Introduction
Given a number of limitations of rodent-based cancer models, coupled with the fact 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. To this end, pigs were created by cloning to contain oncogenic KRASG12D and dominant-negative p53R167H, two commonly mutated genes in human cancers. They were cloned downstream of a LoxP-polyA (STOP)-LoxP sequence (LSL) and CAG promoter, such that exposure to Cre-recombinase would induce their expression in any desired tissue.

1 Vector construction and validation

Cre recombinase-mediated KRASG12D and p53R167H expression was significantly induced in porcine fibroblasts transfected with Ad-Cre-GFP (CRE+) compared with Ad-GFP (CRE-) control, which provides an in vitro proof of functional test of the “oncopig” construct (Figure 1).

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).

3 In vitro assays

- Fibroblast cell strains generated from four such clones were infected with adenovirus vector (CRE+) encoding Cre recombinase and GFP protein or control vector (CRE-) with GFP alone. Upon infection with CRE+, but not CRE-, all four cell strains expressed KRASG12D and p53R167H mRNA, as assessed by RT-PCR (Figure 3a). Preliminary RNAseq data show that KRASG12D and p53R167H reads increase by 300 fold in the CRE+ cell lines, while wildtype message remains the same.
- CRE+ treated cells start changing morphology at about 3 days post infection. They became small and round, while the CRE- treated cells maintain the pretreatment characteristics (Figure 3b).
- In vitro migration capability of CRE+ treated cells was significantly greater than control cells. In a migration time of 24h, the mean cell number in the wound area for the CRE+ cells was 184 as for the CRE- cells was only 67 (p-value ≤ 0.01) (Figure 3c).
- Within a 73h time period, CRE+ treated cells divided twice as many times than CRE- cells (p-value ≤ 0.01) (Figure 3d).
- Control cells were unable to form colonies in soft agar, while the CRE+ cells formed over 100 colonies (p-value ≤ 0.05).

4 In vivo assays

Conclusions and Future Implications
Present results demonstrate that the oncopig construct is functional. Moreover, demonstrates that the induction of the transgenes in these porcine cells triggered a tumorigenic phenotype. In addition, 2 clones have reached 1 year 10 months of age with no development of tumors or other abnormalities demonstrating that the transgene expression remains suppressed without re-combination.

In the future, offspring of these founder 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 anticancer drugs and therapies.

References