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Review

Pigs as models of human cancers

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A B S T R A C T

Recent decades have seen revolutionary advances in our understanding of cancer, with the molecular mechanisms underlying many human cancers now reasonably well understood. The challenge now is to bridge the gap between laboratory and clinical oncology, so these accomplishments can be translated into practical benefits for human patients. Although genetically modified mice are powerful tools to investigate the molecular basis of many human diseases, they are less suitable for many preclinical studies. Other animals can provide important complementary resources to aid the development, validation, and application of new medicines and procedures. Powerful methods of genetic engineering have now been extended to physiologically more relevant species, particularly the pig, opening the prospect of more representative, genetically defined, cancer models at human scale. Here, we provide a brief review of the genetically modified porcine cancer models described in the scientific literature.

1. Introduction

Recent decades have seen revolutionary advances in our understanding of cancer, with the molecular mechanisms underlying many human cancers now reasonably well understood. Nevertheless, effective means of early diagnosis and therapies are often lacking. This rich body of knowledge should be exploited to fulfill unmet clinical needs, and preclinical studies with animal models can play an important role translating basic research into benefit for patients. Most cancer models are in rodents, particularly genetically engineered mice. Although mice and humans share many fundamental similarities as mammals, there are clear differences in cancer biology. For example, murine cells are more easily transformed *in vitro* than human cells [1,2], and the set of genetic events required for mouse tumorigenesis differs from humans [3]. Differences in protein interactions, physiology, and anatomy can thus lead to significantly different disease phenotypes from similar genetic lesions. Metastatic human cancers are particularly difficult to reproduce in mice. As a consequence, basic studies in murine models often do not translate into success in clinical trials. Only 5% of anticancer agents developed in preclinical studies on the basis of traditional mouse models demonstrate sufficient efficacy in phase-III trials [4].

Evidence of the limitations and shortcomings of mouse models is also accumulating in other disease areas. Three independent research groups recently published phase-III studies of new anti-tuberculosis drug regimens showing that despite positive results in mouse studies, the new drugs completely failed in humans [5–7]. Similar discrepancy between mouse and human trials has been shown for amyotrophic lateral sclerosis treatment [8]. The drawbacks of mouse models are also evident in some basic research, e.g., drug metabolism [9], cystic fibrosis [10], breast cancer [11], and colorectal cancer [12]. A systematic study into inflammatory disease highlighted the lack of correlation between results in mice and human conditions [13], and provoked critical commentary in the popular press.

Although the mouse is an invaluable tool for basic disease research, other animals are clearly required as complementary resources. No single species is likely to provide the best model for all human disease, each has advantages and disadvantages. Interspecific studies provide broad

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insight into the genetic bases of disease and disease predisposition. Comparative analysis of gene expression data can identify evolutionarily conserved networks of expression and gene regulatory regions and unravel the complex interactions between genetic, environmental, and lifestyle factors that influence disease pathology. Canine oncology is already providing a useful complementary perspective. Dogs develop spontaneous tumors with histopathological and biological feature similar to human cancers (reviewed by [14]). This has allowed veterinary oncologists to develop substantial repositories, biobanks, of canine cancer samples, and findings show direct relevance to human treatments [15].

Although, pigs have not so far played a major role in experimental oncology, their usefulness is evident in a wide range of preclinical research. Pigs are very similar to humans in terms of body size, anatomy, and their physiological and pathophysiological responses. As such, they are used to study newly designed human-sized equipment and instruments and to develop procedures such as endoscopic and laparoscopic surgery [16]. Pigs are also relatively long-lived, enabling longitudinal studies of disease initiation and progression in individual animals under conditions that mimic the human patient.

As in humans, spontaneous cancer caused by natural mutations is rare in pigs, the most common forms being lymphomasarcoma in young animals [17,18] and melanoma in adult pigs [19]. For many years, only two spontaneous pig tumor models on the basis of germline mutations were available for biomedical research. These are the Libechov and Sinclair minipigs, both predisposed to melanoma. These however, differ from humans because the melanomas spontaneously regress at high frequency (reviewed by [14]). Unfortunately the causative genetic lesions are undefined, making it difficult to draw parallels with human melanoma.

In the absence of genetically based pig tumors, a variety of strategies have been adopted to aid development of tumor therapies in pigs. Adam et al., [20] reported a porcine cancer model based on autologous transplantation of primary porcine cells transduced with retroviral vectors carrying oncogenic complementary DNAs. These studies revealed important similarities in tumorigenesis between pig and human. However, this model falls somewhat short as a representation of human cancer. The use of viral complementary DNA constructs does not reliably reflect the expression and regulation of endogenous genes. Tumors arising from grafted cells also differ in important respects from autochthonous tumors. Tumors arising from grafted cell lines also tend to be poor predictors of clinical efficacy, e.g., anti-cancer drugs found to be effective on such grafts can be ineffective on real tumors [21].

However livestock genetic engineering holds the real key to produce representative pig cancer models. The key techniques for generating genetically modified large animals, nuclear transfer [22], and gene targeting [23], were developed some time ago but it has taken time to refine and improve these sufficiently to produce genetically modified pigs “to order”. The rate of advance is now increasing rapidly with the development of highly specific synthetic endonucleases, transcription activator-like effector nucleases [24,25], zinc finger nucleases [26], and RNA-guided endonucleases [27], together with the availability of the porcine genomic sequence [28]. Generating genetically modified pigs does take more time and effort than mice, but the potential benefits for preclinical research and human welfare are substantial.

The number of pigs genetically modified to replicate human diseases has increased dramatically [29]. Valuable models such as cystic fibrosis and diabetes are established [30,31]. Work is also proceeding toward genetically defined porcine cancer models [32]. Here we provide a brief review of the genetically modified porcine cancer models described in the scientific literature (Table 1).

2. Genetically modified porcine cancer models

The first transgenic pigs designed to model cancer carried the *v-Ha-ras* oncogene directed by the mouse mammary tumor virus long terminal repeat promoter, but no phenotype was observed [39]. Constitutive expression of the Gli2 transcriptional activator in keratinocytes resulted in basal cell carcinoma-like lesions in young pigs, but these were euthanized because of bacterial infection before fuller investigation could be carried out [38]. The first gene-targeted pigs for cancer were generated by adeno–associated virus-mediated gene inactivation of breast cancer associated gene 1 in fibroblasts [40]. Animals were produced by nuclear transfer, but none survived beyond 18 days, but the same group has reported a 2-year-old sow with morphologic changes in the mammary gland (Transgenic Animal Research Conference IX, Lake Tahoe, 2013).

Our group is engaged in program to model human cancers in pigs as accurately as possible. Similar genetic

Table 1
List of genetically modified pigs for cancers model.

Cancer type	Gene	Genetic modification	Reference
Colorectal cancer	*APC*	Targeted truncating mutation at positions 1061 and 1311	[33]
		TALEN-mediated knockout	[25]
Various cancers	*TP53*	Conditionally activated targeted mutation	[34]
		Targeted mutation	[35]
		Inducible mutated trangene overexpression	[36]
	KRAS	Conditionally activated targeted mutation	[37]
		Inducible mutated trangene overexpression	[36]
Basal cell carcinoma	*GLI2*	Human transgene	[38]
Breast cancer	*V-H-Ras*	MMTV directed V-H-Ras transgene	[39]
	BRCA1	Knockout	[40]

Abbreviations: MMTV, mouse mammary tumor virus; TALEN, transcription activator-like effector nucleases.