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Editorial

Use of immune-deficient hosts to study human development and pathogenesis

This collection of reviews and novel research studies constitutes a special issue on "Use of xenografts for studying human development, organ repair and carcinogenesis" and is being published in a new format called the "virtual special issue". In the past special issues have appeared as a single issue dedicated to a particular subject. The virtual special issue is a collection of papers, typically published at about the same time, but possibly in separate volumes accompanied by an editorial. Virtual special issues can be accessed online as a coherent collection of reports that will allow the reader to view a spectrum of invited reviews and research papers as well as relevant papers previously published by Elsevier in Differentiation. Table 1 is a list of relevant previously papers on this topic.

Immune-compromised mice have been used for many decades as hosts for growing human cells and tissues in vivo, initially through use of athymic nude mice (Povlsen et al., 1974; Manning et al., 1973). In the ensuing years a variety of additional immune-deficient mice have been developed and used in xenotransplant studies that include Scid (Sandhu et al., 1996), Rag-deficient (Potocnik et al., 1997) and NOD/SCID mice (Fibbe et al., 2001).

This special issue deals with the use of immune-deficient hosts to study human development and pathogenesis. In 1982 we reported the growth of human fetal female reproductive tracts (containing the developing uterine tube, uterus, cervix and vagina) in athymic nude mice that were untreated or treated continuously with the synthetic estrogen, diethylstilbestrol (DES) (Robboy et al., 1982). This study was undertaken to establish an experimental model of malformations within their reproductive tracts elicited in women exposed in utero to DES. At the time of these studies, little was known of the molecular mechanisms of female reproductive tract development as molecular biology was in its infancy and useful antibodies were limited. Accordingly, we present a trilogy of human female reproductive tract development in this special issue.

The first paper of our trilogy by Robboy et al. (2017) reviews the decades-old classic literature on development of the human female reproductive, which till now has been the basis of our understanding of human female reproductive tract development. In this paper we present a modern compendium of the morphogenetic events involved in human female reproductive tract development. This study, richly illustrated in color plates, extends and improves our understanding of human female reproductive development. An important aspect of Robboy et al. is resolution of the long-standing question of the derivation of human vaginal epithelium through use of antibodies to PAX2 (a Mullerian duct marker) versus FOXA1 (an endodermal marker for urogenital sinus).

The second paper of our trilogy (Cunha et al., 2017a) uses immunohistochemistry to assess developmental markers (keratins, steroid receptors and transcription factors) involved in the developing human female reproductive tract. This study has demonstrated a remarkable congruence between molecular mechanisms and differentiation marker expression in mouse and human female reproductive tract development.

The third paper of our trilogy (Cunha et al., 2017b) is a study of human female fetal reproductive tracts grown in ovariectomized athymic adult female mouse hosts that were either untreated or treated continuously with DES via subcutaneous pellet. Normal morphogenesis and normal patterns of differentiation marker expression were observed in xenografts grown in untreated hosts and mimicked observations of non-grafted specimens of comparable age reported in the second paper of our trilogy (Cunha et al., 2017a). DES treatment elicited several notable morphological affects: (a) induction of endometrial/cervical glands, (b) increased plication (folding) of tubal epithelium, (c) stratified squamous maturation of vaginal epithelium and (d) vaginal adenosis. DES also induced estrogen receptor alpha (ESR1) in epithelia of the uterine corpus, cervix and globally induced PGR in most cells of the developing human female reproductive tract. DES induced vaginal adenosis was devoid of TP63, keratin 14 and RUNX1, while DES-induced mature vaginal epithelium was positive for both transcription factors. These results define for the first time IHC protein markers of DES action on the developing human reproductive tract and provides bio-endpoints of estrogen-induced teratogenesis in the developing human female reproductive tract for future testing of estrogenic endocrine disruptors.

Human endometrium undergoes extensive morphological, biochemical and molecular changes under the influence of sex steroid hormones such as estrogen and progesterone. While the basic biology of female sex steroid action has been elucidated in animal models the precise molecular mechanisms underlying human endometrial biology remain to be determined. The review by Kuokkanen et al. summarizes recent developments and work utilizing immune-deficient mice as hosts for human endometrial tissues (Kuokkanen et al., 2017). Such studies have confirmed the significance of paracrine signaling between the epithelium and stroma in the growth regulation of the endometrium, and have uncovered distinct species differences between the mouse and human. Xenotransplants of the reconstructed human endometrium in immunodeficient host mice have proven as highly promising tools for in vivo research of endometrial functions and highlight the opportunity for the technology application of genome engineering, such as targeted ablation of endometrial genes for example by using CRISPR/CAS9 system. An important advantage of xenotransplantation models of the human endometrium are (a) its use to investigate endometrial effects of new compounds and drugs

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Table 1
Historic papers on the use of human xenografts.

Title	Reference
Effects of human fetal gastroenteric mesenchymal cells on some developmental aspects of animal gut endoderm	(Lacroix et al., 1984)
Human breast epithelial xenografts: An immunocytochemical and ultrastructural study of differentiation and lactogenic response	(Dubois et al., 1987)
Human intestinal development in a severe combined immunodeficient xenograft model	(Savidge et al., 1995)
Three distinct keratinocyte subtypes identified in human oral epithelium by their patterns of keratin expression in culture and in xenografts	(Lindberg and Rheinwald, 1990)
Comparative tumor morphogenesis of two human colon adenocarcinoma cell clones xenografted in the immunosuppressed newborn rat	(Remy et al., 1993)
Conventional patterns of human intestinal proliferation in a severe-combined immunodeficient xenograft model	(Shmakov et al., 1995)
Exploration of Shh and BMP paracrine signaling in a prostate cancer xenograft	(Shaw et al., 2010)
Mesenchyme-mediated and endogenous regulation of growth and differentiation of human skin keratinocytes derived from different body sites	(Boukamp et al., 1990)
Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model	(Hayward et al., 1998)
Mesenchymal reprogramming of adult human epithelial differentiation	(Aboseif et al., 1999)
Cross-species stromal signaling programs human embryonic stem cell differentiation	(Taylor and Risbridger, 2014)
Methods for studying human organogenesis	(Cunha et al., 2016)
Subrenal capsule grafting technology in human cancer modeling and translational cancer research	(Wang et al., 2016)
Defects of basement membrane and hemidesmosome structure correlate with malignant phenotype and stromal interactions in HaCaT-Ras xenografts	(Tomakidi et al., 1999)
Tumor-host interactions contribute to the elevated expression level of α1-antichymotrypsin in metastatic breast tumor xenografts	(Montel et al., 2005)

and elucidating the molecular mechanisms underlying endometrial functions to prevent and cure disease as well as enhance endometrial function and fertility.

The paper by Isaacson et al. continues the theme of the use of immune-deficient hosts in the study of human organogenesis, namely development of external genitalia (Isaacson et al., 2017). This paper is a xenograft study of human penile urethral and clitoral development in which human fetal penes and clitorides were grown under the renal capsules of gonadectomized athymic mice that were untreated or treated with a subcutaneous pellet of dihydrotestosterone (DHT) or diethylstilbestrol (DES). Non-grafted specimens of similar fetal age were sectioned and immunostained for a variety of differentiation markers. Normal morphogenesis and normal expression patterns of cytokeratins, uroplakin and the androgen receptor were observed in xenografted specimens. DHT treatment reliably produced tubularization of nascent urethral and vestibular structures and male patterns of androgen receptor expression in grafts of both genetic sexes, while estrogenic (DES) or hormonally absent conditions reliably resulted in a persistent open urethral/vestibular groove and female patterns of androgen receptor expression. The validation of this model enables further study into causal pathways by which endocrine-disrupting and endocrine-mimicking substances may directly cause disruption of normal human urethral development or hypospadias.

The paper by Salgado et al. reviews xenograft models to study skin physiology (Salgado et al., 2017). Xenograft models are particularly useful due to the lack of clinically relevant animal models in predicting drug effectiveness in humans. Species differences in skin biology are especially evident for complex skin conditions, such as psoriasis and eczema, where interactions between the immune system, epidermis and the environment likely occur. While animal models are still considered the gold standard for systemic toxicity studies, alternative xenograft models are now used for certain applications. Accordingly, research efforts have focused on “humanizing” mouse models to better recapitulate human skin physiology. In their review, Salgado et al. outline the different approaches undertaken to study skin biology using human tissue xenografts in mice and the technical challenges involved. Recent developments are described to generate humanized multi-tissue compartment mice that carry both a functioning human immune system and skin xenografts. Such composite animal models provide promising opportunities to study drugs, disease and differentiation with greater clinical relevance.

The paper by Hutka et al. (2017) describes the use of immune-deficient hosts to study human testicular development. Testicular transplantation has been employed as a ‘direct’ approach to understand the development of human fetal and prepubertal testis in health and disease. In their review Hutka et al. describe the potential use of human testis xenotransplantation to study human testicular development and its application for assessing the effects of environmental exposures in humans and establishing fertility preservation options for prepubertal boys with cancer.

The paper by Sasaki et al. (2017) deals with the interaction of prostate carcinoma-associated fibroblasts with human epithelial cell lines in vivo. The authors emphasize that stromal-epithelial interactions play a crucial and poorly understood role in carcinogenesis and tumor progression. Tumor stroma is a complex mixture of cells that includes a fibroblasts often referred to as cancer-associated fibroblasts (CAF), desmoplasia or “reactive” stroma. In this paper Sasaki et al. discuss the approaches to understand these CAF-carcinoma interactions in prostate cancer in vivo models using human cells and tissues. A complex mixture of signaling molecules has been revealed acting both within the stromal tissue and between the stromal and epithelial tissues. Understanding the molecular basis of these cell-cell interactions may provide a basis for new medical approaches to prostatic carcinogenesis.

McLean et al. (2017) discuss the use of prostate cancer xenografts and hormone induced prostatic carcinogenesis. Despite the advent of transgenic and gene knockout animal models in the prostate cancer, species differences in the carcinogenic process continue to raise the issue of relevance of animal models for human prostatic carcinogenesis, thus raising the need for utilizing xenograft models. Xenografts of human cells and tissues provide an approach with undeniable relevance to human pathogenesis with potential clinical significance. Human xenograft models that progress from one pathologic state to another are commonly used to address important scientific questions including malignant transformation, metastatic spread, and castration resistance. McLean et al. review the utilization of xenografts to assess the biology and genetics of prostate cancer, as well as, for therapeutic benefit. In addition, xenografts are also utilized as a tool in precision personalized medicine where patient derived xenografts (PDXs) can be grown in multiple animals and assessed for therapeutic efficacy. The popularity of such xenograft models and PDXs has led to availability of these resources through public and commercial institutions. McLean et al. describe both traditional and emerging models of prostate cancer and their potential uses. Further development of current models and introduction of new models will likely provide new insights and better understanding of prostatic carcinogenesis and progression.

Strand et al. (2017) discusses the use of immuno-deficient mouse hosts in targeting phenotypic heterogeneity in benign prostatic hyperplasia (BPH). Benign prostatic hyperplasia and associated lower urinary tract symptoms are difficult to treat medically, resulting in surgery performed in elderly men to relieve obstructive symptoms. New medical therapies using alpha blockers and 5-alpha reductase inhibitors have not improved clinical outcomes. An under-appreciated confounder to identifying novel targets for BPH therapy is pathological heterogeneity associated with prostatic enlargement. Accordingly, individual patients display unique pathologic phenotypes, composed of distinct cell types. We have yet to develop a cellular or molecular understanding of these unique phenotypes, which has led to failure in developing targeted therapies for personalized medicine. Strand et al. cover the strategic experimental approach using immune-deficient mouse hosts to unravel the pathogenesis of discrete BPH phenotypes and discuss how to incorporate these findings into the clinic to improve outcomes.

Liver diseases afflict millions of patients worldwide, and costly and invasive liver transplantation is the only long-term treatment for liver failure. The review by Tan et al. (2017) describes that state of the art for intravenous transplantation of suspensions of human hepatocytes as a less-invasive approach to reconstitute lost liver functions. This review emphasizes outstanding questions associated with intravenous hepatocyte transplantation. While adult primary human hepatocytes are the gold standard for transplantation, hepatocytes are heterogeneous, which raises the question of whether all hepatocytes engraft equally and what specifically defines an “engraftable” hepatocyte capable of long-term liver reconstitution. To this end, mouse models of liver injury enable assessment of human hepatocytes and their behavior upon transplantation into host mice. While mouse models may not be fully representative of the injured human liver, valuable lessons have been learned from transplantation of human hepatocytes into mouse models. Future studies must eventually deal with the optimal biological source (whether in vivo- or in vitro-derived) and presumptive heterogeneity of human hepatocytes to understand the mechanisms through which they engraft and regenerate liver tissue in vivo.

One important aspect of experimental analysis of human organogenesis, pathogenesis and physiology through the use of xenotransplantation models is an appreciation of the similarities and differences between human biology/pathobiology versus the animal models that currently are used as surrogates. It is critically important to know those biological processes that are congruent in humans and animal models as well as those biological processes for which important species differences are at play. The use of human xenotransplantation models is an excellent way of revealing species differences and similarities.

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