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Development of the "Onco-Pig": An Inducible Transgenic Porcine Model for Human Cancer

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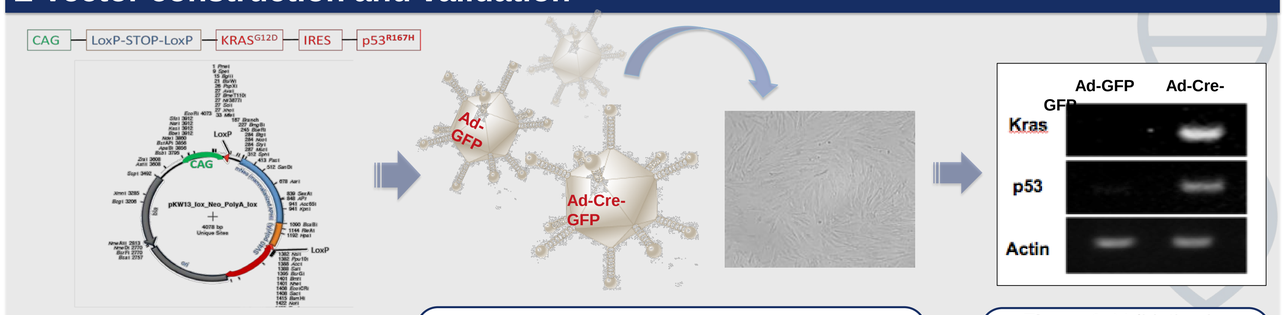
Introduction

Common rodent-based models have limitations in terms of modeling human cancers. Given that pigs share many genetic and physiological similarities with humans, we investigated the potential of developing genetic porcine models of cancer. In this regard, we previously reported that activation of oncogenes such as Ras in conjunction with inhibiting tumor suppressor pathways like p53 were required, in part, to convert normal porcine cells to a tumorigenic state. Based on this, we chose to generate transgenic pigs that can be induced to express oncogenic *Kras* and dominant-negative p53. Porcine *Kras* and p53 wild-type genes were cloned, sequenced and aligned with porcine, human and murine homologues to identify porcine-specific mutation sites corresponding to those commonly found in human cancers. Porcine *Kras* mutation occurs at the 12th glycine (G) to aspartic acid (D), whereas p53 arginine (R) at 167th position was mutated to histidine (H).

1 Vector construction and validation

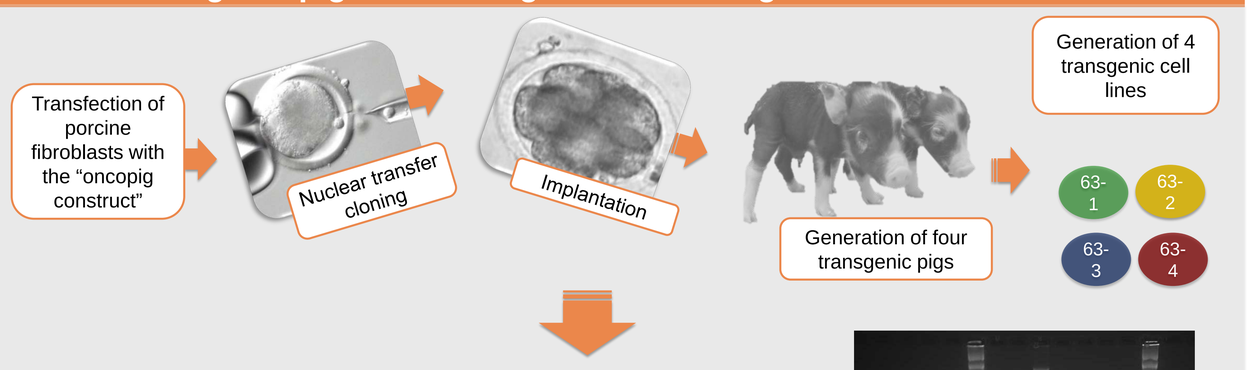


Transfection of porcine fibroblasts with vector construct

Infection with adenovirus (Ad) vectors encoding Cre recombinase (Ad-Cre-GFP), which deletes the LSL sequence and permits transgene expression, or control Ad vectors (Ad-GFP).

Figure 1: Validation by RT-PCR of the deletion of LSL sequence and induction of Kras^{G12D}-p53^{R167H} expression.

2 Cloned transgenic pigs and transgenic cell lines generation



The four cloned piglets were born on May 21st 2012. The four transgenic fibroblast cell lines (63-1; 63-2; 63-3; and 63-4) generated from each present the "oncopig" construct containing both p53 and Kras mutant genes (Figure 1).

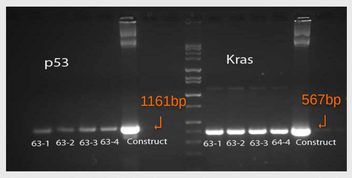
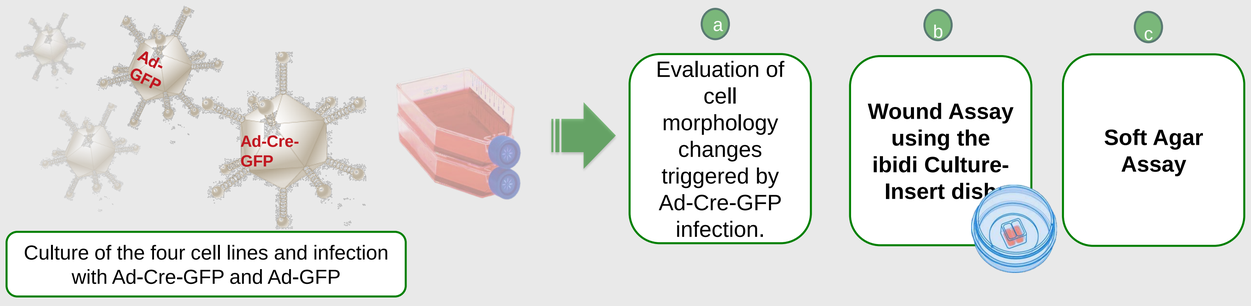


Figure 2: Validation of presence of the "oncopig" construct in the transgenic cell lines.

3 In vitro assays



a. Ad-Cre treated cells start changing morphology at about 3 days post infection. The Ad-Cre cells become small and round, while the Ad-GFP treated cells maintain the pretreatment characteristics.

b. *In vitro* migration capability of Ad-Cre-GFP treated cells was significantly greater than Ad-GFP control cells. In a migration time of 24h, the mean cell number in the wound area for the Ad-Cre-GFP cells was 184 as for the Ad-GFP cells was only 67 (p-value ≤ 0.01) (Figure 3.a.).

c. Ad-GFP cells were unable to form colonies in soft agar, while the Ad-Cre-GFP cells formed over than 100 colonies (p-value ≤ 0.05). As the 4440 and PF161 positive control cells (both transgenic cells expressing 6 oncogenic genes), the Ad-Cre-GFP cells are malignant transformed (Figure 3.b.).

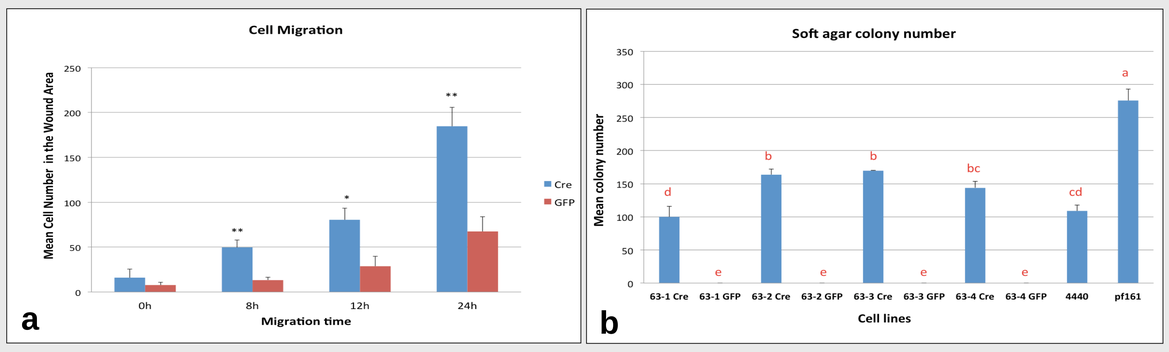
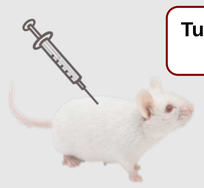


Figure 3: a) Wound Assay (* p-value ≤ 0.05; ** p-value ≤ 0.01; all data points are the mean of the 4 cell lines); **b)** Soft Agar Assay (a,b,c,d,e : p-value ≤ 0.05; and a>b>c>d>e - colony number of the cell lines)

4 In vivo assays

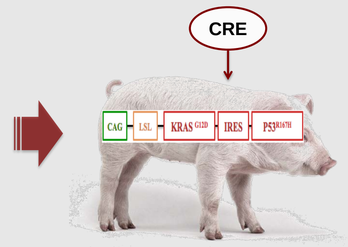


Tumor Growth in Mice

5 ×10^6 Ad-Cre-GFP and Ad-GFP cells in BD Matrigel^{TM} were injected subcutaneously into **NOD.CB17-*Prkdc*^{scid}/J** mice (Jax stock 001303).

Future experiments

Tumor Growth in Pigs



Production of the inducible transgenic porcine model for human cancer

Tumor Growth in mice: 12 of the 14 mice injected have developed measureable tumors. Five mice had been already euthanized and the tumors collected for histopathology, culture and expression analysis (Figure 3). Mice were euthanized when tumors reached the size of approximately 3000mm^2. Histopathological analysis has revealed three sarcomas, with one effacing the renal parenchyma. (Figure 3.1).

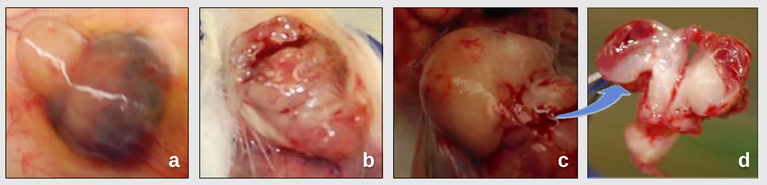


Figure 4: Tumors developed in the mice injected with the Ad-Cre-GFP cell lines. **a)** Mice injected with the cell line 63-1. Tumor reached the size of 2880mm^2 at 51 days post injection. All attached to the skin with no effacement of body wall. **b)** Mice injected with the cell line 63-3. Ulceration was observed when tumor reached the size of 2050mm^2 at 51 days post injection. All attached to the skin with no effacement of body wall; **c)** Cell line 63-4. Tumor reached 2016mm^2 at 90 days post infection and was highly involved both outside and inside the body wall. **d)** Same mouse from Figure 4.c. Tumor was found invading the kidney. No other organs presented malignant cells.

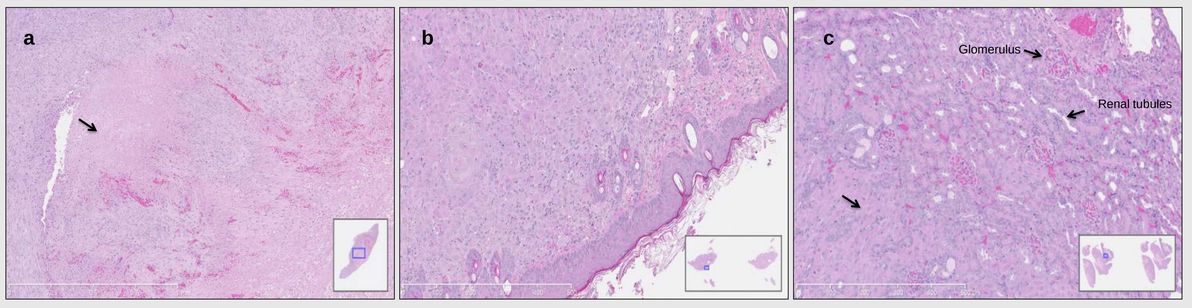


Figure 4.1: Histopathological analyses. Samples were stained with H&E. **a) Sarcoma**. Developed from cell line 63-1. Presence of a nonencapsulated, densely cellular, and locally infiltrative neoplasm with central necrosis (arrow) and acute hemorrhages. **b) Sarcoma.** Developed from cell line 63-3. The dermis is expanded and effaced by an infiltrative neoplasm (as described in Figure 4.1. a.) **c) Sarcoma with renal metastasis.** Tumor from cell line 63-4. Presence of infiltrative neoplasm (as described in Figure 4.1. a.). Neoplastic cells are effacing the renal parenchyma (arrow).

Conclusions and Future Implications

Present results demonstrate that the "oncopig" construct is functional. Moreover, demonstrate that the induction of the transgenes in these porcine cells triggered a transformed phenotype and that they are potentially tumorigenic. In the future, molecular analyses of the tumor samples collected from the mice will be made with the aim to prove that these tumors developed from the Ad-Cre-GFP treated cells. Also, pigs will be monitored for tumor incidence following site-specific transgene induction. Such an approach could provide a porcine model to study cancer etiology and the development of anti-cancer therapies.

References

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2. Adam, S. J.; Rund, L. A.; Kuzmuk, K. N.; Zachary, J. F.; Schook, L. B.; and Counter, C. M. Genetic induction of tumorigenesis in Swine. *Oncogene* (2007) 26,1038-1045

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