Transcriptome-wide analysis reveals novel insights into tumor suppressor function of 1B3 (a chemically modified miR-193a-3p mimic)

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INTRODUCTION

MicroRNAs (miRNAs) are a family of naturally-occurring small, non-coding RNAs that regulate gene expression. Due to their inherent ability to concurrently modulate several molecular pathways and consequently affect important cellular processes, miRNAs represent a potential novel therapeutic modality against cancer. Emerging data show that miR-193a-3p has a suppressive role in many cancer types and is often downregulated in tumors. Therefore, introducing miR-193a-3p into cancer cells could be used as a novel therapeutic approach in oncology. Currently, we are developing a synthetic miR-193a-3p mimic (1B3) which inhibits viability of a broad range of human cancer cell lines *in vitro* by inducing apoptosis, cell cycle arrest and senescence. Parallel development of a lipid nanoparticle-based formulation (INT-1B3) resulted in efficient *in vivo* delivery, marked anti-tumor activity, induction of immune surveillance and increased survival in a variety of murine models. In order to identify important target genes and signaling cascades regulated by 1B3 and to describe its potential mechanism of action leading to the observed phenotypes *in vitro* and *in vivo*, we performed RNA-sequencing of six different human tumor cell lines transfected with 1B3. The differential expression profiles at 24h and 72h post-transfection ($P < 0.05$) were subsequently used in an unbiased analysis to characterize affected cellular pathways.

CELL LINES

#	Name	Cancer Type
1	**A2058**	Skin (Melanoma)
2	**A549**	Lung (NSCLC)
3	**BT549**	Breast (TNBC)
4	**H460**	Lung (NSCLC)
5	**HEP3B**	Liver (HCC)
6	**HUH7**	Liver (HCC)

1B3

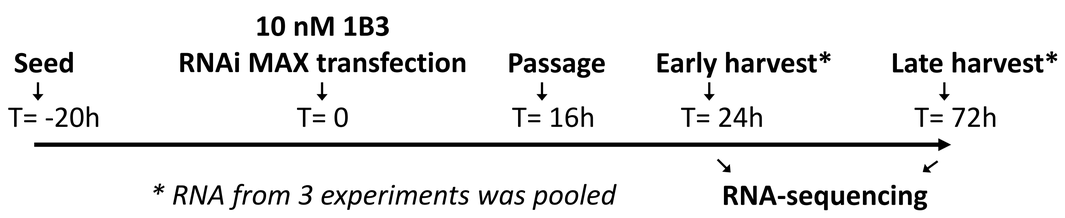
Synthetic double-stranded chemically modified miR-193a-3p mimic (1B3)

3′- **TTUUGACCGGAUGUUUCAGGGU** -5′ Sense (passenger)

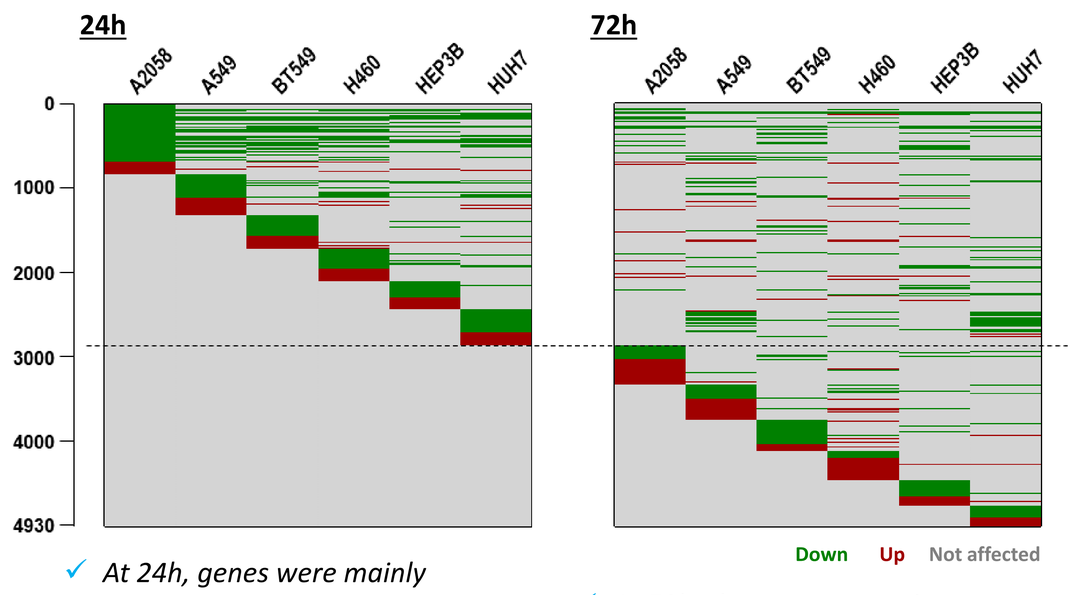
5′- **AACUGGCCUACAAAGUCCCAGU** -3′ Antisense (guide)

Seed

EXPERIMENTAL DESIGN

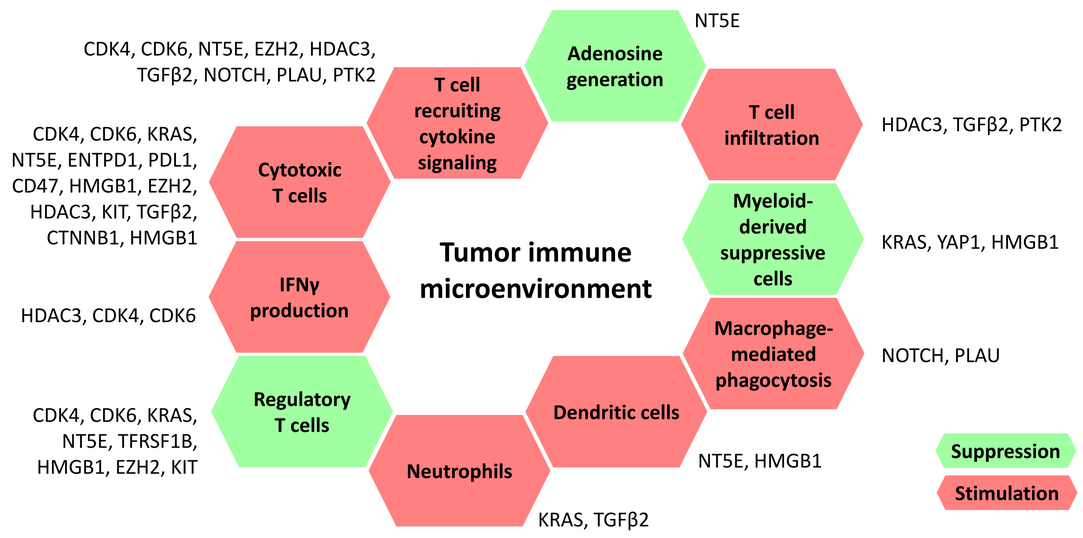


DIFFERENTIAL GENE EXPRESSION PROFILES



- ✓ *At 24h, genes were mainly downregulated by 1B3, consistent with direct effects on mRNA targets*
- ✓ *Hundreds of genes affected in each cell line*
- ✓ *At 72h, the gene expression pattern drastically changed compared to 24h*
- ✓ *Downstream effects triggered by the initial gene downregulation*

IMMUNO-ONCOLOGY POTENTIAL

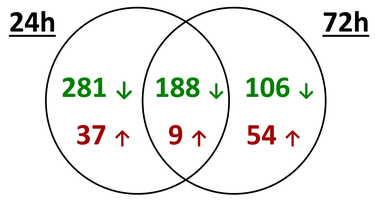


- ✓ *Immune-modulatory genes downregulated by 1B3 in at least 1 cell line at 24h or 72h*
- ✓ *1B3 may turn 'cold' (immuno-suppressed) into 'hot' (immuno-stimulated) tumors*

INGENUITY PATHWAY ANALYSIS IPA

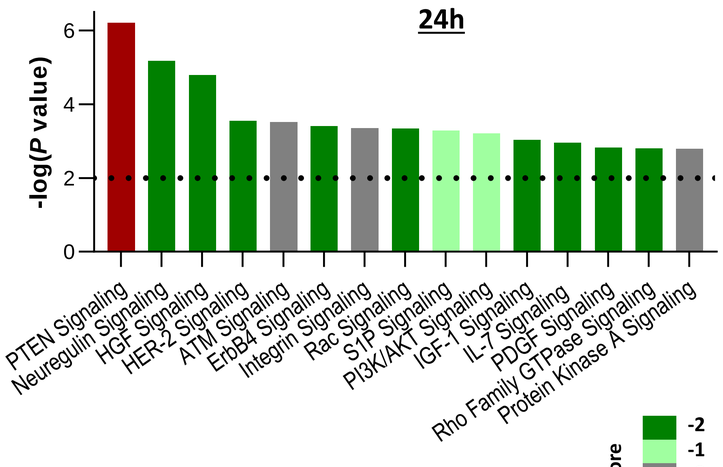
INPUT

- ✓ *Genes differentially expressed ($P < 0.05$) in at least three cell lines*
- ✓ *24h: 469 genes down, 46 up*
- ✓ *72h: 294 genes down, 63 up*

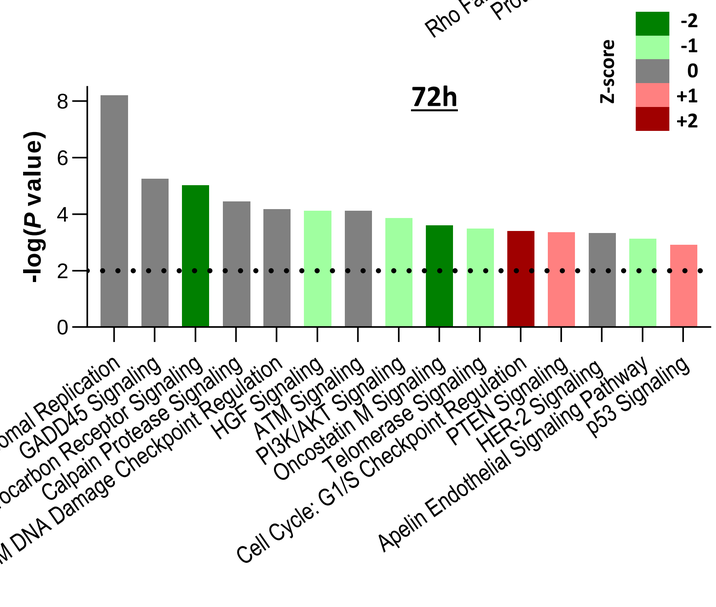


CANONICAL PATHWAYS

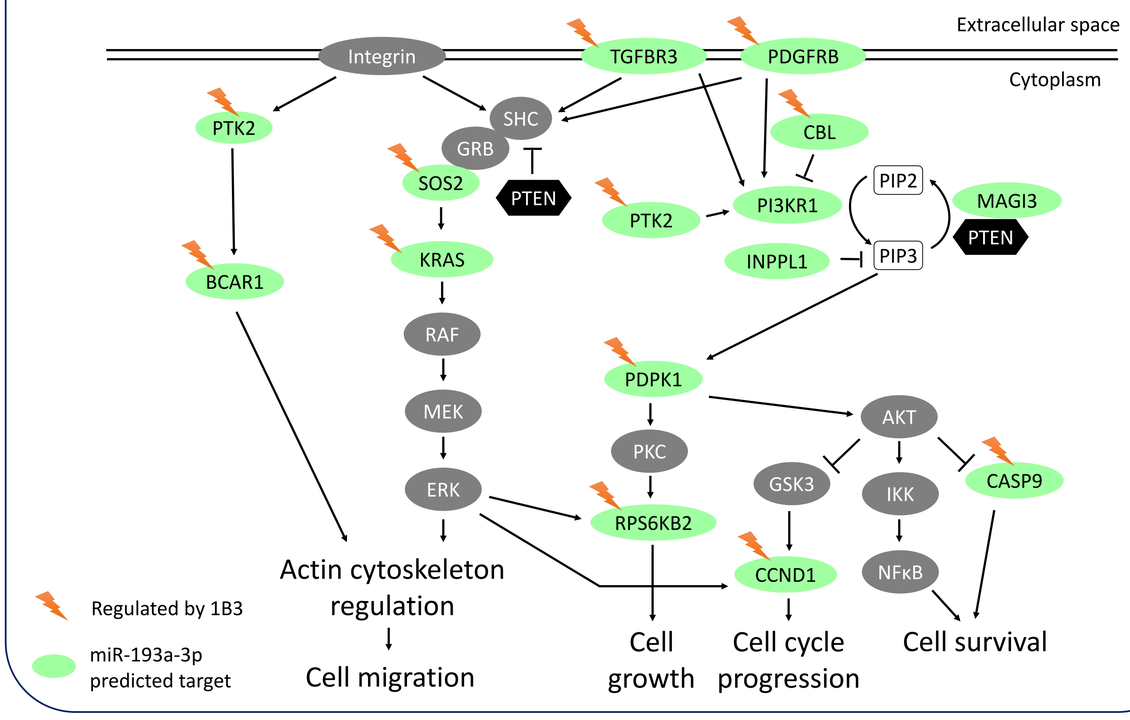
- ✓ *At 24h post-transfection, 1B3 strongly upregulates the tumor suppressive PTEN pathway*
- ✓ *Many growth factor and cell signaling pathways are downregulated*



- ✓ *At 72h post-transfection, 1B3 modulates multiple cell cycle-related pathways*
- ✓ *The tumor suppressive PTEN and p53 pathways are upregulated*



1B3 ACTIVATES THE PTEN PATHWAY



CONCLUDING REMARKS

- ✓ 1B3 targets hundreds of genes, with critical roles in cell signaling, growth and survival, in multiple cancer cell lines. Further downstream differential gene expression is more specific to individual cell lines.
- ✓ 1B3 downregulates various modulators of the immune system and may therefore induce a tumor suppressive microenvironment.
- ✓ Ingenuity Pathway Analysis showed that 1B3 strongly upregulates the tumor suppressive PTEN pathway and downregulates important growth factor signaling pathways.

Taken together, our data demonstrate that 1B3 represents a potent tumor suppressor which directly and indirectly targets a wide variety of oncogenic pathways across cancer types. Therefore, introducing a miR-193a-3p mimic into tumor cells is a promising new strategy for cancer treatment.