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Comparative Genomics

EFFECTS OF ONCOGENE KRASG12D and p53R167H EXPRESSION AND GENOTYPE ON CHEMOTHERAPY DRUG RESISTANCE IN PORCINE CELLS

K. R. Begnini[4] , L. A. Rund[1], and L. B. Schook[1,2,3]

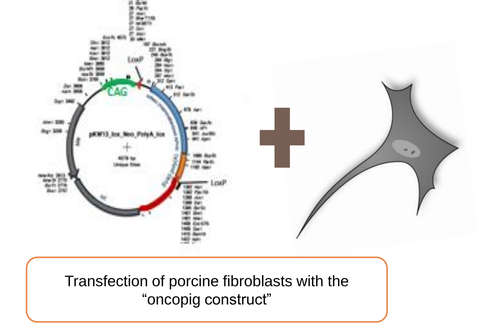
[1]Department of Animal Sciences, [2]Nutritional Sciences, [3]Veterinary Pathobiology, University of Illinois, Urbana, IL, United States;
[4] Technology Development Center, Biotechnology Unit, Federal University of Pelotas, Pelotas, RS, Brazil

Introduction

Mutations in p53 and KRAS genes are present in many human cancers. Both genes play important roles in tumor initiation and in the acquiring of oncogenic properties that enable the tumor to increase its proliferation, become invasive, and metastatic. Although the role of these genes in tumorigenesis and tumor invasiveness is well established, their effect on drug sensitivity are still unclear. Herein we evaluated the response of MN minipigs and Crossbred transgenic swine cells containing *KRAS*^{G12D} and *p53*^{R167H} mutations to the chemotherapeutic drugs doxorubicin and 5-fluorouracil.

Methods

1. Vector construction and validation



Transfection of porcine fibroblasts with the "oncopig construct"

Cre recombinase-mediated Kras^{G12D} and p53^{R167H} expression was 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 "oncopig" construct (Figure 1).

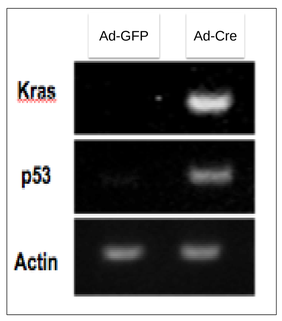
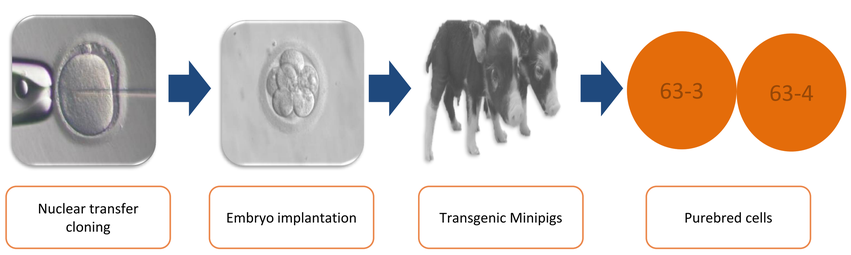
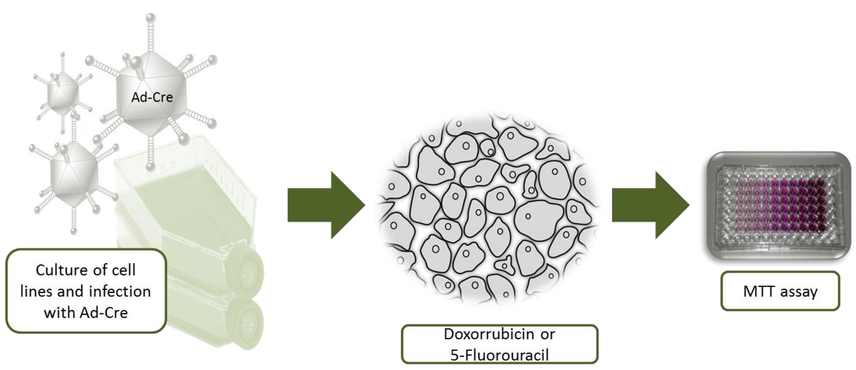


Figure 1: Validation of the deletion of LSL sequence and induction of Kras^{G12D}-p53^{R167H} expression.

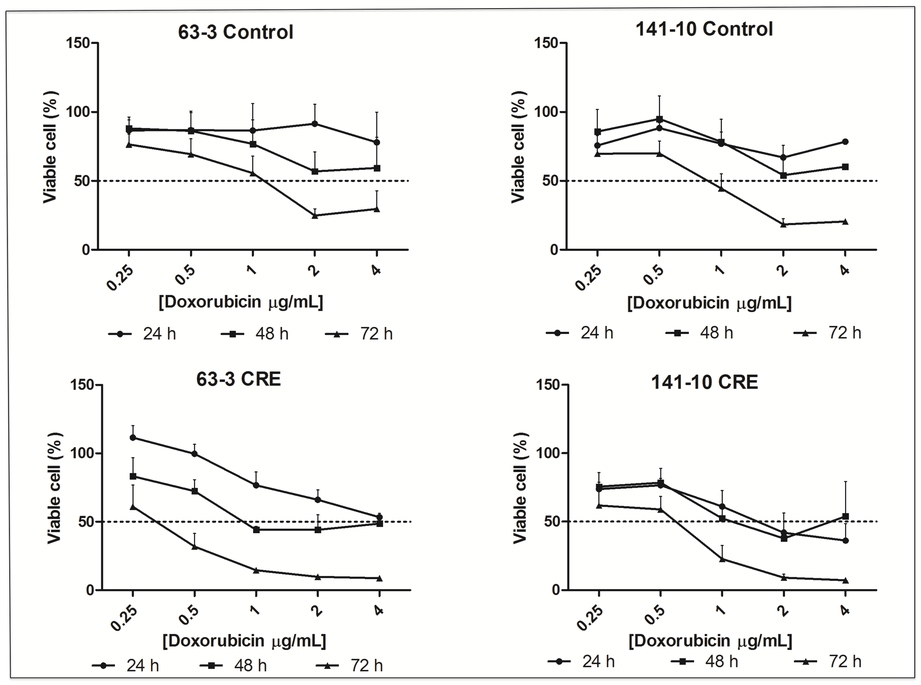
2. Cloned transgenic pigs and transgenic cell lines generation



3. In vitro assays



Results



Doxorubicin treatment reduced the number of viable cells in both *KRAS*^{G12D}/*p53*^{R167H} expressing lines (CRE) to less than 50% at concentrations from 1 μg/mL after 48 hours of treatment and upon concentrations from 0.5 μg/mL after 72 hours of treatment. In the controls lines doxorubicin just was able to reduce significantly the cell viability upon doses from 2 μg/mL in 72 hours. Both purebred minipigs (63-3) and crossbred (141-10) cells showed the same drug response pattern to doxorubicin (Fig 1). There are a few reports in the literature about doxorubicin chemotherapy resistance in the presence of oncogenic *KRAS*^{G12D} or dominant-negative *p53*^{R167H} alone; but our data suggests that the combination of these two point mutations does not affect the cell response to doxorubicin.

Figure 1: Doxorrubicin response pattern for pure (63-3) and crossbred (141-10) porcine cells.

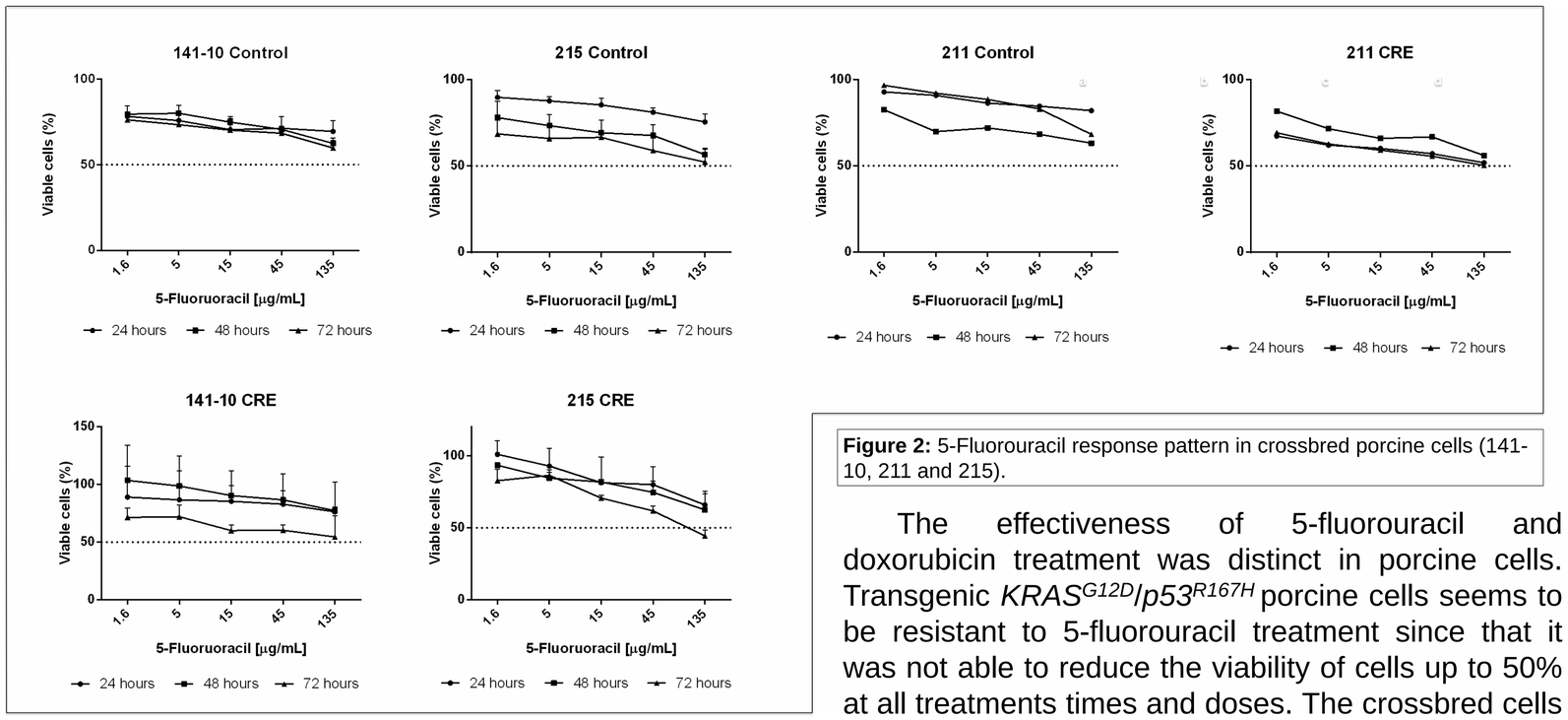


Figure 2: 5-Fluorouracil response pattern in crossbred porcine cells (141-10, 211 and 215).

The effectiveness of 5-fluorouracil and doxorubicin treatment was distinct in porcine cells. Transgenic *KRAS*^{G12D}/*p53*^{R167H} porcine cells seems to be resistant to 5-fluorouracil treatment since that it was not able to reduce the viability of cells up to 50% at all treatments times and doses. The crossbred cells showed a little more resistance to 5-fluorouracil both in CRE and control cells, with the number of viable cells equal or higher than 50% (Fig 2 and Fig 3).

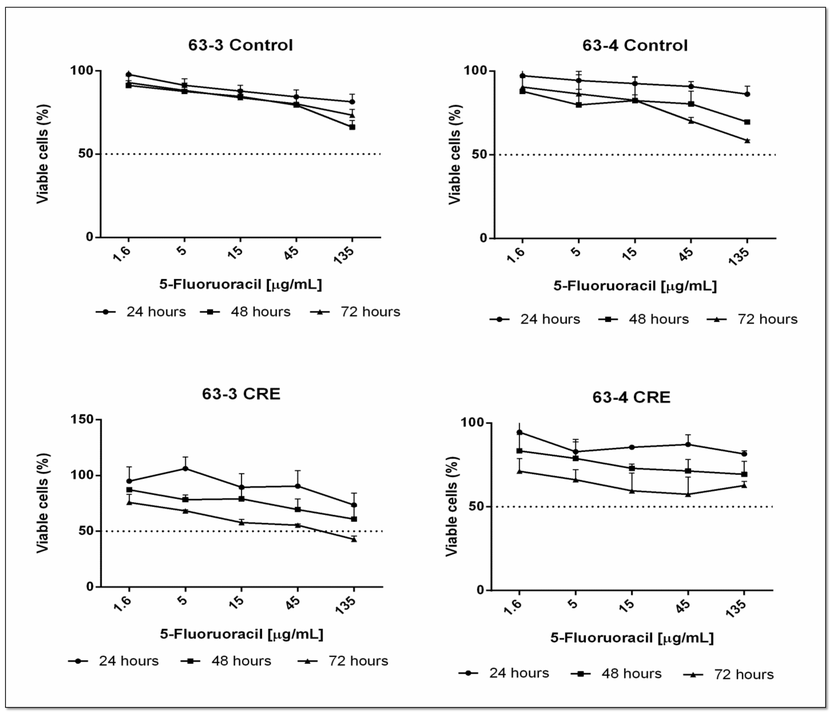


Figure 3: 5-Fluorouracil response pattern in purebred (63-3 and 63-4) porcine cells.

Conclusion

The overall results suggest that the expression of *KRAS*^{G12D} and *p53*^{R167H} mutated proteins does not promote chemotherapy resistance to doxorubicin and may increase the sensitivity to 5-fluoruoracil in transgenic minipig cells. There may be genotypic differences in drug metabolism.

Acnowlegments

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