CHAPTER I

INTRODUCTION

1. Background and Rationale

The cornea on the front surface of the eye is our window to the world, it is responsible for preventing the entry of deleterious agents into the intraocular space and also provides the majority (two thirds) of the total refractive power of the eye. The transparency of the cornea and visual acuity are dependent upon the integrity and functionality of the outermost layer, the corneal epithelium. Throughout life, corneal epithelial homeostasis is dependent upon the self-renewing properties of a small population of putative stem cells located in the basal region of the corneoscleral junction or limbus. Stem cells are primitive cells, which provide an unlimited supply of proliferating cells and can self-renewal, produce at least one of the daughter cells remaining a stem cell during cell division. They also divide asymmetrically to produce daughter transient amplifying cells (TACs) that acquire excessive proliferative activity as they migrate centripetally to replace terminally differentiated cells sloughed from the ocular surface as a consequence of injury or physiological aging, progressively become more differentiated, eventually becoming terminal differentiated cells (1).

Currently, several obstacles for use of adult stem cells as therapy exist. First, the ability to identify most adult stem cells is impeded by lack of stem cell markers. Second, in vitro systems for manipulating adult stem cell populations are often not well defined. Finally, our understanding of how adult stem cells are regulated within their niche is in its infancy. Next to the hematopoietic stem cell, epithelial stem cells are one of the most widely studied stem cell populations.

In recent years, one of the major advances in management of patients with ocular surface failure resulting from limbal stem cell deficiency (LSCD) is by the transplantation of the limbal epithelial stem cells (LESCc) expanded from the limbal explants cultured on human amniotic membrane (HAM) with or without the feeder layer (2). Feeder cells are especially effective for the support of cell growth and play a role in

maintaining the limbal stem cell niche. Feeder cells also provide a suitable environment in the co-culture with a variety of cell types through different mechanisms, including cell to cell and cell to extracellular matrix (ECM) interaction, production of soluble growth factors and removal of toxicants from the culture medium (3).

Like those in other tissues, limbal stem cells are supported by a unique stromal microenvironment called the *stem cell niche*, which consists of certain extracellular matrix components, cell membrane-associated molecules, and cytokine dialogues. Although the diversity between epithelial functions and the unique features of each epithelial stem cell niche in different organs make it difficult to determine whether their common microenvironment in regulating these related stem cells, they likely share many common aspects of regulation.

Depending upon the nature of the injury, stem cells become activated to exit from quiescent stage in their niche and migrate upward to repair the damaged zone. One of the most well-known cytokines involved in wound healing process is Transforming growth factor-β (TGF-β). It also plays a critical role in the epithelial-to-mesenchymal transition (EMT) of normal mammary cells and lens epithelial cells. Indeed, skin keratinocytes and corneal epithelial cells display the same two physiological responses to TGF-β during wound healing; cell migration and growth inhibition (8). Nevertheless, the effects of TGF-β on LSECs properties have not been fully clarified.

In this study, we first aimed at investigating the effect of TGFβ1 and it's inhibitor (SB431542) treatment on clonogenic potential and proliferative capacity of the cultured LESCs. We found that TGF-β1 treatment induced limbal epithelial stem cells to transdifferentiate into fibroblast-like cells as described in epithelial-to-mesenchymal transition (EMT). In contrast, treatment with SB431542, TGFβ-inhibitor, promoted clonogenic capacity of LESCs as well as increased a proliferation rate of LESCs colonies. Our results suggested that TGFβ-inhibitor may be useful in promoting corneal wound healing and improve corneal transparency in patient with corneal injury. Moreover, we found the signaling crosstalk between TGF-β and BMP signals. We

demonstrated that TGF-β1 and the environmental culture system could up-regulate BMP antagonist expression.

Several signaling pathways have emerged as key regulators of stem cells that involved in shaping and maintaining the stem cell niche and therefore act as indirect regulators of the stem cell (5). It is still unclear which signals in the limbal niche play a role in regulating self-renewal and fate decision of limbal epithelial stem. One candidate signaling pathway is bone morphogenetic protein (BMP). BMPs are secreted signaling molecules of the transforming growth factor (TGF)-β superfamily and function as multifunctional regulators of vertebrate development controlling cell proliferation, differentiation, and apoptosis in different body tissue. BMP activity is regulated by diffusible BMP antagonists that prevent BMP interactions with BMP receptors thus modulating BMP effects in tissues. During skin development, BMPs, its receptors and its antagonists show stringent spatiotemporal expression patterns to regulate of cell proliferation and differentiation in the epidermis and hair follicle. Moreover, BMPs are implicated in a variety of pathobiologic processes in skin, including wound healing, psoriasis, and carcinogenesis (6). From previous evidences, BMP signaling is essential for promoting self-renewal and maintaining quiescence of various stem cells.

Understanding, amniotic membrane is an ideal biological substrate that can help maintain and support the expansion of limbal epithelial stem cells (7). Interestingly, human amniotic epithelial cells have shown that synthesize and release various biologically active substances including Noggin (8). However, the function of BMP signaling in LESCs niche remains unknown.

Therefore, the second aim of our work was to study the effect of BMP and it's antagonist secreted from feeder stromal cells on LESCs maintenance and propagation by generating the new feeder system that overexpressed BMP and BMP antagonist to secrete BMP4 and Noggin respectively. We determined the LESCs behaviours such as clonogenic potential and clonal morphology. We further analyzed the gene expression profile of the cultured limbal colonies in order to dissect the molecular mechanisms in LESCs niche. Finally to confirm an exclusive effect of feeder stromal cells-secreted proteins. We more tested whether recombinant protein treatments of BMP2 and BMP

antagonist (Noggin) in the culture media could affect LESCs properties in the same manner as feeder-derived proteins.

2. Research Questions

How and whether BMP and TGF-β signaling can affect limbal epithelial stem cells maintenance *in vitro*?

3. Objectives

1. To study how injury signals affect LESCs and their niche .
2. To investigate the role of BMP and TGF-β signalings in the regulation of LESCs maintenance and differentiation.
3. To develop ex vivo culture system for long-term LESCs expansion and maintenance.

4. Hypothesis

1. An increase in TGF-β signal triggers the change in LESCs niche which may help to promote LESCs recruitment.
2. Balance between BMP and BMP antagonist regulate LESCs maintenance and differentiation.