An Inducible Transgenic Porcine Model for Human Cancer
F. M. Rodrigues1,5, W. Hu1,4, L. A. Rund1, Y. Liang2, C. Counter3, and L. B. Schock1
1Department of Animal Sciences, University of Illinois, Urbana, IL, United States
2Department of Internal Medicine, University of Kentucky, Lexington, KY, United States
3Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC, United States
4State Key Laboratory of Agricultural Biotechnology, China Agricultural University, Beijing, P. R. China
5Technology Development Center, Biotechnology Undergraduate Program, Federal University of Pelotas, Pelotas, RS, Brazil

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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 porcine models of cancer. In this regard, we previously reported that activation of oncogenes like Kras 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 and human 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 175th position was mutated to histidine (H).

1 Vector construction and validation

Cre recombinase-mediated Kras12G12D and p53R175H 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 "oncogenic" construct (Figure 1).

2 Cloned transgenic pigs and transgenic cell lines generation

The four cloned sires 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 "oncogenic" construct containing both p53 and Kras mutant genes (Figure 2).

3 In vitro assays

- 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 3a).
- 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 10.4 as for the Ad-GFP cells was only 0.7 (p-value ≤ 0.001) (Figure 3b).
- Within a 72h time period, Ad-Cre-GFP cells divided twice as many times than Ad-GFP cells (p-value ≤ 0.001) (Figure 3c).
- Ad-Cre-GFP cells were unable to form colonies, while the Ad-Cre-GFP cells formed over 100 colonies (p-value ≤ 0.05). As the 4444 and PF181 positive control cells (both transgenic cells expressing 6 oncogenic genes), the Ad-Cre-GFP cells are morphologically (Figure 3d).

4 In vivo assays

- Tumor Growth in mice: within 14 mice injected, 12 developed measurable tumors (Figure 4).
- Tumors were excised and the tumors collected (Figure 4.1). Mice were euthanized when tumors reached the size of approximately 3000mm3. Histopathological analysis has already revealed several sarcomas, with one affecting the renal pelvis (Figure 4.2).

Conclusions and Future Implications
Present results demonstrate that the "oncogenic" 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 tumors samples collected from the mice will be made with the aim to prove that these tumors are from transgenic porcine 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