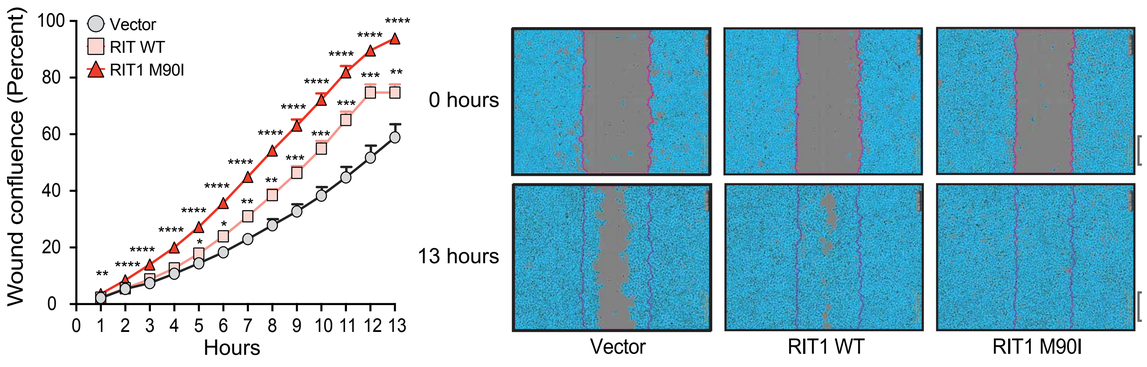
Gene set overlap analysis of differentially abundant proteins suggested an upregulation of EMT proteins across KRAS variant and RIT1 cells, including **increased abundance of key EMT markers Vimentin (VIM), Fibronectin (FN1), and N-Cadherin (CDH2).**



Transcription factor target enrichment analysis using Enrichr libraries in ChEA3[2] showed an increase in the activity of EMT transcription factors Snail (*SNAI1)* and Slug (*SNAI2)* in RIT1^{M90I} cells.



Quantification of Vimentin, Snail, and Slug mRNA by qRT-PCR indicate these EMT genes are indeed upregulated at the transcript level.



In line with a functional EMT, RIT1-transformed cells show enhanced migration in a scratch assay, with RIT1^{WT} and RIT1^{M90I} closing the scratch wound up to 1.5x and 1.9x faster than vector control cells respectively.

Conclusions

- RIT1 and KRAS signaling networks exhibit overall similarity, suggesting that **oncogenic RIT1 can hijack and activate canonical Ras effector pathways.**
- **Amplification of wild-type RIT1 expression phenocopies oncogenic RIT1 activation**
- Both **RIT1^{WT} overexpression and RIT1^{M90I} promote an EMT phenotype**

Future Directions

- Investigate oncogenic properties of amplified wild-type RIT1 in human tumors
- Further determine how RIT1 regulates EMT and whether it does so *in vivo*
- Analyze RNA-seq and phosphoproteomic data further to determine how RIT1 and KRAS affects alternative splicing processes

Acknowledgements

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References

1. TCGA, Nature, 2014. 2. Keenan et al., NAR, 2019.

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