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Review

Capturing epidermal stemness for regenerative medicine

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ABSTRACT

The skin is privileged because several skin-derived stem cells (epithelial stem cells from epidermis and its appendages, mesenchymal stem cells from dermis and subcutis, melanocyte stem cells) can be efficiently captured for therapeutic use. Main indications remain the permanent coverage of extensive third degree burns and healing of chronic cutaneous wounds, but recent advances in gene therapy technology open the door to the treatment of disabling inherited skin diseases with genetically corrected keratinocyte stem cells. Therapeutic skin stem cells that were initially cultured in research or hospital laboratories must be produced according strict regulatory guidelines, which ensure patients and medical teams that the medicinal cell products are safe, of constant quality and manufactured according to state-of-the art technology. Nonetheless, it does not warrant clinical efficacy and permanent engraftment of autologous stem cells remains variable. There are many challenges ahead to improve efficacy among which to keep telomere-dependent senescence and telomere-independent senescence (clonal conversion) to a minimum in cell culture and to understand the cellular and molecular mechanisms implicated in engraftment. Finally, medicinal stem cells are expansive to produce and reimbursement of costs by health insurances is a major concern in many countries.

Contents

1. Introduction

Stem cells are instrumental for renewal, regeneration and repair, and hold great expectations for disease modeling, drug discovery and regenerative medicine. Transplantation of bone marrow derived stem cells (hematopoietic or mesenchymal) is part of the therapeutic arsenal to treat hematopoietic diseases and a variety of disabling conditions [1], including inherited skin diseases [2]. Stem cells or stem cell-derived products are also used in dermatology and reconstructive surgery. Furthermore, stem cell therapy is often regarded as the therapy of the future for diabetes, cardiac and neurological diseases. But it is necessary to capture stem cells before they are used in regenerative medicine and it is worth emphasizing that some cells display stem cell capabilities only when challenged by tissue repair and regeneration, stress or cell culture [3]. For instance, the pluripotent cells of the inner cell mass only undergo a few round of divisions in blastocyst but their pluripotency can be captured in cell culture to generate embryonic stem cells that

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can indefinitely self renew under appropriate conditions [4]. Similarly, cardiac stem cells can be captured from normal or diseased mammalian heart [5–7] and expanded in culture for therapy [8]. To the opposite, hematopoietic stem cells are directly captured from the donor marrow or from the blood stream after mobilization from their niche, and used without or little ex vivo expansion. In any case, long-term therapeutic success is achieved only if the transplanted adult stem cells can permanently engraft, self-renew and properly perform the function for which they are specified. Maintenance of stem cell specification in cell culture is thus critical for a successful cell therapy. Yet stem cell specification can be manipulated in culture in a process termed reprogramming [3] that consists of removing cells from their natural microenvironment and exposing them to a proper combination of transcription factors [9–11], small molecules [12] or even to a completely different microenvironment [13]. Reprogramming of skin cells (fibroblasts, keratinocytes) to generate induced pluripotent stem (iPS) cell is now a common procedure useful for disease modeling, drug testing or to generate differentiated cells other than skin.

Stem cells do not mean the same if you are a scientist, a physician or a patient. Obviously, scientists think self-renewal and potency, symmetric and asymmetric divisions, fate and niches, growth factors and small molecules, molecular markers and signalization, ex vivo expansion and banking [14] whereas physicians think successful therapy of diseases [15] and patients hope for a better health. But stem cells have acquired another dimension besides the scientific and medical breakthroughs as they are expected to create jobs and wealth. Hence, stem cell research and its clinical outcome are under close scrutiny from politicians, the media, regulatory affairs and health insurances as well as from the biotechnology and pharmaceutical industry [16]. The story on how keratinocyte stem cells made it from bench to bedside is a perfect illustration.

2. A short stem cell story

In October 1983, one of us (YB) joined the laboratory of Professor Howard Green at Harvard Medical School as a post-doctoral fellow. It was an exciting and amazing time in the Green laboratory as the first cultured epidermal autografts (CEAs) were prepared to treat two young brothers with extensive third degree burn wounds covering over 90% of their body [17]. The areas to repair with the cultured cells were extremely large, nothing to compare with a small transplant onto the back of a mouse or a human arm as it had already been done by the laboratory [17,18]. To permanently restore a functional epidermal barrier with autologous cultured keratinocytes transplanted on burn wounds excised to muscular fascia with the ultimate hope to save the patients life was a major challenge both in term of biology and medicine. Many questions were opened: can massive expansion of human keratinocytes from a small skin biopsy harvested in an unburned area be achieved in a minimal time and in an emergency context? Can patients be maintained alive during the time of preparation of cultured cells? Can cultured keratinocytes permanently engraft, form an epidermis and restore the skin barrier function? All lab members were aware that the life of two young children was at stake and that Howard Green's decision was based on his faith that cultures of human epidermal keratinocytes contained stem cells [19]. Indeed, the notion that cultured keratinocytes could be useful for cell therapy was first stated in the conclusion of the Rheinwald and Green's seminal paper [19]. Follow-up experiments performed in the Green laboratory had then demonstrated that cultured human keratinocytes transplanted onto athymic mice could generate an epidermis [18] and were able to heal small burn wounds [20]. Nonetheless, moving from bench to bedside overnight was certainly a jump in the unknown but medical breakthroughs often happen in circumstances in which there is no alternative than pushing the limits to save a patient's life. The most emotional time was at the first dressing, usually a week after CEA transplantation (take down). The entire laboratory eagerly waited for news from the hospital; how were the patients doing? Did the cultures engraft? Sometimes news were good and greeted with joy, other times there were bad and everyone was sad with a feeling of catastrophe. Finally, the young brothers went out of intensive care. Several months later, Howard Green and YB visited the children at the Schriners Burn Hospital and found them playing with a basketball in the corridor of the hospital ward. This was an amazing moment, full of emotion. The kids were severely handicapped and surely not at the end of suffering, but they were full of life with twinkles of fun in their eyes. Undoubtedly, CEA had contributed to save the children life and from here, it was evident that the transplanted CEA had to contain stem cells. This was how adult keratinocyte stem cells were captured for the first time ever for therapeutic purposes. From here, several other burn children were treated and the Green lab turned into a cell factory, mixing production of CEA, academic research, media exposure and training of colleagues from all over the world. Unknowingly, the Green lab was bringing cultured adult stem cells from bench to bedside and experiencing translational medicine [21]. Yet, the goal of an academic laboratory was to perform experiments and not to be a cell factory. In 1986, the cell culture technology was transferred to a start-up company, Biosurface Technology Inc., located in Kendall Square in Cambridge (Massachusetts) within a stone's throw from MIT where Howard Green had started his research on cultured keratinocytes in the seventies. With this move, CEA became a product and the scientists discovered that it was another ball game ruled by the FDA (Food and Drug Administration), the health insurances and the amount of cash in the bank. Biosurface Technology Inc. eventually became part of Genzyme (Sanofi). Thirty years later, little has changed and the Rheinwald and Green culture system remains the gold standard and most, if not all, commercial companies providing the service of culturing stem cells for the treatment of extensive third degree burns still rely on it (e.g. Genzyme, Cambridge, MA, USA; Tego Science, Seoul, South Korea; J-TEC, Aichi Japan; Holostem Terapie Avanzate, Modena, Italy).

3. Capturing stemness in human skin

Considerable progress in understanding the biology of skin stem cells has been accomplished in the mouse and there are excellent recent reviews that describe these advances [22,23]. Lineage tracing experiments and fonctional skin reconstitution assays in the mouse have unambiguously demonstrated that the interfollicular epidermis [24–26], the upper constant region of pelage hair follicles [27–29] and the ducts of sweat glands of the foot pad [30] contain cells with stem cells properties. These stem cells are multipotent and clonogenic [27,29], slow-cycling [31,32], or express cell surface proteins like Lgr5 [33] and Lgr6 [34], CD34 [35], Plet1 (MTS24) [36] and Lrig1 [37]. Similarly, multipotent stem cells have been identified in whisker follicles of the mouse and the rat by clonal analysis and serial transplantation [38,39]. Furthermore, the classical scheme of epidermal renewal based on a hierarchy of slow-cycling stem cells and rapidly cycling transient amplifying cells [40] is a matter of discussion with opposing opinions [24–26]. But how much of this knowledge can be translated in human stem cell therapy? A pessimistic but fair answer is very little, and the clinical situation is pretty much the same to day that it was thirty years ago. There are several reasons for that, first stem cell markers described in the mouse do not apply to human, second label retaining, lineage tracing or genetic manipulation experiments cannot be performed in human for obvious ethical reasons and third clonogenic assays remains the sole reliable read-out to capture stemness in human epidermis or epidermal appendages [41–44]. Ultimately a human keratinocyte is considered a stem cell if it forms a colony that can