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Porcine cancer models for translational oncology

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Large-animal cancer models are needed to advance the development of innovative and clinically applicable tumor diagnostic, therapeutic, and monitoring technologies. We developed a genetically modified porcine model of cancer based on a TP53 mutation, and established its utility for tracking tumorigenesis *in vivo* through non-invasive clinical imaging approaches.

The p53 tumor suppressor, encoded by the *TP53* gene, is vital for cancer prevention. Considered the guardian of the genome, p53 responds to genotoxic stresses by transcriptionally regulating numerous genes that suppress cancer initiation and progression.[2] More than half of all human tumors have sporadic mutations of *TP53* and the overall risk of cancer in people with inherited *TP53* mutation is exceedingly high.[3]

In the study by Sieren et al.[1] the porcine *TP53* gene was mutated in Yucatan miniature pigs, resulting in the first gene-targeted large-animal model of cancer. The porcine TP53 mutation (R167H) used is orthologous to a naturally occurring mutation found in many human cancers.[4] Characterization of tumorigenesis in homozygous ($TP53^{R167H/R167H}$) and heterozygous ($TP53^{R167H/+}$) pigs was facilitated by clinical imaging technology, including computed tomography (CT) and magnetic resonance imaging (MRI). Non-invasive medical imaging successfully detected solid tumors, in addition to guiding tissue harvest for histopathological validation and molecular genetic analyses. $TP53^{R167H/R167H}$ pigs developed a variety of cancers during early phenotypic evaluation (within[1,5] years of birth) including lymphoma, osteogenic tumors (osteosarcoma), and Wilms tumor (nephroblastoma). As predicted from studies of the analogous p53 mutation in mice (R172H) and humans (R175H), the porcine p53-R167H protein failed to mediate protective checkpoint responses in cells and promoted significant chromosomal instability in tumors from $TP53^{R167H/R167H}$ pigs. By comparison, no solid tumors have been detected in $TP53^{R167H/+}$ pigs during 2.5 years of observation, which makes them highly suited to testing the cooperative effects of secondary oncogene activation or tumor suppressor gene loss. Indeed, *TP53* mutant pigs represent a valuable platform on which to build new large-animal cancer models. The next milestone for porcine cancer models will be targeted tumor formation through conditional and/or tissue-specific expression of mutant cancer genes, either alone or added to a primed $TP53^{R167H/+}$ background.[5-7]

Importantly, *TP53* mutant pigs and other future porcine tumor models provide an unparalleled opportunity to impact cancer management in humans. For example, pigs allow the development and validation of new diagnostic, monitoring, and treatment approaches that employ clinical technology in an animal cancer model that is a comparable size to humans (**Fig. 1**). At maturity, Yucatan pigs reach a body weight of 60–75 kg, and have a thoracic and abdominal structure similar to that of an adult human, enabling these animals to be imaged by clinical instruments used for humans. The small size of rodents prevents mouse cancer models from being useful for direct translation of techniques requiring clinical technologies (CT, MR, minimally invasive surgical techniques, and/or radiation therapy). Additionally, pigs have a lifespan, circulatory volume, and metabolic/heart/respiratory rate in concordance with that of humans. Another advantage is that research using porcine cancer models can be performed in tightly controlled populations, without co-morbidities, and with or without therapeutic intervention, which is not possible in human cancer patients.

As outlined in **Figure 1**, there is tremendous potential for improving clinical cancer management as a result of research using porcine cancer models. This is true not only for diagnostic imaging, but also for surgical and radiation treatments with accompanying response monitoring. Cross comparison of multiple imaging modalities and/or many variations of data acquisition parameters within a given modality on the same cancer subject is possible with the porcine cancer model. In addition, valuable advancements may be made in utilizing medical imaging to probe early pre-cancerous changes and to follow tumor formation and growth over time in the presence or absence of intervention. One of the current clinical challenges in cancer management is the optimal time for post-treatment positron emission tomography (PET) to detect remaining cancer in patients who have undergone radiation therapy while avoiding false positive signals caused by the inflammatory response.[8] Development and validation of novel radiation therapy and imaging strategies will be assisted by porcine cancer models because of their similar

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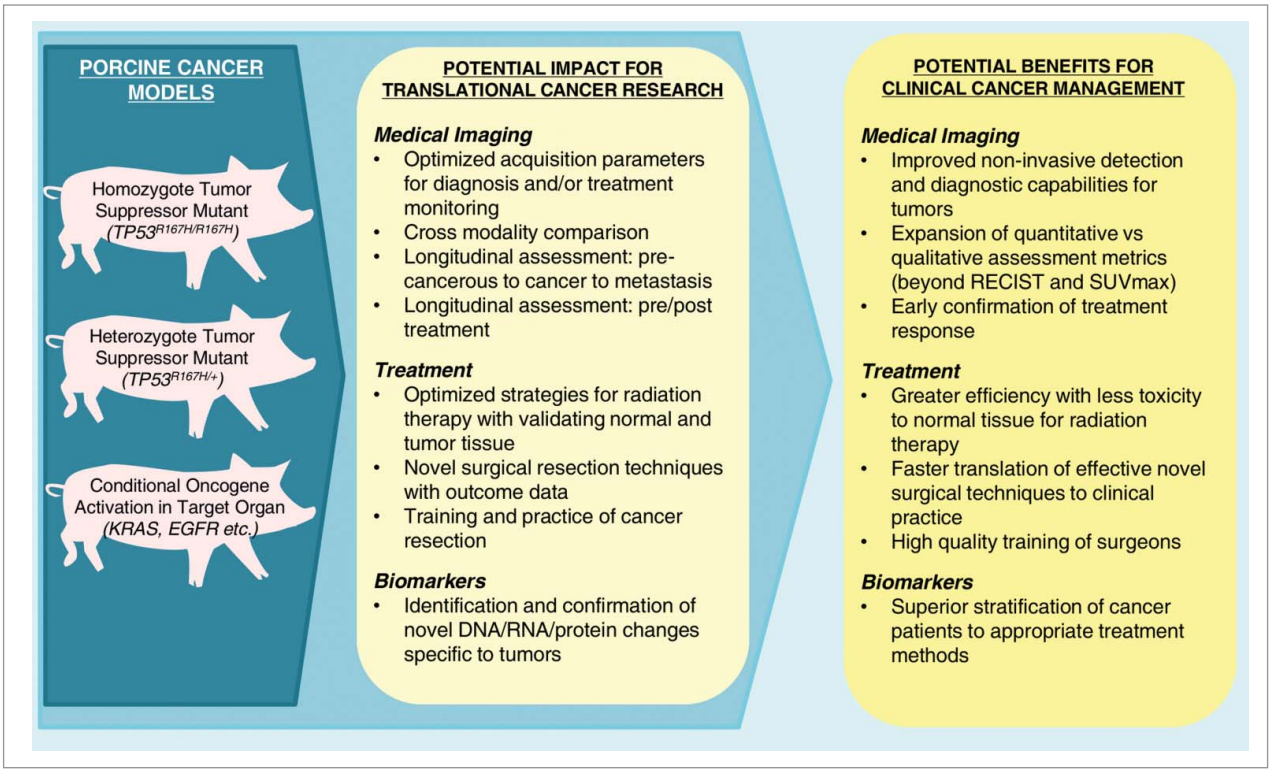

Figure 1. Porcine cancer models provide unique opportunities to advance a number of key translational cancer research areas (examples are listed), which in turn would impact clinical cancer management for human patients. We have reported tumorigenesis in $TP53^{R167H/R167H}$ pigs[1] and continue to characterize $TP53^{R167H/+}$ pigs for possible tumor development. Conditional oncogene activation in target porcine organs or at particular times in development may be implemented independently or in conjunction with the *TP53* mutation. At present, much of the data acquired in human cancer patients through clinical medical imaging techniques is underutilized. For example, clinical imaging systems currently collect volumetric dynamic datasets, however diagnostic and response criteria remain dependent on 2-dimensional measures [Response Evaluation Criteria in Solid Tumors (RECIST)] and single threshold values [maximal standardized uptake value (SUVmax)]. Challenges arise in clinical trials for the investigation of novel surgical and/or radiation treatment approaches due to late stage disease, diversity in cancer origin/subtype, and co-morbidities in the patient population. The porcine cancer model permits data collection in a tightly controlled cancer cohort, of comparable physical size and anatomy to humans, and with unrestricted access to corroborating biospecimens.

anatomy to humans and the unrestricted ability to collect biospecimens for confirmatory analyses. Moreover, we envision the porcine cancer model being useful as both a developmental and educational tool for surgeons, enabling them to optimize techniques for complex surgeries (e.g., the Whipple procedure for pancreatic cancer) and/or novel cancer resection techniques. Notably, the impact of surgery on porcine cancer models with and without alternate neoadjuvant or adjuvant therapies could be studied in a controlled cohort.[9]

Cancer is a complex disease for which targeted therapies based on the unique genetic makeup of each patient and their tumors may yield optimal treatment outcomes. Biomarkers, specific factors, or molecular alterations that predict tumor development (diagnostic), survival (prognostic), and response to therapy (predictive), are the key to personalizing anticancer treatments. Comprehensive genetic and proteomic analyses of tumors from porcine cancer models, followed by cross-species comparisons to molecular data obtained from human and mouse tumors, should facilitate identification of commonly altered genes, RNAs, and/or proteins essential for tumorigenesis. This integrated approach could streamline the discovery of meaningful cancer biomarkers, which can be characterized and corroborated in porcine tumor models prior to their testing and confirmation in the clinic. Such information should ultimately help stratify cancer patients for enrollment in new clinical trials and for treatment with the most appropriate therapies and doses, thereby improving patient survival and quality of life.

Our understanding of human diseases has been profoundly advanced by animal models. Although mice have traditionally served as useful cancer models, large animals such as pigs have been indispensable in studying other pathologies such as cystic fibrosis.[10] The unique translational capabilities of pigs for cancer research, as revealed by the porcine TP53 mutant study, will ideally stimulate additional development and study of porcine tumor models in the coming decade.

Disclosure of Potential Conflict of Interest

Christopher S. Rogers in an employee of Exemplar Genetics, a company that has applied for a patent related to the work reported herein. Jessica C. Sieren, Dawn Quelle and David K. Meyerholz have no conflicts of interest.

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