CELL-BASED THERAPY OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELL EXPRESSING ANGIOPOIETIN-1 IN AN EXPERIMENTAL MODEL OF AIRWAY INFLAMMATION

by

NUR SHUHAIDATUL SARMIZA BINTI ABDUL HALIM

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LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
AB-PAS	Alcian blue-periodic acid-schiff
Ad	Adenovirus
AECs	Airway epithelial cells
AHR	Airway hyperresponsiveness
ALI	Acute lung injury
ALUM	Aluminium hydroxide
ANGPT1	Angiopoietin 1
ANGPT2	Angiopoietin 2
ANOVA	One-way analysis of variance
ARDS	Acute respiratory distress syndrome
ARF	Animal research facility
ASM	Airway smooth muscle
ATI	Alveolar epithelial type I cell
ATII	Alveolar epithelial type II cell
BAL	Bronchoalveolar lavage
BEBM™	Bronchiole Epithelial Basal Medium
BEGM™	Bronchial Epithelial Growth Medium
bFGF	Basic fibroblast growth factor
BM-MSC	Bone marrow-derived mesenchymal stem cells
BSA	Bovine serum albumin
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
CB	Chronic bronchitis
CCSP	Clara cell secretory protein
cDNA	Complementary Deoxyribonucleic acid
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CK14	Cytokeratin 14
CK18	Cytokeratin 18
CK5	Cytokeratin 5
CMV	Cytomegalovirus
CO_2	Carbon dioxide
COPD	Chronic Obstructive Pulmonary Disease

DAVID	Database for Annotation, Visualization and Integrated Discovery
DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
ECs	Endothelial cells
EDTA	Ethylenediaminetetraacetic acid
EGF	Epithelial growth factor
TurboGFP	Turbo Green Fluorescent Protein
EMT	Epithelial-mesenchymal transition
FBS	Fetal bovine serum
FDR	False discovery rate
FGF2	Fibroblast Growth Factor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GM-CSF	Granulocyte macrophage colony-stimulating factor
H&E	Haematoxylin & Eosin
hAD-MSCs	human adipose-derived mesenchymal stem cells
HCl	Hydrochloric acid
HRCT	High-resolution CT scans
HRP	Horseradish peroxidase
HRQoL	Health-related quality of life
HSV	Herpes simplex virus
hUCB-MSCs	Human umbilical cord blood-derived mesenchymal stem cells
i.m	Intramuscular
i.p	Intraperitoneal
i.v	Intravenous
IAA	Iodoacetamide
ICAM-1	Intercellular adhesion molecule 1
ICS	Inhaled corticosteroids
IFN-γ	Interferon gamma
IgE	Anti-immunoglobulin E
IL-1	Interleukin 1
IL-10	Interleukin 10
IL-13	Interleukin 13
IL-1β	Interleukin 1 beta
IL-33	Interleukin 33

IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
ISCT	International Society for Cellular Therapy
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LABA	Long-acting β2-agonists
LB	Luria Bertani
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LFQ	Label-free quantification
LPS	Lipopolysaccharide
LV	Lentivirus
MHC	Major histocompatibility complex
MMP9	Matrix metalloproteinase 9
MSC-pANGPT1	Mesenchymal stem cell expressing angiopoietin 1
MSCs	Mesenchymal stem cells
Muc5ac	Mucin 5ac
NGFR	Nerve Growth Factor Receptor
ORF	Open reading frame
Ova	Ovalbumin
p63	Transcription factor 63
PANTHER	Protein Analysis Through Evolutionary Relationships
PBS	Phosphate buffered saline
PECAM-1	Platelet endothelial cell adhesion molecule 1
PGE2	Prostaglandin E2 synthases
qRT-PCR	Qualitative real time polymerase chain reaction
RNA	Ribonucleic acid
RT	Room temperature
Scgb	Secretoglobin
SM	Smooth muscle
sST2	Soluble IL-1 receptor-like 1
TBST	Tris-buffered saline
TCA	Trichloroacetic acid
TGF-β	Transforming growth factor-β
Th1	T helper 1
Th17	T helper 17
Th2	T helper 2

Tie2	Tyrosine kinase receptor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
UV	Ultraviolet
VCAM-1	Vascular cell adhesion molecule1
VEGF	Vascular endothelial growth factor
WEGO	Web Gene Ontology
WHO	World Health Organization

TERAPI PENGHANTARAN SEL TUNJANG MESENKIMA DARIPADA TISU LEMAK MANUSIA YANG MENGEKSPRESKAN ANGIOPOIETIN-1 DALAM MODEL UJIKAJI KERADANGAN PADA SALUR PERNAFASAN

ABSTRAK

Terapi berasaskan sel tunjang mesenkima (MSCs) didapati mampu membantu mempercepatkan proses pemulihan dalaman dengan meningkatkan kapasiti regeneratif paru-paru yang terhad akibat penyakit paru-paru kronik serta akibat kecederaan. Walau bagaimanapun, mekanisme tindakan MSCs dalam membantu proses regenerasi serta pemulihan salur pernafasan pada peringkat sel dan molekul adalah masih tidak jelas. Kajian ini bertujuan untuk menyiasat kesan penghantaran sel MSCs yang dipencilkan daripada tisu lemak manusia (hAD-MSCs) serta menilai kesan selanjutnya yang melibatkan kombinasi antara MSCs dan gen ANGPT1 (MSC-pANGPT1) dalam model keradangan salur pernafasan pada arnab menggunakan teknik penghantaran secara aerosol. Gen ANGPT1 memiliki sifat anti-keradangan, anti-ketelapan serta pelindung kepada sel endotelial. Oleh yang demikian, gabungan bersama antara MSCs dan ANGPT1 berpotensi dalam merawat keradangan pada salur pernafasan. Transfeksi hAD-MSCs dengan gen ANGPT1 dilakukan dengan menggunakan teknik mikroporasi. Dalam kajian haiwan, arnab pada awalnya disensitifkan dengan suntikan gabungan ovalbumin (Ova) dan alum, dan diikuti dengan sedutan Ova. Tindakan ini adalah untuk mencetuskan keradangan pada salur pernafasan yang mempunyai ciri-ciri yang dikaitkan dengan penyakit asma. MSCs dan MSC-pANGPT1 telah dihantar ke dalam salur pernafasan arnab yang tercedera melalui peranti *MicroSprayer® Aerosolizer* 48 jam selepas kecederaan. Penilaian histopatologi keradangan pada salur pernafasan serta tindakbalas keradangan tempatan telah diukur secara kuantitatif pada hari ketiga selepas penghantaran sel. Untuk menyiasat kesan fungsi MSCs terhadap regenerasi dan pemulihan pada salur pernafasan, satu model kultur bersama *in vitro* tidak langsung telah dibangunkan. Tisu salur pernafasan yang telah tercedera telah dikulturkan

bersama atau tanpa MSCs. Pendekatan proteomik telah digunakan untuk mengenalpasti protein yang dirembeskan oleh MSCs hasil tindakbalas kepada epitelium salur pernafasan yang tercedera akibat induksi oleh Ova. Strategi ini adalah untuk mengenalpasti faktor-faktor yang dirembeskan oleh MSCs dimana ianya memainkan peranan penting dalam proses regenerasi dan pemulihan epitelium. Analisis spektrometri massa kromatografi-tandem dilakukan untuk menganalisis faktor-faktor yang dirembeskan oleh MSCs dan penglibatannya dalam proses regenerasi dan pemulihan epitelium telah dinilai menggunakan kaedah histologi. Kajian ini membuktikan bahawa teknik mikroporasi merupakan teknik yang hebat berbanding teknik lain dalam kemampuannya untuk transfek hAD-MSCs tanpa mengganggu proses proliferasi dan pembezaan sel hAD-MSCs. Penghantaran MSCs dan MSC-pANGPT1melalui kaedah aerosol mampu mengakibatkan pengurangan yang ketara pada keradangan salur pernafasan, seperti yang dapat dilihat oleh penyusutan ketebalan dinding salur pernafasan dan pengurangan penyusupan sel-sel granulosit pada bahagian sekitar bronkiol dan salur darah. Terapi oleh kedua-dua sel ini, MSCs dan MSC-pANGPT1 dilihat mampu mengurangkan penyusupan sel-sel yang terlibat dalam keradangan pada salur pernafasan serta dalam cecair "bronchoalveolar lavage" (BAL) dan hiperplasia sel-sel goblet. Manakala, penghantaran MSC-pANGPT1 melalui aerosol memberi impak tambahan dengan mengurangkan kadar ekspresi pelbagai gen pro-keradangan kepada kadar normal. Penilaian histologi kultur *in vitro* bersama membuktikan bahawa sifat stimulasi MSCs berupaya meningkatan kebolehan sel-sel epitelium pernafasan (AECs) bermigrasi, berproliferasi serta melakukan pembezaan bagi memulihkan kecederaan epitelium pada salur pernafasan. Sebatian yang dirembeskan oleh MSCs juga menunjukkan proses peralihan epitelium kepada mesenkima (EMT) sebagai proses awal pembaikan epitelium salur pernafasan. Pengenalpastian 54 protein yang dirembeskan oleh MSC yang mana kira-kira 43 daripadanya didapati terlibat dalam proses mempercepatkan regenerasi serta pemulihan epitelium pada salur pernafasan. Protein-protein yang dirembeskan ini merangsang kebolehan mekanisme pemulihan dalaman bermigrasi, berproliferasi dan melaksanakan pembezaan. Di samping itu, protein-protein ini juga mencetus proses awal pemulihan iaitu

peralihan epitelium-mesenkima (EMT). Kajian ini memberikan pemahaman tentang terapi berasaskan MSC pada peringkat sel dan molekul sebagai asas untuk terapi bagi rawatan kecederaan paru-paru pada masa hadapan.

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ABSTRACT

Cell-based therapy of mesenchymal stem cells (MSCs) has been shown to enhance the endogenous repair process by increasing the limited regenerative capacity of the lung in chronic lung disorders as well as following injury. However, the underlying cellular and molecular mechanisms of MSCs to enhance airway regeneration and repair are remain unclear. This study was aimed to investigate the effect of delivering human adipose-derived MSCs (hAD-MSCs) alone and in combination with vasculoprotective factor, the ANGPT1, by aerosol technique in a rabbit model of asthma related-airway inflammation. Given the anti-inflammatory, anti-permeability, and endothelial-protective characteristics of ANGPT1, we hypothesise that, combining MSCs with ANGPT1 offers promise in the treatment of airway inflammation. Transfection of hAD-MSCs with ANGPT1 was performed using microporation technique. For *in vivo* study, the rabbit were sensitised with combination of ovalbumin (Ova) and alum injection and further challenged with Ova inhalation to induce asthma-related airway inflammation. The MSCs and MSC-pANGPT1 were aerosolised directly into the airway using the MicroSprayer® Aerosolizer 48 h after injury. Histopathological assessments of the airway inflammation along with local inflammatory responses were quantitatively measured at three days after cell delivery. To investigate the functional effect of MSCs on airway regeneration and repair, an indirect *in vitro* co-culture model of injured airway epithelium explant with MSCs was developed. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed to determine the factors secreted by MSCs and their involvement in epithelium regeneration and repair was evaluated by histopathological assessment. The present study provides evidence that the microporation technique is superior to the others in terms of its ability to

transfect hAD-MSCs without affecting the proliferation and differentiation capabilities of MSCs. Administration of aerosolised MSCs and MSC-pANGPT1 markedly reduced inflammation of the airways, as reflected by decreased of airway wall thickening and infiltration of granulocytes at the peribronchiale and perivascular regions. They also alleviated the number of airway inflammatory cells in the bronchoalveolar lavage (BAL) fluid and goblet cell hyperplasia. Administration of aerosolised MSC-pANGPT1 provided an additional effect merely in reducing the expression levels of various pro-inflammatory genes to the baseline values. In the co-culture assay, histological analysis provided strong evidence of the stimulatory behaviours of MSCs to enhance the migratory, proliferative and differentiation abilities of airway epithelial cells (AECs) in repairing the injured airway epithelium. Compounds secreted by MSCs also markedly initiated the epithelial-to-mesenchymal transition (EMT) process during early wound repair. The identification of 54 of MSC-secreted proteins of which approximately 43 of them were found to be involved in airway epithelial cell migration, proliferation, differentiation and initiation of EMT process. This occurrence further supports the evidence of MSCs stimulatory ability in accelerating airway regeneration and repair. This study provides cellular and molecular insights of MSC-based therapy to form a basis evidence for future treatment to treat lung injuries.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Anatomy of the respiratory airway

1.1.1 The branching of respiratory airway

The lung is made up of complex branches of airway tubes linked to a trachea that carry air to and from the alveoli, where gas exchange occurs (Hogan *et al.*, 2014). Lung can be divided into two general parts which are the conducting and the respiratory parts (Figure 1.1). In human, the conducting airway from the trachea to bronchioles is a set of tubular structure lined by pseudostratified epithelium, comprises of three main cell types: ciliated cells, non-ciliated secretory cells, and basal cells. This cellular composition of the conducting airway differs along its proximal-distal axis (Figure 1.2) (Rock, Randell and Hogan, 2010). This respiratory tract decreases in luminal diameter as the airway tree branching down from proximal to distal. The respiratory part encompasses respiratory bronchioles that branch into alveolar ducts and alveolar sacs at the terminal (Donne, Lechner and Rock, 2015).

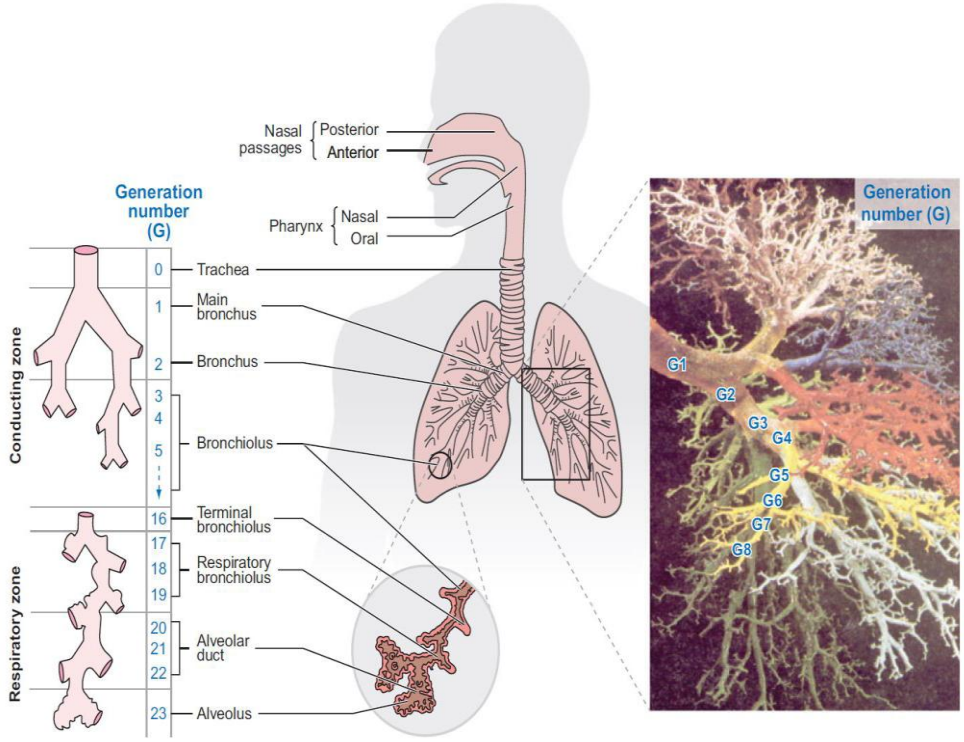

Figure 1.1 Schematic of the human respiratory system. Respiratory airway is divided into two general zones, the conducting zones and the respiratory zone. Adapted from (Kleinstreuer, Zhang and Donohue, 2008).

1.1.2 Respiratory airway structure and cellular composition

1.1.2(a) Proximal airway

The proximal part of conducting airway termed as tracheobronchial tree (airway structure involving the trachea and bronchi) is made up of C-shaped rings of hyaline cartilage composed of chondrocytes. These cartilaginous rings are to support trachea rigidity and provide protection to the trachea from collapsing owing to extreme condition including cough and forced expiration (Fernandez, Norwood and Berne, 2008). Tracheobronchial surface is lined by a pseudostratified mucociliary epithelium that primarily includes multiciliated, secretory/goblet, basal cells and neuroendocrine cells (Figure 1.2). Ciliated cells are the major cellular constituents lining the tracheobronchial surface, followed by basal and fewer number of secretory/goblet cells (Plopper *et al.*, 1983). Ciliated cells are terminally differentiated cell population that are marked by the expression of cytokeratin 8

(CK8) and 18 (CK18) (Rawlins and Hogan, 2008; Cole *et al.*, 2010). Basal cells lined the pseudostratified epithelium, tightly anchored the epithelium to the basement membrane via hemidesmosomes and other adhesion molecules. They constitute approximately`6 – 30% of the total epithelial cells in human conducting airway, characterised by the expression of transcription factor-63 (p63), CK5, CK14, and Nerve Growth Factor Receptor (NGFR) (Nakajima *et al.*, 1998; Rock *et al.*, 2009; Kotton and Morrisey, 2014). However, the proportion of basal cells differs along the proximal-distal axis. In human airway, basal cells are abundant in the trachea and in the first six generations of the respiratory tract. Accumulating evidences suggest that basal cells are stem cells that are capable to self-renew and differentiate into ciliated and secretory/Club cells during homeostasis or following injury (Rock *et al.*, 2009; Crystal, 2014; Weiss *et al.*, 2015).

The secretory/goblet cells populate the tracheobronchial surfaces, express mucin-5ac (Muc5ac) that produces and secretes mucus to create a barrier that inhibit the pathogen from penetrating to the conducting airway. The secretion of mucus by secretory/goblet cell together with ciliated cell's sweeping motion orchestrate the clearance of mucociliary, the process by which inhaled microorganisms and particulates are cleared from the lung. The invagination the airway epithelial layer is a layer of sub-mucosa which comprises of sub-mucosa gland, smooth muscle and blood vessel. These sub-mucosa glands are further specialised into mucus cells or serous cells (Puchelle *et al.*, 2006). Goblet cells, Club cells, and sub-mucosa gland are identified as mucus-producing cells/structures due to their physiological function in producing mucus, which is vital for defensive barrier (Puchelle *et al.*, 2006).

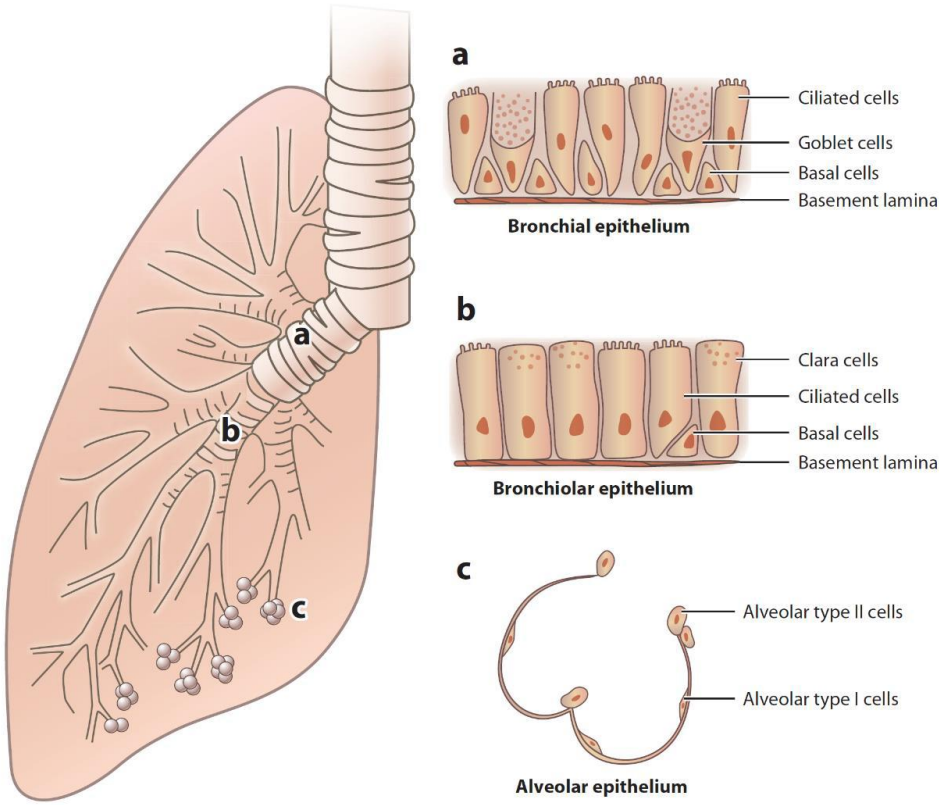

Figure 1.2 Epithelial cell types in the conducting airways (nasal, trachea, bronchi, and bronchiole) and respiratory airways (alveoli). (**a**) Tracheobronchial of the proximal cartilaginous airway are lined with a pseudostratified epithelium that consist of ciliated, basal, and secretory/goblet cells, (**b**) In the more distal airways, the bronchiole region lined with a simple cuboidal epithelium consisting of ciliated, basal, and secretory/club cells, and (**c**) gas-exchanging airspace (alveolar region) consisted of two major distinct epithelial cell types, ATI and ATII. Adapted from (Wetsel, Wang and Calame, 2011).

1.1.2(b) Distal airway

As the bronchi branches into bronchioles and to terminal bronchioles in more distal airway, the cartilaginous bronchi vanishes and substituted for smooth muscle bordering the bronchiole epithelium. This airway area is termed as bronchiolar epithelium. The epithelium gradually modifies from pseudostratified to simple cuboidal epithelium and the number of ciliated, goblet, and basal cells gradually diminishes distally. In addition, a non-ciliated cell named Club cells, marked by the expression of secretoglobins, Scgb1a1 and Scgb3a2 appear to be the main cell type in the bronchiolar epithelium (Nettesheim *et al.*, 1990; Reynolds *et*

al., 2002). Under steady state conditions, airway epithelium in proximal and distal regions is relatively in quiescent state. The lung maintenance and repair under steady state or in response to mild injury is performed by distinct cell population of the airway epithelium known as endogenous lung stem/progenitor cells (Kotton and Morrisey, 2014).

1.1.3 Stem cell population in lung

Lung has an intrinsic capacity to regenerate itself. This endogenous repair mechanism have very slow cellular turnover which is less than 1% of epithelial cells that are proliferating at any given point in time under normal circumstances (Boers, Ambergen and Thunnissen, 1999) . This phenomenon suggests that lung is relatively quiescent under steady state conditions. The maintenance of conducting airway epithelium and its repair is dependent on the activity of endogenous lung stem cells. These endogenous stem cells reside in distinct microenvironment that defined as a 'niche' along the airway tree from proximal-to-distal axis (Hegab *et al.*, 2015). These stem cells are critically influenced by extrinsic signal within their niche that comprises of extracellular matrix and other cell types including epithelial cell, endothelial, mesenchyme and/or hematopoietic cells. The interactions between of these cell-cell and cell-extracellular matrix are mediated via signalling components such as Wnt and Notch (Flozak *et al.*, 2010; Xing *et al.*, 2012). Both of these signalling pathways are known to play key roles in self-renewal and differentiation of the stem cells. They are also associated with airway regeneration and repair. Previous studies have shown that the activation of canonical Wnt signalling pathway in various lung compartments that are undergoing vigorous regrowth and regeneration suggests its important involvement in lung regeneration and repair (Hashimoto *et al.*, 2012; Aumiller *et al.*, 2013). While Notch signalling has been shown to play a critical role in differentiation of basal cells into secretory lineages in respond to sulphur dioxide induced airway epithelial injury (Xing *et al.*, 2012). The interplay between endogenous stem cells and their niches creates dynamic

system in regulating long-term maintenance of stem cells. This is achieved by modulating self-renewal and/or differentiation capability of stem cell or to cause reversion of differentiated cells to stem cell-like functions (Volckaert and De Langhe, 2014a).

The respiratory airway is equipped with epithelial cell lining to provide structural integrity and as the first line of defence mechanism against the external stimuli. Secretory products, tight junction between the epithelial as well as movement of cilia are among defence mechanisms at the respiratory airway wall (Blanpain, Horsley and Fuchs, 2007; Whitsett and Alenghat, 2015). The loss of epithelial cells in response to injury requires the presence of stem cells to persistently replenish the epithelium lining. This continuous cell replacement process is critical in maintaining the adult tissue homeostasis (Blanpain, Horsley and Fuchs, 2007). Initial study by Randell et al (1991) revealed the potential of basal cells in generating a complete pseudostratified epithelium containing basal, ciliated and secretory cells when seeded into a denuded trachea grafted. More recently, *in vivo* genetic lineage tracing experiments provided evidence that basal cells have the ability of long-term self-renewal and differentiate into multiple airways epithelial cell types such as secretory and ciliated cells (Hong *et al.*, 2004; Teixeira *et al.*, 2013). These findings indicated that basal cells function as stem cells that are capable of self-renewal and differentiate into epithelial lineages to support tissue homeostasis. Apart from basal cells, a subset of epithelial cells that make up a population of stem cell of the airway includes Club cells in bronchioles and alveolar type II cells (ATII) in the alveoli (Hong *et al.*, 2004; Rock *et al.*, 2009).

In the proximal airway, previous studies have demonstrated that cytokeratin (CK5) and (CK14) positive basal cells have the ability to give rise to ciliated and secretory cells in response to chlorine and cystic fibrosis (CF) induced airway injury (Hong *et al.*, 2004; Musah, Chen and Hoyle, 2012). In addition, a study Rock et al (2011) showed that basal cells proliferate and differentiate into a multipotent p63-CK8+ luminal cells that comprise of ciliated and secretory cells following exposure to sulphur dioxide. In the distal bronchioles, Club cells are the cell population that functions as stem cells in maintaining the facultative

progenitor cell pool and differentiate to ciliated cells. This is because the Club cells are abundant in the distal bronchioles than the ciliated cell, whereas basal cell population gradually decreases in number distally (Morrisey and Hogan, 2010). According to previous work, a rise of proliferative Club cells that express Clara cell secretory protein (CCSP) at the terminal bronchiolar airway following naphthalene-induced injury can promote epithelial renewal (Reynolds *et al.*, 2000; Hong *et al.*, 2001). In the alveoli region, ATII cells are endogenous stem cell population that have the capability to self-renew and give rise to ATI during lung development and repair following injury. Following injury, ATI cells have been shown to release an epithelial growth factor (EGF) that activated the ATII to differentiate into ATI (Desai, Brownfield and Krasnow, 2014).

The airway epithelial can undergo phenotypic switches in response to damage tissue. In the ablation of airway basal cells, the differentiated airway epithelial cells such as secretory cell can revert into functional basal stem cells (Tata *et al.*, 2013). A study by Randell and co-workers using denuded rat tracheas indicated that the luminal secretory cells have the capability to regenerate the entire epithelium as lectin+ basal cells (Liu, Nettesheim and Randell, 1994). Subsequent lineage tracing study by Tata et al (2013) demonstrated that differentiated secretory cells expressing secretoglobin 1a1 (Scgb1a1) underwent dedifferentiation into Trp63+/CK5+ basal cells in the ablation of CK5-expressing basal cells in the mouse trachea *in vivo*. The generated basal cells persist over the long term and indistinguishable from normal CK5+ stem cells which suggests that differentiated cells can revert into stem cells and function similarly as their endogenous stem cell counterpart in repairing injured epithelial.

In the context of acute injury, the endogenous stem cell populations of the airway epithelium are capable of impacting effective repair (Perl, Riethmacher and Whitsett, 2011). However, chronic injury and inflammation due to repeated airway epithelial injury frequently associated with aberrant repair. The disruption of reciprocal interaction of epithelial stem cell-niche unit and their dysregulation may lead to pathological airway

remodelling and serious impairment of lung function as observed in chronic lung diseases such as chronic obstructive pulmonary disease (COPD) and chronic asthma (Rock and Hogan, 2011; Ganesan and Sajjan, 2013a).

1.2 Lung-related disorder

Lung diseases have become a major concern in medical care and caused an immense global health burden. According to World Health Organization (WHO) statistics, non-communicable diseases including chronic respiratory diseases were responsible for almost 68% of all death worldwide in 2012. Chronic respiratory diseases are global health challenge that is defined as chronic inflammatory conditions of the conducting airways and lung parenchyma (Jeffery, 2001). Two primary debilitating chronic lung diseases are asthma and COPD. The current global estimation of people suffering from chronic respiratory disease is over 1 billion. Asthma has the highest incidence among the chronic respiratory diseases, with about 300 million people were reported to have asthma (Global Initiative for Asthma, 2018), while 210 million people suffer from COPD and patients suffer from these diseases can be fatal if left untreated. These diseases are predicted to become the third leading cause of death in the world by 2030 (World Health Organization, 2008). This imminent fact may contribute to the global burden on healthcare system, economy, and capital human resources, hence the development of better treatment aimed to prevent and cure chronic respiratory diseases are urgently needed.

1.2.1 COPD

COPD is a complex disorder characterised by progressive irreversible airflow obstruction that interferes with normal breathing, persistent systemic and airway inflammation. Patients with COPD usually come together with various co-morbid diseases

such as cardiovascular (Crisafulli *et al.*, 2008; Decramer, Janssens and Miravitlles, 2012). The predominant cause of this disease is cigarette smoke, however other factors including air pollution and occupational exposures to dusts and fumes (Harber *et al.*, 2007; Thorley and Tetley, 2007; Johannessen *et al.*, 2012; Omland *et al.*, 2014; To *et al.*, 2016). COPD primarily manifests by emphysema and chronic bronchitis - they frequently coexist. Other clinical manifestations of COPD are mucus hypersecretion, systemic inflammation and, acute exacerbation that lead to decrease in lung function and breathe shortness (Hogg, 2004; Wright, Cosio and Churg, 2008; Baraldo, Turato and Saetta, 2012; Tuder and Petrache, 2012). Emphysema is a condition of an abnormal permanent enlargement of alveolar spaces due to the destruction of alveolar septa. The damage of the alveolar walls reduces the overall elasticity of the lung which leads to the collapse of the bronchioles obstructing airflow out of the alveoli (Shaker *et al.*, 2009; De Torres *et al.*, 2011). This incidence causes difficulties in breathing due to obstructed airflow.

1.2.1(a) Chronic bronchitis

Chronic bronchitis (CB) is a common but variable phenomenon in COPD defined as the incidence of a chronic, productive cough and production of sputum no less than 3 consecutive months in 2 consecutive years (Kim and Criner, 2013). The clinical phenotype of CB in COPD is associated with an increased exacerbation rate, accelerated decline in lung function, increased risk of respiratory infections, worse health-related quality of life (HRQoL) and increased risk of mortality (Vestbo *et al.*, 1996; Kim *et al.*, 2011; Ramos, Krahnke and Kim, 2014). Accumulating data suggest that the secretion from goblet cells and mucous gland variably described as mucus overproduction and hypersecretion is a pathologic foundation for CB (Kim and Criner, 2013; Kim *et al.*, 2015). Mucus overproduction and hypersecretion by goblet cells lead to airflow obstruction by several mechanisms including luminal occlusion of small airways, airway narrowing caused by

thickening of epithelial layer, epithelial remodelling, inflammation and swelling of the lining of the airways (Hogg *et al.*, 2004; James and Wenzel, 2007). The inflammation starts to stimulate production of mucous and in longer time it may change to sputum, which can cause further obstruction of the airways. Obstruction of the airways, especially with mucus, increases the possibility of bacterial lung infections (Garcia-Bellmunt *et al.*, 2013). Previous study demonstrated that chronic mucus hypersecretion in hospitalised COPD patients increased mortality risk associated with pulmonary infection (Prescott, Lange and Vestbo, 1995).

1.2.1(b) Bronchiectasis

Bronchiectasis is another abnormality that can be found in patients with COPD (Martínez-García *et al.*, 2011). Both bronchiectasis and COPD share numerous characteristics including fixed airway obstruction, chronic cough and production of sputum. Accumulating data suggest that COPD coexisting with bronchiectasis indicated by the discovery of bronchiectasis on high-resolution CT scans (HRCT) in patient with COPD is associated with poorer outcome. Coexisting of COPD and bronchiectasis resulting in advance lung dysfunction, frequent exacerbation and colonisation of bacteria (Martínez-García *et al.*, 2013). In bronchiectasis, serious and recurrent infections of the lung as well as abnormal development of the lung results in permanent damage to the airways (McShane *et al.*, 2013). The damaged airways become enlarged tubes or, in more severe cases, large sacs. These segments of lung can impair clearance of secretions. The damaged, mucus-filled airways often become infected, resulting in further inflammation and damage to the airways (Boyton, 2012).

1.2.2 Asthma

Asthma is a complex, reversible chronic airway inflammatory disorder orchestrated by complex interactions between genetic factors and environmental agents likes aeroallergens and respiratory viruses (Gregory and Lloyd, 2011; Reddel *et al.*, 2015). Asthma affects approximately 300 million people of all age groups worldwide and its prevalence is increasing (Global Initiative for Asthma, 2018). Of these, 10 – 15% were reported to have severe asthma, which is refractory to commonly drug therapies (WHO, 2007; Croisant, 2014). However the cost of health care for severe asthma is estimated to account for more than 60% of the costs related to asthma treatment and management, which are mainly for medications (Sadatsafavi *et al.*, 2010). In Malaysia, approximately 1.6 million to 2 million people were reported to have asthma and the number is still increasing, especially among children (Ngui *et al.*, 2011; Roslan *et al.*, 2011). The majority of asthmatic patients experience recurring episodes of nocturnal coughing, breathlessness, wheezing and chest tightness, together with variable expiratory airflow limitation due to the inflammation and constriction of muscle surrounding the airway (Lambrecht and Hammad, 2012). Besides, a small proportion of asthmatic patients suffer from persistent symptoms and a progressive decline in lung function (Lee *et al.*, 2011a). The factors that lead to declining lung function remain uncertain, but chronic progressive airway wall remodelling may be at work (Lee *et al.*, 2011). Asthma remains a health threat and has become a major cause of morbidity and mortality worldwide as it also associated with exacerbations whereby asthmatic patients have worsening of their symptoms often precipitated by infection. These exacerbations magnify the morbidity and considerably contribute to the mortality rate of asthma (George and Brightling, 2016).

1.2.2(a) Pathophysiology of asthma

Asthma is a phenotypically heterogeneous chronic airway disease characterised by airway inflammation, airway-wall remodelling, airway smooth muscle contraction and hyperreactivity as well as increased mucus production. Airway inflammation is hallmark in mild-to-moderate asthma which is common and known as type 2 inflammation. This type 2 inflammation is driven by inflammatory cytokines (interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) secreted by type 2 helper T (Th2) cells (Figure 1.3) and generally promptly resolves after treatment with glucocorticoid (Israel and Reddel, 2017). These cytokines are also produced by innate lymphoid cells in response to infectious agents. Since the production of eosinophils is promoted by IL-4 and IL-5, this inflammation is often characterised by eosinophils (Nakagome and Nagata, 2011).

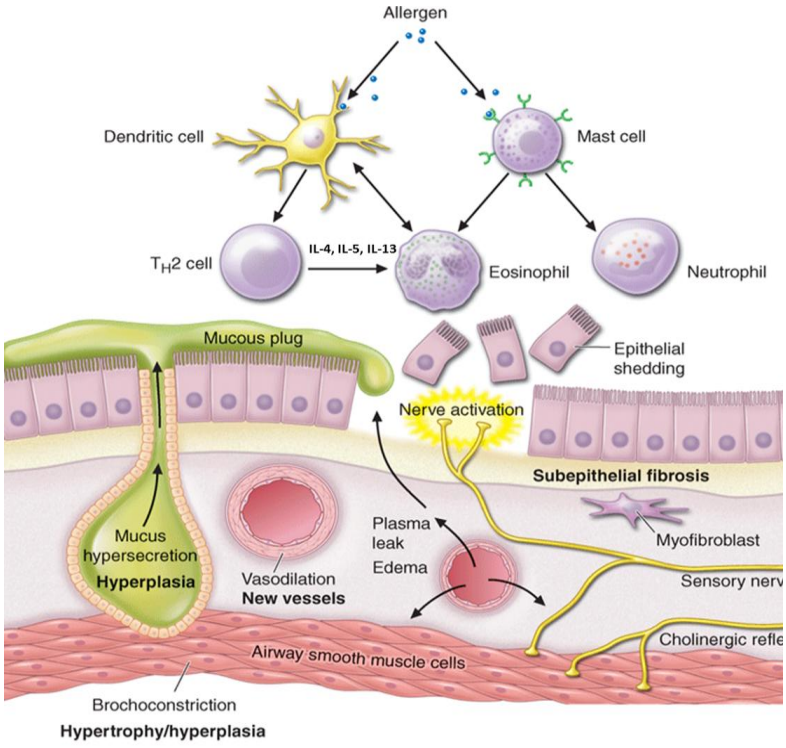

Figure 1.3 Overview of asthma pathophysiology as illustrated by the interaction between different inflammatory cells and the external environment including allergens. Key players in this mechanism include the T helper type 2 (Th2) cells which secrete cytokines including IL-4, IL-5 and IL-13, that activate inflammatory cells such as eosinophils and mast cells. Ultimately, airway smooth muscle cells are activated and airway epithelial cells over-secrete mucus (Jameson and Weetamen, 2012)

1.2.2(b) Mild-to-moderate asthma

Th2-mediated eosinophilic airway inflammation is associated with varying degree of asthma severity, ranging from mild-to-moderate to severe uncontrolled disease (Pelaia *et al.*, 2015). Analysis of sputum or bronchoscopic sampling suggested that eosinophilic airway inflammation is believed to play essential roles in the pathogenesis of asthma via the secretion of various inflammatory mediators together with cytotoxic eosinophils-derived granule proteins and lipid mediators in the airway (Amin, Janson and Bystrom, 2016). These mediators promote persistent inflammation that ultimately leading to the asthmatic exacerbations and declined lung functions which is generally observed in biopsies of asthmatic patients lung (Young *et al.*, 1986; Trautmann *et al.*, 2002; Acharya and Ackerman, 2014). Increasing evidences established that eosinophils essentially contribute to airway remodelling in asthma (Nakagome and Nagata, 2011). A study by Halwani et al (2013) reported that eosinophils promote airway remodelling during asthma by triggering airway smooth muscle (ASM) cell proliferation and thus increasing ASM mass. Another study by Humbles (2004) reported that allergen challenge of mice with selective ablation of the eosinophil lineage showed that eosinophils were required for an increment in ASM cells and collagen deposition suggesting an important role of eosinophils in the development of airway remodelling. Similar findings were also reported by Flood-Page et al (2003) and Lee et al (2004).

1.2.2(c) Severe asthma

Nonetheless, in the context of severe asthma, there are compelling evidences which suggest that a neutrophilic inflammation, orchestrated by type 1 helper T (Th1) and especially type 17 helper T (Th17) lymphocytes is more profound in patients with severe asthma (Baines *et al.*, 2011; Wood *et al.*, 2012; Simpson *et al.*, 2014). Wenzel and co-workers reported that there is a high neutrophils number in the airway lavage of patients with

severe asthma compared to patients with mild and moderate asthma (Wenzel *et al.*, 1997). It is thought to be involved in the progression of more severe irreversible airway obstruction leading to sudden death (Shaw *et al.*, 2007). This statement was supported by the evidence of an excess neutrophils in airway secretions and sub-mucous glands in biopsy sample from patients with fatal asthma (Carroll, 2002). There were also evidences stated that mast cells have been linked to the pathophysiology of asthma and often associated with more severe asthmatic phenotypes (Galli and Tsai, 2012; Andersson *et al.*, 2016). Abundant of mast cells are found to be localised within the bronchial smooth muscle bundles in severe asthma and trigger airway remodelling by secreting inflammatory mediators likes amphiregulin (Lei *et al.*, 2013; Andersson *et al.*, 2016). Therefore, if left untreated, asthma can lead to the airway irreversibility such as airway wall remodelling which is another key feature of asthmatic airways that has been shown to correlate with the disease severity.

1.2.2(d) Airway remodelling

In severe asthma, the term "airway remodelling" denotes the structural alteration that occurs in conjunction with, or as a consequence of, chronic inflammation, contributing to airway obstruction. Airway remodelling refers to a compilation of structural modification characterised by epithelial damage and hyperplasia, deposition of extracellular matrix (ECM) factors below the basement membrane (making the airways less compliant), proliferation of airway smooth muscle, angiogenesis and excess mucus production contribute to the thickening of the airway wall (Figure 1.4) (Evans *et al.*, 2009; Halwani, Al-Muhsen and Hamid, 2010; Lambrecht and Hammad, 2012; Martin and Verma, 2012). Jeffery et al (2001) described that the asthmatic airway walls are generally thickened between 50 and 300% relative to the normal airway walls due to airway remodelling that is attributed to alteration of airway epithelium, basement membrane and sub-mucosa. This phenomenon leads to luminal narrowing. Furthermore, the epithelium layer of the nasal and lower airways in the

asthmatic individuals appears to be more fragile and easily detach from the basement membrane. The consequence of these structural changes may cause partially reversible airway narrowing in mild asthma but mostly irreversible in chronic severe asthma airway together with airway hyperresponsiveness (AHR), mucus hypersecretion and airway oedema leading to a progressive decline in pulmonary function. There is growing support for a role of aberrant airway epithelial repair processes in response to persistent inflammatory response implicated in the development of pathological airways remodelling (Bai, 2010; Ganesan and Sajjan, 2013a). In addition, the accumulation of fibroblasts which leads to ECM deposition is also a causal factor (Pain *et al.*, 2014). Hence, airway remodelling in asthma may results to asthma exacerbations and even death from airway obstruction caused by contraction of smooth muscle, airway oedema, and mucus plugging. It is unclear that current drug therapies such as inhaled corticosteroid (ICS), long-acting B-adrenoreceptor agonist and tiotropium that are used for the treatment of inflammation are adequate for airway remodelling treatment as well. These drugs are effective in alleviating inflammation and preventing airway remodelling in animal models (Alrifai *et al.*, 2014). However, there is no currently available therapy targeting airway remodelling in human, even though reversibility of airway remodelling is suggested by studies in animal models of disease.

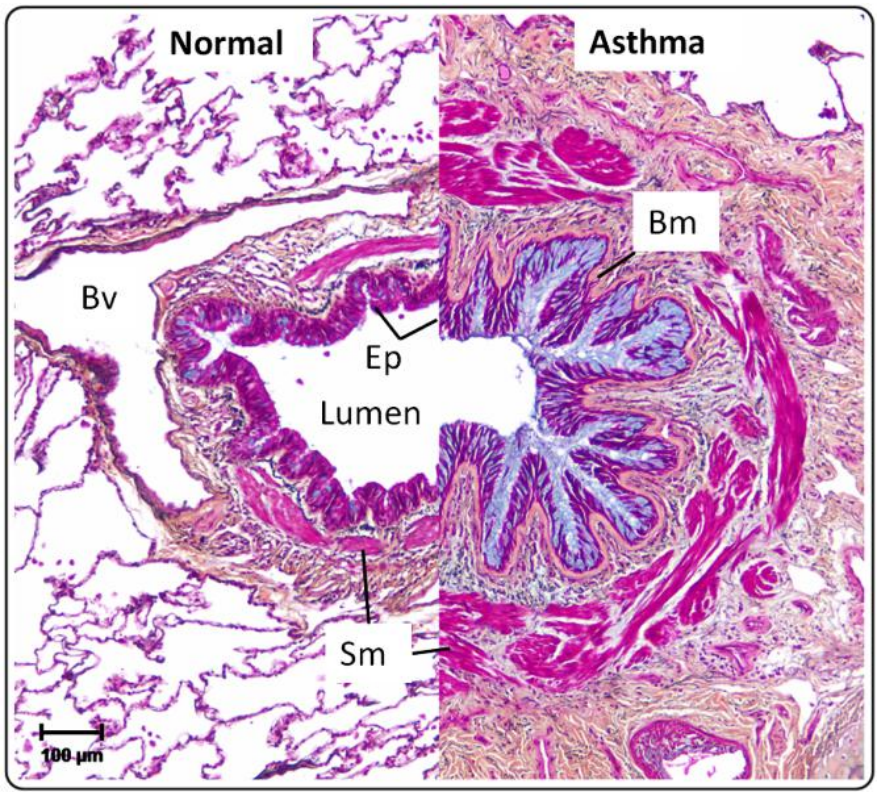

Figure 1.4 A significant structural remodelling of airway walls in asthmatic patients relative to normal individual. Histological section of a medium sized airway from a non-asthmatic patient and a patient with severe asthma stained with Movat's pentachrome stain. In asthma the epithelium (Ep) displays mucus hyperplasia and hypersecretion (blue), and a significant thickening of the basement membrane (Bm). Smooth muscle (Sm) volume is also increased in asthma. Bv=blood vessel. Adapted from (Wadsworth, Yang and Dorscheid, 2013).

1.2.2(e) Vascular permeability in asthma

Vascular component of airway remodelling contributes to the alteration of the airway wall in asthma which may be associated with disease progression. The vessels present in the sub-mucosa region of asthmatics are more permeable (Nagy *et al.*, 2008). Some reports confirmed the relevant occurrence of this phenomenon in airways of asthmatic patients (Kanazawa, Hirata and Yoshikawa, 2002; Asai *et al.*, 2003; Kanazawa, Nomura and Yoshikawa, 2004). In these studies, the permeability of microvascular was examined using the airway vascular permeability index. This index is measured by the level of albumin in induced sputum/albumin in serum (Kanazawa, Hirata and Yoshikawa, 2002), or as fibronectin concentrations (Meerschaert *et al.*, 1999) or alpha 2-macroglobulin (Svensson *et*

al., 1995) levels in the BAL fluid (James, Paré and Hogg, 1989). Increased microvascular permeability is a criterion for airway inflammation, since the extravasation of inflammatory cells to the airway requires vascular leakage and leukocytes transmigration through the endothelium. This plasma leakage can also lead to mucosal oedema and bronchial wall thickening, thereby reducing the airway lumen, which in turn causes airflow limitation and may contribute to AHR. Plasma extravasations can compromise epithelial integrity and contribute to formation of luminal mucus plugs (James, Paré and Hogg, 1989). These phenomena are common features during vascular remodelling in bronchial asthma (Zanini *et al.*, 2010). McDonald (1990) described a role of intercellular gaps between endothelial cells (ECs) of post capillary venules in microvascular permeability using animal models. Increase in vascular permeability results from the gaps in the endothelium that permit the extravasation of plasma proteins into the mucosal connective tissue and even into the airway lumen.

Besides, the immature and unstable nature of new capillaries during the chronic inflammation process lead to increased permeability of the microvascular (Otani *et al.*, 2004). The increased microvascular permeability is attributable to the secretion of inflammatory mediators, growth factors, neuropeptides, cytokines, eosinophil granule proteins, and proteases. A pro-leakage factor, vascular endothelial growth factor (VEGF) plays critical role in vascular permeability of the airway. The expression of VEGF has been shown to be increased in asthma and correlated with alteration in cell-cell junction, an increase in vessel permeability and leakage of plasma constituents into the airway (Kanazawa, Hirata and Yoshikawa, 2002; Kanazawa, Nomura and Yoshikawa, 2004). The anti-permeability factors such as Angiopoietin-1 (ANGPT1) on the other hand, function to stabilise the nascent vessels, making them resistant to leakage by increasing the vascular integrity. For example, mice transgenic for ANGPT1 have leakage-resistant blood vessels (Thurston *et al.*, 1999). The development of new therapy using this ANGPT1 might be beneficial in maintaining the integrity of ECs and thus reduce the airway inflammation.

1.2.2(f) Treatment options in managing asthma

Current drug therapy for asthma has been successful at managing asthmatic symptoms as up to 75% of individuals suffering from asthma can attain sufficient asthma control on inhaled corticosteroids (ICS with and without long-acting β2-agonists (LABA) (Bateman *et al.*, 2004; Royce and Tang, 2009). Development of asthma medication is to prevent and control asthma symptoms, reduce the frequency and severity of asthma exacerbation and reverse airflow obstructions (Dahl, 2006). Asthma medication can be classified as either bronchodilator for airway SM relaxing or as anti-inflammatory drugs to suppress airway inflammation. In current practice, asthma medications are categorised based on their roles in controlling the disease – either as quick relieve or long-term control (Fanta, 2013). An example of quick relief medication is short-acting β-agonist which is effective in quick reversal of airflow obstruction and offers rapid relief of asthmatic attack (Fanta, 2013). Salbutamol, levelbuterol and maeprotenerol are drugs that were grouped under such medication. Conversely, corticosteroids (or glucocorticosteroids), LABA, leukotriene modulators, anti-immunoglobulin-E (IgE) are examples of controller asthma drug (Fanta, 2013). The most common used of corticosteroids nowadays is inhaled corticosteroids (ICS). ICS is the most effective therapy available nowadays and used as the first-line therapy for persistent asthma in adults and children in many countries (Dahl, 2006). Clinical evidences suggest that ICS is capable of significantly diminishing airway inflammation and AHR, preventing the acute exacerbations, improving lung function and decreasing the symptoms severity (Georgitis, 1999). These factors are the key reasons for the widespread use of ICS as the frontline in managing asthma which also has been approved by both national and international guidelines (Dahl, 2006).

Apart from the positive results of ICS, there were also debates concerning the role of ICS in restoring the airway structure (Georgitis, 1999; Dahl, 2006). As mentioned earlier, asthma is characterised by changes in the epithelial structure which include epithelial damage. Several *in vitro* studies demonstrated that corticosteroids could increase the

apoptosis of epithelial cells which is in the case of asthma, this could further leads to chronic epithelial damage (Mauad, Bel and Sterk, 2007). In contrast, in a retrospective biopsy study, prolong treatment of ICS (10 years) did augment the inflammation and moderately improved the epithelial damage (Lundgren *et al.*, 1988). The chemical constituent of ICS may also cause adverse effects to the human beings. Although most of the currently available ICS have been designed to undergo first-pass metabolism inactivation in the liver subsequent to absorption from the gastrointestinal tract before reaching the systemic circulation (Wood and Barnes, 1995; Fanta, 2013). Yet, it is important to note that prolonged usage of ICS and at high doses can cause adverse effects such as accelerated loss of bone mass, osteoporosis, oral candidiasis, dysphonia, cataract and many others (Guilbert *et al.*, 2006; Aun *et al.*, 2009). Furthermore, systemic absorption of ICS may have deleterious effects over long term use (Barnes and Adcock, 2003). Despite ICS is highly effective at controlling asthmatic symptoms, at practical level, only 30-40% of individuals with asthma attained adequate asthma control with ICS with or without LABA (Reddel, 2012). This suboptimal asthma control occurred due to various causes and poor adherence with asthma medications is the main cause. This factor can cause persistent asthma symptoms that finally may contribute to severe asthma (Bardin *et al.*, 2017).

There is a significant unmet need for the therapy of severe asthma that affect approximately 20% of patients with asthma (Hetherington and Heaney, 2015). The major obstacle in this small proportion of patients with severe asthma is that they are resistance to therapeutic effect of corticosteroids. Current treatment guidelines advise high-dose ICS and/or systemic corticosteroid to avoid severe asthma from becoming ‘uncontrolled’, however it is now accepted that many aspects of asthma are not corticosteroid responsive (Chung, 2015). Hence this therapy may not provide clinical benefit in many patients (Hetherington and Heaney, 2015). These problems highlight the need to find alternative therapy with fewer side effects such as stem cell-based therapy.

1.2.2 (g) Asthma model

Asthma led to thousands of death annually worldwide (Global Initiative for Asthma, 2018). Therefore, the development of prevention strategies and new therapy methods is a must. Animal has been used in drug discovery study to eliminate the needs on manipulating human in research (Van der Velden and Snibson, 2011). In remodelling of human respiratory system, the reason in using the animals as a model study is that the animals provide experimental settings that allow us to understand the interaction between immune system with functioning respiratory systems. In order to better mimic the pathophysiology of a human lung disease, animal with large size is the suitable to be used since the complexity of the lung structure is more similar and identical to human as compared with small size animals (Van der Velden and Snibson, 2011). There are many asthma models described in the literature, using various methods to better mimic human asthma. They represent a scenario to understand disease pathophysiology and for discovery of potential novel treatments and to improve the existing medications. An ideal animal model of asthma must have the capability to reproduce both anatomical and physiology features mimic to human asthma. This includes IgE-mediated sensitivity to antigens, increase airway resistance, acute bronchoconstriction, chronic airway inflammation, eosinophils influx, mucus hypersecretion, production of Th2 cytokines, airway-wall remodelling and many others. In developing animal model of asthma-related airway inflammation, rabbit is a suitable candidate due to its ability to develop disease from the point being sensitised with allergen for a prolonged period (Matute-Bello, Frevert and Martin, 2008). Rabbits also shared similarities with human airways and it is phylogenetically closer to human than any other rodents (N A Kamaruzaman *et al.*, 2013). Both rabbit and human have abundant sub-mucosa glands and mucus secreting goblet cells in the upper airways in contrast to mice(Koehler *et al.*, 2005). Previous study had used rabbits as a model to investigate the efficacy of lung reduction surgery in the treatment of emphysema and COPD (Keir and Page, 2008a). The treatment

was found successful and being use at clinical setting in recent years. Sharing similarities with human lungs make rabbits a useful tool as model to study asthma.

The nature of the inflammatory model may be influenced by the choice of the allergen, the sensitisation and challenge protocol and the choice of animal strain (Zosky and Sly, 2007). To mimic the pathogenesis of human asthma, protocols for development of animal models must include a sensitisation and a challenge phase. Generally, repeated doses of systemic allergen administration together with adjuvants, such as aluminium hydroxide are used to increase immune response. Allergens that have been used in animal models including ovalbumin (Ova), house dust mite (HDM), fungi (*Aspergillus fumigatus*, *Alternaria alternata*), cockroach extracts, *Ascaris* antigens, cotton dust, ragweed and latex (*Hevea brasiliensis*) (Zosky and Sly, 2007). The choice of allergen is depending on the condition to be replicated and it can be used separately or in combination. Classically, Ova has been widely used to induce an allergic reaction in animals, and whilst it is possible to reproduce many of the features of the asthmatic lung, i.e. specific immunoglobulin (Ig)E levels, T-helper cell (Th)2-associated eosinophilic inflammation, early and late asthmatic responses (EAR and LAR, respectively), and AHR and airway remodelling (Fuchs and Braun, 2008). Ova, however, is seldom implicated human asthma, and other groups have used alternative allergens that may have greater clinical relevance, such as HDM and cockroach extract (Aun *et al.*, 2017). HDM is an aeroallergen that has been successfully used to induce asthma in animal models. Three characteristics of this allergen that make it suitable are they have an intrinsic enzymatic activity, immunogenicity and direct activation through the Dectin-2 receptor of innate immune cells that promote allergic inflammation are the three characteristics that make HDM suitable to be used as allergen to induce asthma (Kim *et al.*, 2006). A large proportion of human asthmatic patients have elevated levels of HDM specific IgE and, after challenge with HDM, exhibit EAR, LAR and increases in airway inflammation (Aun *et al.*, 2017).Though, HDM is quite dangerous as exposure to HDM by

any genetic susceptible individuals can cause symptoms from as mild as atopic dermatitis to bronchial asthma.

1.3 Angiopoietin 1 and 2

Angiopoietins are major vascular regulators that play critical roles in regulating vascular homeostasis via vascular endothelial tyrosine kinase receptor, Tie2 signalling that highly expressed on ECs under normal or pathologic conditions (Eklund and Olsen, 2006; M. Kim *et al.*, 2016). Tie2 is primarily involved in angiogenesis, in conjunction with VEGF. In addition to angiogenesis, Tie2 receptor is also essential in vascular integrity maintenance in the embryo and in the adults. Both Angiopoietin-1 (ANGPT1) and angiopoietin-2 (ANGPT2) are ligands that share similar structures and compete for Tie-2 receptor with equal affinity but leading to opposing effects (Fukuhara *et al.*, 2008).

ANGPT1 belongs to the endothelial growth factors that are predominantly expressed by pericytes and vascular smooth muscle cells located within the vascular basement membrane (van Meurs *et al.*, 2009). ANGPT1 plays critical role in embryonic vascular development since deletion of either ANGPT1 or Tie2 has been associated with embryonic fatality (Suri *et al.*, 1996). While in the adult's vasculature, ANGPT1 is essential for the maintenance of microvascular endothelium integrity. ANGPT1 triggers Tie2 phosphorylation signalling in promoting normal quiescent vascular phenotype of vascular ECs, reducing endothelial permeability and alleviating vascular inflammation (Gamble *et al.*, 2000; Fukuhara *et al.*, 2010). ANGPT1 is also involved in promoting ECs survival, stabilising the growing vessels as well as attenuating the activation of vascular endothelial barrier to prevent capillary leakage and leukocyte transmigration into the tissues by altering the ECs adhesion molecules and cell junction (Augustin *et al.*, 2009; Moss, 2013). ANGPT2 on the other hand generally has an opposing action. Despite binding of ANGPT1 to Tie2 primarily activates the receptor, the antagonist ANGPT2, may counteract these functions by

binding to Tie2 without inducing phosphorylation, disrupts the connections between the endothelium and perivascular cells and thus reduce vascular integrity, provoke inflammation, and promote vascular leakage (Fiedler and Augustin, 2006; Saharinen *et al.*, 2008; Hakanpaa *et al.*, 2015). ANGPT2 functions as a context-dependent Tie2 antagonist, generally incapable to activate Tie2 except presented at high concentration or in prolonged time under *in vitro* settings. Tabruyn et al (2010) suggested that ANGPT2 competitively suppressed the ANGPT1 action by increase binding to Tie2 resulting in vascular remodelling and inflammation. Though, there is also conflicting evidence indicating that ANGPT2 may possibly induce the activation of Tie2 under certain conditions such as in stressed ECs (Daly *et al.*, 2006; Koh, 2013).

There are accumulating studies that are investigating the role of ANGPT1/Tie2 system in the pathophysiology of respiratory diseases. This is primarily due to the anti-inflammatory, anti-permeability, and endothelial-protective effects of ANGPT1. Impaired ANGPT1/Tie2 system allows migration and infiltration of inflammatory cells from circulation into the airways tissue that will cause inflammation (Makinde and Agrawal, 2008). High circulating ANGPT2 level was observed in exacerbated asthma patients indicating that the serum level of pro-angiogenic factors may correlate on-going asthmatic exacerbated state and serve as a potential biomarker of the disease severity. In contrast, circulating levels of ANGPT1 in plasma of asthmatic patients were found to be reduced (Lee *et al.*, 2016). ANGPT1 has been shown to supress leukocytes adhesion to ECs by reducing the expression of VEGF-induced adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin (Kim *et al.*, 2001). The binding of leukocytes to ECs via these molecules is an early and important step towards inflammation (Ismail *et al.*, 2012). Simoes et al (2008) further demonstrated that administration of ANGPT1 via intranasal route reduced airway inflammation and hyperreactivity in Ova-induced asthma model. In a separate study, Xu et al (2008) showed that the expression of ANGPT1 reduced the permeability of ECs and inflammation in

pathogenesis of acute respiratory distress syndrome (ARDS). In term of vascular permeability, treatment with ANGPT1 has been shown to support ECs junctions tightening by increasing the localisation of platelet endothelial cell adhesion molecule-1 (PECAM-1) to these junctions and attenuate leukocyte transmigration (Gamble *et al.*, 2000). In a separate study, Kanazawa et al (2007) described an inverse correlation between ANGPT1 level and vascular permeability index in asthmatics patients. They found that the vascular permeability was associated with increased VEGF level (pro-leakage) and decreased ANGPT1 level (anti-leakage). Mei et al (2007) showed that pre-treatment with ANGPT1 in endotoxin-exposed mice suppressed the vascular leakage leading to decrease in granulocytes influx and pro-inflammatory mediators in animal model of lipopolysaccharide (LPS)-induced acute lung injury (ALI). This result provides further evidence suggesting the protective effect of ANGPT1 in protecting the adult vasculature against plasma leakage. Agonist activity of ANGPT1 on the Tie2 receptor has vast potential for being a candidate of gene therapy in treating airway inflammation and previously been studied in the ALI by other groups (Mei *et al.*, 2007; Xu *et al.*, 2008). In general, most findings suggest that ANGPT1 provides beneficial effect in the treatment of lung diseases.

1.4 Cell-based therapy using MSCs

Cell-based therapy as a treatment for lung diseases is a rapidly growing field in regenerative medicine. Several preclinical studies reported promising results supporting the use of stem cells to treat lung diseases such as pulmonary hypertension (Wang *et al.*, 2007), ALI (Wang, Li and Wang, 2013), inflammatory and immune-mediated lung conditions (Lama *et al.*, 2007). MSCs represent the most frequently used stem cell type in preclinical reports and clinical trials conducted so far. As of April 2016, more than 33 clinical trials utilising MSCs to treat lung diseases were registered on the ClinicalTrials website (U.S. National Institutes of Health, 2016).