CHAPTER I

INTRODUCTION

1.1. Background

Skin depigmentation disorders are among one of the most common dermatological disorders. One particular example of such disease is vitiligo, which affects at least 1% of the world's population (Ezzedine et al., 2012). Vitiligo is characterized by the appearance of white patches in the skin, which arises from the lack of melanin. Up until today, the exact cause that leads to development of vitiligo remains unclear. However, several theories have been developed as an attempt to explain the etiology of vitiligo and one of the most probable explanation is the autoimmune theory (Ianella et al., 2016). The autoimmune theory generally speculates that the lack of melanin in vitiligo patients arises as a result from progressive destruction of melanocytes by the immune cells (Ianella et al., 2016). In general, vitiligo can affect both men and women with similar prevalence, meaning that there is no particular gender pattern involved (Allam & Riad, 2013). As for the onset, vitiligo can develop at any age, however typically the disease arises before the age of 30, which comprise 70% - 80% of vitiligo cases (Alikhan et al., 2011). Due to apparent and visible features of the disease, it is often that vitiligo patients also experience psychological stress.

Currently there are several treatment options available to treat vitiligo. Phototherapy is one of the widely used treatment for vitiligo patients, which involves the exposure of narrowband UVB on affected areas of the skin to induce skin repigmentation. Results from clinical observations and research suggest this occurs due to UVB-induced migration of melanocytes from the hair follicle stem cell niche that trigger interfollicular repigmentation (Tang et al., 2018; Wu et al., 2004). However, despite its efficacy, the process of phototherapy treatment itself may be considered as rather time consuming and the outcome varies

between individuals. The reason being is that this particular treatment requires at least 30 to 60 treatments that are usually given three times a week in order for the result to be seen ("Phototherapy Treatment", 2018). Therefore, this study aims to enhance the UVB-induced follicular repigmentation during vitiligo.

In the previous research performed by Clavel et al. involving the study on how Sox2 in the dermal papilla (DP) niche controls hair growth, the role of bone morphogenetic protein (BMP) signaling in regulating melanocyte function was first observed (Clavel et al., 2012). In the study, following the knock-out of Sox2 in the DP niche, leads to increased BMP expressions, which was due to reduced Sostdc1 expression, one of the BMP antagonists. The increased in BMP expressions subsequently leads to reduced hair growth speed. At the same time, Clavel et al. also discovered an interesting phenomenon whereby a pigment switch event was observed along with increased BMP expression. This then leads to the speculations that BMP signaling might played an implicating role in regulating melanocytes. Furthermore, other study conducted by Kaiser et al also showed the implication of BMP signaling in affecting cell migration, where it was observed that increased BMP signaling appears to inhibit cell migration (Kaiser et al.,1998). With the following observations, it appears that BMP signaling does not only implicate hair growth but in fact it may also regulate melanocyte functions. Subsequently, the following findings leads to the idea of further scrutinizing the other implication of BMP signaling in regulating melanocyte functions, whereby it can be used as a potential target to induce skin repigmentation in vitiligo.

Therefore, it is hypothesized that down regulation of the BMP signaling, will likely result in the activation of the melanocyte stem cell (MeSC). This will eventually result in the differentiation and migration of the melanocyte stem cell into the epidermis hence leading to an enhanced skin repigmentation when coupled with UV induction. Therefore, the objective of this research is to identify the role of bone morphogenetic protein (BMP) signaling in melanogenesis, melanin transfer and melanocyte migration both *in vitro* and *in* vivo. Having

said that, identifying and understanding the role of BMP signaling will be of great importance, since this could provide knowledge on understanding follicular repigmentation. The significance of this newly profound knowledge could then later on be implemented as a potential therapeutic strategy to treat vitiligo that could serve as a more efficient and reliable treatment option. Thereby relieving the psychological burden in vitiligo patients.

In the proposed study, several approaches will be implemented to justify the objectives of the following research. In general, the research is divided into two parts, *in vitro* and *in vivo*. The *in vitro* part of the research will mainly involve several cell culture methods such as 2D keratinocyte and melanocyte co-culture system to study melanogenesis and melanin transfer at the functional level by incorporating different cytokine treatments. In addition to 2D co-culture, migration assay will also be integrated as part of the *in vitro* aspect. The *in vivo* part of the research will mainly involve mice work using genetically modified mouse models. Fluorescence-based analysis approaches, including immunofluorescence analysis using confocal microscopy and fluorescence-activated cell sorting (FACS) will also be incorporated. In addition to that, downstream experiments like melanin assay and qRT-PCR will also be included in the research to further study the effects of BMP signaling *in* vivo, the gene expression of melanocytes signature genes will also be observed.

1.2. Objectives of the study

The main objective of the following project is to develop a novel strategy to enhance UVB-induced follicular repigmentation in vitiligo. In order to test our hypothesis and achieve our objectives, we are proposing the following specific aims:

Aim 1: To analyze BMP signaling in the human hair follicle stem cell niche

Aim 2: To determine the functional role of BMP signaling in melanogenesis, melanin transfer and melanocyte migration in vitro

A. Analyze the functional consequences of BMP signaling in melanogenesis, melanin transfer, and melanocyte migration in-vitro

Aim 3: To determine the role of BMP signaling in follicular repigmentation in vivo

A. Prospective isolation of the MeSC compartment from BMP4 mice model

B. Analyze the functional consequences of BMP signaling in melanogenesis, melanin transfer and melanocyte migration in BMP4 mice model