ADVANCED CELLULAR DYNAMICS

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LIFE SCIENCE INNOVATION NORTHWEST

Discovery starts here.

The Observation

Recombinant tyrosine kinases transform Ba/F3 cells to IL3-independence. Not all kinases induce transformation with equivalent efficiency, resulting in unique phenotypic traits (growth rate, appearance, etc.).

The Hypothesis

Ba/F3 cells are atypically susceptible to oncogenic transformation, possibly via compensatory mutations and/or epigenetic alterations that impact the expressed transcriptome, facilitating adaptation to the transgene.

The Approach

Next-generation sequencing was used to examine the expressed transcriptomes of several kinase-addicted cell lines. Multidimensional data analyses were used to examine transcriptional heterogeneity among cell lines.

Experimental Data

Multidimensional Scaling Leading Dimensions

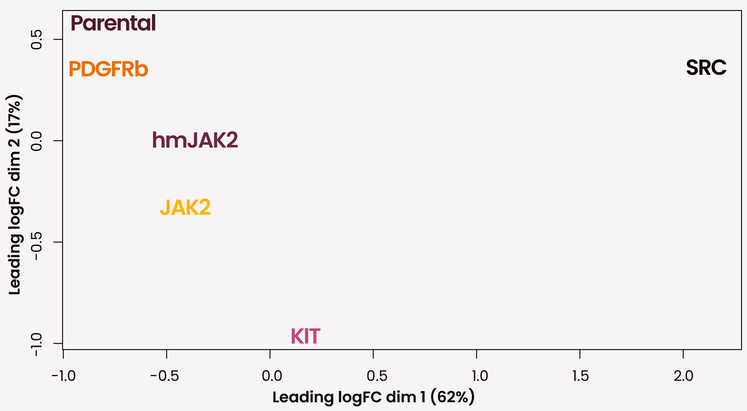

Fig 1. MDS plot showing the variation among samples based on normalized RNA-seq data. The leading logFC (base 2 logarithm of fold change) is the average of the logFC between each pair of samples. Dimension 1 explains 62% of the variance between samples while dimension 2 explains 17% of the variance.

MSigDB Hallmark Gene sets

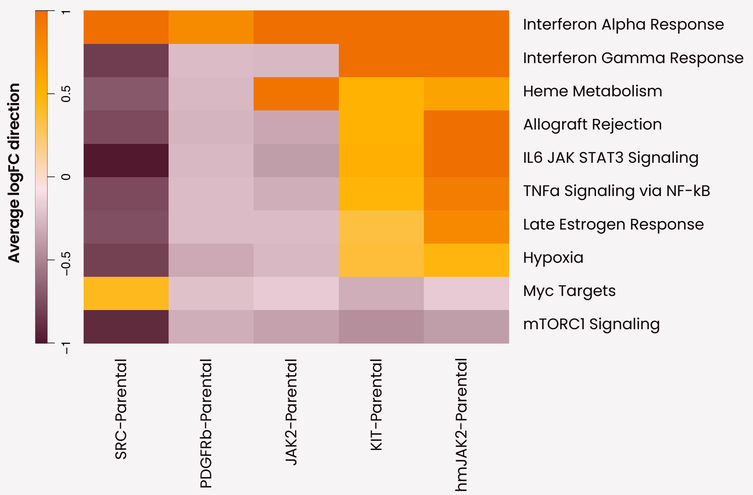

Fig 2. Heatmap showing statistically-significant differences in expressed gene sets (from MSigDB) for each kinase-transformed cell line relative to parental Ba/F3 cells. Negative logFC represents underexpression and positive logFC represents overexpression.

SRC vs Parental Top 20 DE Genes

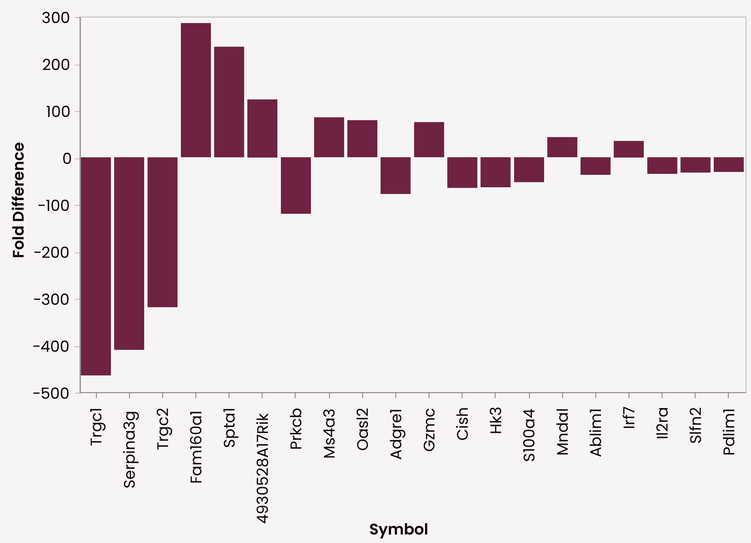

Fig 3. The top 20 differentially expressed genes between SRC and Parental cell lines. All differences are statistically significant ($p < 0.05$) and ordered by absolute fold difference.

Conclusion

Transformed Ba/F3 cells often appear to adapt to the transgenic kinase, with variable degrees of efficiency. PDGFRβ most readily transforms Ba/F3 cells, with an expressed transcriptome very similar to parental cells. However, SRC kinase transformed cells require an extended recovery period before exhibiting robust growth, suggesting additional genetic adaptations are required for full transformation. Consistent with this interpretation, SRC kinase-addicted cells exhibit the greatest degree of transcriptional heterogeneity relative to parental cells. The underlying cause of these transcriptional difference remains to be elucidated.

Next Steps

- Whole genome sequencing to examine genetic composition.
- Bisulfite sequencing to explore potential epigenetic impact of methylation
- Temporal evaluation of genetic adaptation(s) during SRC kinase-mediated transformation.