Development of an Inducible Transgenic Onco-Pig Model

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In order to take full advantage of the potential for new therapeutics and biotechnology applications, there is an urgent need for new animal models supporting cancer research. Common rodent-based models have limitations in terms of modeling human cancers. Because pigs have many genetic and physiological similarities with humans we have been investigating the potential of developing genetic porcine models of cancer. Our previous studies have shown that overexpression of several human oncogenes led to tumor development in pigs. Transgenic pigs were engineered to contain oncogenic $KRAS^{G12D}$ and dominant-negative $p53^{R167H}$, downstream of a LoxP-polyA (STOP)-LoxP sequence (LSL) and CAG promoter, such that exposure to Cre-recombinase induces their expression in desired tissues. Fibroblast cell strains generated from four such clones were infected with an adenovirus vector (Ad-Cre-GFP) encoding Cre recombinase and GFP protein or control vector (Ad-GFP) with GFP alone. Cells were analyzed for cell migration rates, cell proliferation, colony formation in soft agar, tumor development in immunodeficient mice, histopathological and RT-PCR characterization of the tumors formed. All four cell strains expressed $KRAS^{G12D}$ and $p53^{R167H}$ mRNA, and exhibited transformed phenotypes such as increased cell migration rates, increased cell proliferation, and growth in soft agar and in mice. Migration rates in a wound assay were significantly different (184 vs 67 at 24 hr ($p \leq 0.01$). CFSE assay determined that CRE cells divided twice as many times as control GFP cells in a 73 hr period ($p \leq 0.01$). Additionally, GFP cells were unable to form colonies in soft agar, while each of the CRE cell lines formed over 100 colonies ($p \leqslant 0.01$). CRE cell lines produced tumors in the mice (13/14) while no tumors developed from the GFP lines. Histopathological analyses revealed the tumors to be sarcomas, while RT-PCR of tumor cDNA confirmed expression of the oncogenic $KRAS^{G12D}$ and dominant-negative $p53^{R167H}$ *mRNA* from each tumor; therefore, demonstrating that induction of the transgenes in these porcine cells triggered a tumorigenic phenotype. Next, we will monitor tumor incidence following site-specific transgene induction in these Onco-pigs.