A c-Src C-terminal Mutant Deregulates Genes Involved in Metastasis and Translation

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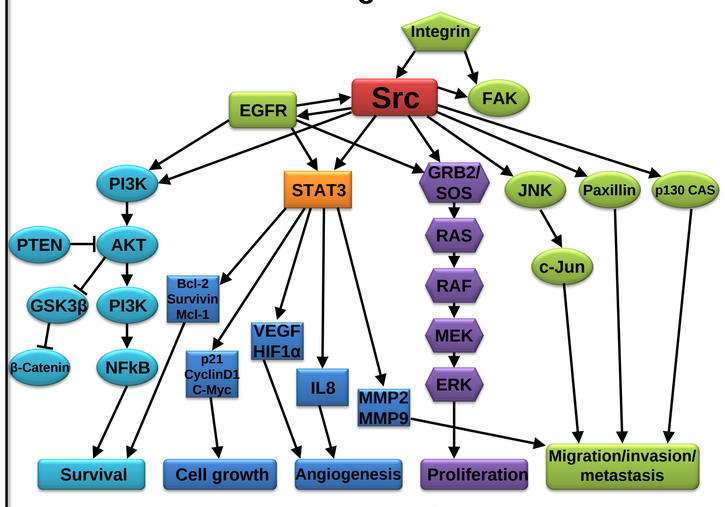
Src is the longest known retroviral oncogene. Activation of its cellular homolog c-Src is characteristic of many cancers. Here we analyzed a pro-metastatic C-terminal mutant of the c-Src protein. This mutant, c-Src(mt), has a point mutation at its very C-terminal amino acid which impairs its binding to PDZ domain-containing tumor suppressors in comparison to the wildtype c-Src, c-Src(wt). c-Src(mt)-expressing cells exhibited increased metastatic activity *in vivo*. Whole-genome transcriptome analysis revealed that c-Src(mt) deregulated the expression of over 400 genes, which were analyzed with respect to functions and signaling pathways. We found that c-Src(mt)-activated genes are mainly involved in metastasis and protein translation and identified about 70 c-Src(mt) target genes not previously associated with metastasis. This study reveals some insight into Src-dependent metastatic behavior and provides putative biomarkers characteristic of metastatic cells. Some of the newly identified genes may serve as potential markers of metastasis and possibly turn out to be useful as targets for therapeutic intervention of metastatic cancer.

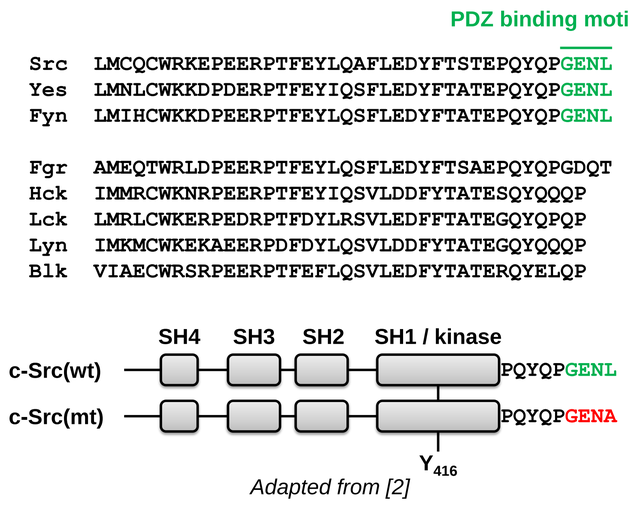
INTRODUCTION

Master Regulator Src



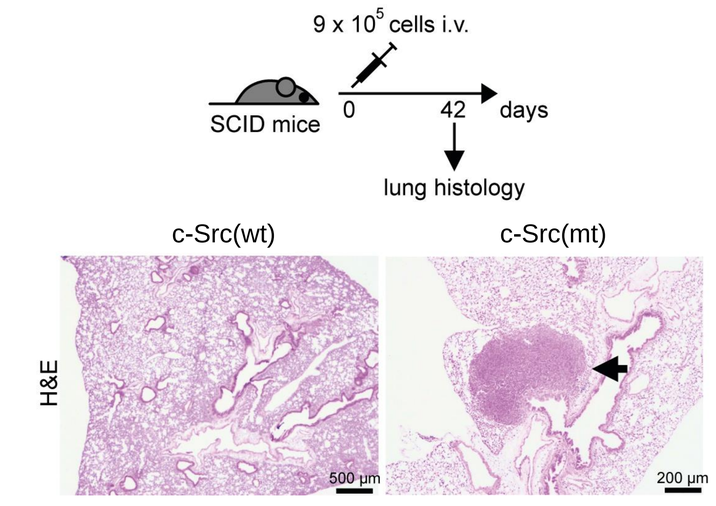
Adapted from [1]

Src Family Kinases and PDZ Binding Motif



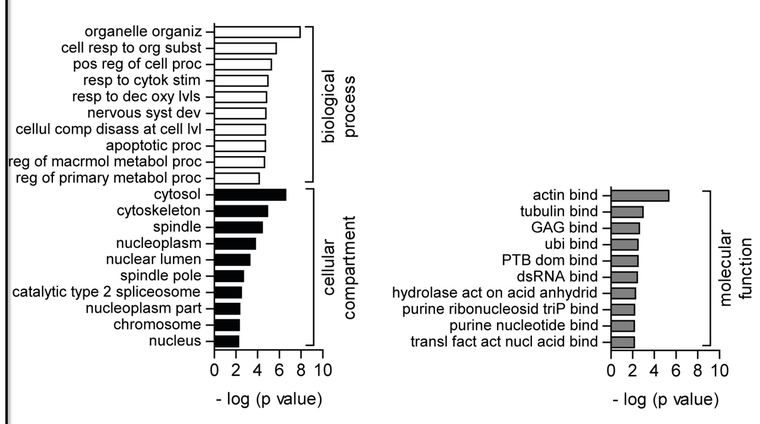
Adapted from [2]

Metastatic Activity of c-Src(mt)-Expressing Cells



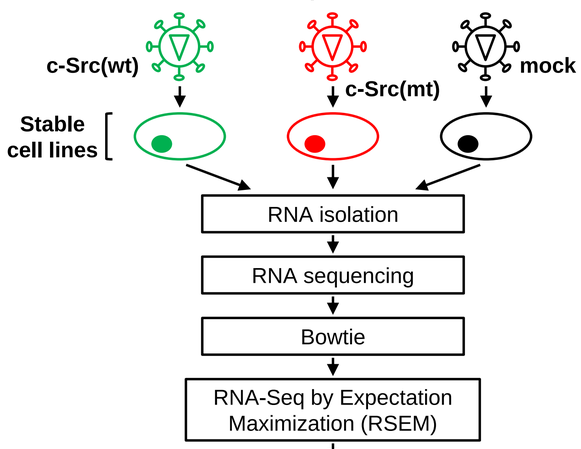
RESULTS

Gene Ontology Enrichment

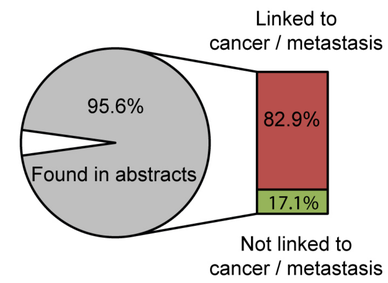


▲Differentially expressed genes (q ≤ 0.05) (c-Src(mt) vs. mock) were assigned to protein functions that are statistically overrepresented in this set of genes.

Lentiviral expression vectors



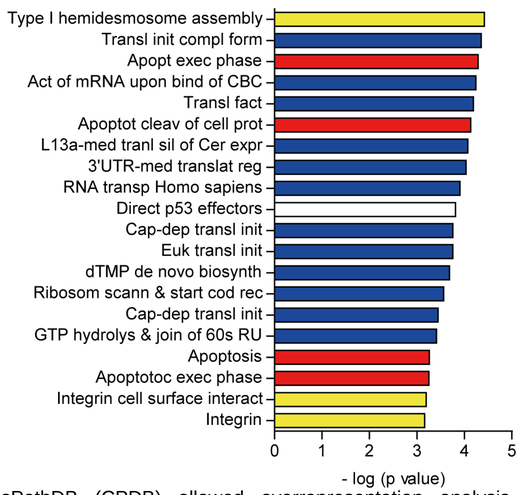
Literature Mining



→ **Novel therpeutic and diagnostic markers?**

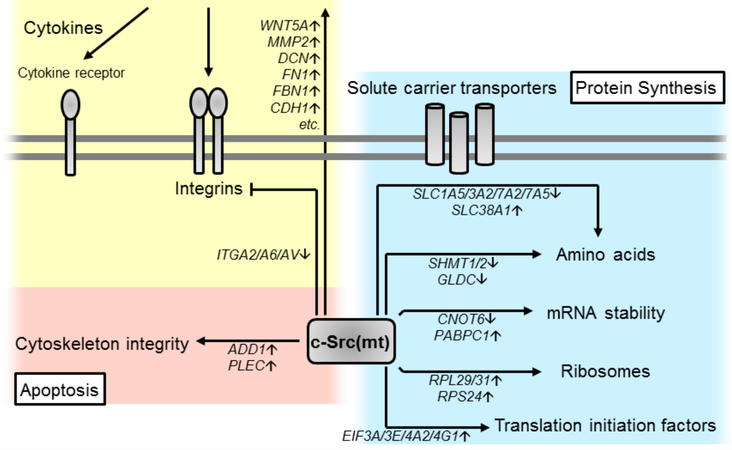
▲Literature mining of PubMed abstracts with a lexicon of metastasis-associated terms allowed identification of novel genes associated with Src-dependent metastasis. This set of genes provides putative novel diagnostic markers and therapeutic targets for metastatic cancer.

Pathway Enrichment

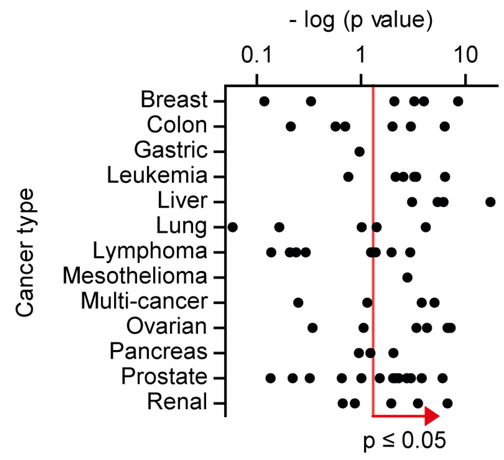


▲ConsensusPathDB (CPDB) allowed overrepresentation analysis in different pathway databases (PID, Reactome, Wikipathways, etc.). The analysis shows that genes involved in adhesion (yellow), translation (blue), and apoptosis (red) signaling pathways are dysregulated in c-Src(mt) vs. mock cells.

Regulation of Metastasis, Apoptosis and Protein Synthesis by c-Src(mt)



Oncomine Analysis



▲Overlap of differentially expressed gene sets of our c-Src(mt)-expressing cells with a wide range of human primary tumors demonstrates that the gene sets are relevant for human neoplastic disease.

CONCLUSIONS

- **Stable cell line expressing c-Src(mt) serves as *in vitro* model for Src-dependent metastasis**
- **Transcription profiling of these cells reveal c-Src(mt) target genes with altered expression on genome-wide scale**
- **These genes have been characterized in detail based on protein function, pathways, promoter analyses and correlation with expression patterns of human primary cancers**
- **Literature mining allowed definition of genes not previously associated with metastasis as novel targets for diagnostic and therapeutic purposes**

REFERENCES

[1] Nagathihalli NS, Merchant NB. Src-mediated regulation of E-cadherin and EMT in pancreatic cancer. *Front Biosci* **2012**, 17, 2059.

[2] Baumgartner M, Radziwill G, Lorger M, Weiss A, Moelling K. c-Src-Mediated Epithelial Cell Migration and Invasion Regulated by PDZ Binding Site. *Mol Cell Biol* **2008**, 28, 642.