Oncopig soft-tissue sarcomas recapitulate key transcriptional features of human sarcomas

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Introduction

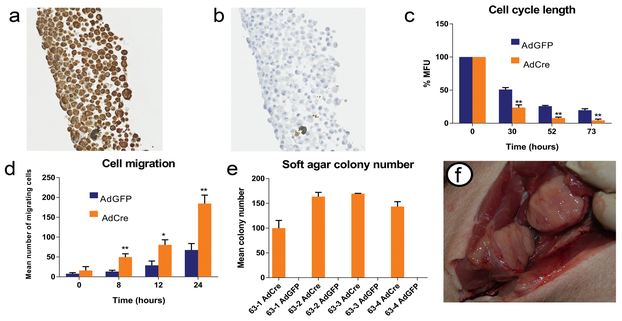
- Human soft-tissue sarcomas (STS) are rare, aggressive mesenchymal tumors with a late stage 5-year survival rate (50-60%) that has for decades remained unchanged.
- Research into STS treatment is hampered by the limited human STS cell line availability and the large number of STS subtypes, highlighting the need for development of STS cell lines and animal models representative of diverse human STS subtypes.
- Pigs represent ideal human disease models due to their similar size, anatomy, metabolism, genetics, and epigenetics compared to humans.
- The Oncopig encodes Cre recombinase inducible porcine transgenes encoding *KRAS*^{G12D} and *TP53*^{R167H}, allowing the Oncopig to model a number of human sarcomas in an inducible and temporal manner.

Materials and Methods

- The purpose of this study was to validate the Oncopig STS model as a viable model for human STS through histological and transcriptional profiling.
- Oncopig fibroblasts were isolated from ear notches of 4 Oncopigs and cultured *in vitro*.
- Cultured Oncopig fibroblasts were transformed via adenoviral vector encoding Cre recombinase (AdCre) to produce Oncopig STS cell lines and characterized *in vitro* in comparison to control fibroblasts treated with GFP (AdGFP).
- Oncopig STS tumors were produced *in vivo* through intramuscular injection of AdCre in 2 Oncopigs (2 sites/Oncopig) to produce 4 tumors blindly characterized as leiomyosarcomas.
- Genome-wide expression of Oncopig STS cell lines and tumors was profiled via RNA-seq.

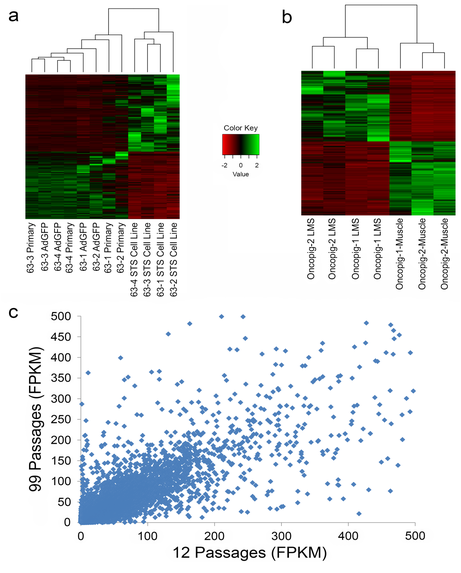
Results

Fig. 1. Characterization of Oncopig STS *in vivo* and *in vitro*



Oncopig primary fibroblasts stained a) positive for vimentin and b) negative for cytokeratin. c) Normalized MFU measured by FACS at time points following Carboxyfluorescein succinimidyl ester (CFSE) dye loading of cells. d) Graphical analysis of the mean number of migrating cells from triplicate plating of each of the 4 cell lines. e) Graphical analysis of the mean number of colonies growing in soft agar for each cell line from triplicate plating. (c-e: all data points are the mean of the 4 Oncopig cell lines; error bars = SD; *p-value ≤ 0.05; **p-value ≤ 0.01). f) Leiomyosarcoma 20 days following intramuscular injection of AdCre.

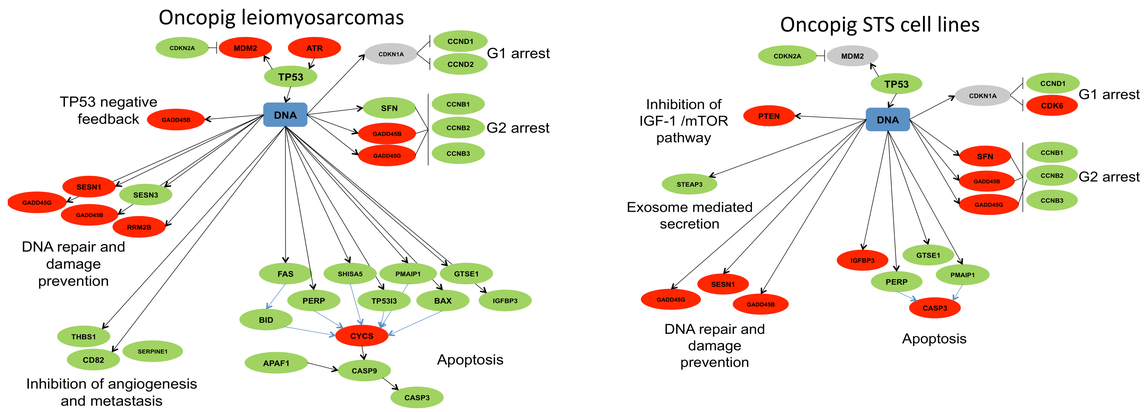
Fig. 2. Reproducible Oncopig STS expression profiles



Heatmap of the normalized expression level of a) 3,360 differentially expressed genes (DEGs) for each cell line, and b) 7,625 DEGs for each *in vivo* sample, represented as z-scores. LMS = leiomyosarcoma. Dendrograms represent relationships between samples based on complete linkage clustering. c) Expression profiles of 1 Oncopig STS cell line maintained in culture for 12 and 99 passages were highly correlated (Spearman's Rho 0.92, p < 1x10-15). Expression values are presented as fragments per kilobase of transcript per million fragments mapped (FPKM).

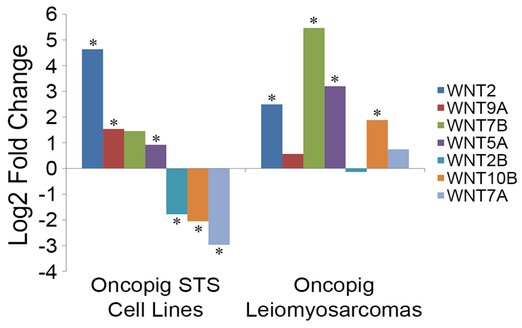
Results (cont.)

Fig. 3. Map of differentially expressed genes (DEGs) in Oncopig STS within the TP53 signaling pathway



Adopted from the KEGG hsa04115 p53 signaling pathway. Green ovals represent genes with elevated expression, red ovals represent genes with reduced expression, and grey ovals represent genes with no expression change in Oncopig STS compared to controls. Black bars represent inhibition, black arrows represent activation, and blue arrows represent indirect effects.

Fig. 5. Differential expression of WNT genes in Oncopig STS



Expression of WNTs in Oncopig leiomyosarcomas and STS cell lines relative to controls, represented as the log2 fold change. * denotes q-value < 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

Conclusions and Future Work

- Oncopig STS cell lines can be produced through *in vitro* transformation of Oncopig mesenchymal cells.
- Oncopig *in vivo* tumors were blindly characterized as leiomyosarcomas.
- Oncopig leiomyosarcoma and STS cell line expression profiles were highly reproducible.
- Oncopig STS cell lines displayed high temporal stability.
- A total of 3,360 and 7,652 DEGs were identified in the Oncopig STS cell lines and tumors, respectively.
- Commonly identified alterations in human STS gene expression and pathway regulation were identified in Oncopig STS, including altered *TP53* signaling and activation of Wnt signaling.
- Master regulators of Oncopig STS gene expression were identified, including *FOSL1*, which was previously identified as a potential therapeutic target for human STS.
- These results demonstrate the Oncopig STS model's ability to mimic human STS on a transcriptomic level, q making the Oncopig a valuable resource for sarcoma research and cell line development.
- Further work is required to produce Oncopig STS cell lines representative of human STS subtypes from additional cell lineages.

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