THE EFFECT OF MICRORNA TARGETING IL17RA IN REGULATING THE EXPRESSION OF RANKL AND OPG IN STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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LIST OF SYMBOLS, ABBREVIATION AND ACRONYMS

±	Plus minus
°C	Degree celcius
µg	Microgram
µL	Microliter
Act 1	Actin-1 precursor
ALP	Alkaline Phosphate
ANOVA	Analysis of variance
BMMSC	Bone marrow mesenchymal stem cell
BMP	Bone morphogenic protein
BMU	Basic multicellular unit
Cbfa1	Core-binding factor alpha 1
CD	Cluster differentiation
cDNA	Complimentary DNA
CFU-F	Colony Forming Unit Fibroblast
c-JUN	Jun Proto-oncogene
CO_2	Carbon dioxide
Col10a1	Collagen Type X Alpha 1 Chain
DEPC	Diethyl Pyrocarbonate
DFSC	Dental follicle stem cells
DNA	Deoxyribonucleic acid
DPSC	Dental Pulp Stem Cells
ECM	Extra cellular matrix
EDTA	Ehylene diamine tetra acetic acid

EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbant assay
ERK	Extra cellular signal-regulated kinase
et al.	Others
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HLA	Human Leucocyte Antigen
hMSC	Human mesenchymal stem cell
IFNγ	Inteferon gamma
IL	Interleukin
JNK	c-Jun NH_2-terminal kinases
MAPK	Mitogen-activated protein kinase
MiRNA	MicroRNA
mL	Milliliter
mRNA	Messenger ribonucleic acid
MSC	Mesenchymal stem cell
ng	Nanogram
OCN	Osteocalcin
OD	Optical density
OM	Osteogenic media
OPG	Osteoprotegerin
PBS	Phosphate buffered saline
PDLSC	Periodontal Ligament Stem Cells

PPARγ2	Peroxisome proliferator-activated receptor γ
qRT-PCR	Quantitative real time polymerase chain reaction
RANK	Receptor activator of nuclear factor kappa-β
RANKL	Receptor activator of nuclear factor kappa-β ligand
rIL17A	Recombinant interleukin 17A
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Reverse transcriptase
Runx2	Runt related transcription factor 2
SHED	Stem cells from human exfoliated decidous teeth
Th17	T helper 17
TNF	Tumor necrosis factor
UTR	Untranslated region
α	Alpha
α-MEM	Alpha-minimum essential medium
β	Beta
γ	Gamma

KESAN MIKRORNA MENSASARKAN IL17RA UNTUK MENGAWAL EKSPRESI RANKL DAN OPG DALAM SEL STEM DARIPADA GIGI SUSU MANUSIA YANG TERLUPAS

ABSTRAK

Kini, IL17A telah menjadi kunci utama dalam pembentukan semula tulang. Pengaturan osteoprotegerin (OPG) dan pengaktifan reseptor factor nuclear kapa-β ligan (RANKL) oleh IL17A telah dijelaskan dalam sel stem mesenchymal manusia (hMSC). Di sini, matlamat kajian ini adalah untuk menentukan potensi mikroRNA yang menyasarkan IL17RA dan kesannya terhadap ekspresi OPG dan RANKL dalam sel stem daripada gigi susu manusia yang terlupas (SHED). MikroRNA yang berpotensi telah diramal dengan menggunakan tiga program algoritma yang terkini iaitu DIANA-micro T CDS, TargetScan v7.1 dan miRWalk v2.0 melalui proses penapisan yang kompleks secara *in silico*. Kepekatan mikroRNA yang mensasarkan GAPDH telah dioptimumkan untuk mendapatkan penurunan ekspresi GAPDH yang paling berkesan. Kepekatan 25, 50 dan 100 nM dengan tempoh pemindahan selama 48 jam diuji dengan mikroRNA-kawalan negatif sebagai kawalan negatif. Keputusan menunjukkan tahap mRNA GAPDH adalah terendah pada kepekatan 50 nM selepas dinilai oleh PCR masa nyata kuantitatif dan dinormalisasi dengan *β-actin*. Kepekatan ini boleh digunakan sebagai kepekatan miRNA umum dalam kajian ini. SHED telah dikulturkan dalam media penting minimum alfa (αMEM) yang lengkap dengan media osteogenik (OM) dan dirawat dengan 50 ng/mL interleukin 17A (rIL17A) rekombinan selama 7 hari (fasa osteogenik awal). Sel yang dirawat kemudiannya ditransmisikan dengan 50 nM mimik kawalan positif, kawalan negatif dan mikroRNA yang diramal

dari kajian silico iaitu hsa-miR-6761-5p dan hsa-miR-4524a-3p. Pengurangan nilai gen sasaran dan kesannya terhadap penanda osteogenik dinilai dengan mengukur ekspresi *IL17RA, OPG, RANKL* dan *GAPDH* oleh PCR masa nyata kuantitatif serta dinormalisasi dengan *β-actin*. Oleh itu, kedua-dua mimik mikroRNA menunjukkan penurunan gen IL17RA dan hsa-miR4524a-3p telah menunjukkan penurunan yang lebih kuat ($p < 0.001$) apabila dibandingkan dengan hsa-miR-6761-5p ($p < 0.01$). Di samping itu, terdapat pengurangan ketara kepada ekspresi *OPG* dari kedua-dua mikroRNA dan penurunan *RANKL* juga diperhatikan dalam kedua-dua SHED yang ditrasfeksikan, namun pengurangan itu tidak ketara. Penemuan ini menunjukkan peranan mikroRNA yang menyasarkan IL17RA dalam ungkapan OPG dan RANKL dalam SHED, dengan itu mencadangkan peranan penting dalam fisiologi tulang SHED.

THE EFFECT OF MICRORNA TARGETING IL17RA IN REGULATING THE EXPRESSION OF RANKL AND OPG IN STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

ABSTRACT

IL17A is now becoming a key role in bone remodelling. The regulations of osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-β ligand (RANKL) by IL17A were briefly explained in human mesenchymal stem cell (hMSC). Herein, the aim of the present study was to determine the potential microRNA targeting *IL17RA* and its effects toward *OPG* and *RANKL* expression in stem cell from human deciduous teeth (SHED). Potential microRNA was predicted by using three latest algorithmically programs such as DIANA-micro T CDS, TargetScan v7.1 and miRWalk v2.0 through a complex filtration process via *in silico*. The concentration of microRNA-targeting *GAPDH* was optimised for the most efficient downregulation of *GAPDH* mRNA. The concentrations of 25, 50 and 100 nM and transfection duration of 48 hours were tested with microRNA-mimic negative control (microRNA-mimic NC) as a negative control. The results showed the GAPDH mRNA level was lowest at concentration of 50 nM after evaluated by quantitative real time PCR and normalised with *β-actin*. This concentration can be used as the general miRNA concentration in this study. SHED was cultured in complete alpha minimum essential media (αMEM) supplemented with osteogenic media (OM) and treated with 50 ng/mL of recombinant interleukin 17A (rIL17A) for 7 days (early osteogenic phase). Treated cells were then transfected with 50 nM of positive control mimic, negative control mimic and predicted microRNAs mimic from *in silico* study which are hsa-miR-6761-5p and hsa-miR-4524a-3p. Downregulation of target gene and its effects towards osteogenic

markers were evaluated by measuring the expressions of *IL17RA, OPG, RANKL* and *GAPDH* by quantitative real-time PCR and normalised with *β-actin*. Hence, transfections of both microRNA mimics showed downregulation of *IL17RA* gene which hsa-miR4524a-3p showed stronger downregulation ($p < 0.001$) when compared to hsa-miR-6761-5p ($p < 0.01$). In addition, there was a significant downregulation of *OPG* expression from both microRNAs and downregulation of *RANKL* was also noted in both transfected SHED, however the reduction was not significant. These findings demonstrate the role of microRNA targeting *IL17RA* in the expression of *OPG* and *RANKL* in SHED, thus suggesting its important role in bone physiology of SHED.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Vital functions of bone are protection, movement, leverage, mineral storage as well as a source of hematopoietic cells and stem cells. Bone formation and resorption are constantly being remodelled in bone metabolism process (Nam and Kampa, 2013). Stiff, light and flexible are important characteristics of the bone to make loading and facilitate movement. Role players in bone remodelling cycle are osteoblast which mediated the bone formation and bone resorption by osteoclasts (Stavre *et al.*, 2016).

The activity of the cycle must be dynamically balance to maintain the integrity of the constituent cell types in bone. Osteoblast produced the organic bone matrix which mediated mineralisation whereas the osteocytes dissolves bone mineral and enzymatically degrades extracellular matrix (ECM) proteins through its unique type of exocrine cell (Crockett *et al*., 2011).

The past decade has seen the immune and skeletal systems are played a complex role by sharing several regulatory molecules such as receptors, cytokines and transcription factors (Rucci, 2008). Wide range of signalling pathways regulated the complex sequence of events in the skeletal development process (Gámez, Rodriguez-Carballo and Ventura, 2014).

The signalling molecules include osteoprotegerin (OPG), receptor activator of nuclear factor-β ligand (RANKL) and receptor activator of nuclear factor-β (RANK) are played important roles in the regulation of bone (Boyce and Xing, 2007). Formation of multinucleated osteoclasts, their activation and survival in normal and pathologic conditions of bone remodelling are regulated by RANKL/RANK signalling. Binding of OPG with RANKL would protect the skeleton from excessive bone resorption and preventing RANKL to bind with its receptor. These are important to determine bone mass and skeletal integrity (Jones, Kong and Penninger, 2002).

Recent developments in the field of bone repair have led to a renewed interest in stem cell research. Self-renewal capacity, osteogenic lineage differentiation and trophic factors secretions are the main characteristics of mesenchymal stem cells (MSCs) that may contribute to cellular therapies in bone repair (Fanganiello *et al.*, 2015).

In recent years, there has been an increasing interest in stem cell from human exfoliated deciduous teeth (SHED) which showed the expressions of mesenchymal stem cell markers such as STRO-1 and CD146 but negative for haematopoietic markers such as CD45, CD11b/c and HLADR. In addition, SHED has been reported to undergo osteogenic differentiation and generated bone *in vivo* which led to high interest for bone tissue regeneration (Rosa *et al.*, 2016).

Previous studies have attempted to explain the role of IL17A in osteogenesis which showed increased in ALP activity and decreased RANKL mRNA expression (Osta *et al*. 2014). Conversely, Lin *et al*. (2014) has reported IL17A could significantly increase the expression of RANKL and downregulated the expression of OPG in human periodontal ligament cells.

IL17A which commonly known as IL17 was one of the six members in the IL17 cytokine family including IL17B, IL17C, IL17D IL17E and IL17F. The biological function and regulation of IL17A and IL17F were best understood (Jin and Dong, 2013). On the other hand, significant events were induced in many tissues after the binding of IL17 to its receptor (Raychaudhuri, Raychaudhuri and Genovese, 2012).

The first IL17 binding protein to be identified was IL17RA. IL17RA is ubiquitously expressed at the mRNA level, although surface expression varies widely. Because the IL-17R superfamily shares only minimal homology with other receptors (Maitra *et al.*, 2007).

The past decade has seen the rapid development of microRNAs (miRNAs) research in osteogenic differentiation where these would provide a unique regulatory layer that is essential for the regulation of gene expression in most metazoans (Brennecke *et al.*, 2005).

RNA-induces silencing complex (RISC) guided by these small RNAs which the size about 22 nucleotides in length to 3' UTR of selected mRNAs target site (Hofacker, 2007). These would provide a new dimension to the post-transcriptional control of cells which were considered the key epigenetic mechanisms in bone remodelling by inhibiting mRNA translation.

Therefore, numerous miRNAs have been found to be involved in the regulation of bone homeostasis, and they play critical roles in bone remodelling (Jing *et al.*, 2015). Collectively, we outline a critical role of microRNA-targeting IL17RA in regulating osteogenic differentiation of SHED.

1.2 Statement of the problem

Six ligands including IL17A to IL17F are exclusively the IL17 cytokine family which signal through five receptors namely IL17RA to IL17RE. A number of researchers have reported the roles of IL17RA in bone-destructive mechanism (Loss *et al.*, 2010), rheumatoid arthritis (Kehlen *et al.*, 2002), glioma stem cell (Parajuli *et al.*, 2015) and recent evidence suggest the major role of inhibition of IL17A and IL17RA in pathological condition (Beringer, Noack and Miossec, 2016).

In addition, IL17A promoting effects on the osteogenic differentiation of isolated human bone marrow-derived mesenchymal stem cells (hMSCs) was reported by Osta et al. 2014. In addition, IL17A promoted osteoblastogenesis in bone regeneration process (Ono *et al.*, 2016). IL17 stimulation in hMSC are important in osteogenic differentiation which suggests the interaction between IL17 and IL17RA in osteogenesis (Kim *et al.*, 2014).

What is not yet clear is the effect of microRNA targeting IL17RA towards the osteogenic differentiation in SHED where the overall mechanism of RANKL/OPG in osteogenic differentiation of SHED has not been studied so far. The physiological role of microRNA targeting IL17RA is still unknown.

1.3 Justification of study

We examined the potential molecular mechanisms regulating the expression of IL17RA. In depth analysis of the regulatory role of these important cytokine and its receptor could provide further understanding on the underlying mechanism in bone remodelling. Some miRNAs play critical roles in immunoregulation and these results provide an insight into the roles of IL17RA in mediating physiological role of bone. In contrast, these microRNA targeting IL17RA may play important role in other physiological or pathological conditions.

1.4 Research objectives

1.4.1 General Objective

a.) To determine the effect of microRNA targeting IL17RA in regulating RANKL and OPG expression in SHED.

1.4.2 Specific Objective

a.) To predict and identify the interaction between microRNAs and human IL17RA mRNAs and to elucidate the sequence-dependent regulation of IL17RA by miRNAs by *in silico* study.

b.) To verify the effect of the predicted microRNAs targeting IL17RA on the levels of IL17RA mRNA transcript by quantitative real-time PCR.

c.) To investigate the effect of microRNA targeting IL17RA on osteogenic markers expression, OPG and RANKL by quantitative real-time PCR.

1.5 Research Hypothesis

MicroRNA targeting IL17RA regulates osteogenic markers expression, OPG and RANKL in SHED.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bone and teeth

There is a large volume of published studies describing the role of bone and teeth in research and its important in orthopaedic and orthodontic field. In response to hormones, growth factors and mechanical loading, bone is continuously formed and absorbed (Nam and Kampa, 2013). The rigidity, hardness as well as power of regeneration and repair were the important characteristics of bone as specialized supporting framework of the body (Fogelman, Van Der Wall and Gnanasegaran, 2012).

Modelling (reshaping) of bone is important to help it adapts with biomechanical forces and influences remodelling of bone to enable it to eliminate old or micro-damaged bone and replace it with new bone. These processes are crucial to enhance mechanical strength of bone (Raisz, 1999).

Cortical bone and trabecular bone were two components of bone which cortical bone is dense, solid and surrounds the marrow space whereas the trabecular bone consist of honeycomb-like network of trabecular plates and rods interspersed in the bone marrow compartment (Clarke, 2008).

Generally, bone protects the vital organs, producing marrow-derived cell for both blood forming and fat storage, acts as a mineral reservoir for calcium homeostasis and a storage of growth factors and cytokines, and also takes part in acid–base balance (Birmingham *et al.*, 2012).

In contrast, development of teeth is crucial at the correct positions in relation to the forming jaw bones. Tooth-associated bone namely alveolar bone enables teeth to attach to the jaw bones via periodontal ligament (Lin *et al.*, 2014). Sequential and reciprocal interactions between the oral ectoderm and neural crest-derived mesenchyme develop an organ namely teeth (Thesleff, 2003).

2.2 Bone remodelling

Bone is a dynamic tissue, subjected to a continuous renewing during the life of each individual by the process of bone remodelling (Fogelman *et al.* 2012) Balanced homeostasis between bone formation and resorption are crucial in maintaining dynamic bone structure and function, eliminate ischemic or micro-fractured bone and to ensure a correct calcium homeostasis (Raisz, 1999). In early foetal life, remodelling of bone begins and involves a series of process of both cell lineages mediated by mesenchymal osteoblastic lineage and the hematopoietic osteoclastic lineage (Crockett *et al.*, 2011).

From Figure 2.1, interaction of osteoclast and osteoblast precursor cells were the initial “activation” stage and led to migration, differentiation and fusion of large multinucleated osteoclasts which initiates resorption to the mineralized bone surface (Rucci, 2008). These cells degrade all the bone matrix components by secretion of hydrogen ions and lysozyme enzymes especially cathepsin K (Teitelbaum, 2000). Whereas, osteoblast cells contribute to deposit the organic components of bone extracellular matrix and control the resorption activity of osteoclasts by monitoring recruitment, differentiation and maturation of osteoclast (Caetano-Lopes, Canhao and Fonseca, 2000).

Meanwhile, Multinucleated cells osteoclasts were formed by the fusion of mononuclear progenitors of the monocyte or macrophage family and played a central role in resorptive of bone (Fogelman, Van Der Wall and Gnanasegaran, 2012). Several factors were expressed by the osteoclast associated with bone resorption that

regulate osteoblast function. Osteocytes and bone lining cells were two components of bone that important in mechanosensor function, osteoblast and osteoclast activity modulation and mineral homeostasis regulation (Teitelbaum, 2000). Both osteoblast and osteoclast have an equal important role in the regulation of bone mass.

Large Golgi apparatus and abundant rough endoplasmic reticulum were visible when mononuclear osteoblasts were activated. In addition, tight junction formation with adjacent osteoblasts facilitate the regions of plasma membrane in vesicular trafficking and secretion (Caetano-lopes and Canhão, 2007). The differentiation led to secretion of bone matrix and giving rise to osteocytes resulted from trapped osteoblasts in bone matrix. Through the plasma membrane of osteocytes, they communicate with each other and their surrounding which act as mechanosensor to control both osteoclast and osteoblast when to resorb or form the bone (Crockett *et al.*, 2011).

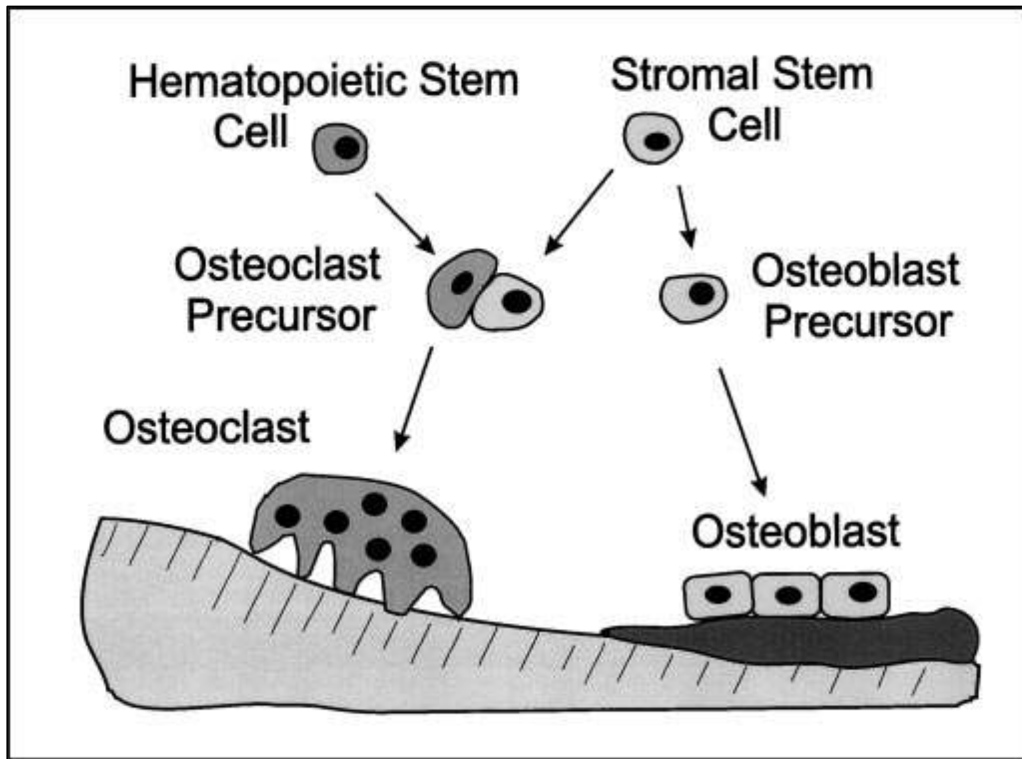

Figure 2.1 The schematic drawing shows the origin and fate of osteoblasts and osteoclast. Adapted from Raisz (1999).

2.3 RANKL/RANK/OPG in bone remodelling

In 1990, the RANKL/RANK/OPG system was discovered for the regulation of bone which accelerated the fundamental understanding of bone remodelling and modelling regulation (Boyce and Xing, 2007). For many years, we have known that osteoblastic stromal cells regulated osteoclast formation and discovered the RANKL/RANK/OPG system would have extensive functions beyond regulation of bone remodelling (Boyce and Xing, 2008).

Formation, activation and survival of osteoclast was regulated by RANKL/RANK signalling in normal remodelling and pathological conditions (Jones, Kong and Penninger, 2002). As showed in Figure 2.2, protection of bone from excessive resorption was mediated by OPG by binding to RANKL which would prevent the ligand to bind with its receptor, RANK (Oshita *et al.*, 2011). The relative concentration of RANKL and OPG in bone is important to determine the mass and strength of bone (Baharuddin *et al.*, 2015).

RANK, RANKL and OPG showed dynamic spatiotemporal expression patterns during odontogenesis and osteogenesis (Ohazama, Courtney and Sharpe, 2004). A critical regulator of osteoblast differentiation in the presumptive dentary bone region, runt-related gene 2 (*Cbfa1/Runx2*) showed similar expression as RANKL from E12 and discussed the role of RANKL in early ossification centre for dentary bone formation. Moreover, these expression pattern showed that RANK and OPG are expressed in tooth bud epithelium and mesenchyme (Otero, García and Wilches-Buitrago, 2016).

At early stages of development, connections between the formation of ossification centre for dentary bone and tooth was developed from the well-established interactions among RANK, RANKL and OPG (Heinrich *et al.*, 2005). These interactions may be crucial in coordinating the temporal spatial development of bone and teeth.

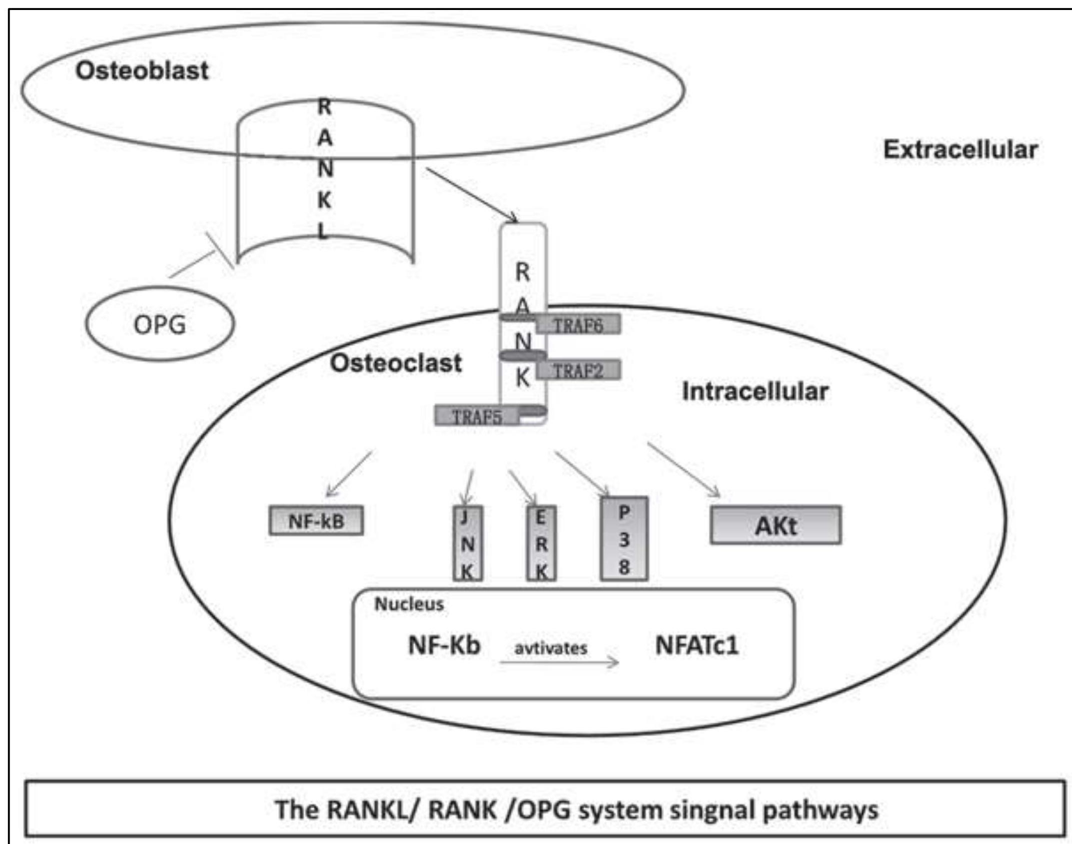

Figure 2.2 The schematic drawing showed the RANKL/RANK/OPG system signal pathways. Adapted from Liu and Zhang (2015).

2.4 Mesenchymal stem cell (MSCs)

Mesenchymal stem cell or stromal stem cells are multipotent stem cells (MSC) mainly reside in bone marrow (BM) and also been isolated from other tissues. MSC are capable to differentiate *in vitr*o and *in vivo* through many pathways such as bone, cartilage, cardiac cells, skeletal muscle, neural cells, tendon, adipose and connective tissue (Ayatollahi *et al.*, 2012).

In 1976, MSCs was first described as the clonal and plastic-adherent cells and these multipotent cells have giving many advantages characteristics such as multilineage potential, immunosuppressive ability and ease to gene modification which would give promising future for tissue engineering, gene therapy applications and immunotherapy (Sotiropoulou *et al.*, 2006).

MSCs have three minimum criteria include 1) MSCs show fibroblast-like morphology and plastic adherent properties under normal culture conditions; 2) MSCs express some markers such as CD24, CD90, CD105, Sca-1, SSEA-4, CD11b, and Sdc4 on their surface while lack expression of others surface markers such as CD133, CD14, CD19, CD34, CD45 and HLA-DR and 3) MSCs have great self-renewal capacity while maintaining their multi-potency (Chen *et al.*, 2009).

Besides their potential in cardiogenic and neurogenic differentiation, they would differentiate into three lineages such as osteoblasts, adipocytes and chondrocytes under appropriate conditions (Sotiropoulou *et al.*, 2006). Therefore, remarkable attention has been arose to use the potential of MSCs in many discipline of science (Sisakhtnezhad, Alimoradi and Akrami, 2017).

2.5 Stem cell from human exfoliated deciduous teeth

Adult stem cell was widely used in research and clinical treatments due to their ability in multilineage differentiation potential while maintaining their unique characteristics. In contrast, adult stem cells were less controversial compared with embryonic stem cells which involving tissue engineering (Lee *et al.*, 2015).

Despite this, dental tissue has becoming a promising source of MSCs and they are easily accessible. There are few of stem cells that can be isolated from orofacial region include stem cells isolated from human exfoliated deciduous teeth (SHED), dental pulp stem cells (DPSC), dental follicle stem cells (DFSC) and periodontal ligament stem cells (PDLSC) as presented in Figure 2.3 (Yildirim *et al.*, 2016).

On the other hand, in spite of knowledge about the types of dental tissues, SHED has giving a new promising benefits in regenerative medicine due to their multipotent stemness (Gazarian and Ramírez-García, 2017). SHED has capability to develop into odontoblasts, adipocytes, neural cells and osteo-inductive cells (Wang *et al.*, 2012).

Recent evidence suggested that SHED displayed high expression of the MSC markers such as CD73, CD90, CD44, the osteoblast markers *Runx2*, *CBFA1* and collagen I, the cartilage cell markers *Col10a1*, the adipose cell markers *PPARγ2* and the neuronal stem cell markers, *Nestin*. In contrast, SHED showed low expression of the hematopoietic stem cell marker CD34 (Zhang *et al.*, 2016). Additionally, SHED

shows negative expression for CD18, CD31, CD34, CD49d, CD49e, CD106, CD133, CD184, CD197, CD146, and HLA II cell surface markers (Yildirim *et al.*, 2016).

Advantages of SHED were explained in previous studies where it exhibits a higher proliferative rate than other types of dental stem cells and shown excellent bone/dentin formation capacity in single colony-derived SHED clones in immune-deficient mice (Zhang *et al.*, 2016). In addition, SHED has been used to reconstruct trabecular bone in systemic lupus erythematosus-like in mice and helped in critical-size calvarial defect regeneration in Wistar rats (Fanganiello *et al.*, 2015).

SHED also may be a suitable resources for regeneration of alveolar and orofacial bone defects due to their similar tissue origin with mandibular bone (Bartosh *et al.*, 2013). Recent study showed the beneficial role of SHED in neurodegenerative disease treatment due to their neurotrophic factors secretion and could be utilized in alleviating Parkinson’s disease, Alzheimer’s disease and cerebral palsy (Esmaeilzadeh, Reyhani and Bahmaie, 2016).

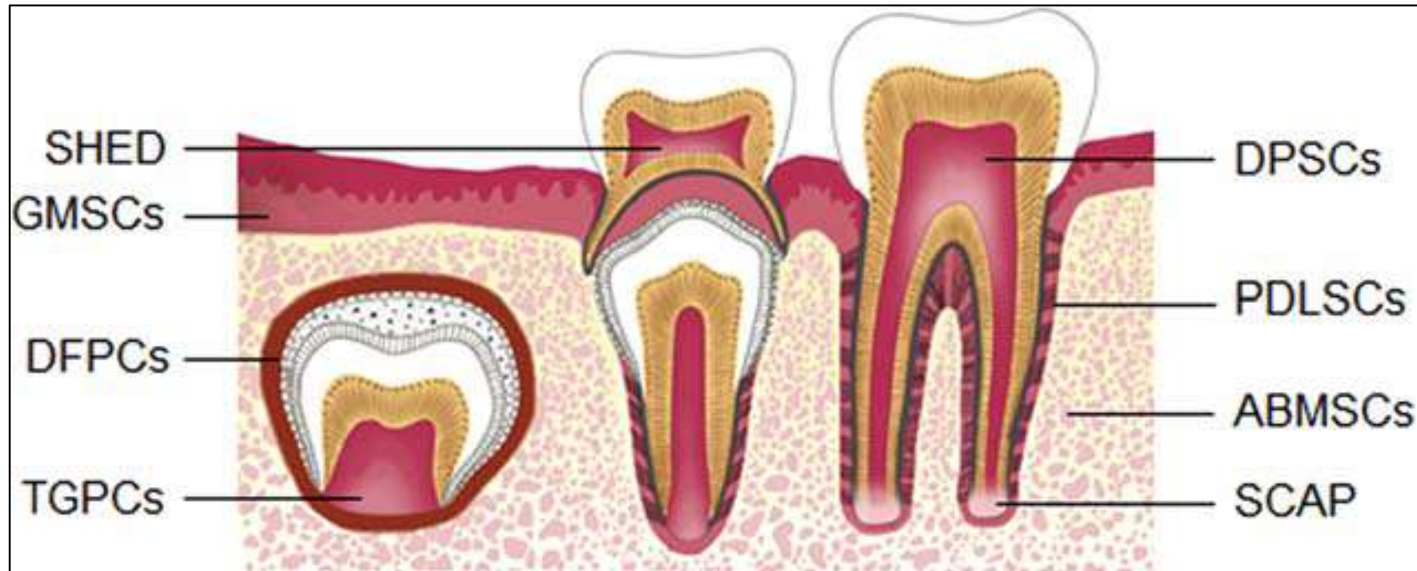

Figure 2.3 Adapted from (Bartosh *et al.*, 2013). The picture shows the sources of human dental tissue-derived MSCs. Abbreviations: ABMSCs, alveolar bone derived mesenchymal stem cells; DFPCs, dental follicle progenitor cells; DPSCs, dental pulp stem cells; GMSCs, gingiva-derived MSCs; PDLSCs, periodontal ligament stem cells; SCAP, stem cells from the apical papilla; SHED, stem cells from exfoliated deciduous teeth; TGPCs, tooth germ progenitor cells.

2.6 IL-17 Cytokines and Receptors

In 1993, IL17 which originally called CTLA8 was cloned and its functional role remained uncertain to a decade until it came into breakthrough with the discovery of a new population of CD4+ T helper (Th) cells that involved in expression of IL17 in 2005 (Liu *et al.*, 2013).

The discovery of this subset became known as type 17 T helper (Th17) cells and has been devoted to comprehending the development, differentiation and regulation of this lineage mechanism (Amatya, Garg and Gaffen, 2017). Characteristic of IL17 gene was studied with subsequent studies include pro-inflammatory cytokines, chemokines, antimicrobial peptides (AMPs), matrix metalloproteinases (MMPs) and inflammatory effectors (Beringer, Noack and Miossec, 2016).

IL17A, IL17B, IL17C, IL17D, IL17E and IL17F were six structurally related cytokines in IL17 family which they are co-expressed on linked genes and co-produced by Th17. Whereas, IL17R family consists of five receptors subunits namely IL17RA, IL17RB, IL17RC, IL17RD and IL17RE. Both IL17A and IL17F exist either as homodimer or as a heterodimer that induce signals through dimeric IL17RA and IL17RC receptor complex (Amatya, Garg and Gaffen, 2017).

Generally, non-haematopoietic cells are the main responders to IL17 even though the IL17R is expressed ubiquitously. IL17 was shown to activate NF-kB and induce NF-kβ-dependent cytokines which first demonstrated the pro-inflammatory role of IL17 in fibroblast (Huang *et al.*, 2009). IL17 also has a role in haematopoiesis and differentiation of hMSC as well as neutrophil recruitment (Sivanathan *et al.*, 2015). Intracellular signal transduction pathways including protein kinase A, JAK/STAT, and mitogen-activated protein kinases MAPKs were triggered when IL17RA activated (Krstic *et al.*, 2015).

Act1 and TNF receptor-associated factor (TRAF) were recruited after binding of IL17A with its receptor and activates MAPK (Lin *et al.*, 2014). In IL17A deficient mice, bone regeneration was impaired due to decrease in osteoblastic bone formation (Huang *et al.*, 2006).

Previous study showed IL17A promoted osteoblastic bone formation (Ono *et al.*, 2016). In human bone marrow derived mesenchymal stem cells, leptin production was significantly increased which inhibited adipogenesis and promoted osteogenesis via JAK/STAT signalling (Mojsilović *et al.*, 2015). In addition, IL17A increased ALP activity during osteogenesis (Osta *et al.*, 2014). Colony frequency and size of individual colonies of hMSC was significantly increased by IL17A *in vitro* in a dose-dependent manner (Jung *et al.*, 2012).

In contrary, other studies have found that the osteoclastogenesis was induced by T_h17 cells through secreting IL17, RANKL, TNF, IL6 and low expression of IFNγ (Ono *et al.*, 2016) and released of RANKL by osteoblast and osteocytes after stimulation of IL17 cytokine which favour osteoclastogenic activity of RANKL by enhancing RANK in bone loss inflammatory conditions (Pacifici, 2016). Meanwhile, pathological conditions such as arthritis or periodontal disease may be due to IL17-dependent RANKL expression in osteoblast which lead to differentiation and activation (Lin *et al.*, 2014).

2.7 MicroRNA biogenesis

In most eukaryotes, the endogenous non-coding 21-23 nucleotide long RNA sequences microRNA or miR or miRNA are found and shown a post-transcriptional ability in gene expression regulation (Hofacker, 2007). In Figure 2.4 (a), RNA polymerase II will transcribe miR genes and two complementary proteins namely Drosha and Pasha will process the primary transcript called pri-miRNA (Friedman *et al.*, 2009).

These proteins will shape and shorten the pri-miRNA into approximately 70 nucleotide hairpin (stem-loop) by removing 5' cap and poly-A tail called pre-miRNA. This process is important in forming pre-miRNA and development of mature miRNA. Next, transport protein Exportin 5 associated with a Ran-GTP will facilitate the pre-miRNA to export out of the nucleus through the nucleus pores (Wahid *et al.*, 2010).

Based on the Figure 2.4 (b), endonuclease Dicer will process the pre-miRNA in cytoplasm which further shortens the pre-miRNA into a 20 - 25 nucleotide double-stranded miRNA molecule with 2 nucleotide overhang on each 3' end (Mohr and Mott, 2015). The loop was removed by Dicer through cleavage so that both ends of the miRNA duplex are exposed. These initiates the formation of RNA-induced silencing complex (RISC) for association of the mature miRNA. One strand of the double stranded miRNA will be integrated into the RISC and the most stable 5' end strand will be selected by the Argonaute protein (Rupaimoole and Slack, 2017).