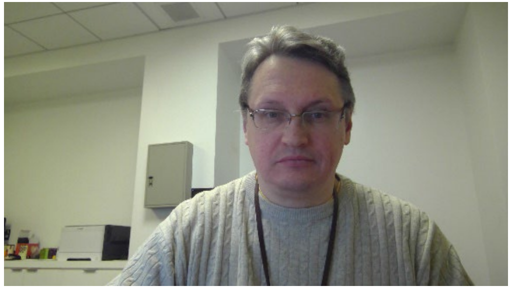
Finding microRNA markers in the African Green Monkey Kidney cells model of neoplastic transformation.



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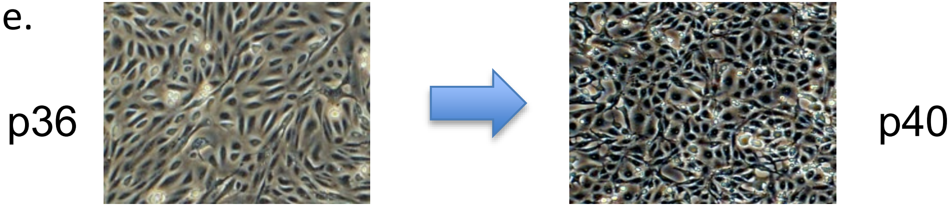
Introduction/Hypothesis

- The African Green Monkey Kidney (AGMK) cell model of neoplasia has been established by serial passaging of the primary kidney cells in culture for over 45 passages.
- At the passage p38-p40 the cells convert from fibroblast-like (mesenchymal) to epithelial phenotype.



- AGMK cells become fully transformed by the passage p40 as evidenced by their ability to produce tumors and metastasis in different strains of immuno-compromised mice.

Passage level	Mouse strain	Tumor incidence	Lung metastases
p22	Nude	0/18 (0%)	0/18 (0%)
p32	Nude	0/15 (0%)	0/15 (0%)
p41-p44	Nude	16/23 (70%)	8/23 (35%)
p44	SCID	45/45 (100%)	36/45 (80%)
p44	CD3epsilon	53/53 (100%)	43/53 (81%)

Objective

- Our objective is to find the microRNA markers of tumor initiation and progression from the ‘omics’ data, using the microRNA and mRNA profiles from different passages of AGMK cells.

Methods

AGMK samples analyzed

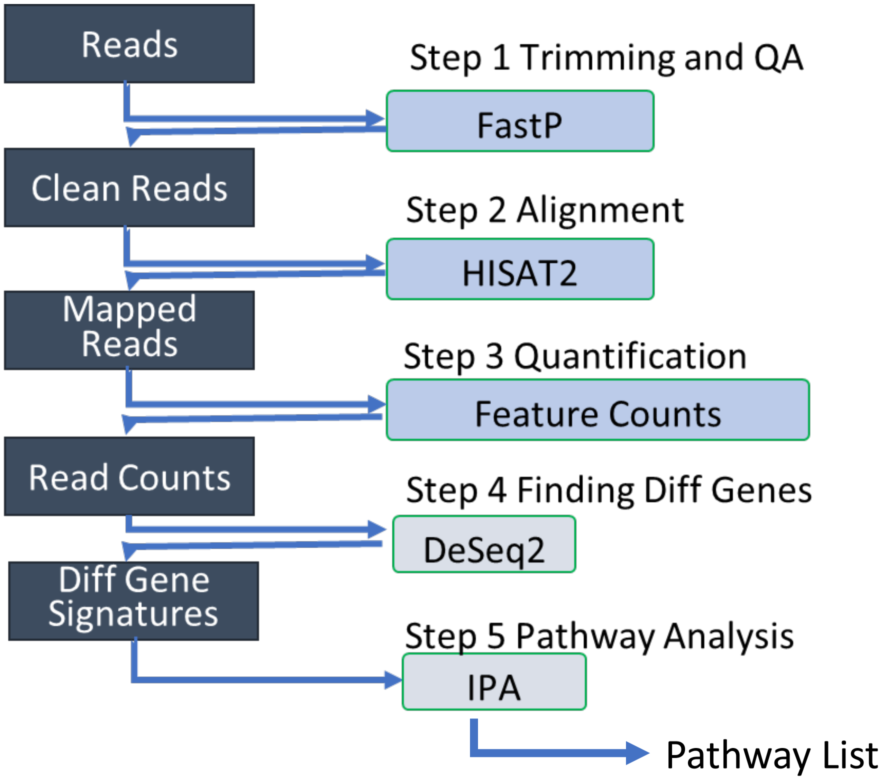
MicroRNA profiling arrays:

- p0, p1, p2 kidney cells from 5 animals
- p13, p24, p34, p45 of AGMK-9T7 cells
- Several tumor/met batches

RNASeq transcriptome:

- p13, p24, p34, p45 of AGMK-9T7 cells
- 4 tumor/met pairs

HIVE RNASeq Analysis Pipeline



Reads

Step 1 Trimming and QA

FastP

Clean Reads

Step 2 Alignment

HISAT2

Mapped Reads

Step 3 Quantification

Feature Counts

Read Counts

Step 4 Finding Diff Genes

DeSeq2

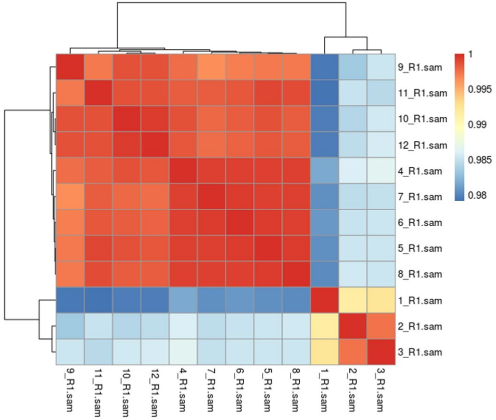
Diff Gene Signatures

Step 5 Pathway Analysis

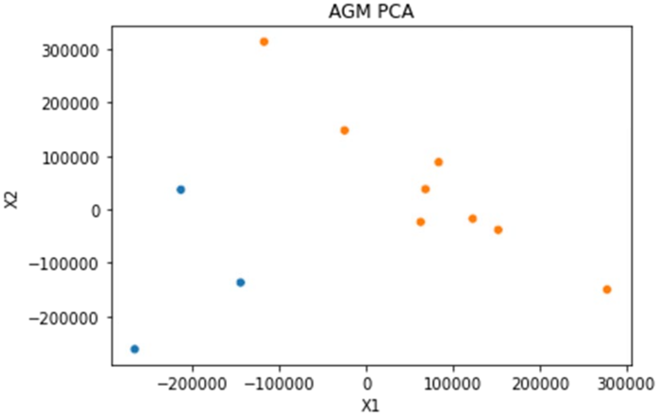
IPA

Pathway List

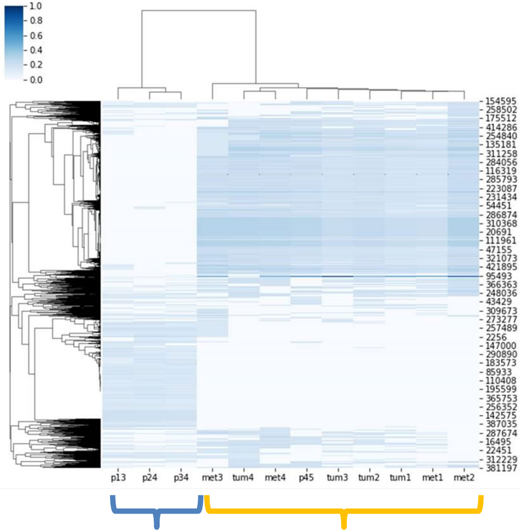
AGMK RNASeq Analysis Results



Correlation of gene expression profiles

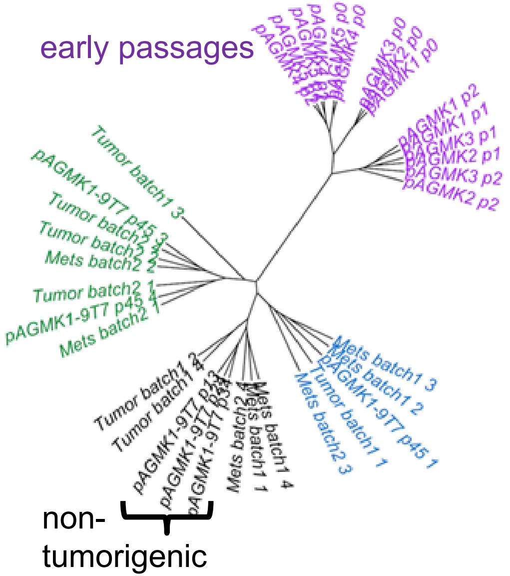


- AGMK-9T7 passages p13, p24, p34
- AGMK-9T7 passage p45 and Mets/Tumors



Clustering of gene mutation profiles

AGMK microRNA Analysis Results



MicroRNA	Expression Log Ratio
miR-141-3p	0.284 ⬆
miR-455-3p	0.09 ⬆
miR-130a-3p	-1.695 ⬇
miR-103-3p	-1.528 ⬇

Significant microRNAs from comparing non-tumorigenic passages p13, p24, p34 vs tumors/metastasis

IPA analysis: Molecular and Cellular Functions

	p-value range	# Molecules
Cellular Development	4.58E-02 - 8.38E-04	3
Cell Death and Survival	2.54E-02 - 1.17E-03	4
Cell-To-Cell Signaling and Interaction	1.47E-02 - 1.34E-03	1
Cell Morphology	1.53E-02 - 1.68E-03	1
Cellular Function and Maintenance	4.90E-02 - 1.68E-03	2

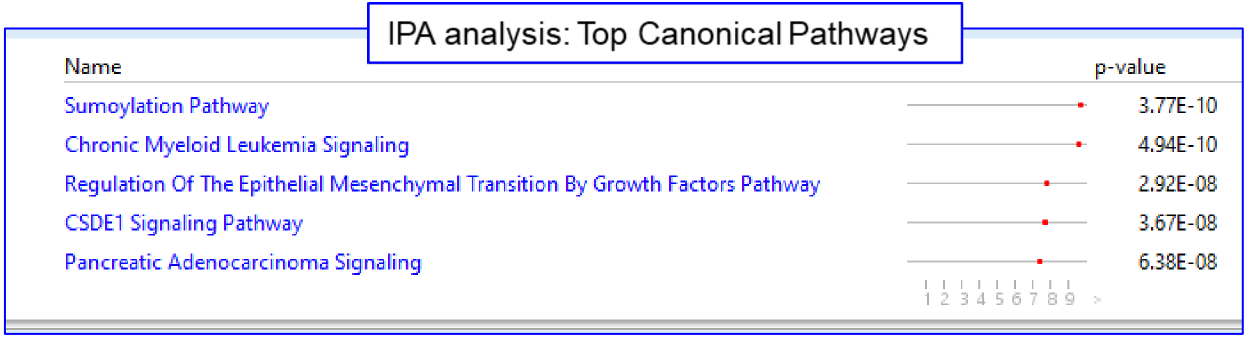
Integrating RNASEq and microRNA Analysis Results

MicroRNA	Expression Log Ratio
miR-141-3p	0.284 ↑
miR-455-3p	0.09 ↑
miR-130a-3p	-1.695 ↓
miR-103-3p	-1.528 ↓

Significant microRNAs from comparing non-tumorigenic passages p13, p24, p34 vs tumors/metastasis

→ 40 DEG Targets

↓



IPA analysis: Top Canonical Pathways

Name	p-value
Sumoylation Pathway	3.77E-10
Chronic Myeloid Leukemia Signaling	4.94E-10
Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway	2.92E-08
CSDE1 Signaling Pathway	3.67E-08
Pancreatic Adenocarcinoma Signaling	6.38E-08

Conclusion

- We observed significant changes in the expression of microRNA, mRNA and mutational profiles of AGMK cells that correlate with the neoplastic process.
- Based on profile dynamics we stratified all passages into 3 groups: (i) 'early' passages p1 and p2, (ii) non-tumorigenic passages p13-p34, and (iii) tumorigenic samples p45 and tumor/metastasis sample.
- We have identified a set of potential microRNA markers of tumorigenesis in AGMK cells based on their differential expression levels, targeting the genes that show the change, and pathway involvement.

- We have observed that microRNA profiles of the early passages (p0-p2) are different from non-tumorigenic passages (p13 –p34) suggesting the stem cell effect that we plan to explore further.

Thank you!

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