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Oncopig carcinoma cell lines: a foundation for co-clinical trials

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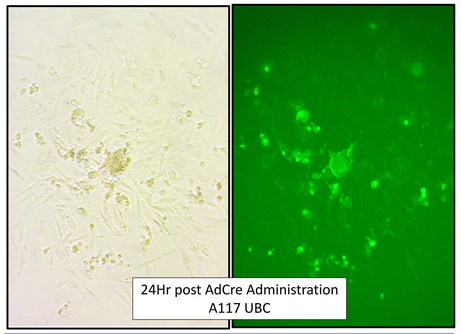
Introduction

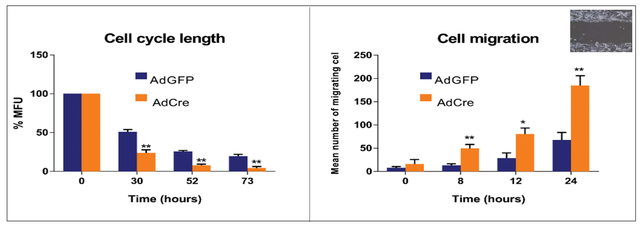
- Pigs represent ideal human disease models due to their similar size, anatomy, metabolism, genetics, and epigenetics compared to humans.
- The Oncopig cancer model (OCM) was developed to support a broad range of solid and liquid tumor malignancies following Cre recombinase induced expression of KRASG12D and TP53R167H transgenes.
- The OCM was designed with the potential to generate cancers of all tissue origins. Therefore, to demonstrate the potential importance of the OCM, we have isolated and transformed multiple progenitor cells of clinically relevant human disease.

Materials and Methods

- Cell type specific cell isolations were performed, and cells cultured under appropriate conditions.
- Within the first 48 hours, isolated cells were treated with adenoviral vector encoding Cre recombinase (AdCre) at 200-400 MOI for 5 hours in low serum (5%) medium.
- Both control (when available) and transformed (AdCre treated) cells were monitored for phenotypic changes characteristic of transformed cell lines including histopathologic screening (H&E staining, immunohistochemical staining for vimentin and cytokeratin), and verification of transgene expression (RT-PCR)
- Each cell line was also evaluated for tumoriginicity in the SCID mouse xenograft assay.

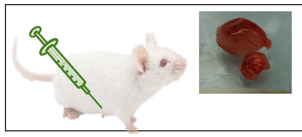


Transformation with Cre Recombinase is monitored by checking GFP expression. GFP remains detectable from few hours after administration through approximately 7 days. This is dependent on the replication rate of the cells.

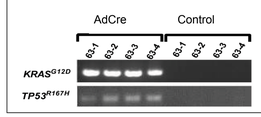


Normalized MFU measured by FACS at time points following Carboxyfluorescein succinimidyl ester (CFSE) dye loading of cells.

The ability of cells to migrate in monolayer cultures was assessed by a scratch wound assay. The number of cell within the cell free zone were compared over



Each cell line was injected into a SCID mouse (2x10^6 to 1x10^7 cells in DMEM and /or Matrigel to determine if tumorigenic. Tumors were collected for further molecular and histologic analysis.

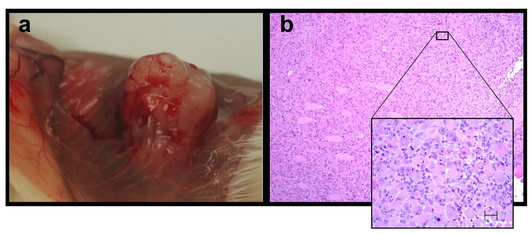


RT-PCR analysis of $KRAS^{G12D}$ and $TP53^{R167H}$ mRNA expression in 4 fibroblast cell lines from 4 oncopigs and treated with AdCre or controls.

The cancer phenotype include expression of the 2 oncogenic transgenes, altered morphology, growth characteristics including increased cell migration rates, shortened cell cycle length and the ability to form tumors. Additional analyses have shown altered transcriptome and drug sensitivity.

Results

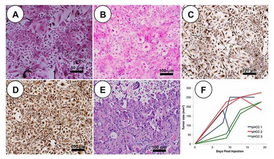
Fibroblasts



Fibroblast lines were the first cell type evaluated. This published work showed that all AdCre lines compared to control lines expressed high levels of both transgenes, had shortened cell cycle length, increased migration, were immortalized (grew more than 100 passages) and produced tumors in SCID mice.
[https://doi.org/10.1371/journal.pone.0128864]

In addition, RNA-seq analysis of these AdCre lines (sarcomas) identified 7,652 altered genes including transcriptional hallmarks of human soft tissue sarcomas. These results demonstrate the Oncopig STS model's ability to mimic human STS transcriptional profiles, providing a valuable resource for sarcoma research and cell line development.
[https://www.nature.com/articles/s41598-017-02912-9]

Hepatocytes

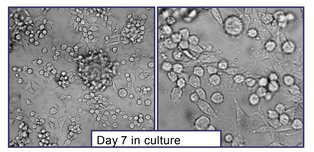


Oncopig HCC lines recapitulate cytologic and histologic features of human HCC both *in vitro* and *in vivo*
(A) Representative porcine hepatocytes in culture show polygonal shape with granular cytoplasm. (B) In culture, pleomorphic elongated pHCC have clear to granular cytoplasm and round to oval pleomorphic nuclei. (C) Positive cytokeratin immunostaining of cultured pHCC cells (D) Positive vimentin immunostaining of cultured pHCC cells. (E) H&E stained SC xenografted tumor. (F) Growth curve of SC xenografted tumors indicative of linear growth kinetics.
[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5609948/]
See Poster **#2776** and **#4904** for more information on Oncopig Hepatocellular Carcinoma

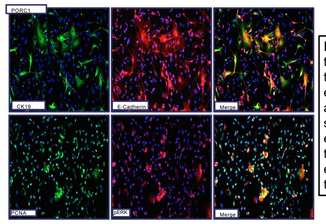
CELL TYPE	NUMBER OF LINES
Fibroblast	15
Hepatocytes	54
Pancreas	2
Ovarian Surface Epithelium (OSE)	3
Fallopian Tube Secretory Epithelium (FTSE)	3
Renal Proximal Tubule Epithelium (RPTE)	4
Bone Marrow	2
Dermis	2
Testis (non specific)	2
Splenocyte	1
Skeletal Muscle	1
Bladder Uroepithelial Cells	4
Bladder Smooth Muscle	1

Oncopig Transformed cell lines. Each of the lines listed in this table has been isolated, transformed, shown to express the transgenes and form a tumor in SCID mice. Each of these lines and whenever available the untreated control cells have been frozen in the biorepository. Further characterizations of and comparison to human cancers are underway.

Pancreatic Ductal Cells

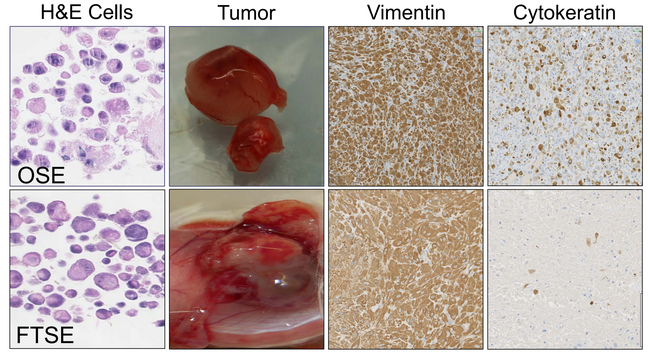


AdCre treated freshly isolated oncopig pancreatic ductal cells express both Kras and TP53 mutated genes. This lead to immortalization and induced tumorigenicity of the cells.

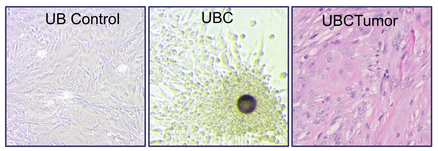


Immunocytochemistry shows that these cells are positive for the duct marker CK19, epithelial marker E-Cadherin, as well as proliferation surrogate PCNA and RAS effector pERK. Introduction of these cells to SCID mice establish large subcutaneous tumors.

Ovarian Cells



Both ovarian surface epithelial cells and fallopian tube secretory cells have been implicated as ovarian cancer progenitor cells. Both types of Oncopig cells have been transformed and generated tumors in SCID mice. The individual neoplastic cells are round to polygonal with abundant light eosinophilic cytoplasm and single to multiple round to oval nuclei with scattered chromatin and nucleolus. The mitotic index is 4 per 10 hpfs. Approximately 20-70% neoplastic cells stain with cytokeratin (epithelial marker) whereas almost all neoplastic cells stain with vimentin (mesenchymal marker) indicating the epithelial – mesenchymal transition a signature of carcinoma. The FTSE lines express KRT19, CD13 and PAX8, but not CD10 a profile the same as human UBC cancer lines. The ACS estimates that in 2018, about 22,240 new cases of ovarian cancer will be diagnosed and mortality rates for ovarian cancer have declined only slightly in the forty years emphasizing the need for new therapies. The oncopig cells could provide a translational tool in the future.



Bladder Uroepithelial Cells

These images demonstrate the drastic change in phenotype of the cells post AdCre. Cells have become smaller, less tightly attached to the surface grow in stacks, having lost their contact inhibition. Xenografted cells formed a tumor which has been stained with H&E showing the neoplastic morphology. About 1 in 3 bladder cancers are diagnosed only after invasion into the deeper layers of the bladder wall and nearby tissues. Development of oncopig urothelial bladder cancer lines may provide a good model to speed development of new therapeutics.

Conclusions and Future Work

- To date 100% of these clinically relevant cell types isolated from the Oncopig have been successfully transformed by the administration of Cre recombinase.
- In depth analysis of several of these cells lines has verified that the oncopig cancer lines are similar to human cancers in gene expression, morphology and drug sensitivity.
- Therefore we conclude that cancer lines generated from the OCM accurately replicate human cancer and will be useful to support and expand preclinical, translational, and co-clinical investigations.