CHAPTER I

INTRODUCTION

1.1. Background

Cell motion has been previously found to predict the fate of epidermal keratinocyte stem cells (Nanba et al., 2015). According to their report, early cell movements acquired through single-cell live imaging technique can effectively determine epidermal stemness and the clonal type they are most likely to form. This is a remarkable discovery that needs further investigation, as in regenerative medicine, maintenance and expansion of stem cells through a series of cultivation is required prior to transplantation and therefore, early determination of cell fate with the highest clonogenic ability possible is necessary to safe time and increase therapeutic success. Colony forming efficiency assay is one of the methods that can be utilized to determine clonal types; however, it takes at least 19 days to complete and it might also limit the usefulness or proliferative capacity of the stem cells (Barrandon et al., 2012). The image-based identification provides invaluable option that can overcome these drawbacks, but unfortunately, the exploration of this powerful tool in other type of stem cells remains limited. Hence, by using the same approach, we evaluated the motion feature of human thymic epithelial cells (hTECs) to understand their characteristics and to investigate if motion also predicts stem cell fate in hTECs population.

Thymus is a primary lymphoid organ of endodermal origin that provides the microenvironment necessary for T cells maturation (Miller, 1961). The stroma of thymus, an essential part of the microenvironment, is composed of epithelial and mesenchymal cells (Miller, 1961). TECs are highly crucial for proper T cell instruction. Deletion of their several main transcription factors in the mouse, such as FOXN1, FOXG1, SIX1, HOXA3, TBX1, and EYA1, results in impaired thymopoiesis due to a highly dysfunctional thymic epithelium or even a complete absence of TECs in the case of FOXN1 (Manley & Capecchi, 1995; Blackburn et al., 1996; Jerome & Papaioannou, 2001; Zou et al., 2006; Cheng et al, 2009). Mainly, TECs are located in two distinct regions: the cortical and medullary regions, each has specific role in the maturation of T cells (Laufer et al., 1999; Prockop & Petrie, 2000).

Thymus eventually involutes with age in healthy individuals and it is an evolutionary conserved event in vertebrates (Shanley et al., 2009). It is characterized by progressive reduction in thymus size due to the decrease in TEC numbers, followed by adipocytes and peripheral lymphocytes infiltration within the perivascular space (Taub & Longo, 2005; Aw et al., 2009). Disorganization of the cortico-modullary junction and loss of integrity in the cortical and medullary regions are also observed in the aged thymus (Taub & Longo, 2005; Gui et al., 2007; Aw et al., 2009). Consequently, this involution results in the tremendous reduction of thymopoesis and hence, significantly hinder the production of naïve T cells (Weng, 2006; Lynch et al., 2009; Chinn et al., 2012). The lack of naïve T cells delays and impairs the immune response in encountering new antigens, causing considerable decline in the function of immune system with age (Holland & van den Brink, 2009; Lynch et al., 2009).

Although in most cases this phenomenon does not significantly affect the overall health of normal individuals, the deterioration of immune system with age has direct etiological linkage with the rising chance of developing diseases, such as cancer, autoimmunity, and opportunistic infections (Chen et al., 2003; Lynch et al., 2009; Palmer et al., 2018). Moreover, thymic involution also induces defective capacity of the adaptive immune system to recover following immune depletion circumstances, which seen in cancer treatments and bone marrow transplantation, leading to an increase in the mortality and morbidity of the aged group (Holland & van den Brink, 2009; Lynch et al., 2009). Hence, the regeneration of thymus would be highly beneficial in wide variety of clinical settings, since it may enhance immune response and strengthen adaptive immunity through the increase of naïve T cells production. Not to mention that this breakthrough will also be extremely helpful for patients with DiGeorge syndrome, a congenital disease that results the absent of or hypoplastic thymus, causing severe immunocompromised condition (Abbas et al., 2015). The goal of thymus rejuvenation involves the restoration of thymus architecture, which primarily constituted by TECs (Figure 1.1).

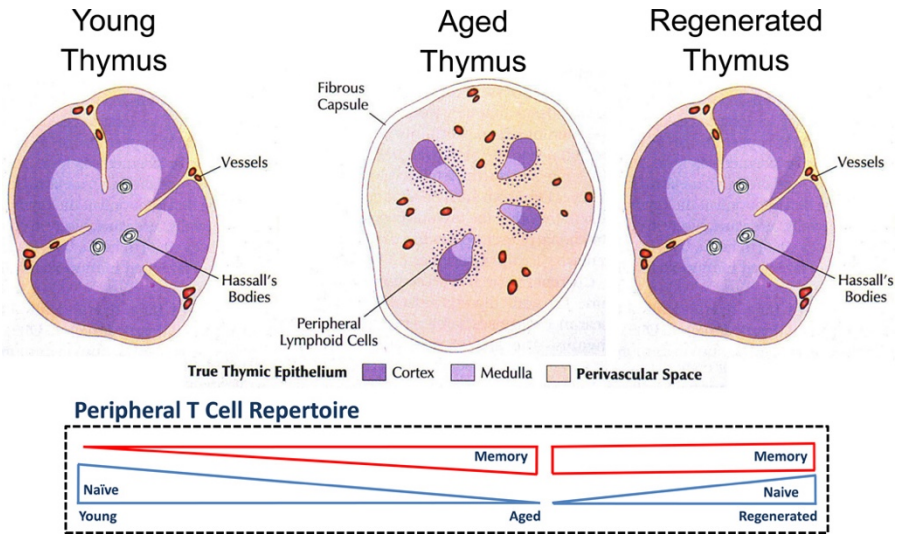

Figure 1.1 Scheme of young, aged, and future goal of regenerated thymus. Young thymus produces extensive naïve self-tolerance T cells, as this is supported by a fully functioned cortex and medulla TECs. With age, involution process leads to massive reduction in naïve T cells and peak of memory T cells. The future goal of regenerated thymus is expected to enhance immune system by producing high level of naïve T cells while at the same time maintaining the previously acquired memory T cells (Ventevogel & Sempowski, 2013).

In vision to regenerate thymus, one of the possible ways is through the application of stem cells and fortunately, in the case of thymus, clonal analysis of hTECs has shown that hTECs are clonogenic. They give rise to a tripotent precursor cells through asymmetric and symmetric divisions in culture (Maggioni et al., paper in preparation). These precursor cells self-renew in the form of long-lived refringent colonies and also generate short-lived scattered and long-lived stratified colonies; each with unique characteristics and distinct proliferative capacity (Maggioni et al., paper in preparation). Furthermore, clonogenic hTECs can be isolated not only from post-natal thymus, but also age-involuted thymus, making them a strong candidate for personalized regenerative medicine applications in all age groups (Arlabosse, et al., paper in preparation). In 2010, an *in vivo* study with rat has also shown that clonogenic TECs can be massively expanded in culture and have been illustrated to retained thymic functionality when transplanted back to thymus (Bonfanti et al., 2010).

More interestingly, further comprehensive gene expression analysis and immunofluorescence on hTECs have displayed many similarities to epidermal keratinocytes. hTECs express tumor protein 63 (TP63), a transcription factor that is well known as a key regulator for epidermal keratinocyte stem

cells functions and differentiation (Senoo et al., 2007; Bonfanti et al. 2010). It is therefore one of the important markers used in the quality assessment of cultured epidermal stem cells prior to transplantation (Pellegrini et al., 2001). In addition, the stratified colonies derived from hTECs show common morphology of which seen in epidermal keratinocytes and they express squamous epithelial differentiation markers (Maggioni et al., paper in preparation). Moreover, Bonfanti et al. (2010) have demonstrated that clonogenic rat TECs have the ability to form hair follicles, sebaceous glands, and epidermis when exposed to skin microenvironment. This significant discovery has opened the opportunity to regenerate epidermal appendages, which has never been possible to recover after transplantation of epidermal keratinocytes stem cells even after the wound healed properly (reviewed in Yang & Cotsarelis, 2010; Balañá, 2015; Brockmann et al., 2018). Moreover, hTECs can grow under conditions used for regeneration of human epidermis developed by Rheinwald & Green (1975). Hence, by understanding hTECs, we might be able to regenerate not only thymus, but also epidermis for patients with minimal or abnormal epidermis function, such as severely burned patients (Hettiaratchy & Dziewulski, 2004) and patients with epidermolysis bullosa simplex (Coulombe et al., 2009), an inherited genetic disease that results in easy tearing and blistering of the epidermis due to faulty keratinization program. Indeed, the fact that TECs is high in intrinstic plasticity as illustrated by Bonfanti et al. gives another reason for us to focus on hTECs, since they exhibit feature of cellular reprogramming when exposed to different microenvironments (Bonfanti et al., 2010).

As remembering the fact that hTECs possess huge potential in the future regenerative application, the main goal of this study was to reveal if motion feature is able to predict the stem cells fate in hTECs. In addition, to understand their motion pattern and its relation to epithelial to mesenchymal transition (EMT) and squamous stratification. This study undoubtedly deepened our understanding on hTECs, but on the top of that, it allowed efficient and effective identification of hTECs fate in very early phase. Hence, the single-cell live imaging will possibly be extensively utilized and become a powerful tool not only for hTECs and epidermal keratinocytes, but also for other types of stem cells in regenerative application.

1.2. Objectives

The objectives of this research were:

1. to investigate whether cell motion predicts the stem cells fate in hTECs.
2. to evaluate the pattern of cell motion in hTECs and its relation to EMT and stratification.

1.3. Hypothesis

It was hypothesized that:

1. Early cell motion of hTECs can reliably predict hTECs clonal types.
2. Each hTECs clonal types have distinctive motion patterns and the hTECs which are more motile are embarking towards EMT rather than stratification.