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Comparative Genomics

An Inducible Transgenic Porcine Model for the Study of Human Cancer

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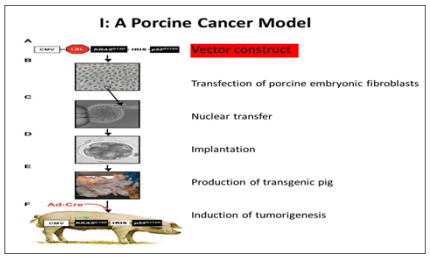
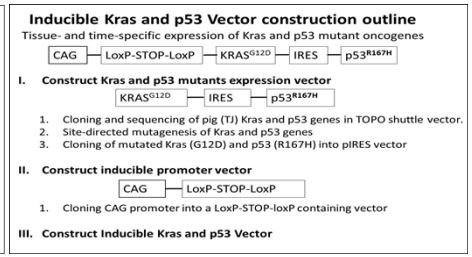
Introduction:

The common rodent-based models are limited in their capability to mimic human cancer development. Given the more similarity in genetics and physiology between human and pig, we aim to generate a novel transgenic porcine oncogenesis model for the study of human cancer[1]. Our previous study has shown that overexpression of several human oncogenes led to tumor development in pigs[2]. We thus chose two major mutant oncogenes, Kras and p53, to generate transgenic pigs that can be induced to simultaneously express both genes. We first cloned and sequenced porcine Kras and p53 wild-type genes. Protein sequence alignment of each gene among pig, human and mouse determined the pig-specific mutation sites that correspond 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). KrasG12D and p53R167H mutants were linked by internal ribosome entry sites (IRES) for their simultaneous expression. The cassette was then inserted into a vector following the LoxP-polyA(STOP)-LoxP sequence. We further tested this vector construct in pig fibroblasts by co-transfection with Cre plasmid, which deletes the polyA "STOP" sequence and allows transgene transcription. We found that expression of KrasG12D and p53R167H mutants was significantly induced upon Cre recombinase introduction. In the future, we will use this vector construct and clone technology to generate inducible KrasG12D and p53R167H transgenic pig, and ultimately to create a more human-like tumor model for the study of cancer etiology, and for the development of anti-cancer therapy.

Materials and Methods:





Results:

I. Construct Kras and p53 mutants expression vector

1. Cloning and sequencing of pig (TJ) Kras and p53 genes in TOPO shuttle vector (**Fig. 1).**
   1) cDNA was made from bone marrow of pig strain TJ 2-14.17.7
   2) Kras and p53 genes were amplified by PCR (Kras: 567bps; p53:1161bps)

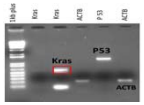
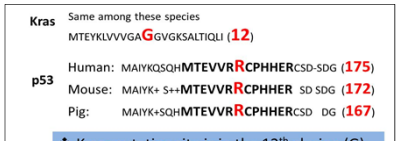


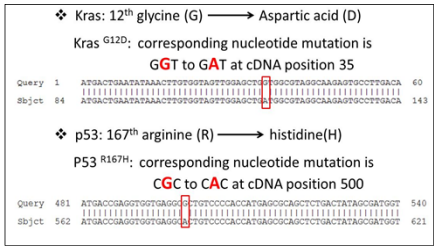
Fig 1. Porcine Kras and P53 PCR.

2. Site-directed mutagenesis of Kras and p53 genes

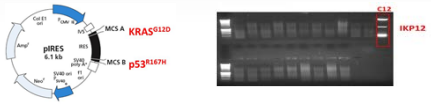
1) Sequence alignment of Kras and p53 genes among human, mouse and pig to determine mutated sites



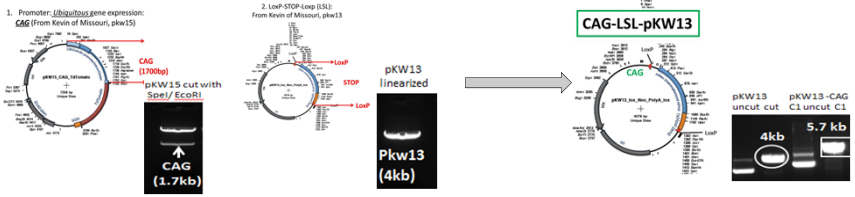
2) Site-directed mutagenesis



3. Cloning of mutated Kras (G12D) and p53 (R167H) into pIRES vector



II. Construct inducible promoter vector: Cloning CAG promoter into a LoxP-STOP-loxP containing vector



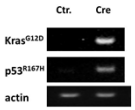
III. Construct Inducible Kras and p53 Vector

All three clones (C) are positive tested by insert size and specific genes (Kras and p53) amplification

IV. Proof-of-test of "CAG-LSL-Kras^{G12D}-IRES-p53^{R167H}" vector construct in porcine fibroblast in vitro

Methods : 1) Transfection of pig fibroblast with LSL- KrasG12D-p53R167H vector
2) G418 selection for successfully transfected (positive) fibroblasts
3) Co-transfection of Adenovirus-Cre or Adenovirus-GFP control to the positive cells
4) Validation of the deletion of induction of KrasG12D-p53R167H expression

Results: Cre-mediated induction of transgenic Kras^{G12D} and P53^{R167H} expression



Conclusions and Implications:

In this study, we cloned and sequenced Kras and p53 genes from porcine strain TJ2-14.17.7. We identified and created porcine-specific Kras and p53 mutants (Kras^{G12D} and p53^{R167H} respectively) which have been shown oncogenic in human and mouse cancers. We produced a Cre-LoxP medicated inducible vector construct in which Kras^{G12D} and p53^{R167H} oncogenes are linked by IRES sequence. We further provided proof-of-test of the function of this vector construct by showing the significant induction of both oncogenes in fibroblasts upon Cre recombinase introduction. In the future, we will use this vector construct and clone technology to generate inducible KrasG12D and p53R167H transgenic pig, and ultimately to create a more human-like tumor model for the study of cancer etiology, and for the development of anti-cancer therapy

References:

1. Pigs as a model for biomedical sciences. KN Kuzmuk and LB Schook. CAB International 2011. The genetics of the pig. 2nd Edn, 426-444.
2. Genetic induction of tumorigenesis in swine. SJ Adam, LA Rund, KN Kuzmuk, JF Zachary, LB Schook and CM Counter. Oncogene (2007) 26, 1038-1045.