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Generation of functional organs from stem cells

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Abstract

We are now well entering the exciting era of stem cells. Potential stem cell therapy holds great promise for the treatment of many diseases such as stroke, traumatic brain injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral-sclerosis, myocardial infarction, muscular dystrophy, diabetes, and etc.. It is generally believed that transplantation of specific stem cells into the injured tissue to replace the lost cells is an effective way to repair the tissue. In fact, organ transplantation has been successfully practiced in clinics for liver or kidney failure. However, the severe shortage of donor organs has been a major obstacle for the expansion of organ transplantation programs. Toward that direction, generation of transplantable organs using stem cells is a desirable approach for organ replacement and would be of great interest for both basic and clinical scientists. Here we review recent progress in the field of organ generation using various methods including single adult tissue stem cells, a blastocyst complementation system, tissue decellularization/recellularization and a combination of stem cells and tissue engineering.

Keywords: Stem cells, Functional organs, Blastocyst complementation, Decellularization, Recellularization, Tissue engineering

Introduction

Stem cells are undifferentiated cells found in the body which have the ability to continuously divide, self-renew themselves and differentiate into various kinds of cells. With the capability of self-renewal, pluripotency and differentiation, stem cells have been believed to be useful for treatment of a wide variety of diseases in the future, including stroke, traumatic brain injury, Alzheimer's disease, Parkinson's disease, spinal cord injury, baldness, blindness, deafness, wound healing, amyotrophic lateral-sclerosis, myocardial infarction, muscular dystrophy, osteoarthritis rheumatoid arthritis, Crohn's disease, and diabetes. Amongst the applications, a number of adult stem cell therapies have already been practiced clinically. As an example, hematopoietic stem cell transplantation has been successfully applied to treat leukemia.

In addition to cell replacement therapy using stem cells, organ transplantation has been successfully practiced in clinics for organ failure of the liver or kidney. However, the severe shortage of donor organs has become the main obstacle to expand the organ transplant program. Generation of biological or semi-biological organs could be an alternative approach to solve the problem of the donor organ shortage. Notably, researchers have been hunting for ways to establish a whole organ using stem cells.

In recent years encouraging approaches for functional organ generation have emerged. The present manuscript provides an overview of organ generation using a single adult tissue stem cell, a blastocyst complementation system coupled with a specific stem cell niche, a method of decellularization and recellularization of bio-scaffold, and a combinatorial approach of tissue engineering and stem cells.

Generation of a functional organ from a single adult tissue stem cell

To demonstrate whether there are true stem cells in a given tissue, one needs to show that a single stem cell purified from the tissue has the capability of generating the entire organ. Up to present, it has been elegantly shown by independent groups that the mammary gland and the prostate can be generated in vivo from a single adult tissue stem cell [1-3].

Two laboratories have reported independently that single stem cells isolated from adult mouse mammary glands are able to produce secretory mammary glands

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when they are transplanted in the fat-pad in mice [1,2]. It is long believed that there are stem cells in the mammary glands because this organ has the capability of undergoing an extensive growth at puberty and a second phase of expansion and retraction during pregnancy under the regulation of estrogen [4]. However, due to the lack of defined markers, there has been no reliable method to isolate mammary stem cells. This hypothesis was not proven until the work by the two groups. Based on some previous work by Clark and colleague [5], Shackleton et al. isolated putative mouse mammary stem cells using specific cell-surface markers (Lin^-, $CD29^{hi}$, $CD24^+$) by FACS. They demonstrated these $Lin^-CD29^{hi}CD24^+$ mammary cells have *in vitro* sphere formation capability and the ability to repopulate all mammary epithelial cells after transplantation into the fat-pad. Importantly, the investigators employed a lineage-tracer in the stem cells so to follow their ultimate phenotypes *in vivo*. They showed elegantly that a single cell within the $Lin^-CD29^{hi}CD24^+$ population of the mouse mammary gland, marked with a LacZ report transgene, can reconstitute a completely functional mammary gland *in vivo*. Notably, the transplanted cell contributes to both the luminal and myoepithelial lineages and generates milk-producing lobuloalveolar units during pregnancy. This is the first report to demonstrate that a single adult tissue stem cell has multi-lineage differentiation capacity to produce a functional organ in an *in vivo* setting.

Similarly, using a colony-formation *in vivo* assay and an *in vivo* renal capsule transplantation approach, Gao and his colleagues have also reported that a single stem cell isolated from the adult mouse prostate epithelium has the capacity to generate a functional prostate [3]. Actually, prostate stem cells were postulated to exist because of its regenerative feature following androgen deprivation and replacement more than twenty years ago [6]. Because cell death mainly occurs in the luminal cell compartment after castration and cell proliferation mainly happens in the basal compartment following androgen replacement, stem cells are generally believed to reside in basal cell compartment and are able to repopulate the entire prostate epithelial cells.

First, this group identified a new marker for prostate stem cells, CD117, based on the following 6 features: 1) It is enriched in proximal region of the prostate; 2) It is mainly expressed in basal cell population; 3) It is upregulated after castration and returned to normal levels following androgen replacement; 4) Only $CD117^+$ cells, but not the $CD117^-$ cells, can form colony structures with lumen *in vitro*; 5) It can generate prostate epithelial glandular structures *in vivo*; 6) CD117+ cells from 2nd and 3rd generation grafts also have the self-renewal capability.

To demonstrate whether a single stem cell has the ability to generate a prostate structure, the investigators first further enriched the stem cell population by sorting the cells expressing multiple stem cell markers, $Sca\text{-}1^+CD133^+CD44^+CD117^+$, into individual wells of a 96-well plate, verified them under a microscope, and transplanted them in a combination with rat embryonic urogenital sinus mesenchymal cells (rUGM) under the renal capsule of athymic nu/nu mouse hosts. Three months later, they removed the kidneys and analyzed the fate of the grafted cells. Of the 97 single-cell transplants, there were about 1/7 $Sca\text{-}1^+CD133^+CD44^+CD117^+$ grafts that demonstrate a branching pattern with epithelial tubules composed of prostatic basal, luminal and neuroendocrine lineages by the histological and immunocytochemical examination.

The generation of mammary gland and prostate acini from single stem cell implants in mice is a major breakthrough. These findings raise the possibility that people who have lost their mammary glands or prostates due to cancer could grow new ones. On the other hand, it is believed that in some tissues, there are cancer stem cells that are tumor-initiating cells. Cancer stem cells might be derived from normal stem cells in which specific tumor suppressor genes are mutated or lost [7]. Therefore, although the prostate glands were grown in mice but the research may aid the identification of markers for cancer stem cells, which may help diagnosis and more efficient treatment of prostate cancer in humans. These studies on reconstruction of mammary and prostate glands using single adult stem cells have important ramifications not only for tissue repair/regeneration, but also for identification of mammary and prostate cancer stem cells. In other words, we envision that the potential use of single stem cells in clinic will ultimately change the treatment paradigm for human disorders more than mammary gland injury and prostatic disease.

Generation of organs using a blastocyst complementation system

In addition to single stem cells prepared from specific adult tissue, embryonic stem cells (ESCs) have been shown to be able to produce specific organs by using a strategy of injection of ESCs from one species into the blastocyst of another species. The development of a specific organ can be precluded by genetic manipulation in the recipient species but still providing a niche for organ development. The pluripotent stem cell-derived cells from the donor species would then developmentally compensate for the defect and produce the missing organ.

The blastocyst complementation system was first reported by Chen et al. by implanting normal mouse embryonic stem cells (mESCs) into the blastocysts derived from $Rag2^{-/-}$ mice to generate T and B lymphocyte lineages [8]. In recent years, this system was applied to generate pluripotent stem cell (PSC)–derived donor organs *in vivo* due to the fact that complex cellular