ESTABLISHMENT AND CHARACTERIZATION OF HUMAN SKINGRAFT MODEL IN IMMUNODEFICIENT MICE

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Introduction

The humanization process of mice with various tissues named Chi-mice aimed to reproduce better the human situation and to be more predictive than conventional models. In order to evaluate new targeting therapies or adverse side effects involving skin components, pre-clinical studies need to be assayed with new molecules in adequate 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 skins of different origins including foreskin, breast and abdomen that were isolated by different methods (1 cm depth sample fully recovered or dermatomized for the 1-2 mm epidermal/dermal layer). These samples were xenografted on immune deficient NOD-SCID mice. After one month implantation, skingrafts were collected and observed microscopically to confirm the preservation of human organization of skin by full histology. The species nature of vessels was characterized by CD31 immunohistochemistry to evaluate the host penetration within human skingraft. In the context of vascular leak syndrome (VLS) induction by IL2 treatment, we have used the wet/dry ratio of skingraft to measure the induced-edema and we have measured the evans blue uptake in those injured skingraft to appreciate the epidermal vasculature leaking. Anapathological comparisons were also performed to reveal the histological modifications observed during VLS.

Material and Methods

Animals

Healthy female NOD-SCID were obtained from Charles River (L'Arbresles, France). Animal experiments were performed according to ethical guidelines of animal experimentation[(1)]. 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 anaesthesized and shaved on a 3 cm^2 diameter of surface on the right flank. The mouse skin was exchanged by a 0.5-1.0 cm^2 fresh human full-thickness or dermatomized skin sample, sutured with Ti-cron 6.0.

After one month, the mice were sacrificed and skin xenografts were collected, together with adjacent mouse skin. Samples were fixed in formalin for 18-24h and then embedded in paraffin. One section of mouse/human junction was stained with haematoxylin and eosin (HE) for histology. Other sections of mouse only, human only or xenograft skin were stained with CD31 species specific antibodies (for human :clone A10, Novocastra, for mouse: MEC13.3, Biolegend) through a standard IHC protocol.

Vascular leak syndrome validation

At D30, the skingraft 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 IL2 at 50 000 U / mouse three times *per* day for 3 consecutive days (at 8h, 16h and 24h) then once the fourth day (at 8h) before sacrifice.
The vascular leak of human skingraft was evaluated by three different methods:

- Wet / dry tissue weight ratio (oedema status): skingraft and mouse healthy skin from the other flank of the animal were collected with 4 mm diameter biopsy punches. Both samples were weighed before and after drying at 55°C overnight. The water content was calculated as ratio of wet weight over dry weight.
- Evans Blue measurement (vascular leakage): 20 minutes before sacrifice, 100 µl of Evans Blue solution (5 mg/ml in saline) were IV injected via the caudal vein on 4 mice per group. After wet and dry weight measurement, they were solubilized in 200 µl of Soluene 3500 overnight at 37°C then completed with 400 µl of Ethyl Acetate and 400 µl of HCl 1N, vortexed and centrifuged. The OD from upper phase was read at 625 nm. Concentration of Evans Blue solution was estimated using a standard curve established with unstained material spiked with known amount of Evans Blue.
- Histological analysis: a fragment of skingraft and mouse skin were fixed in formalin for 18-24h, then embedded in paraffin. One slide was issued per sample, stained with HE-Safran and analyzed by histology for comparison between vehicle and IL2 treated conditions.

[(1)]Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988, Directive 2010/63/EU.

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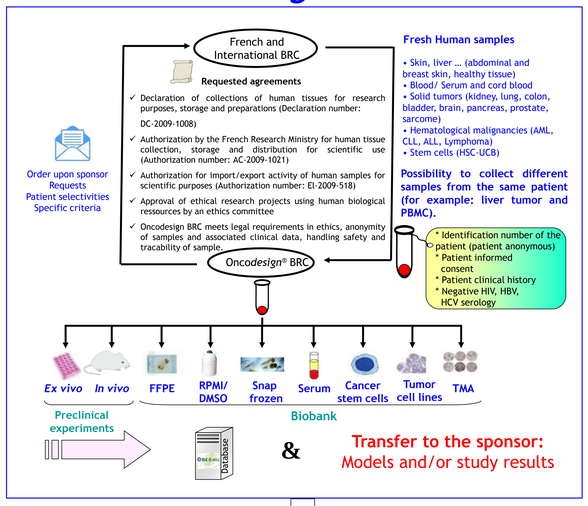
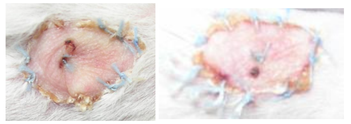
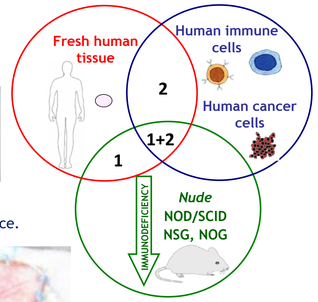


Chi-mice® platform to establish new predictive models

Human skin used for xenograft. This skin originating from abdomen was dermatomized.

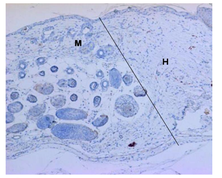
Human skin graft on NOD-SCID mice.





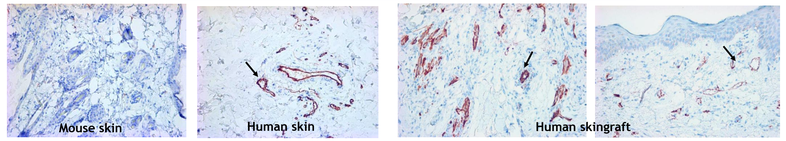
Results

Skingraft histology

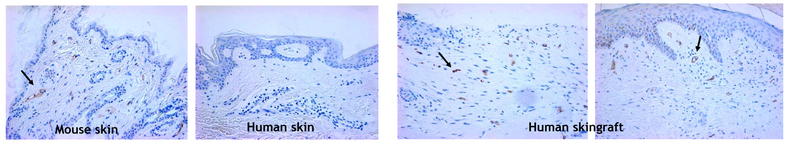


Cross-section of junction between human skin (H) and mouse skin (M) : histological analysis showed a well differenciated and healthy human skingraft in mouse.

Characterization of human skingraft vascularization



→ Human anti-CD31 IHC: human vessels are localized only in human tissue with a marked staining in the center of the graft

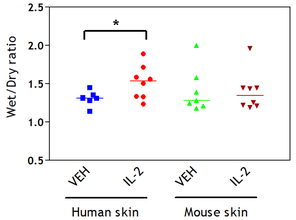


→ Mouse anti-CD31 IHC: mouse vessels are localized in human tissue, mostly at the periphery of the graft

Human vasculature is preserved within the skingraft, co-existing with invasion of host vasculature.

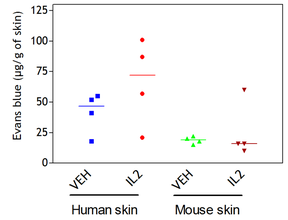
Vascular Leak Syndrom study

Wet/Dry ratio



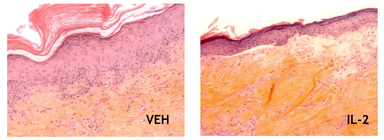
The amount of water was significantly increased in human skingrafts isolated from IL2 treated compared to vehicle treated mice. No significant difference of water content was observed in skin mice.

Evans Blue accumulation



The accumulation of Evans blue was increased in human skingrafts isolated from IL2 treated compared to vehicle treated mice. No significant difference of Evans blue accumulation was observed in skin mice.

Histology analysis



We observed an increase of the ulceration epidermis in IL2 versus vehicle treated mice.
No significant difference was observed at the microscopic level in dermis and epidermis for other lesions (spongiosis, hyperkeratosis, number of vacuolated basal cells, inflammation, oedema and vascularisation).

In human skingraft, the Vascular Leak Syndrom (VLS), a very harmful adverse effect induced by some drugs, could be detected and characterized through different parameters. Thus, skingraft might be a powerful pre-clinical tool to identify and to study the VLS 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 skingraft model mice that retained the human characteristics of the primary material.
- The co-implantation of other human tissue (tumor) with skin on same mice which refined better the humanized model is in course, considering HLA matching.
- The use of fresh skin and various tissues in drug discovery and early preclinical development of new therapies aimed at corroborating results with clinical reality.
- Altogether, these processes from the clinical sample collection to the *in vivo* drug efficacy study through *ex vivo* assays should help the preclinical drug selection, development and clinical positioning as well as companion biomarker identification.