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Laboratory of Comparative Genomics

An Inducible Transgenic Porcine Model for Human Cancer

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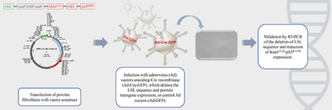
Introduction

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 like 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


Ad-GFP
Ad-Cre-GFP
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).
Validation by RT-PCR of the deletion of LSL sequence and induction of Kras^{G12D}-p53^{R167H} expression.

Cre recombinase-mediated Kras^{G12D} and p53^{R167H} expression was significantly induced in porcine fibroblasts transfected with Ad-Cre-GFP virus compared with Ad-GFP control, which provides an *in vitro* proof of functional test of the "oncopig" construct (Figure 1).

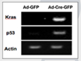


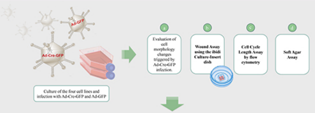
Figure 1: Validation of the deletion of LSL sequence and induction of Kras^{G12D}-p53^{R167H} expression.

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 2).

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

3 In vitro assays


Ad-GFP
Ad-Cre-GFP
Culture of the four cell lines and infection with Ad-Cre-GFP and Ad-GFP
Evaluation of cell morphology changes triggered by Ad-Cre-GFP infection.
Wound Assay using the ibidi Culture-Insert dish
Cell Cycle Length Assay by flow cytometry
Soft Agar Assay

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 (Figure 3.a.).
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.b.).
c. Within a 73h time period, Ad-Cre-GFP cells divided twice as many times than Ad-GFP cells (p-value ≤ 0.01) (Figure 3.c.).
d. Ad-GFP cells were unable to form colonies, 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.d.).

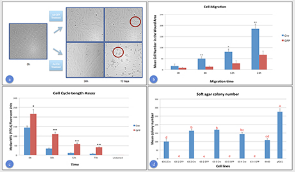
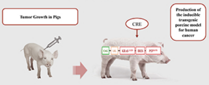


Figure 3: a) Cell morphology changes triggered by Ad-Cre infection; **b)** Wound Assay (* p-value ≤ 0.05; ** p-value ≤ 0.01; all data points are the mean of the 4 cell lines); **c)** Cell Cycle Length Assay (* p-value ≤ 0.05; ** p-value ≤ 0.01); **d)** Soft Agar Assay (a,b,c,d,e : p-value ≤ 0.05; and a>b>c>d>e - colony number of the cell lines)

4 In vivo assays

5 ×10[6] Ad-Cre-GFP and Ad-GFP cells in BD Matrigel™ were injected into the subcutaneous region of the mice's right and left sides, respectively, of the **NOD.CB17-*Prkdc*^{scid}/J** mice (Jax stock 001303).

Future experiments



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Tumor Growth in mice: within 14 mice injected, 12 developed measureable tumors (Figure 4). Five mice had been already euthanized and the tumors collected. (Figure 4.1). Mice were euthanized when tumors reached the size of approximately 3000mm². Histopathological analysis has already revealed three sarcomas, with one effacing the renal parenchyma. (Figure 4.2).

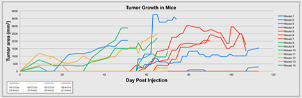

Figure 4: Increasing of tumor area (mm²) and days post injection.

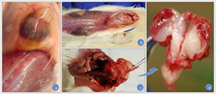

Figure 4.1: Tumors developed in the mice injected with the Ad-Cre-GFP cell lines. **a)** Mice injected with the cell line 63-1. Tumor reached the size of 2880mm² at 51 days post injection. All attached to the skin with no effacement of body wall. **b)** Mice injected with the cell line 63-3. Ulceration was observed when tumor reached the size of 2050mm² at 51 days post injection. All attached to the skin with no effacement of body wall; **c)** Cell line 63-4. Tumor reached 2016mm² at 90 days post infection and was highly involved both outside and inside the body wall. **d)** Same mouse from Figure 4.1.d. Tumor was found invading the kidney. No other organs presented malignant cells.

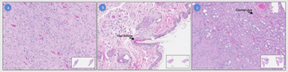

Figure 4.2: Histopathological analyses. **a) Sarcoma**. Developed from cell line 63-1. Presence of a nonencapsulated, densely cellular, and locally infiltrative neoplasm. **b) Sarcoma.** Developed from cell line 63-3. The dermis is expanded by an infiltrative neoplasm (as described in Figure 4.2. a.) Neoplastic cells are continuous with overlying epidermis; **c) Sarcoma with effacement of renal parenchyma.** Tumor from cell line 63-4. Presence of infiltrative neoplasm (as described in Figure 4.2. a.). Neoplastic cells are effacing the kidney.

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 cancer etiology and the development of anti-cancer therapy.

References

1. Kuzmuk, K. N. & Schook, L. B. Pigs as a model for biomedical sciences. *CAB International* (2011). The genetics of the pig. 2nd Ed,426-444.
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