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Review article

Human reconstructed skin xenografts on mice to model skin physiology

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ABSTRACT

Xenograft models to study skin physiology have been popular for scientific use since the 1970s, with various developments and improvements to the techniques over the decades. Xenograft models are particularly useful and sought after due to the lack of clinically relevant animal models in predicting drug effectiveness in humans. Such predictions could in turn boost the process of drug discovery, since novel drug compounds have an estimated 8% chance of FDA approval despite years of rigorous preclinical testing and evaluation, albeit mostly in non-human models.

In the case of skin research, the mouse persists as the most popular animal model of choice, despite its well-known anatomical differences with human skin. Differences in skin biology are especially evident when trying to dissect more complex skin conditions, such as psoriasis and eczema, where interactions between the immune system, epidermis and the environment likely occur. While the use of animal models are still considered the gold standard for systemic toxicity studies under controlled environments, there are now alternative models that have been approved for certain applications. To overcome the biological limitations of the mouse model, research efforts have also focused on "humanizing" the mice model to better recapitulate human skin physiology.

In this review, we outline the different approaches undertaken thus far to study skin biology using human tissue xenografts in mice and the technical challenges involved. We also describe more recent developments 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.

1. Introduction

The use of the laboratory mouse, *Mus musculus,* has emerged as the cornerstone of *in vivo* preclinical research. The mouse genome has been sequenced for numerous extensively-used laboratory mouse strains, and approximately 99% of mouse genes have a corresponding human homolog (and *vice versa*) (Mouse Genome Sequencing et al., 2002). Due to their genetic similarity to humans and relative ease of handling, they are the foremost mammalian model used to gain insights into human development, disease and drug testing. With the advent of genetic engineering in the 1970s, it was not long before transgenic mice became a reality (Palmiter and Brinster, 1986). The mouse model became an evolving paradigm to investigate the systemic biological functions of genes, and consequently, human pathology. There are now more than 5000 mouse genotypes modeling more than 1000 human diseases (Blake et al., 2017), including many inherited human skin diseases, skin cancers and inflammatory skin disorders (Chen and Roop, 2008; DiTommaso et al., 2014; Haase et al., 2004; Liakath-Ali et al., 2014).

As an important preclinical model, the laboratory mouse is used to test for drug efficacy and toxicity. This is often a compulsory drug regulatory requirement, where the systemic effects of the drugs are studied under controlled conditions in a model organism. However, major challenges still remain when translating and applying the results from mouse experiments to humans, as evidenced by the majority of novel drugs being unsuccessful despite promising preclinical animal trials (Adams and Brantner, 2006; FDA, 2004; Hackam and Redelmeier, 2006). Unsatisfactory translation has been attributed to several reasons, including suboptimal experimental design, lack of methodological rigour and poor matching of disease phenotypes in

Abbreviations: ECM, Extracellular matrix; EGFR, Epidermal growth factor receptor; GvHD, Graft *versus* host disease; HLA, Human leukocyte antigen; HSC, hematopoietic stem cells; LAB, Laser-assisted bioprinting; NOD, Non-obese diabetic; NSG, NOD SCID gamma; PBMC, Peripheral blood mononuclear cells; SCID, Severe combined immune deficiency; SCC, squamous cell carcinoma; DMBA, 7,12-dimethyl[*a*]benzanthracene

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mice and humans. The last factor is more apparent in more complex human diseases that are difficult to mimic fully in a mouse model. A classic example is the amytrophic lateral sclerosis (ALS) mouse model with mutations in the RNA binding protein TDP-43 that was purported to display neurodegenerative traits similar to human ALS (Wegorzewska et al., 2009). Drugs developed and tested in human trials ultimately failed due to the model more closely representing gastrointestinal neurodegenerative disease than ALS (Hatzipetros et al., 2014; Perrin, 2014). This example highlights the need for scientists to proceed with caution and due diligence when using mouse models for disease modeling.

Comparison of human and mouse skin tissue reveals that significant species differences exist that are important to consider when using the mouse as a substitute for human physiology and disease (Gerber et al., 2014; Khavari, 2006). The skin comprises at least three specialized tissue compartments in both human and mouse: (i) The epithelial compartment that includes the stratified epidermis, hair follicles and sweat glands, consisting mainly of keratinocytes and home to the pigment producing melanocytes and resident specialist immune cells such as Langerhans cells and CD8+ T cells (Pasparakis et al., 2014). (ii) The underlying dermal layer, which is a complex network of extracellular matrix (ECM) with its own specialized cells and structures, including the mesenchymal fibroblasts, various immune cell subsets and both vascular and lymphatic structures. (iii) The subcutaneous adipose tissue, which contains mainly adipocytes but also fibroblasts, endothelial cells, and immune cells, and has now been recognized as being rich in stem cells with various regenerative capacities (Klar et al., 2017). A key difference between humans and mice is the detailed composition of these compartments and the functions that they deliver. For example, the most visually obvious difference is dense fur coverage on murine skin, with a hair cycle that undergoes a defined synchronous cycle of hair growth that is different from the asynchronous human hair growth cycle (Paus et al., 1999). Histologically, there are structural differences in the skin of the mouse compared to the human, such as a significantly thinner epidermis and dermis in mice, lack of sweat glands (except on the paws) and looser skin attachment (Fig. 1). Additionally, murine skin has a thinner subcutaneous adipose layer with a distinct panniculus carnosus, a thin skeletal muscle layer, below the adipose tissue. This layer is limited in humans to the platysma muscle of the neck. In relation to this, in terms of wound healing, unlike human skin which heals by re-epithelization, murine skin heals with a major contraction component (Wong et al., 2011). Moreover, other systemic differences between the human and mouse immune system exist that impact on the skin; these have been reviewed in great detail elsewhere (Mestas and Hughes, 2004; Pasparakis et al., 2014; Sellers, 2017). Due to these differences, the murine genomic response is unrepresentative of human inflammatory diseases (Seok et al., 2013). One clear example for skin is that the major T cell type found in human dermis is the conventional αβ T cells, however, in mice the γδ T cell predominates in the dermis (Bos et al., 1990; Cai et al., 2011; Pasparakis et al., 2014). Due to these highlighted differences between mouse and human skin, it is understandable that many transgenic animal models to study skin physiology or mimic skin diseases are unable to fully recapitulate the corresponding human condition.

As the outermost barrier under constant environmental exposure, it is perhaps not surprising that the skin is under a stronger evolutionary pressure than other tissues. This is reflected in the comparison of 30 tissue-specific gene sets between mouse and human, which found skin to be the one of the topmost evolutionally divergent tissues, along with the eye and testis (Monaco et al., 2015). Only 30% identity exists between human and mouse skin associated genes (Gerber et al., 2014). For example, epidermal growth factor receptor (EGFR) was found to be elevated in human skin compared to murine skin, leading the authors to postulate this underlies the lack of harmful side effects in mice in response to EGFR inhibition, unlike what was observed in humans. In relation to the abovementioned predominance of γδ T cells in mice, the authors also report Skint proteins, which have been linked to maintenance of γδ T cells, as being exclusive to mice (Gerber et al., 2014).

Given the concerns regarding the differences and deficiencies in traditional mouse model, efforts have been directed at tackling these problems by engineering “human-like” or “humanized” mouse models. These humanized mouse models express human transgenes or are engrafted with functional human cells or tissues. Instead of relying on mouse skin as a surrogate tissue to understand human responses, such models can more accurately reflect human physiology and better predict relevant clinical response. The scope of this chapter focuses on recent progress in the development of such humanized animals for use in skin research and is divided into 2 major approaches: (1) human skin xenografts and (2) human immune-responsive, humanized skin xenografts. While such complex animal models may be technically challenging, they provide great opportunities to study drugs, disease pathology and tissue differentiation with greater clinical relevance.

2. Human skin xenografts

Human skin xenografts were made possible in the early 1970s with the advent of immune-deficient mice, reducing host-graft rejection and thereby allowing grafted skin to potentially remain *in situ* for the lifetime of the mouse (Reed and Manning, 1973; Rygaard, 1974). Today there is a large number of immune-deficient mouse strains, substrains and gene-deficient transgenic models that are amenable as recipients for human skin xenografts; a number of these have now been assayed for this purpose. From the numerous strains and specific

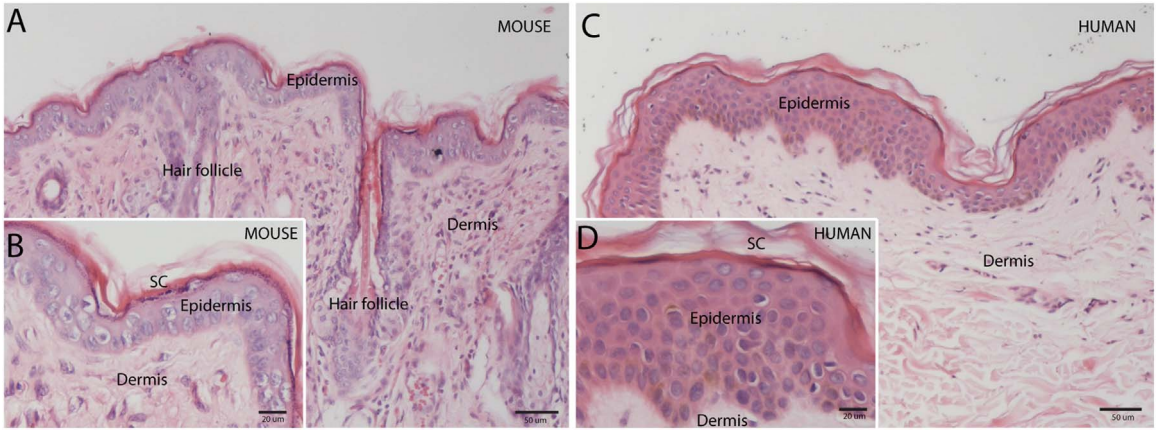

Fig. 1. Structural comparisons between mouse and human skin. Histology of the skin is shown at 10 × and inlay box 20 × magnification for (A, B) mouse and (C, D) human skin tissue. The epidermis and dermis is thicker in the human skin as compared to the mouse, and the mouse skin has the presence of a high density of hair follicles. SC = stratum corneum.