CHAPTER II
LITERATURE REVIEWS

1. Hair

Skin is the second largest organ in the body after the skeleton and it consists of three structural layers including epidermis, dermis and subcutis. Epidermis is a terminally differentiated, stratified squamous epithelium composed of predominantly keratinocytes. Dermis is a complex tissue comprising many different cell types. Subcutis is the fatty tissue below the skin and consists of spongy connective tissue distributed with the energy-storing fat cells (McElwee & Sinclair, 2008). Within these generalized layers of the skin are specialized derivatives structures of skin including hair, nails, sensory nerves, arrector pili muscle, blood supply, sebaceous, sweat and apocrine glands (Figure 1) (Ro and Dawson, 2005).

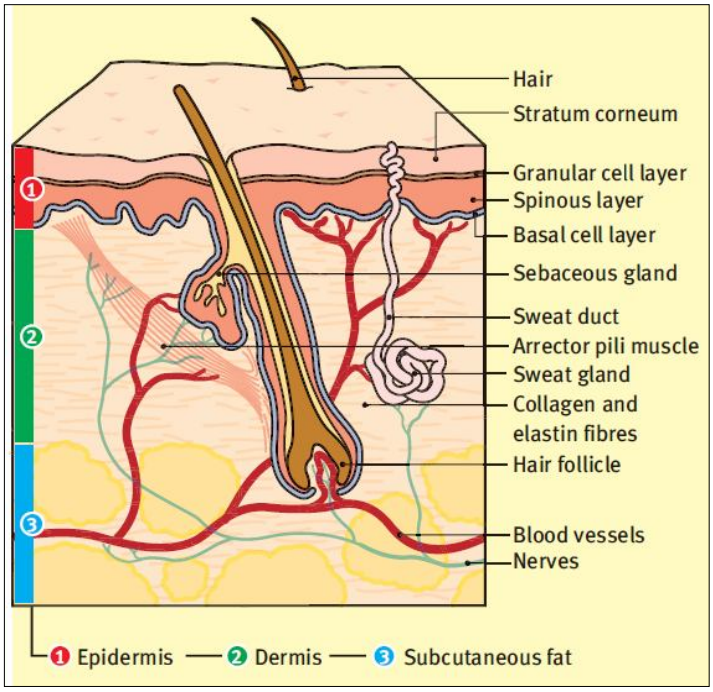

Figure 1 The structure of human skin (Quinn, 2004)

Hair is a basic form of skin. The role of hair growth in human is merely cosmetic in significance, although the arrector pili muscle may cause body hair to

erect in response to cold or fear *via* the sympathetic nervous system (Chen and Chuong, 2012). Hair is able to protect the skin from dust, germs and other small particles. Hair follicles first start to form in the embryo skin from the 8^{th} to 12^{th} week of gestation. Hair follicle formation begins on the face and then spreads over the body (McElwee & Sinclair, 2008). It has been suggested that after birth there are no new hair follicles develop and there is no increase in the number of hair follicles (Yoo et al., 2009). Hair is divided into shaft and root parts. Each hair shaft consists of three layers including medulla, cortex and cuticle. Terminal hairs are composed of medulla, cortex and cuticle while vellus hairs lack of medulla. A few rows of the incompletely keratinized cells form medulla, which is in the middle of the hair shaft. The cortex is built with several rows of completely keratinized fusiform cells; it gives strength to the hair. Cortex is covered with the cuticle which is a row of flat, keratinized cells arranged like tiles on the roof (Jankovic and Jankovic, 1998). The hair shaft is surrounded with the inner root sheath and consists of cuticle, Henley and Huxley layers. It is bordered by the outer root sheath layer that contains proliferating cells derived from stem cells in the bulge that feed into the matrix compartment of the bulb (Sennett and Rendl, 2012). Hair root is enclosed within the structure called follicle. The base of the hair follicle is dermal papilla, which is fed by the bloodstream which carries nourishment to produce new hair. The dermal papilla is very important to hair growth because it contains receptors for male hormones and androgens (Figure2) (Kaufman, 2002). Embryonic hair follicle induction and formation are regulated by mesenchymal-epithelial interactions between specialized dermal cells and epidermal stem cells. Adult hair follicle is regenerated in the hair cycle and it is thought to be controlled by activating signals originating from the mesenchymal compartment and acting on hair follicle stem cells (Sennett and Rendl, 2012; Yang et al., 2010). Stem cells of the hair follicle are gathered in the basal layer of the outer root sheath bulge and formed matrix cells (Wilson et al., 1994). Growth and differentiation of the matrix cells are under the influence of substances produced by dermal papilla cells (Rochat et al., 1994).

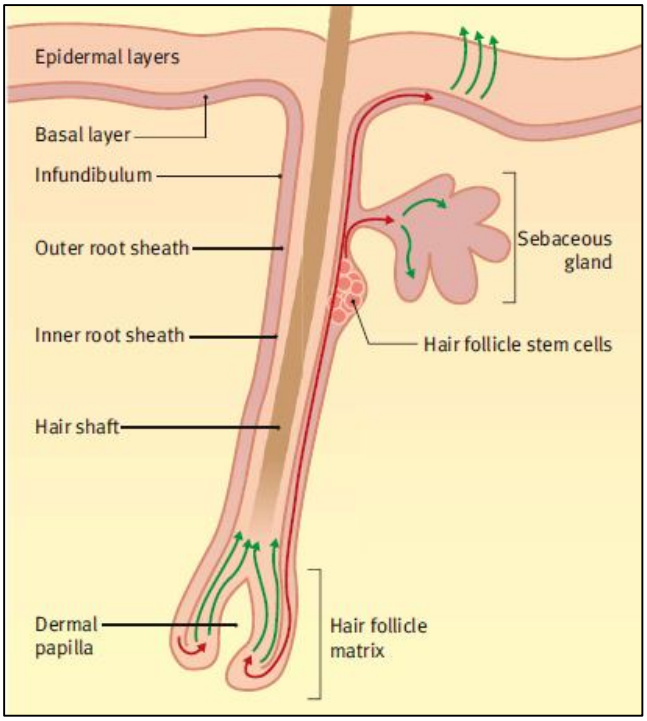

Figure2 Diagrammatic drawing of the hair (Lai-Cheong and McGrath, 2009)

1.1 The growth of hair follicle

Each hair undergoes life-long cycles of rapid growth (anagen), regression (catagen) and resting periods (telogen). The purpose of hair cycling in mammals is not as obvious, but may include cleaning the skin surface and excretion of dangerous chemical (Stenn and Paus, 2001). In addition, follicle cycling may act as a regulator of hormones and growth modulators produced within the follicle and secreted into the skin (Krause and Foitzik, 2006). Approximately 85% of all hairs are in the anagen phase. The anagen phase can vary from two to six years. Hair grows approximately 10 cm per year. At the end of the anagen phase, the hair enters into a catagen phase which remains about one or two weeks. During the catagen phase, the hair follicle shrinks to about 1/6 of the normal length. The lower part is destroyed and the dermal papilla breaks away to rest below. The resting phase follows the catagen phase and normally remains about 5-6 weeks. During this time, the hair does not grow but stays attached to the follicle while the dermal papilla stays in a resting phase below. Approximately 10-15 percent of all hairs are in this phase. At the end of the telogen phase, the hair follicle re-enters the anagen phase. The dermal papilla and the follicle join together again and a new hair begins to form (Tiede et al., 2007).

Hair follicle is composed of epidermal (epithelial) and dermal (mesenchymal) compartments and their interactions play important roles in the morphogenesis and growth of the hair follicle (Yang et al., 2010). Hair follicle and hair shaft are complex organs composed of several cells including dermal papilla cells, dermal sheath cells, outer root sheath cells, keratinocyte, melanocyte and sebocyte (Yoo et al., 2009). The cross-talk between dermal papilla cells in dermal papilla portion and keratinocyte in outer root sheath area is thought to be a key for successful reconstitution of hair follicles (Fujie et al., 2001). The structure of hair follicle in the hair growth cycle is shown in Figure 3.

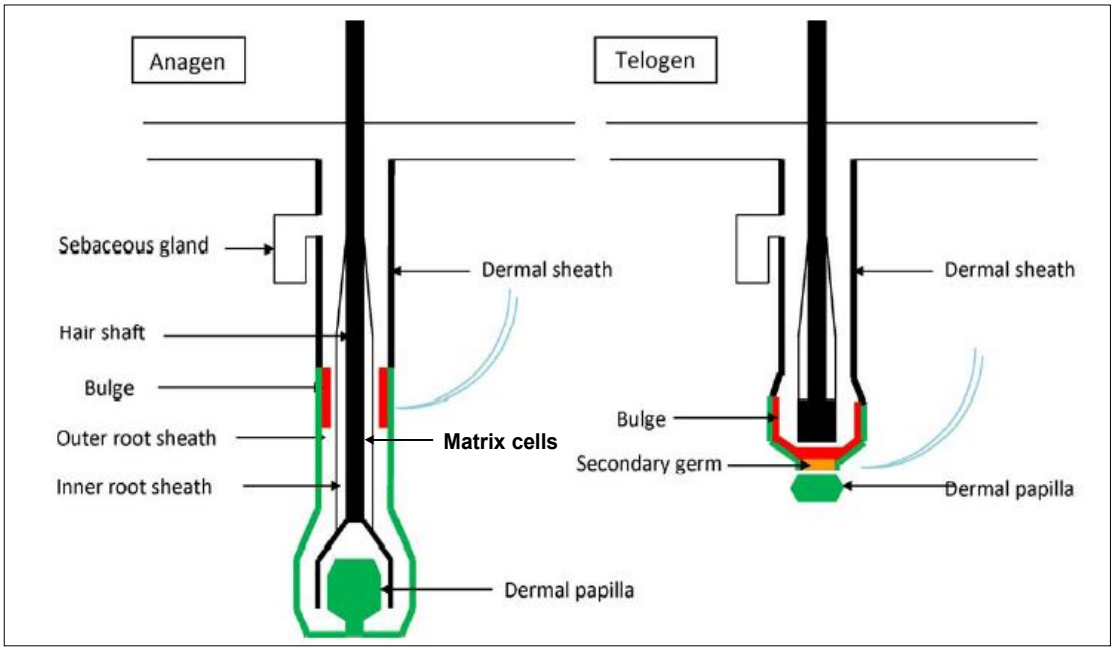

Figure 3 The structure of hair follicle (Yang et al., 2010)

Many signaling molecules are believed to be involved in hair cycle as shown in Figure 4.

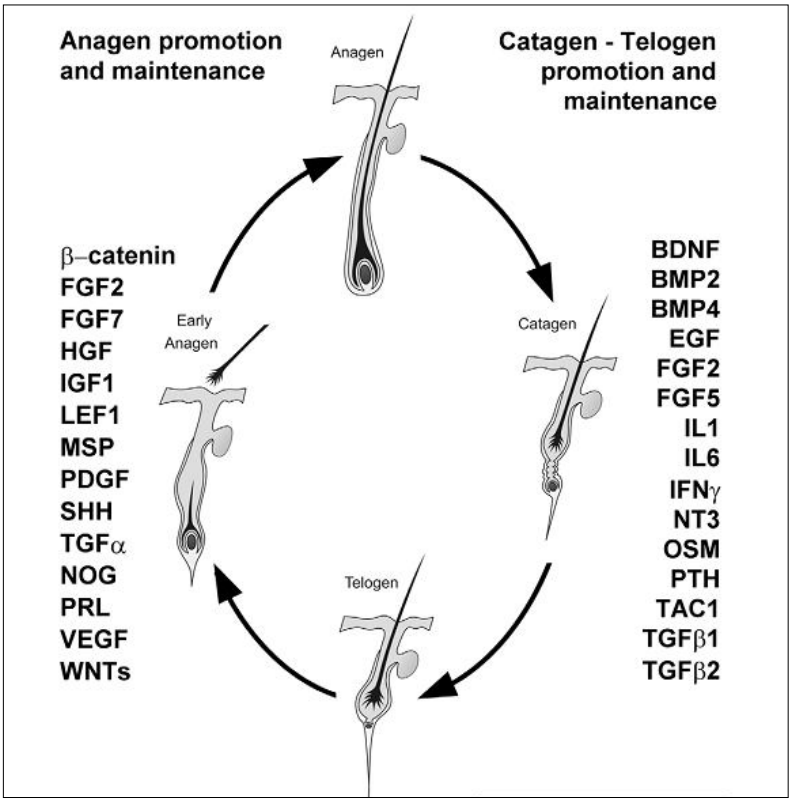

Figure 4 Signaling molecules controlling hair growth (McElwee & Sinclair, 2008)

In Figure 4, there are several anagen promoters including, β-catenin, fibroblast growth factor2 (FGF2), fibroblast growth factor7 (FGF7) or keratinocyte growth factor, hepatocyte growth factor (HGF), insulin-like growth factor I (IGFI), lymphoid enhancer binding factor I (LEF I), macrophage stimulating factor (MSP), platelet–derived growth factor (PDGF), sonic hedgehog (SHH), transforming growth factor alpha (TGFα), noggin (NOG), prolactin (PRL), vascular endothelial growth factor (VEGF) and multiple WNT factors (WNTs).

The catagen-telogen promoters included brain-derived neurotrophic factor (BDNF), bone morphogenetic protein 2 (BMP-2), bone morphogenetic protein 4 (BMP-4), epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), fibroblast growth factor 5 (FGF5), interleukin 1 (IL-1), interleukin 6 (IL-6), interferon

gamma (IFNγ), neurotrophin3 (NT3), oncostatin M (OSM), parathyroid hormone (PTH), Tachykinin I (TACI), transforming growth factor beta I (TGFβI) and transforming growth factor beta 2 (TGFβ2). In addition, aside from growth factors, androgens both testosterone and dihydrotestosterone (DHT) are known to involve in this stage. DHT is considered to be more potent in triggering hair growth and / or hair loss (Hoffmann et al., 2000). 5α-reductase is an enzyme that converses testosterone to DHT and induces the production of transforming growth factor-β1 (TGF-β1). TGF-β1 is responsible for androgen induced epithelial cell growth inhibition (Inui et al., 2002).

Growth factor or signaling molecule is a substance capable of stimulating cellular growth, proliferation and cellular differentiation. Usually it is a protein or a steroid hormone. Growth factors are important for regulating a variety of cellular processes. Growth factors typically act as signaling molecules between cells. The members of the epidermal growth factor and fibroblast growth factor families, as wells as hepatocyte growth factor and insulin-like growth factor, play central roles in hair cell proliferation, while transforming growth factor-β, vitamin D3, and interferon-γ are important inhibitors of hair growth.

The epidermal growth factor (EGF) family of growth factors comprises of EGF, transforming growth factor (TGF)-α, heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, epiregulin, neuregulin. All members of the EGF family share six similarly spaced, conserved cysteine residues, and have one or more EGF domains, as well as a transmembrane domain. EGF family members activate an intracellular signaling cascade upon binding to their receptors, collectively known as the ErbB family. The EGF family can be divided into two groups according to receptor binding specificity. First group includes EGF, TGF-α, HB-EGF, amphiregulin, betacellulin, and epiregulin, which bind to ErbB1, while the second group is neuregulins that bind to ErbB3 and ErbB4. Each EGF member induces not only its mRNA (auto-induction) but also those of other EGF members (cross-induction). These auto-and cross-induction mechanisms may be very effective in the stimulation of keratinocyte proliferation (Hashimoto, 2000) (Figure 5).

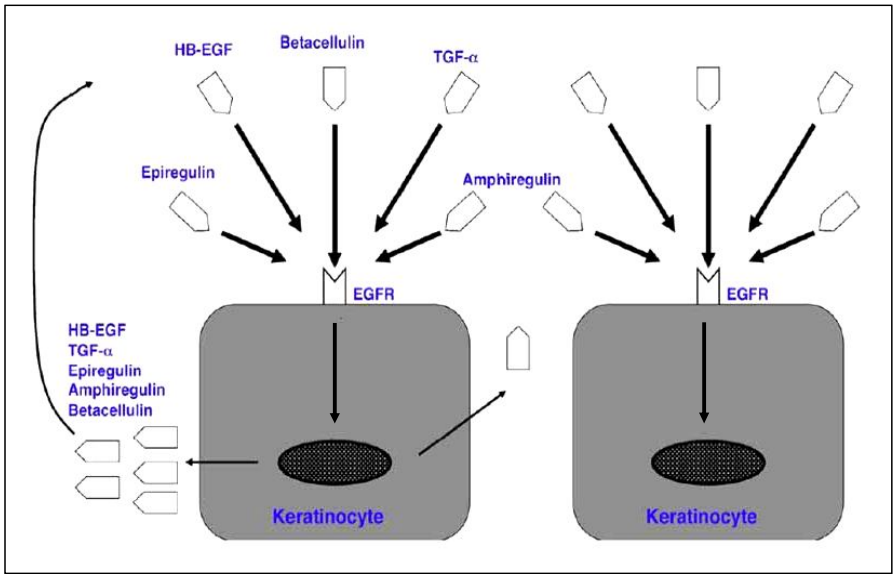

Figure 5 Auto-and cross-induction of TGF-α, amphiregulin, HB-EGF, epiregulin, and betacellulin in normal human keratinocytes (Hashimoto, 2000)

Fibroblast growth factor (FGF) was first characterized as a mitogen for cultured fibroblasts. To date, 22 FGF family members have been identified (Eswarakumar et al., 2005). FGF7 (also referred to as keratinocyte growth factor (KGF)) and FGF10 are expressed in mesenchymal cells, while FGF2, FGF4, FGF6, FGF8, FGF9 and FGF17 are expressed in epithelia. FGFs signal through four high-affinity transmembrane protein tyrosine kinase, FGF receptors 1-4 (FGFR1-4). FGF2, FGF7 and FGF10 have been extensively studied in keratinocytes. Several studies have reported that FGF2 is less active in keratinocytes than FGF7 or EGF family members. However, FGF2 stimulates keratinocyte migration (Sogabe et al., 2006). Of the FGFs, the most potent keratinocyte mitogen is FGF7, which is secreted by dermal fibroblasts and acts on epidermal keratinocytes in a paracrine fashion. FGF7 is an important factor in wound healing.

KGF is successfully used for the treatment of chemotherapy- and radiotherapy-induced oral mucositis in cancer patients. Cytoprotection of keratinocytes by KGF is not a direct anti-apoptotic effect but requires *de novo* protein synthesis. The *in vitro* findings are clinically relevant because KGF protected keratinocytes in organ-cultured human scalp hair follicle from the toxicity of the xenobiltic menadione (Braun et al., 2006).

Hepatocyte growth factor (HGF) synthesized by mesenchymal cells and signals via a high-affinity transmembrane tyrosine kinase receptor called c-MET. HGF enhances cell migration and growth, and DNA synthesis, in keratinocytes cultured in physiological Ca^{2+} levels. Both HGF and FGF7 promote the migration of keratinocytes in low-calcium medium. HGF activates a signal transducer and activator of transcription-3 (STAT3), coincident with its induction of keratinocyte migration.

Insulin-like growth factor (IGF)-I and –II, also referred to as somatonedin C and somatomedin A, respectively, are peptides that produce both mitogenic and insulin-like effects. Theirs effects are mediated by the insulin receptor and IGF receptors (IGF-IR and IGF-IIR). IGF-I and IGF-II bind IGF-IR with high affinity; IGF-IR binds insulin with low affinity. IGF-IIR binds IGF-II with high affinity and IGF-I with low affinity, and does not bind insulin. The functions of IGFs are controlled by important regulatory molecules known as IGF-binding proteins (IGFBPs). IGF-I has been shown to promote the clonal proliferation of cultured keratinocytes. Indeed, IGF-I stimulated keratinocyte proliferation (in serum-free culture) at concentrations up to 100 nM without producing any morphological changes (Figure 6). Recently, the function of IGF-IR signaling was analyzed using a gene-targeting technique. Though IGF-IR-disrupted mice die at birth, IGF-IR-null keratinocytes reportedly exhibit accelerated differentiation and decreased proliferation (Sadagurski et al., 2006).

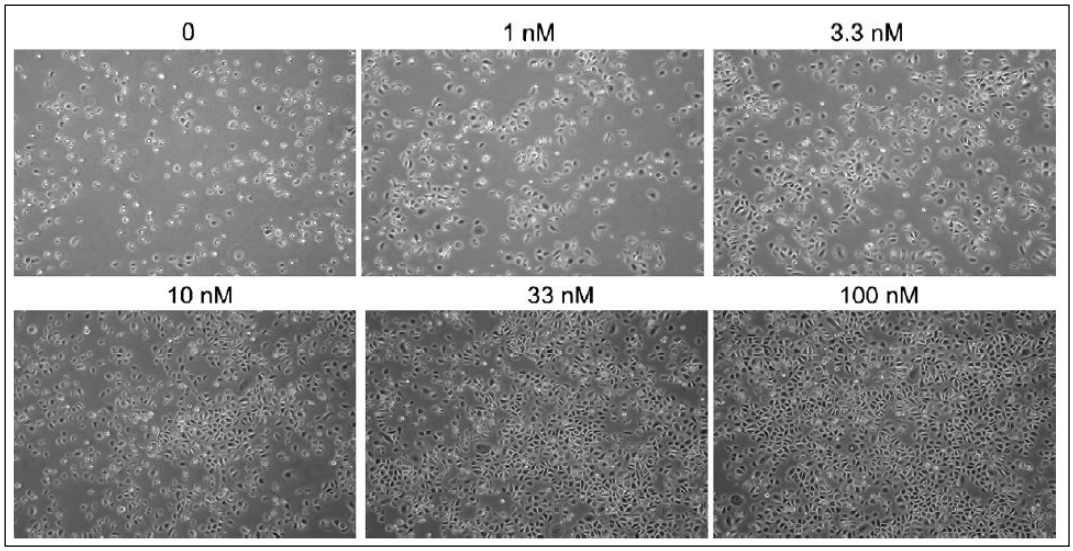

Figure 6 Effect of insulin-like growth factor-I on keratinocyte growth (Shirakata, 2010)

Normal human keratinocytes were cultured with or without recombinant IGF-1 under serum- free conditions for 4 days. IGF-1 promoted keratinocyte proliferation in a dose-dependent manner without inducing any obvious morphological changes.

In contrast, there are several factors that inhibit the growth of hair such as transforming growth factor (TGF) β, vitamin D_3 and interferon-γ. Transforming growth factor (TGF) β superfamily is a large group of proteins that comprises the various isoforms of TGF- β, bone morphogenetic proteins (BMPs), the nodals, activins and inhibins, anti-Mullerian hormone, and many other structurally related factors. TGF- β is a 25-kDa protein that has a wide range of biological effects. Three different isoforms of TGF- β have been described in mammalian cells, the expression of which is regulated in different ways. TGF- β superfamily members signal by binding a receptor complex comprising two transmembrane serine/threonine kinase known as the type I and type II receptors. TGF- β has been shown to inhibit keratinocyte growth. This growth inhibition depends on its rapid repression of c-myc expression. TGF- β inhibits the growth of keratinocytes at concentrations greater than 2 ng/ml under conditions of low Ca^{2+}. A decrease in ^{3}H-thymidine incorporation was observed as early as 3 h after treatment with TGF- β. Expression of c-myc mRNA decreased within 30 min of TGF- β treatment. This rapid reduction in the level of c-

myc mRNA may be one of the key processes of the inhibition of human keratinocyte growth by TGF- β. The examination of the effects of BMP2 and activin, as well as TGF- β1, on the growth of epidermal keratinocytes show the difference inhibitory effect among them (Figure 7).

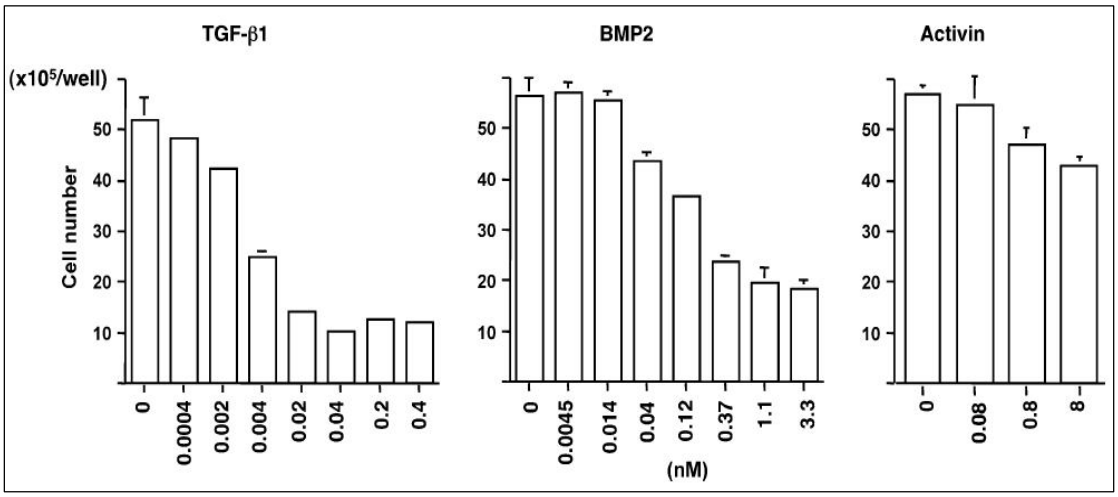

Figure 7 Effects of TGF- β1, BMP2, and activin on keratinocyte growth (Shirakata, 2010)

1α, 25-Dihydoxyvitamin D3 (1, 25$(OH)_2D_3$) is the active form of vitamin D_3. Vitamin D_3 is converted from 7-dehydrocholesterol (7-DHC) in keratinocytes by UV radiation (Figure 8). It plays roles in many biological processes, including calcium homeostasis and bone formation.

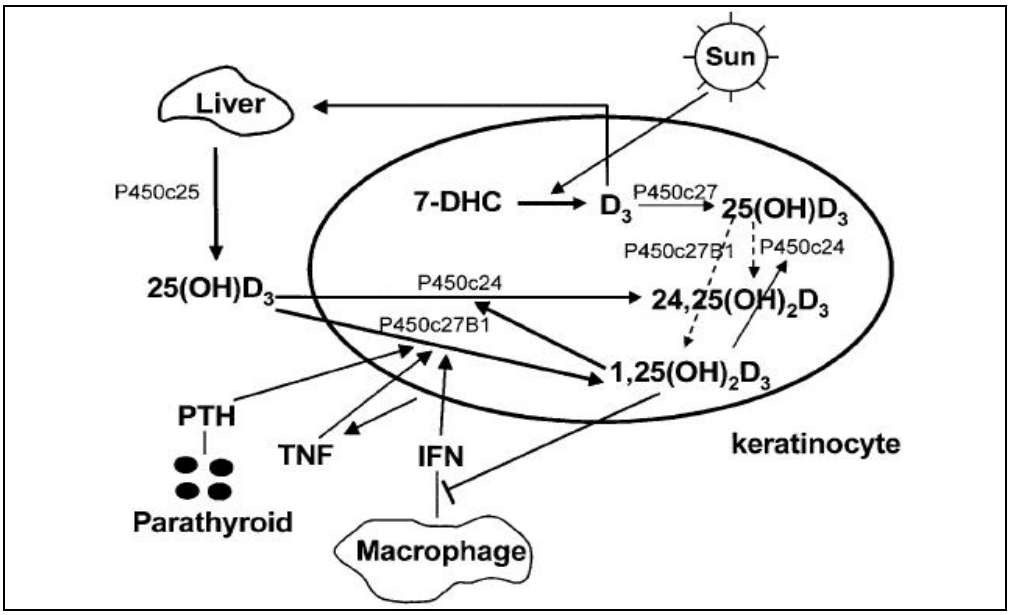

Figure 8 Vitamin D production and metabolism (Bikle, 2004)

Figure 8 showed the production and conversion of vitamin D_3 to $1,25(OH)_2D_3$ in the keratinocyte. 7-Dehydrocholesterol (7-DHC) is converted to vitamin D_3 by a photochemical reaction. The produced vitamin D_3 is either transported out of the keratinocyte to the liver, where it is converted to $25(OH)D_3$, or metabolized directly to $25(OH)D_3$ in the keratinocyte by the enzyme 25-hydroxylase (CYP27). $25(OH)D_3$ is metabolized either to 24, $25\ (OH)_2D_3$ or to $1,25(OH)_2D_3$ by the enzymes 24-hydroxylase (CYP24) and 1α-hydroxylase (CYP27B1), respectively.

$1\alpha,25(OH)_2D_3$ binds to and activates the vitamin D receptor (VDR) in nucleus, which modulates physiological events, such as cellular differentiation and proliferation. $1\alpha,\ 25\ (OH)_2D_3$ has been shown to decrease the proliferation of NHEK cells. It decreased the numbers of S-phase cells within 6 h, and also induced G0/G1 arrest (Shirakata et al., 2004). The active Vitamin D_3 analogs tacalcitol, calcipotriol, and maxacalcitol also suppress keratinocyte proliferation and stimulate its differentiation, with similar potencies to Vitamin D_3 itself (Takahashi et al., 2003). In low calcium (0.03 mM) condition, most keratinocytes are proliferative with little evidence of differentiation. Increased calcium (1.2 mM) inhibits proliferation and induces the onset of terminal differentiation accompanies by increased expression of early differentiation markers such as keratin 1 and keratin 10. The activation of

vitamin D receptor (VDR) by $1,25(OH)_2D_3$ potentiates the action of calcium by inhibiting cell proliferation and inducing differentiation. VDR controls the proliferation and differentiation of keratinocytes. In proliferating keratinocytes, VDR bound preferentially to the DRIP complex, whereas in differentiated keratinocytes the SRC complex was preferred. These results are consistent with the differential localization of DRIP205 and SRC-3 in skin (Oda et al., 2007).

Interferon-γ (IFN-γ) is a 14-kDa polypeptide that is secreated by activated T cells. It is well known to affect a wide number of different cellular processes, including antiviral responses, cell growth, and differentiation, as well as immunoregulatory functions. In addition to its immunological function, IFN-γ plays an important role in regulating proliferation and differentiation in the epidermis. Treatment of keratinocytes with 1000 U/ml recombinant IFN-γ increased the percentage of attached cells displaying a mature, differentiated appearance. IFN-γ-treated keratinocytes were enlarged, with an increased cytoplasmic volume, and inhibition of the proliferation of keratinocytes in a dose-dependent manner (Karlsson et al., 2010).

1.2 Hair pigmentation

The color of hair depends on the presence of melanins. Melanin is the product of melanogenesis that is catalyzed by three melanocyte-specific emzymes including tyrosinase, tyrosinase-related protein (TRP-1) and , tyrosinase-related protein (TRP-2) (Krause and Foitzik, 2006; Yang et al., 2006). Melainin is produced by specialized dendritic cells known as melanocyte. The microphthalmia associated transcription factor (MITF) is thought to act as a master gene within melanocytes because it can convert fibroblasts to melanocyte-like cells (Kim et al., 2006). Melanogenesis is a characteristic of melanocyte differentiation (Sato et al., 2011). It is a multistage process involving melanin synthesis, melanin transport, and melanosome release (Hirata et al., 2007). Melanocytes are highly responsive cells and modulate their levels of melanogenesis or proliferation according to extrinsic signals (such as ultra-violet light, UV) and factors derived from other cell types (Lei et al., 2002). UV stimulates the secretion of prostaglandin E2, α-melanocyte stimulating hormone (α-MSH), adrenocorticotropin-melanocyte-stimulating hormone (ACTH), endothelin-1

and nitric oxide from keratinocytes, which induces melanogenesis (Choi et al., 2005). Melanogenesis of follicular melanocytes is the process that strictly couple to the growth stage of the hair cycle and switch off at the earliest of catagen, and absent throughout telogen (Slominski et al., 1994). Melanocytes are divided into three distinct subpopulations. The first is located in the hair follicle bulge, the second is presented in the outer root sheath and the third is stayed in the hair matrix above the dermal papilla. Some reports suggested that the genetic program of formation of the hair pigmentary unit is organized as a multistep transition of undifferentiated melanocytes progenitors to fully differentiated cells actively producing and transporting melanin to hair shaft keratinocytes as showed in Figure 9 (Botchkareva et al., 2001). The important regulators are melanocortin-1 (MC-1) receptor and adrenocorticotropic hormone, melanocyte stimulating hormone, agouti protein ligands (in rodents), c-Kit, and the endothelin receptors with their ligands. Melanin itself has a wide range of bioactivities that extend far beyond its determination of hair color (Slominski et al., 2005).

Proliferation and differentiation of the hair follicle melanocytes during the hair cycle is controlled by SCF/c-kit receptor signaling, while pigmentation in the hair follicle melanocytes is regulated by signaling through the c-kit and melanocortin type 1 (MC-1R) receptors (Slominski et al., 2005; Barsh et al., 2003). The variation of hair color derives from different relative amounts of brown/black eumelanins and yellow/red pheomelanins (Neste and Tobin, 2004). Pheomelanin synthesis in hair follicle melanocytes occurs when MC-1R signaling is inhibited by Agouti signal protein that competes with α-MSH in binding to MC-1R, while eumelanin is synthesized when α-MSH stimulates MC-1R (Thody and Graham, 1998).

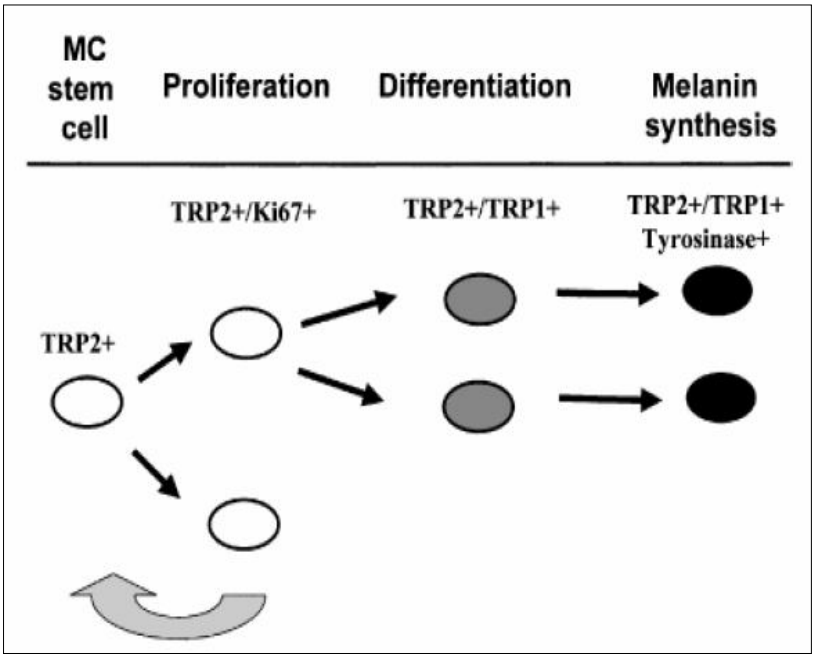

Figure 9 Fate of melanocytes (MC) during morphogenesis of hair follicle pigmentary unit (Botchkareva et al., 2001)

Tyrosinase family has been known to play the role in the regulation of melanogenesis. Tyrosinase family consists of tyrosinase, TRP-1 and TRP-2. They are glycoproteins embedded in the melanosome membrane that share 70-80% nucleotide sequence homology with 30-40% amino acid identity (Jung et al., 2001). Tyrosinase is the rate-limiting enzyme in melanin biosynthesis and catalyzes tyrosine to form 3,4-dihydroxy-phynylalanine (L-DOPA) and oxidation of DOPA to produce DOPA-quinone. TRP-2 acts as a DOPAchrometautomerase and catalyzes the rearrangement of DOPA-chrome to form 5, 6-dihydroxyindole-2-carboxylic acid (DHICA), and TRP-1 oxidizes DHIDA to produce carboxylated indole-quinone (Figure 10) (Matsuyama et al., 2009; Sato et al., 2011). Thus, TRP-1 and TRP-2 also function in the biosynthesis of melanin downstream of tyrosinase. Without melanogenesis, the new hair growing has no pigment, and appearing gray, white, or silver. The stimulation of tyrosinase activity is thus related to melanogenesis. The lacking of tyrosinase and TRP-1 expression may correlate with poorly differentiated malanocytes (Neste and Tobin, 2004)

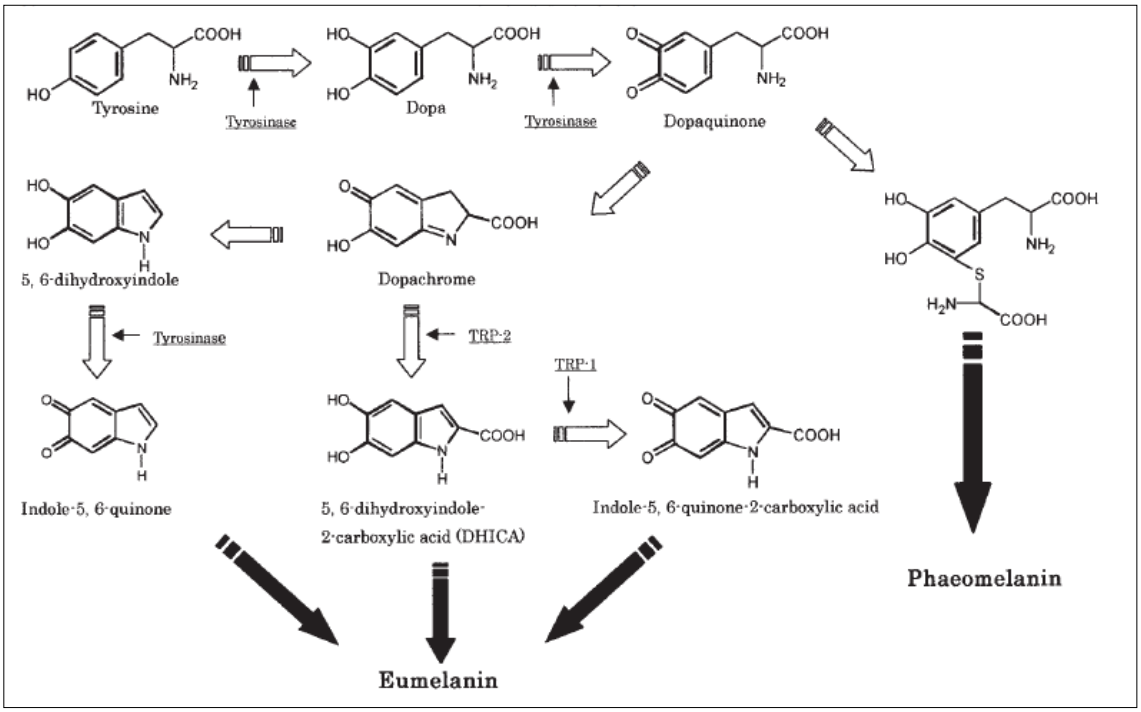

Figure 10 Melanin synthesis pathways (Itoh and Furuichi, 2005)

During hair follicle morphogenesis, melanocytes migrate into the hair follicle, produce and transport melanin to the keratinocytes then differentiate to form the pigmented hair shaft. Therefore, there are some reports on the effort to develop a melanocyte-keratinocyte coculture protocol that allows testing of compounds for potential effects on pigmentation (Lei et al., 2002).

Molecular mechanisms in regulating pigmentation suggest that cyclic adenosine-3',5'-monophosphate (cAMP) and protein kinase C (PKC) are two major intracellular signaling molecules critical for pigmentation. Extracellular signaling increases cAMP levels and activates PKA which controls tyrosinase activity during melanogenesis (Itoh and Furuichi, 2005). The pigmentation of hair fibers is affected by various intrinsic factors including general metabolism and nutritional status, hair-cycle dependent changes, body distribution, racial and gender differences, variable hormone, genetic defects and age-associated changes. Graying first appears at the temples, and spreads to the vertex and then the remainder of the scalp, affecting the occiput last. Beard and body hair is affected later. The cyclic re-construction of hair

follicle pigmentary unit occurs during only the first 10 hair cycles that mean by approximately 40 years of age (Neste and Tobin, 2004). The earliest sign of balding is occasionally scalp sunburn. Hair bulb melanocytes appear to survive for only a single hair growth cycle and are destroyed by apoptosis during early catagen (Tobin et al., 1998).

1.3 Hair cells

The hair follicle consists of the dermal papilla (DP) and dermal sheath (DS). DP is enveloped by the DS in the bulb of a hair follicle. The DP composed of dermal papilla cells (DPCs) derived from the mesoderm during early-stage dermal condensation, occurring beneath in the skin of an embryo (Inamatsu et al., 2006). The DPCs show aggregative behavior and provide the inductive signals to the epidermal portion of the follicle during cyclic regeneration. DPCs produce and secrete paracrine factors such as insulin-like growth factor-I (IGF-I) and hepatocyte growth factor (HGF) (Fujie et al., 2001). DPCs dominantly composted of type IV collagen, laminin, and smooth muscle alpha-actin. DPCs express some markers which have been widely used to identify these cells, these markers including alkaline phosphatase, versican, α-smooth muscle actin (α-SMA) and CD133 (Yang et al., 2010).

Alkakine phosphatase (ALP) is an enzyme that contained in every cell. The level of ALP activity is a well-established DP marker. In anagen, highly active DPCs exhibit strong ALP activity while telogen, DPCs are resting with reduced ALP activity. Thus, ALP activity is an important indicator of DP activity (Hsia et al., 2011). Versican is an aggregating proteoglycan involved in extracellular matrix and in cell adheshion. It plays important roles in anagen induction and maintenance of normal hair growing phase (Soma et al., 2005). α-smooth muscle actin (α-SMA) is an isoform typical of smooth muscle cells and present in *in vitro* culturing DPCs. CD133 is a known hematopoietic stem cell marker that is strongly expressed in DP of developing stage of hair follicles (Yang and Cotsarelis, 2010). Therefore, these markers can be used for DPCs characterization.

The papilla is primarily composed of connective tissue and a capillary loop. DPCs tend to regress between cycles 15 and 20 due to heredity, hormones and stress.

DSCs are the progenitor of DPCs. The DPCs and DSCs were spindle shape cells seem like skin fibroblast (Figure 11A, 11B).

The epidermal cells arising from hair follicle stem cells in the outer root sheath (ORS) at the bulge region are connected to each other via desmosomes and gap junctions. These cells seem like cobble-stone shape (Figure 11C) and proliferate rapidly and move downward, differentiating into postmitotic matrix cells and inner root sheath (IRS) cells, and finally into a keratinized hair shaft.

Melanocytes are cells of neural crest origin. In the human epidermis, they form a close association with keratinocytes via their dendrites (Figure 11D). Melanocytes are key components of the skin's pigmentary system through their ability to produce melanin. These cells are found at many locations throughout the body. In the skin they are associated with the hair follicle and in some mammals including human are also found in the basal layer of the interfollicular epidermis. Pigmentation of the hair results from the activity of melanocytes, which reside in the hair follicle bulb and deposit pigment granules into the hair shaft as it forms.

The pilosebaceous unit consists of a sebaceous gland in association with a hair follicle. Sebaceous glands develop in the 13^{th} to 15^{th} week of gestation from a bulge in the follicular promordium. The sebocytes have cobble-stone shape but size is bigger than outer root sheath cells (Figure 11E)

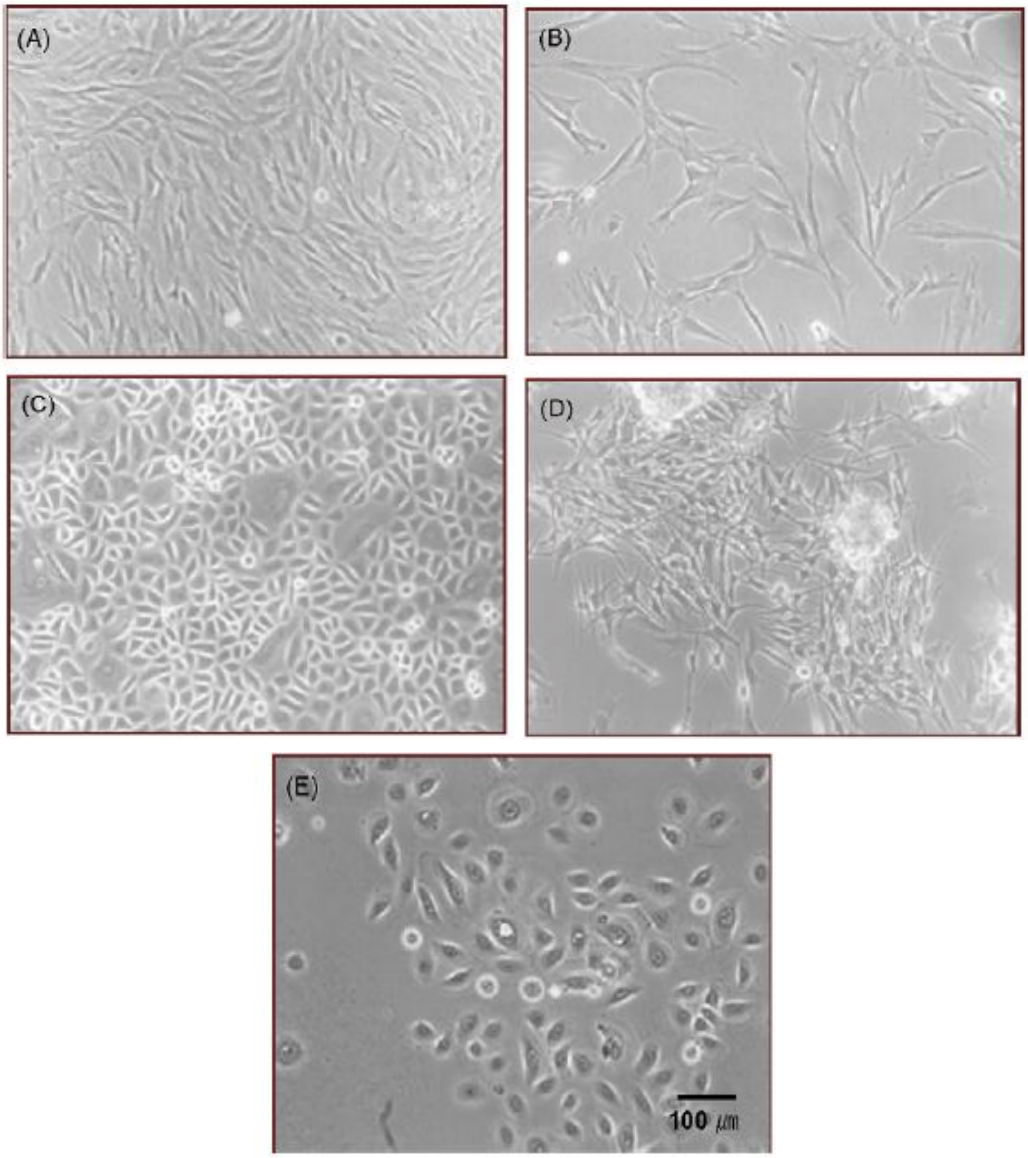

Figure 11 Various isolated and cultivated hair follicular cells (Yoo et al., 2009) (A=human hair dermal papilla cells, B=human hair dermal sheath cells, C=human outer root sheath cells, D=human scalp hair follicle melanocyte, E=human sebocytes)

1.4 Hair loss

Hair loss is not life threatening but often causes severe mental stress and becomes a medical problem when it is excessive, premature and distressing to a patient. It may be caused by genetic factors, follicular microinflammation, precipitating factors, stem cell apoptosis and possibly others (Trueb, 2002). Hair loss affects at least 50% of men by the age of 50 years and up to 70% of all males in later life. Its prevalence in women has varied widely, and 6% of women aged less than 50 years are affected, increasing to 30-40% of women aged 70 years and over (Norwood et al, 2001). Currently, there are two commercially available methods employed to treat this problem: drug therapy and human hair transplantation. However, these two methods still have a number of limitations.

In men, hair loss does not present until after puberty. Male pattern hair loss typically begins with bitemporal recession, followed by progressive thinning in the frontal and vertex areas of the scalp, in which the recession of frontal hairline is common. Over time, the frontal and vertex thinning areas may merge, resulting in near complete visible hair loss over the top of the scalp. In contrast, pattern of hair loss in female may present as late as the sixth decade of life and is characterized by diffused thinning in the frontal and parietal areas of the scalp (Kaufman, 2002) (Figure 12).

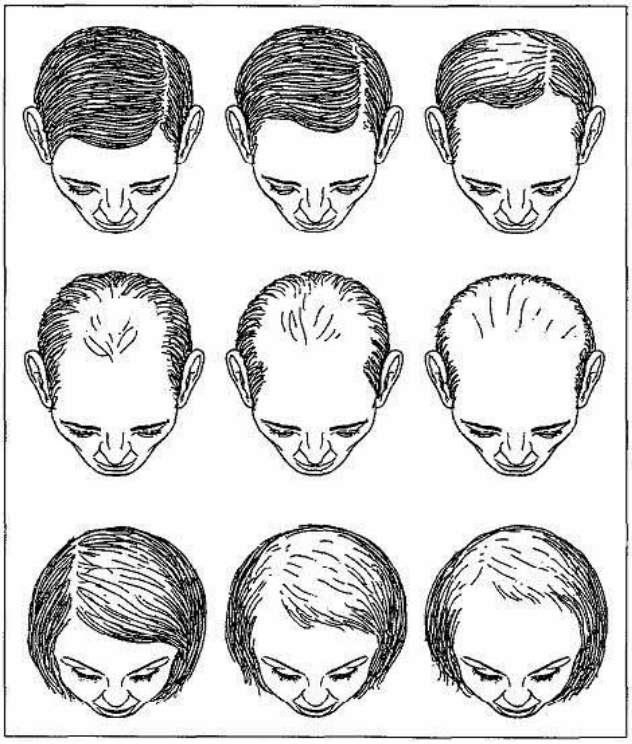

Figure 12 Clinical patterns of hair loss in male and female (Bergfeld, 1995)

The three distinct aims of therapy for hair loss are 1) to arrest further progression, 2) to stimulate re-growth and 3) to conceal the hair loss. A focus of hair disorder development research has been on preventing dermal papilla cell responses to androgens or overcoming their response by an increase in hair follicle growth activity.

Alopecia or hair loss is the medical description of the loss of hair from the head or body, sometimes to the extent of baldness. Alopecia tends to be involuntary and unwelcome. It can also be an important sign of systemic disease. It may be caused by hereditary characteristic, a psychological compulsion to pull out one's own hair or the unforeseen consequences of voluntary hairstyling routines or other causes. From its causes and symptoms, alopecia can classify into several types as shown in the Table 1.

Table 1 Types of alopecia

1. Alopecia Areata	Hair loss occurring in patches anywhere on the body.
2. Alopecia Totalis	Total loss of the hair on the scalp.
3. Alopecia Universalis	Total loss of all hair on the body.
4. Alopecia Barbae	Loss of facial hair (for a man) especially in the beard area.
5. Alopecia Mucinosa	A type of alopecia which results in scaley patched.
6. Androgenetic Alopecia	Also know as male pattern baldness. It is a thinning of the hair to an almost transparent state, in both men and women. It is thought to be a hereditary form of hair loss.
7. Traction Alopecia	Traction alopecia is usually due to excessive pulling or tension on hair shafts as a result of certain hair styles. It is seen more often in women, particularly those of East Indian and Afro-Caribbean origin. Hair loss depends on the way the hair is being pulled. Prolonged traction alopecia can stop new hair follicles from developing and leads to permanent hair loss.
8. Anagen Effluvium	This hair loss is generally caused by chemicals such as those used to treat cancer. Initially it causes patchy hair loss, which often then leads to total hair loss. When stop using these chemicals the hair normally grows back (usually about 6 months later). Many drugs also can cause hair loss.
9. Scarring Alopecia	A form of alopecia which leaves scarring on the area of hair loss.
10. Telogen Effluvium	A form of hair loss where more than normal numbers of hair fall out. There is a general 'thinning' of the hair. Unlike some other hair and scalp conditions, it is temporary and the hair growth usually recovers

(Barahmani et al., 2009; Reid et al., 2012)

1.4.1 Androgen metabolism

Androgen is a steroid hormone that stimulates or controls the development and maintenance of masculine characteristics in vertebrates by binding to androgen receptors. Androgens are mediators of terminal hair growth throughout the body. Without androgens or their activity, scalp hair grows constitutively while body hair growth is inhibited. The pathway of steroid hormone metabolism studied most in relation to hair growth is the peripheral conversion of testosterone to dihydrotestosterone (DHT), a reaction catalyzed by the enzyme 5α-Reductase (5αR). Compared to testosterone, DHT has approximately fivefold greater affinity for the androgen receptor. In some androgen-sensitive target tissues (e.g. prostate), DHT rather than testosterone appears to mediate aspects of androgen action. There are two distinct forms of 5αR, referred to as types 1 and 2, which differ in their tissue distribution. Type 1 5αR is prominent in sebaceous glands, while type 2 5αR is prominent in the genitourinary tract and within hair follicles, in the outer root sheath and proximal part of the inner root sheath. Type 2 5αR plays an essential role in normal male genital development in utero, in adulthood it appears to have no beneficial physiological role, but rather is implicated in the pathogenesis of a variety of androgen-dependent disorders in adult men (Faragalla et al., 2003). At present, little is know about the role of type 1 5αR on hair growth. Unlike type 2 5αR, a human type 1 5αR genetic deficiency syndrome has not been identified, and the role of this isoenzyme in human physiology is unclear (Kaufman, 2002).

In males, testosterone is the major precursor of dihydrotestosterone (DHT) while dehydroepiandrosterone (DHEA) is more likely to be the major precursor in female. The conversion of these precursors to DHT is the important step in production of the peripheral androgens that result in the clinical signs of androgen excess, such as alopecia, hirsutism and acne. The conversion of these androgens is facilitated by increased activity of three enzymes: 5α-reductase, aromatase and 3*β*-hydroxysteroid dehydrogenase isomerase (3β-HSH). Specifically, testosterone is converted to DHT by 5α-reductase. In the female, the major precursor, DHEA, is converted to androstenedione by 3β-HSD and to testosterone by 17β-ol-dehydrogenase. Testosterone is then converted to DHT by 5α-reductase.

The present increased activity of aromatase in the female scalp facilitates conversion of androstenedione and estrone and is greater than its conversion to testosterone and DHT. This appears to protect the female from severe AGA since aromatase is markedly decreased in the bald or balding areas of both males and females. Increased activity of 3β-HSD isomerase has been identified in balding scalp and sebaceous glands. This enzyme is responsible for conversion of 5-androstene,3β,17-β-diol to testosterone, and DHEA to androstenedione, then to testosterone and DHT. Increased circulation of androgens and precursors can affect peripheral metabolism and precipitate signs of androgen excess. The mechanism primarily results in increased androgen diffusing into cell nuclei and binding to cytosolic or nuclear receptor proteins (Figure 13).

Androgen Receptors

Androgen receptors vary in number and are dependent on site, i.e., the beard is greater than the scalp in males. These cytosolic or nuclear receptor proteins are of two types: monomer and tetramer. In the balding areas of males, the monomer/tetramer ratio is increased. Anagen receptors in follicles are mainly identified in the dermal papillae and within the pilosebaceous keratocyte but are absent from the bulge area and the matrix. Extrafollicular androgen receptors are seen in many tissues and cells.

Androgens: Shortened Growth Cycles

DHT inhibits adenyl cyclase activity, interfering with the formation of cyclic adenosine monophosphate (cAMP), while estrone stimulates it. Glucose-6-phosphate dehydrogenase (G6PD) activity is then stimulated by cAMP, which increases during the anagen cycle. DHEA inhibits G6PD, which shortens the anagen cycle. Both androgens, DHT and DHEA, are able to shorten the anagen cycle and are partially responsible for the miniaturization of the follicle in AGA.

Androgen Production

Central and peripheral androgen production and metabolism is similar in both sexes but differs in type, amount, and enzyme activity. In females, 40-50% of testosterone is produced by the ovaries and adrenals, while 50-60% is produced by peripheral conversion of androgen precursors. The results are lower plasma levels of tertosterone and higher levels of DHEA and estradiol. Any elevated androgen plasma level produces greater peripheral metabolism. Sex hormone binding globulin binds

androstenediol, testosterone and estradiol in plasma with different binding affinities. Unbound testosterone, or free testosterone, produces greater peripheral metabolism and increased production of DHT.

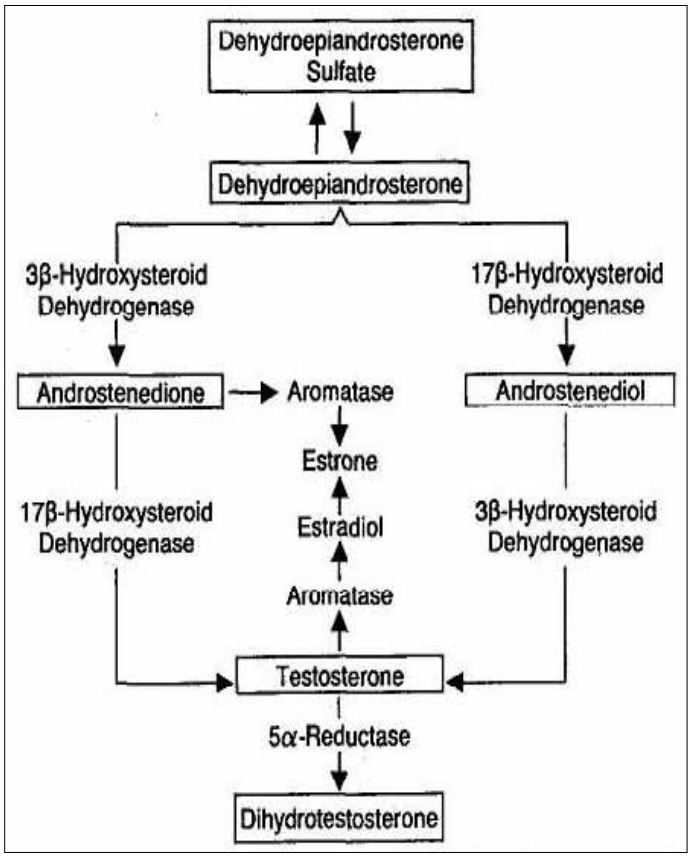

Figure 13 Pathways for androgen metabolism by scalp hair follicle (Bergfeld, 1995)

1.4.2 Genetic involvement

The genetic involvement of AGA is pronounced but poorly understood. Genetic factors modify the magnitude of the hair follicle response to circulating androgens and the baldness gene behaved in an autosomal dominant manner in men and an autosomal recessive fashion in women (Sinclair, 2004). To date, there have been no genes confirmed as predisposing to AGA. Studies are ongoing, and with the rapid advancement of molecular biological techniques for identifying disease genes. Identification of such genes will allow a more thorough understanding of the mechanisms that cause hair loss and, therefore, provide a strong basis upon which to design effective treatments. Of the estimated 40,000 genes in the human genome,

there are many candidates for involvement in AGA. Therefore, it may not be until a full genome-wide scan is completed that we are able to uncover the complete polygenetic predisposition to hair loss (Ellis and Harrap, 2001). The candidate genes for involvement in AGA include:

5α-Reductase

DHT appears to be the more relevant androgen in AGA. An increase in DHT in scalp appears to be an important determinant of this condition. Therefore, genes that may play a role in regulating levels of DHT are potential candidates for AGA. The enzyme 5α-reductase is responsible for the reduction of testosterone to the more potent DHT. The genes encoding 5α-reductase were considered to be the strongest candidates for involvement in AGA. Pseudohermaphrodites, who lack a functional type II 5α-reductase gene, do not develop AGA. In addition, the more recent pharmaceutical treatment for AGA, finasteride, is a strong and selective inhibitor of 5α-reductase.

Insulin

There appears to be a strong association between levels of insulin and levels of sex hormone binding globulin, and possibly between levels of insulin and levels of testosterone. Thus, the insulin and androgen pathways may interact in a regulatory mechanism that is yet to be clearly established. Also, insulin has been shown to be important in the regulation of the timing of the growth (anagen) and rest (telogen) cycles of the hair follicle. A mutation in the regulatory region of the insulin gene that affects the amount of insulin that is expressed could have the capacity to affect the anagen/telogen balance.

Androgen Receptor

Response to androgen in the form of gene regulation is attenuated by the androgen receptor. For this reason, it is possible that the gene encoding androgen receptor may play a role in regulating the potency of androgen available to the hair follicle, and thus may be a candidate for involvement in AGA predisposition. The androgen receptor has been shown to be increased in balding scalp, however, the androgen receptor is located on the X chromosome, and it would not help to explain the father-to-son transmission of AGA.

Aromatase

The enzyme aromatase is responsible for the conversion of C19 androgens such as testosterone to estrogens. Aromatase has been demonstrated to be decreased in balding scalp. Reduction of activity of aromatase may result in an increased level of testosterone available for conversion to DHT. Therefore, the autosomal gene encoding aromatase is worthy of investigation.

1.5 Medication management

Drugs not only have side effects but they also accelerate hair loss when stop using after prolong treatment. The limitation of hair transplantation is the deficiency of transplantable hair (McElwee & Sinclair, 2008). The restriction of transplantable hair number in hair transplantation can resolve by hair multiplication technique. Hair multiplication or hair cloning is a developed technology to harvest donor hair cells, duplicate them and then re-implant into balding areas. The results exhibit the benefits of using this technique; however, it was found that when dermal papilla cells are 3-4 passages cultured *in vitro*, they gradually lose their innate hair follicle-inducing ability (Reynolds and Jahoda, 1996). Stem cells therefore is an optional cell source in hair multiplication technique since bone marrow and umbilical cord stem cells can be induced and reconstructed to be dermal papilla-like tissue (Yoo et al., 2010a)

1.5.1 Drug therapy

At present, there are 2 approaches in the hair loss therapies, drug and non-drug related treatments. However, these two methods still have a number of limitations. Among the non-drug therapy, hair follicle transplantation is the most popular. However, the limitation of this method is the deficiency of transplantable hair (McElwee and Sinclair, 2008). Many researches have attempted to multiply hair follicle *in vitro* and re-implant to balding area (Yoo et al., 2009; Yoo et al., 2010b; Qiao et al., 2008). *In vitro* culturing of hair follicle and re-implanting showed positive result but still faces the problem of loosing in hair follicle-inducing ability of dermal papilla cells (Reynolds and Jahoda., 1996). For drug therapy, the limitations are not only having side effects but also increasing of hair loss when stop using after prolong treatment. There are 2 commercially available drugs: topical minoxidil and oral finasteride. Minoxidil promotes hair growth through increasing the duration of

anagen. It causes hair follicles at rest to grow. Unfortunately, the efficacy of minoxidil is variable and temporary. Finasteride is a competitive inhibitor of type2 5 α-reductase and inhibits the conversion of testosterone to dihydrotestosterone. Finasteride is contraindicated in women; it may cause malformation of the external genitalia of male fetuses (Sawaya and Shapiro, 2000; Sinclair, 2004). From this information, therefore, it is of great importance to develop new drugs or substances for preventing hair loss and enhancing hair growth. Recently, dutasteride, a dual inhibitor of both type I and type II 5α-reductase drug is in phase III study. It is approximately 3 times as potent as finasterides at inhibiting type II 5α-reductase and more than 100 times as potent at inhibiting the type I enzyme (Olsen et al., 2006). However, only finasteride and minoxidil solution are approved by the Food and Drug Administration for treating hair loss, while few studies have been conducted on dutasteride for the treatment of hair loss (Eun et al., 2010).

1.5.2 Hair transplantation

The most effective treatment of hair loss developed to date is auto hair transplantation. The transplantation of human hair involves taking plugs of natural hair from areas in which occipital hair is growing and transplanting them to bald areas. Although the transplanted hair settles at the transplant area as a complete hair follicle and becomes a permanent hair, the transplantation is severely limited. Only 2000 hairs were transplanted per operation, three operations are possible and also leave the donor area scarring.

Due to the limitation of transplantable hair in hair transplant therapy, hair multiplication technique therefore is developed. There are many *in vitro* hair growth models such as monolayer hair cell culture systems and three dimensional bioartificial skin models that employ hair cells (Yoo et al., 2009). These models are useful in place of animal tests for cosmetic pharmaceutical studies that are related to hair follicles. The important technique in hair multiplication is isolating and culturing dermal cells. The most widely used method to isolate dermal cells is surgical micro-dissection (Moll, 1996).

At the beginning of this technique, dermal papilla cells were cultured and successfully implanted into ear skin wounds and the back of rats (Jahoda, 1984).

However, it was found that *in vitro* cultured dermal papilla cells gradually lose their innate hair follicle-inducing ability after about 3-4 passage numbers (Reynolds and Jahoda, 1996). Qiao et al. (2008) have attempted to prepare mixed aggregates of murine follicular cells and found that the mixed aggregates can form proto-hairs that retain ability to fully develop into hair follicles after implantation. Due to the restriction of *in vitro* cultured dermal papilla cells, stem cells therefore are an optional cell source in hair multiplication technology. Recently, dermal papilla-like tissue that obtained from mesenchymal cells of bone marrow and umbilical cord exhibited the same hair bulb structure as natural dermal papilla-like tissue *in vitro*. Moreover, the transplanted dermal papilla-like tissue can induce new hair follicle in athymic mice (Yoo et al., 2010a). In summary, at present, there are several therapies of hair loss (Table 2).

Recently, medical researchers believe that stem cell therapy has the potential to dramatically change the treatment of human disease. A number of adult stem cell therapies already exist; particularly bone marrow transplants are used to treat leukemia (Gahrton and Bjorkstrand, 2002). In the future, medical researchers are able to use technologies derived from stem cell research to treat a variety of diseases including cancer, Parkinson's disease, spinal cord injuries, multiple sclerosis, and muscle damage (Lindvall, 2003; Goldman and Windrem, 2006).

Table 2 Targets and related therapies

	Strategic approach to target	Expected outcome of intervention at target	Therapies in trial
Drug promotion of hair growth	Minoxidil-based treatments in multiple topical formulations	Increased duration of anagen hair growth and increased hair follicle size	All trials complete, commercially available
Drug inhibition of androgen-mediated hair loss	Oral finasteride inhibitor of Type II 5 alpha reductase	Reduced action of dihydrotestosterone in androgenetic alopecia. Increased duration of anagen hair growth and increased hair follicle size	All trials complete, commercially available
Drug inhibition of androgen-mediated hair loss	Blockade of androgen production or androgen receptors using antiandrogen drugs (spironolactone, cyproterone acetate)	Reduced action of testosterone and dihydrotestosterone in androgenetic alopecia. Increased duration of anagen hair growth and increased hair follicle size	Limited clinical studies complete, commercially available
Drug inhibition of androgen-mediated hair loss	Oral dutasteride inhibitor of Type I and Type II 5 alpha reductase	Reduced action of dihydrotestosterone in androgenetic alopecia. Increased duration of anagen hair growth and increased hair follicle size	Phase III trials active
Drug modification of hair follicle size	Activation of the sonic hedgehog (Shh) gene pathway using agonists	Increased duration of anagen hair growth and increased hair follicle size	Preclinical research
Drug modification of hair follicle size	Ammonium trichloro (dioxoethylene-O,O'-tellurate compounds)	Increased duration of anagen hair growth and increased hair follicle size	Phase II clinical trials
Drug modification of hair follicle size	Tetrapeptide aldehyde proteasome inhibitors	Increased duration of anagen hair growth and increased hair follicle size	Phase II clinical trials
Hair follicle formation	New hair follicle induction using promoters of WNT gene activity	Increased number of new hair follicles per unit area	Phase II clinical trials
Hair follicle regeneration	Hair follicle replication and/or reactivation using cell implantation	Increased number of new hair follicles and/or increase size of miniaturized hair follicles	Pilot clinical trials complete, Phase II clinical trials
Hair follicle redistribution	Surgical transplantation of hair follicles from donor regions to alopecia scalp skin	Modification of hair follicle density in alopecia scalp regions	Available commercially

(McElwee and Sinclair, 2008)

Stem cells are biological cells found in all multi-cellular organisms, that can divide through mitosis and differentiate into specialized cell types and can self renew to produce more stem cells. In mammals, there are two types of stem cells that are divided by its origin; embryonic stem cells and adult stem cells. Embryonic stem cells, derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency (Takahashi et al., 2003). The direct utilization of embryonic stem cells still has the limitation by the ethic. Adult stem cells are found in various tissues after embryonic development and are undifferentiated cells. Besides, stem cell can be divided by its potency into five types; totipotent, pluripotent, multipotent, oligopotent and unipotent. Totipotent stem cells can differentiate into embryonic and extraembryonic cell types. Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells. Multipotent stem cells can differentiate into a number of cells, but only a closely related family of cells. Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells. Unipotent stem cells can produce only one cell type but have the property of self-renewal which distinguished them from non-stem cells (Mitalipov and Wolf, 2009). The origin of each stem cell type is shown in Figure 14.

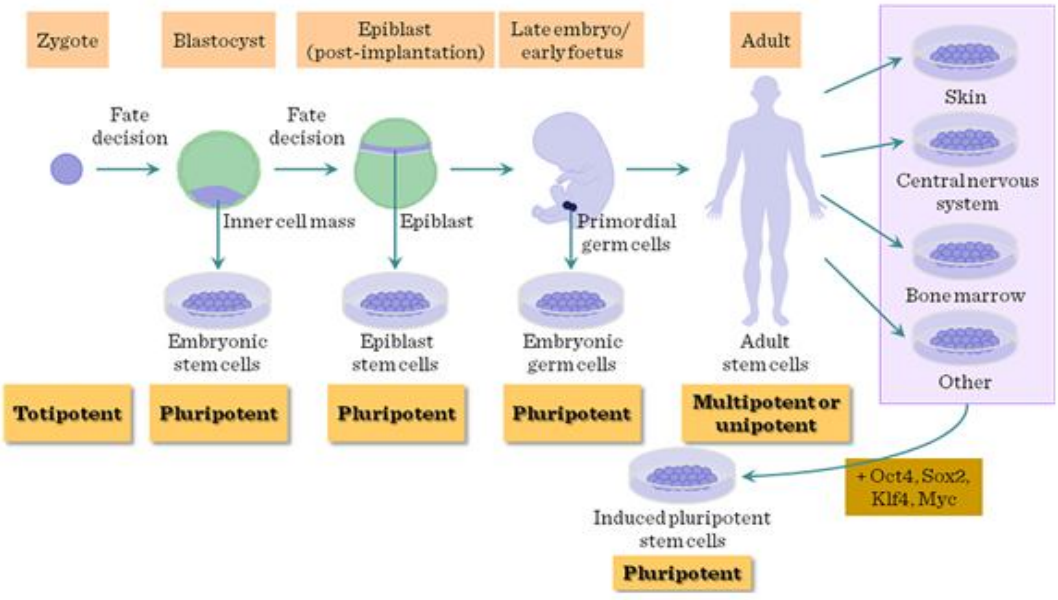

Figure 14 Stem cell diagram (Balogh and Engelmann, 2011)

Mesenchymal stem cells (MSCs) are multipotent stem cells. They are undifferentiated cells that are able to self-renew and have a high proliferative capacity. These cells comprise of multipotent precursors that are capable of supporting hematopoiesis. Moreover, several reports suggest that MSCs were able to differentiate into various cell types including chondrocytes, osteocytes, adipocytes, myocytes, and neurons (Karahuseyinoglu et al., 2007; Kern et al., 2006). MSCs can be isolated from different tissues such as bone marrow, adipose tissue, dental pulp, placenta, umbilical cord and from a variety of fetal tissues such as spleen, lung, pancreas and kidneys (Secco et al., 2008). Phenotypic and genetic evidences suggest that MSCs are a potentially useful model for developmental biology studies. Therefore, MSCs from umbilical cord was used in the research since it exhibited differentiate potency to be dermal papilla cells and induced hair growth *in vivo* (Yoo et al., 2010b).

Clinical and investigative advances have helped us to understand some of the pathogenic steps leading to hair loss (Figure15). Besides androgens and genetic imbalance, additional pathogenic factors are suspected, such as microbial flora, endogenous and exogenous stress, microinflammation, and possibly others.

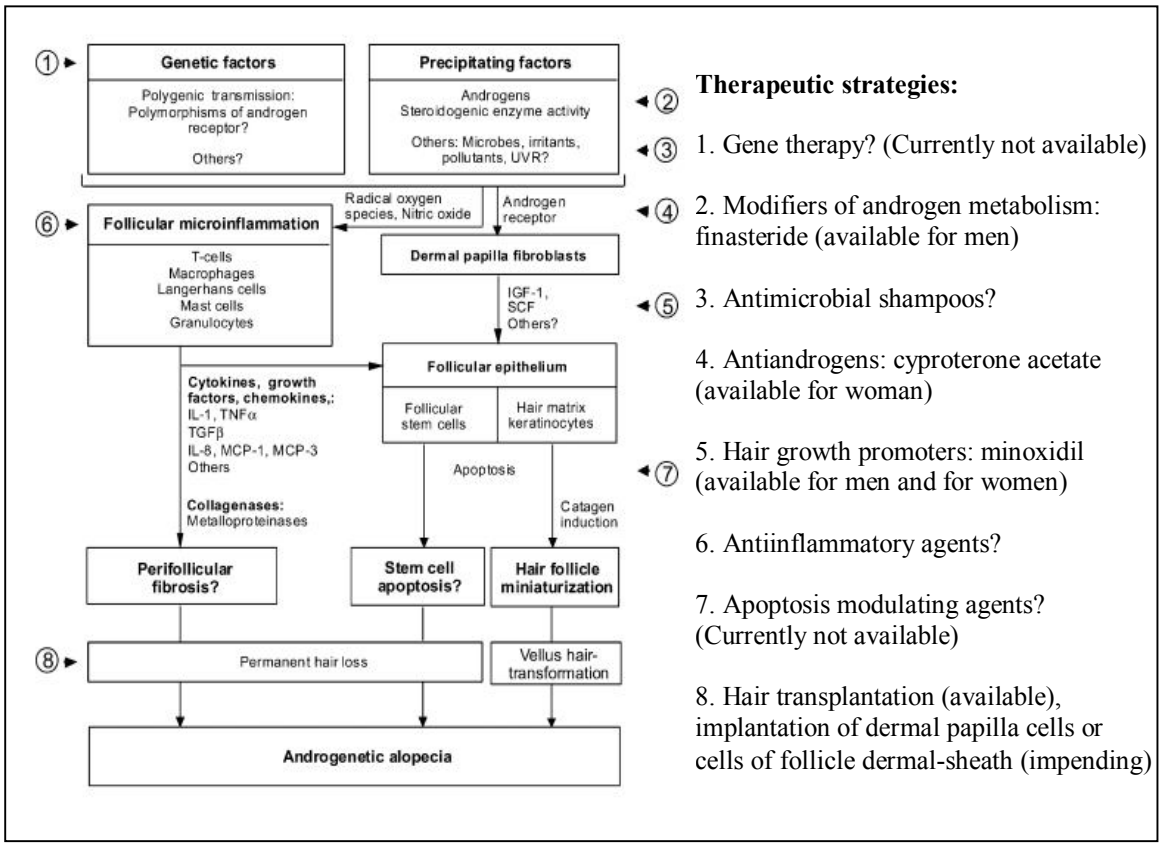

Figure 15 Androgenetic alopecia: Pathogenic mechanisms and therapeutic strategies (Trueb, 2002)

2. Report on plants affecting hair growth

Hair loss is the common problem that has affected both men and women. Nowadays, many hair loss treatment contained natural or synthetic based products. The natural products are popular because synthetics based product may cause unpleasant side effects. Several hair growth promoting natural substances influenced the expression of hair growth factors such as isoflavone and capsaicin promoted hair growth by increasing IGF-I production in mice and in humans with alopecia (Harada et al., 2007). The essential oils of *Chamaecyparis obtusa* promoted hair growth through the induction of VEGF gene (Lee et al., 2010) The methanolic extract of *Sophora flavescens* promoted hair growth by influencing the expression of IGF-I and KGF in dermal papilla cells (Roh et al., 2002). The extract of *Asiasari radix* showed the potent hair growth stimulation in C57BL/6 and C3H mice experiments and also

increased the protein synthesis in vibrissae follicle cultures and the proliferation of both keratinocytes and dermal papilla cells *in vitro* (Rho et al., 2005). Some of herbal plants revealed anti-hair loss effect by the inhibition activity of 5α-reductase type 2 such as the extract of *Thujae occidentalis* (Park et al., 2003)

Recently, the hair care products which are available on the market mostly contained herbal substances. The traditional system of medicine in India acclaims a number of herbal drugs for hair growth promotion including *Hibiscus rosa-sinensis* Linn, *Cuscuta reflexa* Roxb, *Asiasari radix*, *Ocimum gratissum* Linn, *Ginseng radix*, *Aloe vera* L., *Rosmarinus officinalis* Linn, *Lawsonia alba* L., *Ginkgo biloba*, *Tridax procumbens* L., *Sophora flavescens*, *Citrullus colocynthis* Schrad, *Emblica officinalis*, *Bacopa monnieri*, *Trigonella foenumgraecum*, *Murraya koenigii*, *Nordostachys jatamansi*, *Eclipta alba* (L) Hassak, *Indigofera Tinctoria*, *Vitex negundo* Linn, *Terminalia bellerica*, *Gmelina arborea*, *Centella asiatica* Linn and *Cardiospermum halicacabum* Linn (Adhirajan et al., 2003; Jadhav et al., 2009).

3. *Carthamus tinctorius* L.

Based on the traditional used of selected plants treating hair loss and promoting hair growth, the preliminary study demonstrated that Kham foi extract exhibited highest proliferative effect on DPCs and HaCaT and also high yield of the extraction. Therefore it was selected for further study.

Carthamus tinctorius L. or Safflower or Kham foi (in Thai) (Figure 16), also was called Flores Carthami, Saffron and Carthamine. It belongs to Asteraceae (Compositae) family. Safflower is an herbaceous with highly branch plant. It is an important oil-seed crop. Plant is 0.5-1.5 m tall with brilliant yellow, orange or red globular flower heads and tubular florets. Each branch usually has 1-5 flower heads which contain 15-20 seeds per head. Seed is bitter in taste and look like orange seed. It is spirally arranged, dark green, sessile, oblong and glossy leaves with many long sharp spines. It has long and strong taper root. Oil is unsaturated with little odor and a pale to rusty yellow in color. Safflower is believed to have originated in the eastern Mediterranean and the Persian Gulf and has been cultivated in Egypt, the Middle East and India. Nowadays, it was found in a warm and moist climate. It is in leaf from May to October, in flower from August to October, and the seeds ripen from

September to October. It is a minor crop today, about 600,000 tons being produced commercially in more than sixty countries worldwide. There are two parts of the plant which can be used; the pale seeds and the red florets. Traditionally, this plant was used for coloring and flavoring foods, medicines, and making red and yellow dyes. In China, this herb is traditionally used to treat coronary disease, thrombotic disorders and menstrual problems. For the last fifty years, the plant has been cultivated for the oil extracted from its seeds for cooking oil, salad dressing and for the production of margarine. Today this crop supplies oil, meal and birdseed.

Several chemicals such as *N*-feruloylserotonin, *N*-(*p*-coumaroyl) serotonin, acacetin, proteins, α-tocopherol, carthamone, lignans, polysaccharide, aracic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid were found in the seed. Its flower contained several chemical ingredients including hydroxysafflor yellow A (Figure 17), 6-hydroxykaempferol 3,6-di-O-β-glucoside-7-O-β-glucoronide, 6-hydroxykaemferol 3,6,7-tri-O-β-glucoside, 6-hydroxykaempferol 3-O-β-rutinoside-6-O-β-glucoside, 6-hydroxykaempferol 3,6-di-O-β-glucoside, 6-hydroxyapigenin 6-O-glucoside-7-O-glucuronide, anhydrosafflower yellow B, kaempferol 3-O-β-rutinoside, guanosine, syringin, 7,8-dimethylpyrazino[2,3-g] quinazolin-2,4-(1H, 3H)-dione, adenosine, adenine, uridine, thymine, uracil, roseoside, 4'-O-dihydrophaseic acid-beta-D-glucopyranoside methylester, 4-O-beta-D-glucopyranosyloxy-benzoic acid and p-hydroxybenzoic acid (Fan et al., 2009 ; Jiang et al., 2008; Lee et al., 2004; Sagiroglu et al., 2009)

Figure 16 Safflower plants (A), florets (B), ethanolic extract (C)

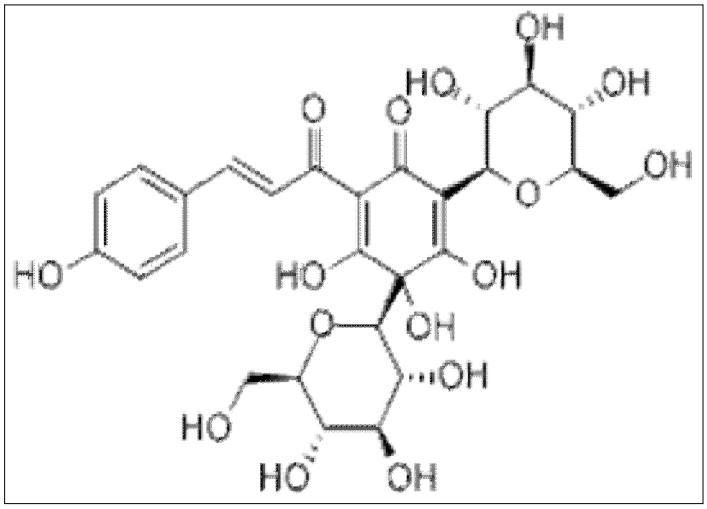

Figure 17 Structure of hydroxysafflor yellow A

Molecular Formula = $C_{27}H_{32}O_{16}$

Formula Weight = 612.534

Synonyms =2,5-Cyclohexadien-1-one,2,4-di-β-D-;glucopyranosyl-3,4,5-trihydroxy-6-[(2E)-3-(4-hydroxyphenyl)-1-oxo-2-propen-1-yl]-

The florets of Safflower have been used as a remedy for stroke, gynecological disease, coronary heart disease, angina pectoris, anti-inflammation, anti-oxidation, cardio-protective activity and hypertension (Fu et al., 2005; Jun et al., 2011; Kanehira et al., 2003; Tien et al., 2010). In China, it has been used as a food additive, a natural pigment and a famous traditional Chinese medicine having the function of promoting

blood circulation by removing blood stasis (Fan et al., 2009). In Korea, the Safflower seed extract have been used for blood stasis treatment and supported the bone formation and prevented osteoporosis (Huh et al., 2001; Kim et al., 2002). In Thailand, Safflower has traditionally been used for the hair growth promoting activity. It is contained in the herbal formulations that effectively promote hair growth and has been reported to exhibit anti-hair loss by inhibiting 5α-reductase function (Kumar et al., 2012). Safflower seed oil has proved beneficial in lowering blood cholesterol, treatment of constipation, support of sexual debility, anti-pain, treatment bronchial asthma and lowered plasma and hepatic lipid (Asp et al., 2011; Moon et al., 2001). Moreover, hydroxysafflor yellow A, a compound of Safflower could protect spinal cords from ischemia/reperfusion injury (Shan et al., 2010). There was no toxicity at dose safflower 10 g/kg with oral administration and intra-subcutaneous injection in mice. However, Safflower extract showed the teratogenic effect on the central nervous system development in mice embryos at doses of 2 mg/kg/day (Nobakht et al., 2000).