First reconstitution of an epidermis from human embryonic stem cells

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Stem cell research is making great strides. This is yet again illustrated by a study carried out by the I-STEM Institute (France), published in the *Lancet* on 21 November 2009. The I-STEM team, directed by Marc Peschanski has succeeded in recreating a whole epidermis from human embryonic stem cells.

The goal is to one day be able to propose this unlimited resource of cells as an alternative treatment in particular for victims of third degree burns and patients with genetic diseases affecting the skin. These studies were financed in particular by Telethon donations.

Cell therapy has radically changed the life of serious burn victims. For more than two decades, physicians have used cell culture techniques in order to obtain a sufficiently large area of skin to reconstruct the destroyed epidermis from a small sample harvested from the patients themselves.

Although this type of graft has been used with success, one of its limits is the time required (three weeks) to produce a sufficient amount of epidermis to cover the affected areas, leaving the patient unprotected during this interval. For a few years, research has led to the development of skin substitutes that help protect patients during the period before grafting. However, these technological means do not rule out the risk of immune rejection and transmission of disease.

Rapid access to an unlimited number of cells capable of yielding a well-

formed epidermis, perfectly controlled in the laboratory before use, would therefore be the ideal solution to the problems posed by existing techniques. For these reasons the I-STEM researchers attempted to reconstruct a whole epidermis using human embryonic stem cells.

A protocol in several stages

Human embryonic stem cells (hES) have two fundamental characteristics: a capacity for unlimited proliferation and pluripotency i.e. the capacity to differentiate into all the cell types in the human body.

The first objective of the team was to obtain skin stem cells (keratinocytes) similar to those naturally present in the human epidermis from hES cells. Keratinocytes, permit the constant renewal of the skin.

Once this stage was achieved, the second objective of the researchers consisted in finalising strategies to isolate keratinocyte stem cells in order to test their capacity to reconstitute a functional epidermis firstly in vitro - then in vivo. "It is these cells that interest us as they are the only cells capable of recreating all the layers of the human epidermis" pointed out Christine Baldeschi.

The transformation of hES cells into epidermal cells was made possible by a combination of cell biology and pharmacological approaches . A "cell niche" was first created around the ES cells to guide them towards an epidermal destiny and an appropriate pharmacological agent was then added to the culture medium. The researchers decided to maintain this treatment for the 40 days that is normally required for an embryo to form its epidermis. By applying this concept of respecting chronobiology, the hES cells engaged in this differentiation process acquired first the markers of a simple epithelium and then finally those of keratinocytes. A population of cells presenting all the characteristic marker of adult keratinocytes was isolated and then amplified. It is

without doubt by maintaining this treatment for 40 days that the I-STEM team succeeded where many others had failed.

Thanks to the complementarity of the research teams working at I-STEM and the sharing of means, the researchers succeeded in rebuilding in vitro a functional epidermis in which keratinocyte stem cells have all the qualities required for satisfactory function (self-renewal, stratification and final differentiation properties). It remained to be shown if these results obtained in vitro could be confirmed in vivo. The final stage therefore consisted in reproducing this protocol in the mouse. For these experiments, I-STEM collaborated with a Spanish research team specialised in the use of such grafts in animals with a weakened immune system to overcome potential graft rejection. Twelve weeks after transplantation, the mice presented localized areas of completely normal and functional adult human epidermis containing all the skin cell types. "Our team is currently the only one to have succeeded in finalising a protocol making it possible to transform human embryonic stem cells into a pure and homogeneous population of keratinocytes able to reconstitute a whole epidermis both in vitro and in vivo." concluded Marc Peschanski, director of I-STEM.

For the researchers, there are numerous future applications of this work. These "ready-to-use" cells will be proposed to produce epidermal cells for the treatment of third degree burn victims and also other skin diseases such as genodermatoses or ulcerations which complicate diabetes in a very large number of patients. "For more than 25 years we have known how to make epidermis with skin stem cells and skin grafts are made in particular for victims of third degree burns. The keratinocytes obtained from human embryonic stem cells therefore have an immediate clinical application. We are now therefore seeking how, in concrete terms, to pass to man." added finally Christine Baldeschi, leader of the team performing this study.

Research on human embryonic stem cells is at the centre of the debate on the bioethics laws. Since the bioethics law of 2004, this research is authorized by special dispensation in exceptional cases after submitting protocols to the Biomedecine Agency for an opinion. The I-STEM team is one of the 28 French teams to have received an authorisation to conduct research on these cells.

More information: "Human embryonic stem cells derivatives enable full reconstruction of the pluristratified epidermis." *The Lancet*, volume 374, number 9703, 21 November 2009

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