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Comparative Genomics

A Genetically Defined Porcine Model of Tumorigenesis

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ABSTRACT

Our objective is to create a porcine model representative of human cancers. Genetically engineered tumors in pigs may prove to be invaluable in the future for determining the efficacy of anti-cancer drugs and to study the process of tumorigenesis and cancer in a genetically compliant animal model that is physiologically more similar to humans than rodents. Genetic engineering of the porcine cancer cells was based on how human cells are driven to a tumorigenic state via the enforced expression of proteins that disrupt the p53 tumor suppressor pathway and activate the c-Myc and Ras pathways, all of which are commonly corrupted in human cancers. Four genes (cyclin D1, CDK 4, c-Myc, and H-Ras) were introduced into cultured porcine fibroblasts using retroviral vectors. Tumorigenic porcine cells were injected into isogenic, immune-compromised pigs to test for tumor growth. Tumors formed but quickly regressed when immune suppression was removed. In an attempt to induce a less immunogenic tumor, pigs were infected directly with two retroviruses carrying these four genes. This method induced lymphosarcoma in all of the animals (n = 6) in the absence of immunosuppression. Future studies will attempt to induce tumors in cloned animals, thus producing tumors that can be transferred to any number of identical animals to study the process of tumorigenesis and cancer phenotypes.

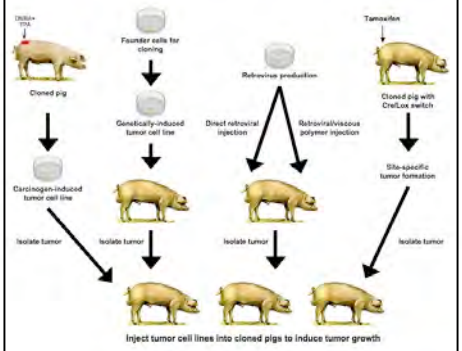
INTRODUCTION

- Current limitations in cancer biology have delayed the transition from basic science to patient benefit.
- Cancer is a complex, heterogeneous disease. Achieving an understanding of the pathogenesis of cancer in a model similar to humans is key to developing successful treatments.
- Within each tissue type there are forms of cancer that demonstrate different clinical behaviors. There is no clear understanding as to why individual tumors differ & current animal models are not sufficient to study the distinct tumor phenotypes that result in the assortment of clinical outcomes.
- Mouse models are the predominant choice currently for cancer studies. However, the differences between murine and human tumorigenesis and pharmacodynamics prevent adequate comparisons to human cancers.
- Current large animal models rely on spontaneous tumor formation and are lacking tumor homogeneity.
- Cancer is a multi-step process that results in genomic alteration. The methodology that is required for the tumor cell to undergo various types & frequencies of mutations can be exploited to create a system that can be genetically manipulated to induce tumor formation.

OBJECTIVES

- Develop approaches to create site/tissue-specific tumor models with designated clinical outcomes.
- Develop methods to induce genetically defined tumor formation by altering pathways commonly disrupted in human cancers.
- Develop protocols to create standardized tumor models analogous to human tumors & support adjunct therapies.

Designing Cancer Models

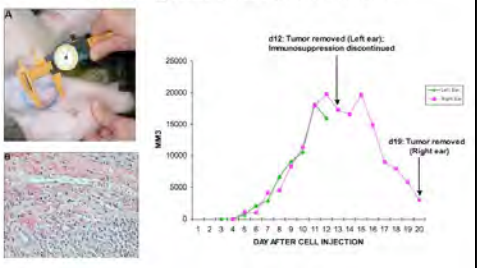


APPROACHES

- Genetically engineer porcine fibroblasts to be tumorigenic by altering the p53 tumor suppressor pathway and activating the c-Myc and Ras pathways, all of which are commonly corrupted in human cancers.
- Inject tumorigenic fibroblasts into an immunosuppressed, isogenic animal to form solid tumors at the site of the injection.
- Infect pigs directly with retroviruses containing cyclin D1, CDK4, c-Myc, and H-Ras to create a model of lymphoma.

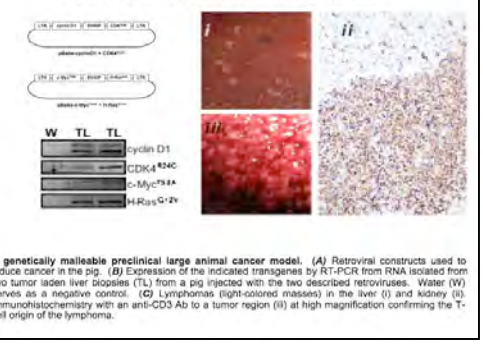
RESULTS

Tumorigenic Cell-Based Model



Genetically defined tumor growth in the pig. **(A)** Photograph of the two tumor masses generated 11 days after 6870T cells, derived from ear notch fibroblasts expressing the 4 transgenes were mixed with isogenic control cells and injected orthotopically behind each ear of the donor pig treated daily with immunosuppressive drugs. **(B)** Hemotoxylin and eosin staining of sectioned-paraffin-embedded mass demonstrates a poorly differentiated tumor, as characterized by large nuclei with multiple nucleoli, with evidence of glandular formation, fibrosis, and vascularization. Magnification, x200. **(C)** Tumor growth curve for isogenic cells expressing the 4 genes.

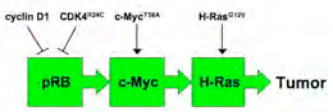
Retroviral Injection-Based Model



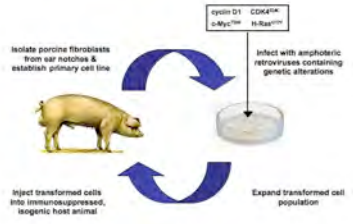
A genetically malleable preclinical large animal cancer model. **(A)** Retroviral constructs used to induce cancer in the pig. **(B)** Expression of the indicated transgenes by RT-PCR from RNA isolated from two tumor laden liver biopsies (TL) from a pig injected with the two described retroviruses. Water (W) serves as a negative control. **(C)** Lymphomas (light-colored masses) in the liver (i) and kidney (ii). Immunohistochemistry with an anti-CD3 Ab to a tumor region (iii) at high magnification confirming the T-cell origin of the lymphoma.

MATERIALS AND METHODS

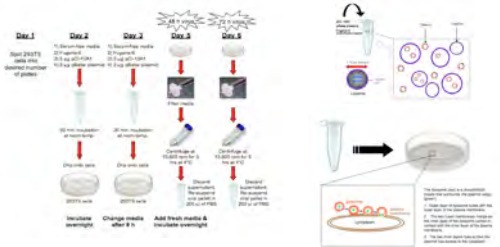
Key Genes in Human & Porcine Tumorigenesis



Tumorigenic Cell-Based Model



Retroviral Injection-Based Model



Retrovirus injection:
1) Subcutaneously into mammary area
2) Intravenously via ear vein

Experimental Design

Pig	Treatment	Site of Injection
1882	1x	Subcutaneously; mammary area
1884	1x	Subcutaneously; mammary area
1448	2x	Subcutaneously; mammary area
1471	2x	Subcutaneously; mammary area
6413	3x	Subcutaneously; mammary area
6412	3x	Subcutaneously; mammary area
4481	1x	Intravenously
4482	1x	Intravenously
1441	2x	Intravenously
1431	2x	Intravenously
6410	3x	Intravenously
6411	3x	Intravenously

1x = 40, 10 cm plates; 2x = 80, 10 cm plates; 3x = 120, 10 cm plates

CONCLUSIONS

- A porcine model of tumorigenesis was successfully created by genetically modifying pathways commonly disrupted in human cancers.
- This genetically compliant animal model is physiologically more similar to humans than rodents and supports adjunct therapies.
- Established site-specific swine tumors in the presence of immuno-suppression.
- Strong immune responses prevent tumor growth; direct retroviral injection circumvents immune response.
- Established a porcine model of T-cell lymphoma.

FUTURE DIRECTIONS

- Expand methods to support a preclinical model for testing of novel therapeutics, experimental therapies and imaging.
- Develop cell-based solid tumor models from known tissue of origin and with designated clinical outcomes in the absence of immunosuppression.
- Establish a porcine model for breast cancer using both cell-based and retroviral injection methods.
- Generate transgenic pig that will be used to induce tissue-specific, conditionally expressed solid tumors.
- Induce tumors in cloned animals, thus producing tumors that can be transferred to any number of identical animals to study the process of tumorigenesis and cancer phenotypes.

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