The humanization process of mice with various tissues named Chi-mice aimed to reproduce better the human situation and be more predictive than conventional models. In order to evaluate new targeting therapy adverse side effects involving skin components, pre-clinical studies need to be assayed with new molecules in advanced and validated mouse models bearing human skin. The collection of skin samples was done under ethically approved master agreements and with the signed consent of each patient. The patient’s clinical history, the serology results and tissue banking were centralized in our internal approved biological resource center. To develop such models, we used skin of different origins including flexion, breast and abdomen that were isolated by different methods (0.1 cm depth sample fully released, dermolethelium for 1.2 mm epidermal/dermal layer). These samples were xenografted on immune deficient NOD-SCID mice. After one month implantation, skin grafts were collected and observed macroscopically for the preservation of the human organization of skin by full histology. The specific nature of vessels was characterized by CD31 immunomarkings to evaluate the host penetration within human skin xenografts in mice. In the case of a vascular leak syndrome (VLS) induction by a three-step protocol, we used the wet/dry ratio of skin grafts to measure the induced edema and we have measured the Evans Blue uptake in these injured skin grafts to appreciate the tissue edematous leakage. Anatomopathological comparisons were also performed to reivew the histological modifications observed during VLS.

Animals

Healthy female NOD-SCID were obtained from Charles River (L’Arbresle, France). Animal experiments were performed according to ethical guidelines of animal experimentation10. All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon).

Skin xenograft validation

The female NOD-SCID mice were anesthetized and shaved on a 3 cm² diameter of surface on the right flank. The mouse skin was exchanged by a 0.5-1.0 cm² fresh human full-thickness or dermolethelium skin sample, sutured with 10/0 Vicryl®

Vascular leak syndrome validation

At day 30, the skin graft bearing mice were randomized in 2 groups of 4 mice according to their body weight. They were IP treated with vehicle (VEH) or recombinant human IL-8 50,000 µl/mice three times per day for 3 days (at day 0, 19th and 24th) and then once the fifth day at day 5 before protocol. The vascular leak of skin graft was evaluated by three different methods:

- Hematoxylin and eosin (H&E) staining: skin graft and mouse healthy skin from the other flank of the animal were collected with 4 mm diameter biopsy punch. Both samples were weighed before and after drying at 55 °C overnight. The water content was calculated as ratio of wet weight/ dry weight.
- Evans Blue measurement (vascular leaksize): 20 minutes before sacrifice, 100 µl of Evans Blue solution (5 mg/ml) in saline were intravenously (i.v) in each mouse. When skin graft was fully perfused, they were harvested in 200 µl of Sobeane 3000 overnight at 37°C then centrifuged at 4000 x g for 10 minutes. The Evans Blue content was read at 625 nm. The concentration of Evans Blue solution was estimated using a standard curve established with unstained material mixed with known Evans Blue amount.
- Histological analysis: a fragment of skin graft and mouse skin were fixed in formalin for 18-24h, then embedded in paraffin. One slide was issued per sample, stained with H&E and analyzed by histology for comparison between human and IL-2 treated conditions.

Results

Chi-mice® platform to establish new predictive models

The human skin used for xenograft, this skin originating from abdomen was dermolethelialized.

Skin graft on NOD-SCID mouse.

The amount of water was significantly increased in human skin grafts isolated from NOD-SCID mice compared to vehicle treated mice, no significant difference of water content was observed in skin grafts.

In skin grafts, the Vascular Leak Syndrome (VLS), a very harmful adverse effect induced by some drugs, could be detected and characterized through different parameters. Thus, skin graft might be a powerful predictive model to study the effects induced by new drugs. Moreover, this example shows that human skin preserves its physiological properties that might be characterized for other purposes.

Conclusions

We successfully developed and validated the human skin graft model which retained the human’s physiological properties. The co-implantation of other human tissues (liver, pancreas, bladder) on the same mice which better the humanized model is in course, considering the use of skin and various tissues in drug discovery and early preclinical development of new therapies aimed at corroborating results with clinical reality.

Moreover, beside the traditional xenografts, these processes from the clinical sample collection to the in vivo drug efficacy study through an in vivo assay should help the predictive drug selection, development and clinical positioning as well as companion biomarker identification.

1. ESTABLISHMENT AND CHARACTERIZATION OF HUMAN SKINGRAFT MODEL IN IMMUNODEFICIENT MICE

Guillaume Serin, Loic Morgand, Marie Leblanc, Francis Bichat - Oncodesign, France

# 2790

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