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Laboratory of **Comparative Genomics**

An Inducible Transgenic Porcine Model for the Study of Human Cancer

W. Hu[2], Y. Liang[1], S. Xu[1], L. A. Rund[2], C. M. Counter[3], E.M. Walters[4], K. D. Wells[5], and L. B. Schook[2]

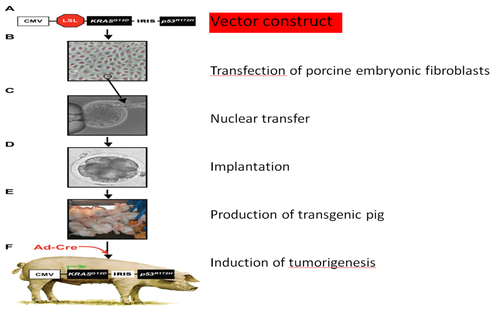
[1] Department of Pathology, University of Illinois, Urbana IL 61801 [2] Department of Animal Sciences, Nutritional Sciences, and Veterinary Pathobiology, University of Illinois, Urbana, IL 61801
[3] Department of Pharmacology and Molecular Cancer Biology, Duke University, Durham, NC 27710 [4] Department of Veterinary Pathobiology, University of Missouri-Columbia, Columbia, MO 65211
[5] Animal Science Research Center, Division of Animal Sciences, University of Missouri-Columbia, Columbia, MO 65211

Introduction:

The common rodent-based models are limited in their capability to mimic human cancer development. Therefore, 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 to generate transgenic pigs carrying two inducible mutant oncogenes, Kras and p53. We first cloned and sequenced porcine Kras and p53 wild-type genes. Pig-specific mutation sites corresponding to those commonly found in human cancers were identified. 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). Kras^{G12D} and p53^{R167H} 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 Kras^{G12D} and p53^{R167H} mutants was significantly induced upon Cre recombinase introduction. This construct is currently being used for nuclear transfer cloning to generate an inducible Kras^{G12D} and p53^{R167H} transgenic pigs.

Materials and Methods:

I: A Porcine Cancer Model



Inducible Kras and p53 Vector construction outline

Tissue- and time-specific expression of Kras and p53 mutant oncogenes

CAG — LoxP-STOP-LoxP — KRAS^{G12D} — IRES — p53^{R167H}

I. Construct Kras and p53 mutants expression vector

KRAS^{G12D} — IRES — p53^{R167H}

1. Cloning and sequencing of pig (TJ) Kras and p53 genes in TOPO shuttle vector.
2. Site-directed mutagenesis of Kras and p53 genes
3. Cloning of mutated Kras (G12D) and p53 (R167H) into pIRES vector

II. Construct inducible promoter vector

CAG — LoxP-STOP-LoxP

1. Cloning CAG promoter into a LoxP-STOP-loxP containing vector

III. Construct Inducible Kras and p53 Vector

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).

a. cDNA was made from bone marrow of pig TJ 2-14.17.7
b. Kras and p53 genes were amplified by PCR (Kras :567bps; p53: 1161bps)

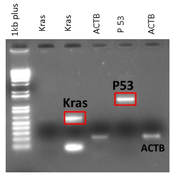


Fig1. Porcine Kras and P53 PCR

2: Site-directed mutagenesis of Kras and p53 genes

a. Sequence alignment of Kras and p53 proteins among pig, human and mouse to determine mutationed sites.

Kras Same among these species
MTEYKLVVVGAGGVGKSALTIQLI (**12**)

p53
Human: MAIYKQSQH**MTEVVRRCPHHER**CSD-SDG (**175**)
Mouse: MAIYK+ S++**MTEVVRRCPHHER** SD SDG (**172**)
Pig: MAIYK+SQH**MTEVVRRCPHHER**CSD DG (**167**)

- Kras mutation site is in the 12th glycine (G)
- P53 mutation site is in the 167th arginine (R)

b. Site-directed mutagenesis

- Kras: 12th glycine (G) ⟶ Aspartic acid (D)

Kras ^{G12D}: corresponding nucleotide mutation is GGT to GAT at cDNA position 35

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Query  1    ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  84   ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGATGGCGTAGGCAAGAGTGCCTTGACA  143
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- p53: 167th arginine (R) ⟶ histidine(H)

P53 ^{R167H}: corresponding nucleotide mutation is CGC to CAC at cDNA position 500

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Query  481  ATGACCGAGGTGGTGAGGGCTGTCCCCACCATGAGCGCAGCTCTGACTATAGCGATGGT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  562  ATGACCGAGGTGGTGAGGGCACTGTCCCCACCATGAGCGCAGCTCTGACTATAGCGATGGT  621
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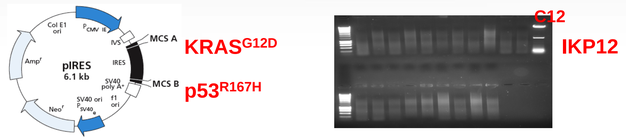
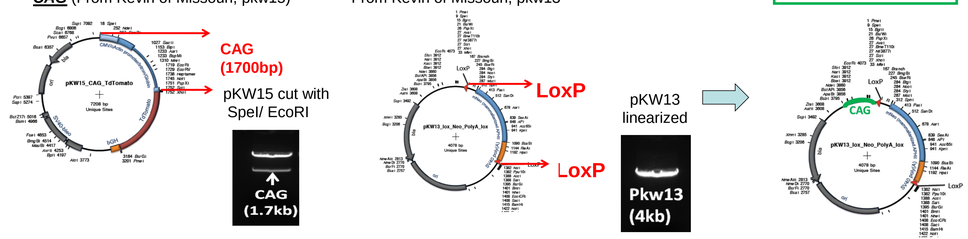
3: Cloning Kras ^{G12D} and p53^{R167H} into PIRES vector



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

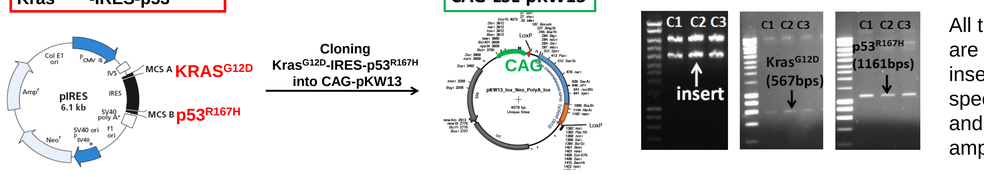
1. Promoter: *Ubiquitous* gene expression: ***CAG*** (From Kevin of Missouri, pkw15)
2. LoxP-STOP-Loxp (LSL): From Kevin of Missouri, pkw13

CAG-LSL-pKW13



III. Construct Inducible Kras and p53 Vector

Kras ^{G12D}-IRES-p53^{R167H} → Cloning Kras^{G12D}-IRES-p53^{R167H} into CAG-pKW13 → **CAG-LSL-pKW13**



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}-p53^{R167H} vector construct in porcine fibroblast in vitro

Methods: 1) Transfection of pig fibroblast with LSL- Kras^{G12D}-p53^{R167H} 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 LSL sequence and induction of Kras^{G12D}-p53^{R167H} 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 mediated 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. Transgenic pigs are now being produced from a minipig cell line carrying this inducible oncogene construct via nuclear transfer cloning. (Piglets Due May 28, 2012.) This animals will be treated with Cre recombinase and monitored for transgene expression and for tumor formation.

References:

1. Pigs as a modlel 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

Funded by NIH grant # 1R01CA153132-01.