Expression of Identical Genetic Mutations Across Oncopig Cell Types Results in Distinct Expression Profiles Recapitulating Transcriptional Hallmarks of Human Tumors

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Introduction

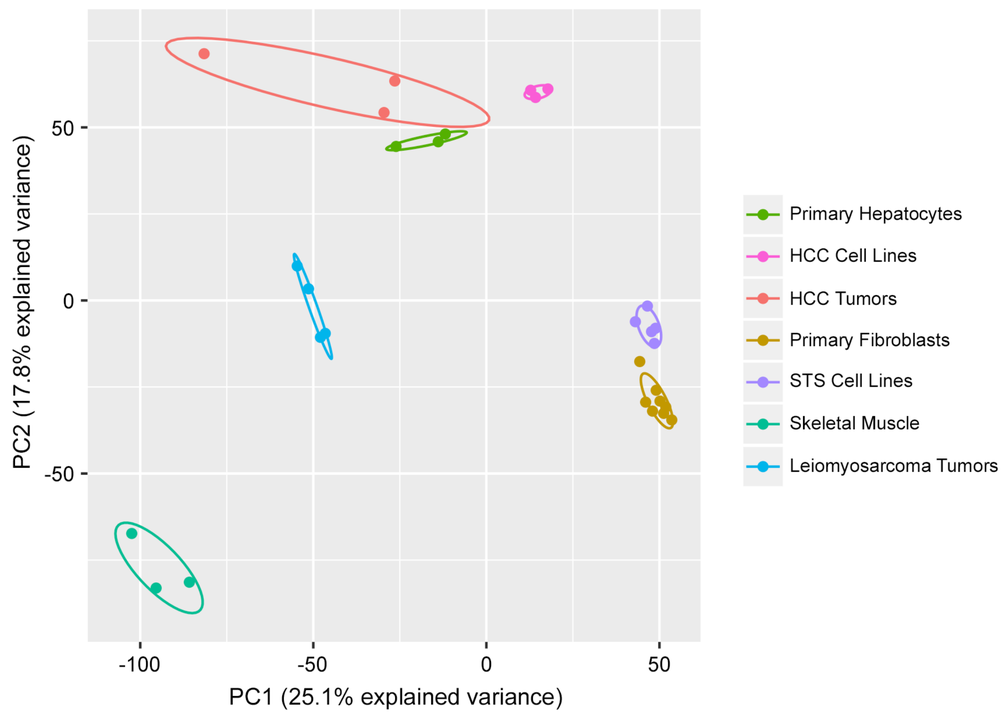
- Difficult questions confront clinicians attempting to improve patient outcomes for a wide range of cancer types.
- A large animal model with genetic, anatomic, and physiologic similarities to humans is required for transitioning between preclinical mouse models and human clinical trials in order to address unmet clinical needs.
- We previously reported the production of an inducible porcine cancer model (Oncopig) encoding Cre recombinase inducible porcine transgenes encoding *KRAS*^{G12D} and *TP53*^{R167H}, which represent a commonly mutated oncogene and tumor suppressor in human cancers, respectively.
- Need for comparative transcriptomic and histologic profiles of human and Oncopig cancers to determine relevance of the model.

Materials and Methods

- Oncopig primary (fibroblasts and hepatocytes) and transformed cell line (soft-tissue sarcoma (STS) and hepatocellular carcinoma (HCC)) expression profiles were produced via RNA-seq.
- Oncopig *in vivo* tumors (leiomyosarcoma and HCC) expression profiles were produced via RNA-seq.
- Gene expression profiles were reverse engineered to identify master regulators of gene expression in Oncopig.
- Gene expression profiles from 18 commonly used human HCC cell lines were downloaded from http://medicalgenomics.org/cellminerhcc for comparison of master regulators.

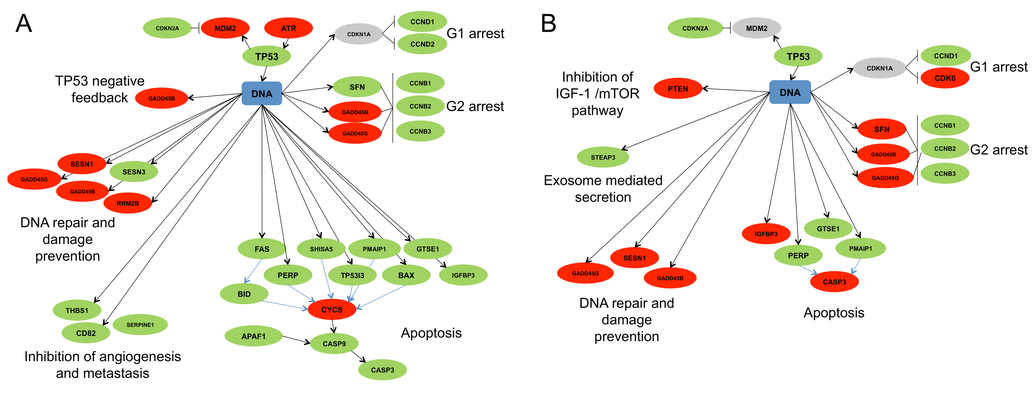
Results

Figure 1. PCA of relative gene expression across Oncopig samples



PCA based on the relative expression of 11,041 known genes for which expression information was available for each sample resulted in samples clustering based on cellular origin.

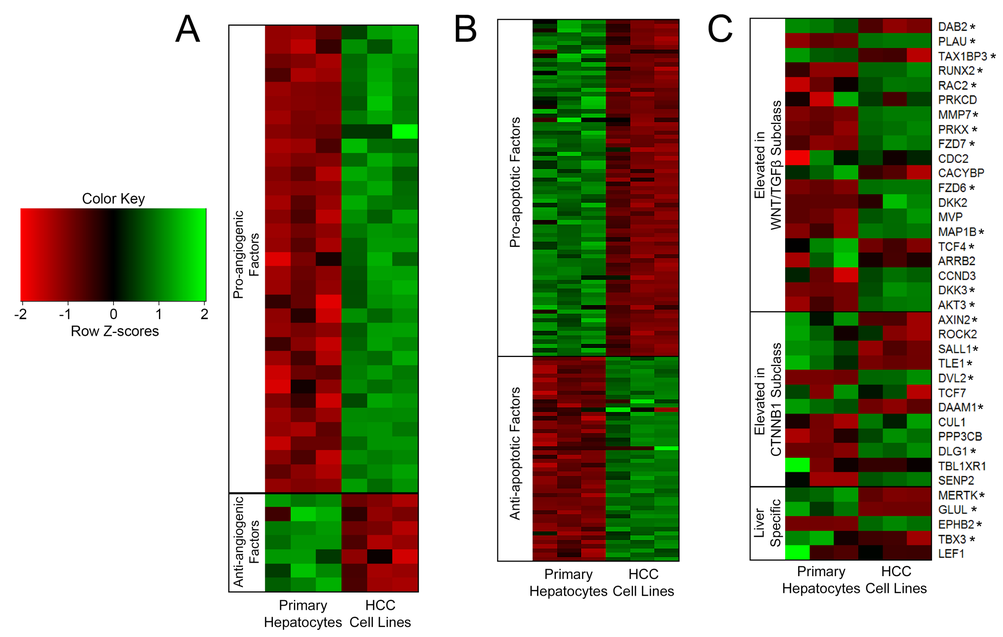
Figure 2. Altered TP53 signaling in Oncopig STS



Map of differentially expressed genes (DEGs) in Oncopig A) leiomyosarcomas and B) STS cell lines and their function within the TP53 signaling pathway. Green ovals represent elevated expression, red ovals represent reduced expression, and grey ovals represent no expression change in sarcoma compared to control samples. Black bars represent inhibition, black arrows represent activation, and blue arrows represent indirect effects.

Results (cont.)

Figure 3. Apoptosis evasion, angiogenesis activation, and Wnt signaling activation in Oncopig HCC



Heatmaps demonstrating increased and reduced expression of A) pro- and anti-angiogenic factors, and B) anti- and pro-apoptotic factors in Oncopig HCC cell lines relative to controls, respectively. C) Expression of genes elevated in human HCC subtypes, indicating Oncopig HCC represent the WNT/TGFβ subclass. * denotes $q < 0.05$.

Table 1. Master regulators of Oncopig STS cell lines

Master Regulators of Elevated Gene Expression		
Transcription Factor	**NES**	**# DE Target Genes**
FOSL1	6.603	873
Master Regulators of Reduced Gene Expression		
SRF	5.721	628
ABCF2	4.015	71

Table 2. Master regulators of Oncopig leiomyosarcomas

Master Regulators of Elevated Gene Expression		
Transcription Factor	**NES**	**# DE Target Genes**
SPI1	7.128	1,977
ETV4	5.322	1,897
UBB	4.702	1,080
HMGA1	4.114	1,145
FOS	4.061	432
EXOSC3	4.022	1,050
Master Regulators of Reduced Gene Expression		
MEF2C	7.593	1,893
HLF	4.332	1,527

Transcription factors whose target genes were enriched amongst the identified DEGs between Oncopig STS and control samples. Normalized enrichment score (NES) > 3 corresponds to a false discovery rate between 3 and 9%.

Table 3. Master regulators of genes with reduced expression in Oncopig and 18 human HCC cell lines

Transcription Factors	Oncopig HCC	7703	Focus	Hep3B	Hep3B-TR	Hep40	HepG2	HLE	HLF	HUH-1	HUH-6	HUH-7	SK-Hep1	SNU-182	SNU-387	SNU-389	SNU-449	SNU-475	PLC/PRF/5
STAT1	769	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EP300	607	431	456	450	438	596	307	545	392	343	379	385	488	270	462	664	652	626	410
FOXA2	541	384	418	394	396	553	298	477	362	312	349	218	451	245	431	638	577	561	386
SPI1	535	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FOXA1	532	448	478	441	452	630	324	559	408	368	398	382	506	286	506	712	657	667	449
HNF4A	522	435	463	421	429	556	304	542	383	347	387	370	477	285	436	672	654	644	408
HNF4G	391	408	424	421	378	550	293	500	364	331	351	382	458	248	445	628	597	584	390
CEBPB	337	372	384	380	276	357	33	442	339	221	235	252	419	244	408	553	527	535	366
HNF1A	274	-	-	-	-	-	167	-	330	-	-	-	-	-	-	-	-	-	-
NFIC	234	215	226	122	179	169	-	257	208	173	114	172	243	128	228	186	179	291	205
HDAC2	223	353	396	369	206	498	120	451	320	157	147	161	396	236	387	497	442	489	346
NR2F2	174	152	149	137	141	183	95	179	141	123	129	124	170	80	153	218	204	198	148
NR3C1	156	-	-	91	-	-	-	-	-	-	-	157	-	-	-	-	-	-	-
FOXA3	115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GATA3	102	-	-	-	-	-	69	-	-	-	-	-	-	-	-	-	-	-	-
E2F1	77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
STAT2	41	-	-	-	-	-	41	-	-	-	-	-	-	-	-	-	-	-	-

The number of target genes with reduced expression for each transcription factor is indicated for each cell line. (-) Indicates that transcription factor target genes were not enriched (i.e. overrepresented) in the list of genes with reduced expression for a given cell line.

Conclusions and Future Work

- Expression of identical genetic mutations (*KRAS*^{G12D} and *TP53*^{R167H}) across Oncopig cell types results in distinct expression profiles recapitulating transcriptional hallmarks of human tumor types.
  - *TERT* reactivation, apoptosis evasion, angiogenesis activation, altered cell cycle regulation, and Wnt signaling activation in HCC samples.
  - Altered *TP53* signaling, Wnt signaling activation, and epigenetic reprogramming in STS samples.
- Identification of master regulators previously implicated in human STS and HCC development.
- These results demonstrate the value of the Oncopig cancer model for modeling of distinct human cancer subtypes.
- Further production and profiling of addtional Oncopig cancer models is warranted.