The Libyan International Medical University

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Save the Tooth Fairy Package – Regenerate Mammary Glands

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Abstract

It has been shown that dental epithelial stem cells (DESCs) are able to generate all epithelial cell populations within incisors during homeostasis. Nevertheless, it has not been clear whether these cells possess the capability to transform into other structures. The plasticity of DESCs was assessed in the context of mammary gland regeneration. The transplantation of DESCs together with mammary epithelial cells into the mammary fat pads resulted in the formation of chimeric ductal epithelial structures in which DESCs adopted all the possible mammary fates including milk-producing alveolar cells. To add onto that, having DESCs inoculated without mammary epithelial cells resulted in the development of small ducts and cysts. This finding could open doors to a new form of reconstructive treatment for breast cancer patients.

Introduction

It is widely known that mastectomy is one of the procedures used to treat breast cancer, a type of cancer known to be the most common in women. Over the years, patients have been offered to undergo breast reconstruction to create their breast contour subsequent to mastectomies. Breast reconstruction surgery is the creation of a new breast shape, or mound, using surgery. It may be done after removal of a whole breast (mastectomy) or part of the breast (breast-conserving) and it usually involves several operations to give you the best outcome possible. Choosing whether or not to have breast reconstruction is a very personal decision. Some women feel reconstruction is necessary to restore their confidence, while others prefer to wear an external breast form; and some women choose not to have reconstruction surgery nor wear a prosthesis.

Various techniques are currently being used for breast reconstructive surgery, with silicone implants and latissimus dorsi flap being the most popular amongst patients. While both of these remain to be, more or less, sound options, the latter has been proven to have an impact on shoulder function, making it less convenient for individuals who are active [1], whereas the U.S. Food and Drug Administration identified a possible association between the former and the development of Anaplastic Large Cell Lymphoma (ALCL)[2]. Recent studies have shown that stem cells of the teeth can contribute to the regeneration of non-dental organs, namely mammary glands. This finding could consequently support post-surgery tissue regeneration in breast cancer patients and so offering them an alternative choice.

In-vivo cell transplantation assays have been commonly used to identify epithelial stem cells and assess their plasticity during tissue regeneration. Mammary gland regeneration is one of the most commonly used reconstitution assays, where mammary gland-derived epithelial fragments are transplanted into an epithelium-free mammary mesenchyme for de novo generation of functional ductal epithelial structures. Previous studies using the mammary reconstitution assay have shown that neuronal[3], testicular[4], bone marrow[5] and cancer cells mixed together with mammary epithelial cells (MECs) can be reprogrammed and integrate into the epithelial ductal outgrowths. However, these cells were never found to possess the ability to generate mammary glands without the support of MECs, whereas dental epithelial cells (DECs) can do so. Therefore, for the first time ever, it has been proven that a non-mammary epithelial cell can regenerate mammary glands, with or without MECs.[6]

Methods and Material

2.1. Cell Isolation

DESCs were isolated from the cervical loop of incisors of mice. The cells were then cultured in collagen-coated plates in Dulbecco's Modified Eagle Medium containing penicillin/streptomycin, epidermal growth factor, and basic fibroblast growth factor. Mouse MECs were also isolated. A treatment with trypsin and DNase followed by filtration through a 40 μm cell strainer was performed in order to get single epithelial cells. Briefly, mammary glands from eight week-old female mice were manually minced and enzymatically digested. The resulting MECs were plated in collagen-coated plates and infected with a lentivirus expressing DsRed prior to the in vivo mammary transplantation assay.[6]

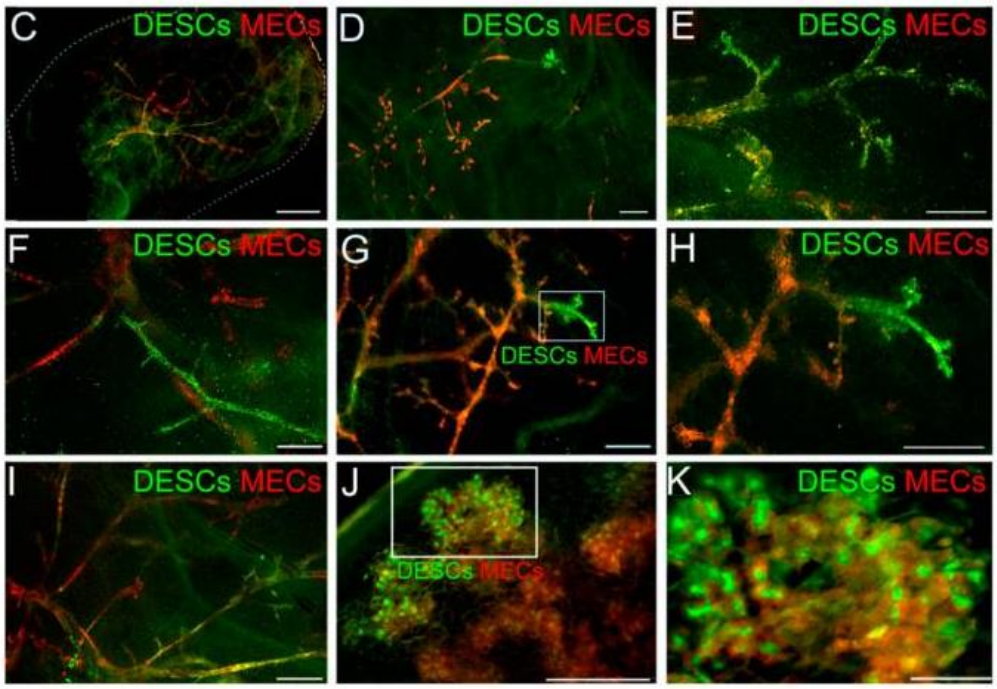
2.2. Animals and Surgical Procedures

Immunocompromised mice were used as hosts for the mammary transplantation experiments. Briefly, mammary fat pad containing endogenous epithelium (from the lymph node to the nipple area) was removed from the fat pads of the mammary glands. Cells were then injected in the remaining fat pads of mammary glands using a Hamilton syringe. The mammary fat pads from virgin host mice were dissected eight weeks after cell injection. At this time point, some of the mice were mated and analyzed at pregnancy day sixteen. Mammary glands were then fixed for thirty minutes in paraformaldehyde 4% at room temperature and processed for paraffin embedding.

The DESCs were mixed with MECs and then subsequently injected into epithelium-free mammary fat pads of immunocompromised mice. The fate of cells derived from these two cell populations was tracked by green fluorescent protein expression for DESCs and lentivirus-induced DsRed fluorescent protein expression for MECs.[6]

Results

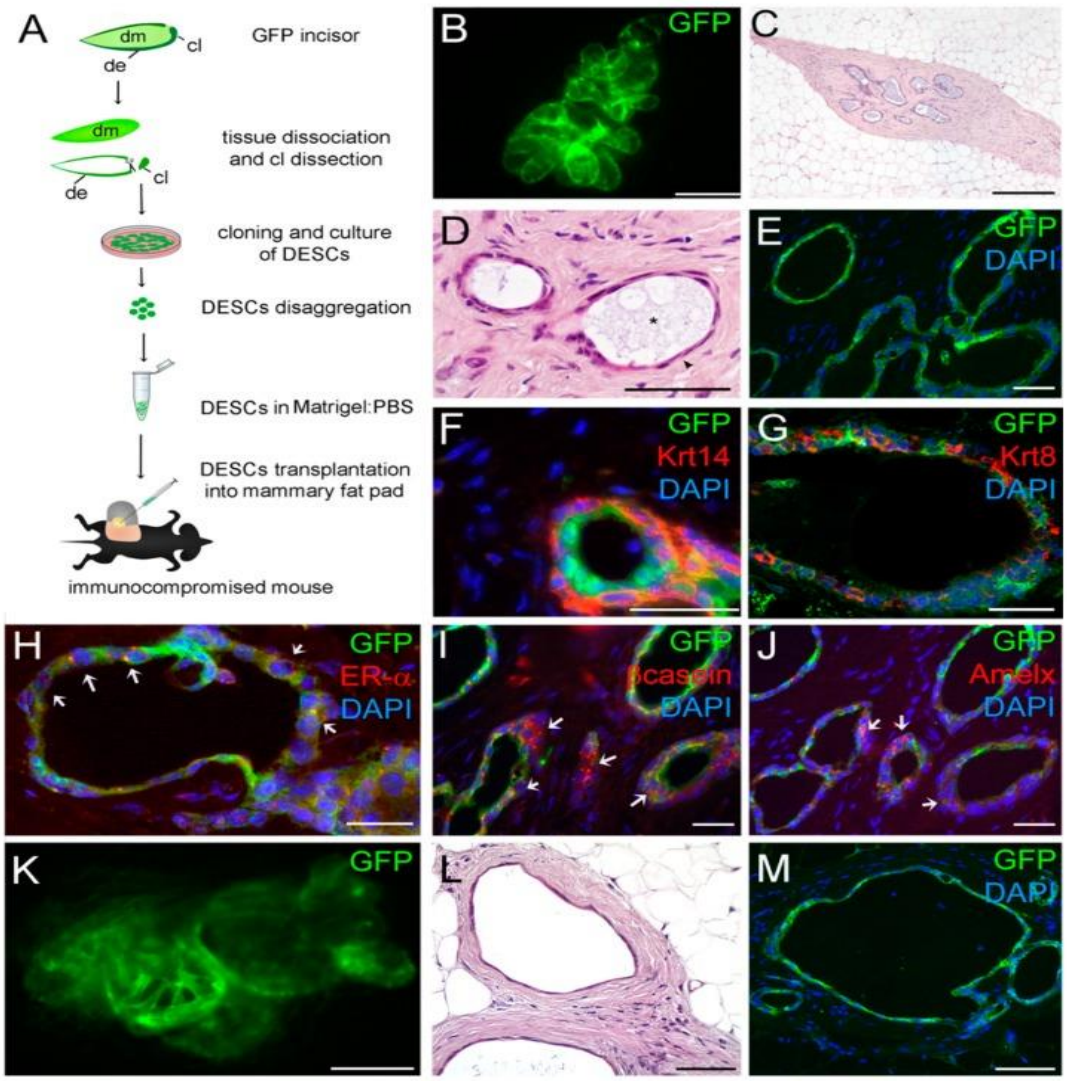
DESCs and MECs cells formed chimeric ductal structures composed by GFP-positive DESCs-derived cells and DsRed-positive MECs in mammary glands analysed eight weeks post-transplantation (figure 1).[6]



(Figure 1)

DESCs-derived cells accounted for approximately 20% of the cells composing the epithelial compartment of the chimeric mammary ducts. Mammary luminal epithelium is complex and composed by various cell populations, grouped in two main subsets named ductal and alveolar cells. Ductal cells are lining the epithelial ducts and among them, oestrogen receptor alpha (ERα) expressing cells are responsible for the activation of the paracrine signalling that is essential for mammary epithelium elongation upon exposure to pubertal oestrogens. On the other hand, alveolar cells constitute the milk-secreting alveolar units that arise during late pregnancy.

It has then been assessed whether DESCs possess the plasticity and reprogramming competence to regenerate ducts in absence of mammary epithelium. For this purpose, the previously described mammary reconstitution assays was performed in absence of MECs by injecting only DESCs into the fat pads. Analysis revealed the formation of GFP-expressing small branched epithelial structures surrounded by a dense fibrotic tissue (figure 2).[6]



(Figure 2)

In some cases, the generated structures were dilated and adopted a cystic appearance (Figure 3K–M), characterized by flattened epithelial cells. These cystic structures were observed in more than 80% of the glands in which only DESCs were engrafted. In association with DESCs transplantation, the formation of dense fibrotic tissue was frequently observed. Fibrosis was detected in a subset of mammary fat pads transplanted with mixed MECs/DESCs, and in all fat pads inoculated with DESCs alone.

Discussion

This study has initially aimed to assess the plasticity of DESCs and whether they had the unique ability to regenerate non-dental organs, specifically mammary glands. When mixed with MECs and incorporated in the mammary gland fat pad, DESCs have been found to be capable of being reprogrammed to a mammary epithelial phenotype, giving rise to a variety

of cell types that constitute the mammary epithelium, inclusive of milk-producing cells. Furthermore, it has been demonstrated that DESCs solely injected can result in the formation of a ductal branching morphogenesis without inputs from mammary epithelium. Previous studies using the mammary reconstitution assay have shown that neuronal, testicular, bone marrow and cancer cells mixed together with MECs can be reprogrammed and integrate into the epithelial ductal outgrowths. However, non-mammary epithelial cells have never showed the ability to grow in fat pads without the support of MECs and so there was a lack of ductal system formation.

As teeth structures and mammary glands belong to the class of ectodermal appendages where they develop through continuous and reciprocal epithelial-mesenchymal interactions, both organs share many morphological similarities during initial stages of development. During those stages, their development depend on the same molecular cues, mostly associated to transforming growth factor β (Tgf-β), fibroblast growth factor (Fgf) and Wnt signalling pathways. At subsequent stages, their development diverges and undergo distinct morphogenesis. Among the signaling pathways involved in mammary gland morphogenesis, the FGF pathway is required from the first phases of mammary gland development and throughout ductal elongation and branching. FGF-10 regulates branch initiation while FGF-2 controls ductal elongation and thus different FGF ligands have different effects during mammary gland development, controlling distinct aspects of ducts formation. Likewise, FGF signalling is a vital trigger of tooth development. While FGF-10 and FGF-3 are not involved in tooth initiation as they are in mammary gland initiation, they regulate tooth morphogenesis and are important for the maintenance of DESCs. DESCs are, thus, responsive to FGF-10 and FGF-3, while no evidence supports any effect of FGF-2 onto these cells. Therefore, while it has been proven for the first time that non-mammary epithelial cells can develop into ductal outgrowths without the use MECs, DESCs might not be able to respond to FGF-2-mediated proliferative stimuli and, thus, fail to give rise to full-sized ducts. That being the case, their transplantation alone could propel the formation of smaller ducts compared to the transplantation of a mixture of DESCs and MECs.

Another drawback displayed by the injection of DESCs alone is the activation of ERα expression. ERα-expressing cells are responsible for the activation of the paracrine signalling that is critical for mammary epithelium elongation, and ERα knock-out mice display severely impaired ductal elongation. Since ERα is expressed at low levels in DESCs, the ductal system

that appeared from DESCs alone had a very limited amount of ERα making them poorly responsive to the hormonal stimuli. Consequently, while DESCs alone can form ducts, MECs provide signals that prompt high expression of ERα in DESCs, making the latter responsive to hormones and so a competent contributor to a fully, well-developed, branched duct.

Lastly, upon the transplantation of DESCs alone, and in a subset of transplantations of mixed MECs and DESCs, the regenerated ducts showed dense fibrotic tissue. DESCs undergo epithelial-to-mesenchymal transition (EMT), differentiate into α-SMA-expressing myofibroblasts and thereby contribute to fibrotic tissue formation. Even though fibrosis was detected in some of the mammary fat pads transplanted with mixed MECs/DESCs, fibrosis was found in all fat pads inoculated with DESCs alone[6]. For that reason, MECs could be fundamental to preventing the EMT of DESCs.

Conclusion

The exceptional plasticity of dental epithelial stem cells to generate, not only dental tissues, but also other tissues of the body should not be overlooked. However, when wanting to regenerate mammary glands, it would be more advantageous to mix DESCs with MECs prior to inoculation instead of utilizing them on their own as this mixture ensures a well-developed, branched ductal system with milk-producing cells, along with a lower chance of forming fibrotic tissue. This recent discovery of dental epithelial stem cells having the ability to replace cells from the mammary gland opens up new paths for developing stem cell-based therapies that could be used for post-surgery tissue regeneration in breast cancer patients.

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