An Inducible Transgenic Porcine Model for Human Cancer

Wenping Hu[1,5], Laurie A. Rund[1], Fernanda. M. Rodrigues[7], Tiago Collares[7], Fabiana. K. Sexas[7], Ying Liang[6], Kuldeep Singh[3], Christopher. M. Counter[4] & Lawrence B. Schook[1,2,3]

([1] Department of Animal Sciences, [2]Nutritional Sciences, [3]Veterinary Pathobiology, University of Illinois, Urbana, IL, United States; [4] Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC, United States; [5]State Key Laboratory of AgroBiotechnology, China Agricultural University, Beijing, China; [6]Department of Internal Medicine, University of Kentucky, Lexington, KY, United States; [7]Technology Development Center, Biotechnology Unit, Federal University of Pelotas, Pelotas, RS , Brazil.)

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.

RESULTS

To this end, pigs were created by cloning to contain oncogenic $KRAS^{G12D}$ and dominant-negative $p53^{R167H}$, two commonly mutated genes in human cancers, 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. Fibroblast cell strains generated from four such clones were infected with adenovirus vector (Ad-Cre-GFP) encoding Cre recombinase and GFP protein or control vector (Ad-GFP) with GFP alone. Upon infection with Ad-Cre-GFP, but not control Ad-GFP, all four cell strains expressed $KRAS^{G12D}$ and $p53^{R167H}$ mRNA, as assessed by RT-PCR, transformed phenotypes such as increased cell migration rates, increase cell proliferation, and growth in soft agar. For example, migration rates in a wound assay were significantly different (184 vs 67 at 24 hr time point ($p \leqslant 0.01$). CFSE assay determined that CRE cells divided twice as many times than control GFP cells in a 73 hr period ($p \leqslant 0.01$). Additionally, GFP cells were unable to form colonies in soft agar, while each of the CRE cell lines formed over 100 colonies ($p \leqslant 0.01$). These lines were then injected into immunodeficient mice to test for tumorigenicity. Tumors from the CRE cell lines developed in the mice (13/14) while no tumors developed from the GFP lines. Histopathological analysis revealed the tumors to be sarcomas, which were non-encapsulated, densely cellular and locally infiltrative with marked cellular and nuclear pleomorphism.

DISCUSSION AND CONCLUSION

These results demonstrate that the induction of the transgenes in these porcine cells triggered a tumorigenic phenotype. In the future, 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 therapies.