Mechanisms of Laser-Induced Hair Regrowth

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1. Alopecia

Male androgenetic alopecia (AGA) is the most frequent type of thinning or loss of hair in males. The condition, also known as male pattern baldness, causes hair loss as early as late adolescence. Polygenic heredity is assumed to be the primary cause, although the male hormone testosterone plays an important role, seemingly independent of genetic predisposition. In the hair follicle cells, testosterone converts into the biologically more active metabolite, 5α-dihydrotestosterone (DHT) catalyzed by the enzyme 5-alpha reductase. This hormone binds to androgenic receptors in the hair follicle and the specific bond triggers cellular processes, which reduce the anagen phase of the hair cycle. For this reason the hair passes earlier into the telogen phase and falls out. Gradually, over succeeding cycles terminal hair converts into thinner and shorter vellus hair (i.e. the retrograde phase of the cycle) and the hair follicle becomes minute. The density of the androgenic receptors in the hair follicles varies according to location and this is genetically determined. Age factors too play an important role in AGA, the first manifestation is usually appearing in the third decade. Further factors are probably involved. In males usually symmetric fronto-parietal retraction of the hair-line occurs. The hair in the central part of the vertex is rarefied and thin, and the skin becomes transparent. The alopecia progresses and sooner or later results in a bald spot on the vertex. The remaining hair is distributed in crown-like pattern above the ears and at the scruff of the neck. However, it also becomes gradually thinner and silky, and growing more slowly. Histological findings of the initial phase are characterized by focal perivascular basophil degeneration of connective tissue around the lower third of the anagen follicle. A perifollicular lymphocyte infiltrate then occurs. In the late stage, involution of all the structures in corium becomes apparent; the terminal hairs turn into subtle, vellus hairs, which are located higher in the dermis.

2. Low-Level Laser (Light) Therapy

In 1967 a few years after the first working laser was invented, Endre Mester in Semmelweis University, Budapest, Hungary decided to test if laser radiation might cause cancer in mice [1]. He shaved the hair off their backs, divided them into two groups and gave a laser treatment with a low powered ruby laser (694-nm) to one group. They did not get cancer and to his surprise the hair on the treated group grew back more quickly than the untreated group. This was the first demonstration of "laser biostimulation". Since then, medical treatment with coherent-light sources (lasers) or noncoherent light (light-emitting diodes, LEDs) has passed through its childhood and adolescence. Currently, low-level laser (or light) therapy (LLLT), also known as “cold laser”, “soft laser”, “biostimulation” or “photobiomodulation” — is considered part of light therapy as well as part of physical therapy. In fact, light therapy is one of the oldest therapeutic methods used by humans (historically as solar therapy by Egyptians, later as UV therapy for which Nils Finsen won the Nobel prize in 1904 [2]). The use of lasers and LEDs as light sources was the next step in the technological development of light therapy, which is now applied to many thousands of people worldwide each day. In LLLT the question is no longer whether light has biological effects but rather how energy from therapeutic lasers and LEDs works at the cellular and organism levels and what the optimal light parameters are for different uses of these light sources.

One important point that has been demonstrated by multiple studies in cell culture, animal models [3] and in clinical studies is the concept of a biphasic dose response with the total delivered light energy density (fluence). The reason why the technique is termed LOW-level is that there exists an optimal dose of light for any particular application, and dose lower than this optimum value, or more significantly, larger than the optimum value will have a diminished therapeutic outcome, or for high doses of light a negative outcome may result.

3. Biological Basis for LLLT

The first law of photobiology states that for low power visible light to have any effect on a living biological system, the photons must be absorbed by electronic absorption bands belonging to some molecular chromophore or photoacceptor [4]. One approach to finding the identity of this chromophore is to carry out action spectra. This is a graph representing biological photoresponse as a function of wavelength, wave number, frequency, or photon energy and should resemble the absorption spectrum of the photoacceptor molecule. The existence of a structured action spectrum is strong evidence that the phenomenon under study is a photobiological one (i.e., cellular photoacceptors and signaling pathways exist).

The second important consideration involves the optical properties of tissue. Both the absorption and scattering of light in tissue are wavelength dependent (both much higher in the blue region of the spectrum than the red) and the principle tissue chromophores (hemoglobin and melanin) have high absorption bands at wavelengths shorter than 600-nm. Water begins to absorb significantly at wavelengths greater than 1150-nm. For these reasons there is a so-called "optical window" in tissue covering the red and near-infrared wavelengths, where the effective tissue penetration of light is maximized (Figure 1). Therefore although blue, green and yellow light may have significant effects on cells growing in optically transparent culture medium, the use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-950-nm).

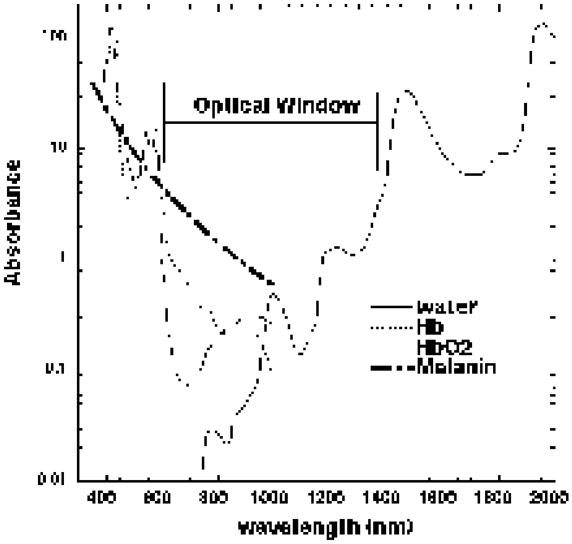

Figure 1. The optical window in tissue between 600 and 1200 nm where absorption of light by tissue chromophores is minimized.

It was suggested in 1989 that the mechanism of LLLT at the cellular level was based on the absorption of monochromatic visible and NIR radiation by components of the cellular respiratory chain [5]. The inner mitochondrial membrane contains 5 complexes of integral membrane proteins: NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), cytochrome c oxidase (Complex IV), ATP synthase (Complex V) and two freely-diffusible molecules ubiquinone and cytochrome c that shuttle electrons from one complex to the next. The respiratory chain accomplishes the stepwise transfer of electrons from NADH and $FADH_2$ (produced in the citric acid or Krebs cycle) to oxygen molecules to form (with the aid of protons) water molecules harnessing the energy released by this transfer to the pumping of protons (H^+) from the matrix to the intermembrane space. The gradient of protons formed across the inner membrane by this process of active transport forms a miniature battery. The protons can flow back down this gradient, reentering the matrix, only through another complex of integral proteins in the inner membrane, the ATP synthase complex.

In 1995, an analysis of five action spectra suggested that the primary photoacceptor for the red-NIR range in mammalian cells is cytochrome c oxidase [6] (Figure 2). It is remarkable that the action spectra that were analyzed had very close (within the confidence limits) peak positions in spite of the fact that these are seemingly different processes. The enzyme contains two iron centres, haem a and haem a_3 (also referred to as cytochromes a and a_3), and two copper centres, Cu_A and Cu_B [7]. Fully oxidized cytochrome c oxidase has both iron atoms in the Fe(III) oxidation state and both copper atoms in the Cu(II) oxidation state, while fully reduced cytochrome c oxidase has the iron in Fe(II) and copper in Cu(I) oxidation states. There are many intermediate mixed-valence forms of the enzyme and other coordinate ligands such as CO, CN, and formate can be involved. All the many individual oxidation states of the enzyme have different absorption spectra [8], thus probably accounting for slight differences in action spectra of LLLT that have been reported.

A recent paper from Karu's group [9] gave the following wavelength ranges for four peaks in the LLLT action spectrum: 1) 613.5 - 623.5 nm, 2) 667.5 - 683.7 nm, 3) 750.7 - 772.3 nm, 4) 812.5 - 846.0 nm.

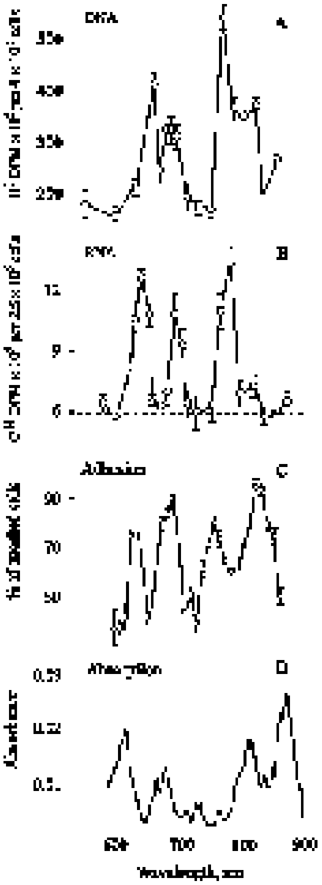

Figure 2.
Action spectra for (A) DNA synthesis, (B) RNA synthesis, (C) cell-plastic adhesion, and (D) absorption spectra of dried cell layer. HeLa (human cervical carcinoma) cells were used.

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Absorption of photons by molecules leads to electronically excited states and consequently can lead to acceleration of electron transfer reactions [10]. More electron transport necessarily leads to increased production of ATP [11]. Light induced increase in ATP synthesis and increased proton gradient leads to an increasing activity of the Na^+/H^+ and Ca^{2+}/Na^+ antiporters and of all the ATP driven carriers for ions, such as Na^+/K^+ ATPase and Ca^{2+} pumps. ATP is the substrate for adenyl cyclase, and therefore the ATP level controls the level of cAMP. Both Ca^{2+} and cAMP are very important second messengers. Ca^{2+} especially regulates almost every process in the human body (muscle contraction, blood coagulation, signal transfer in nerves, gene expression, etc.).

In addition to cytochrome c oxidase mediated increase in ATP production, other mechanisms may be operating in LLLT. The first of these we will consider is the "singlet-oxygen hypothesis." Certain molecules with visible absorption bands like porphyrins lacking transition metal coordination centers [12] and some flavoproteins [13] can be converted into a long-lived triplet state after photon absorption. This triplet state can interact with ground-state oxygen with energy transfer leading to production of a reactive species, singlet oxygen. This is the same molecule utilized in photodynamic therapy (PDT) to kill cancer cells, destroy blood vessels and kill microbes. Researchers in PDT have proposed that very low doses of PDT can cause cell proliferation and tissue stimulation instead of the killing observed at high doses.

The next mechanism proposed was the "redox properties alteration hypothesis" [14]. Alteration of mitochondrial metabolism and activation of the respiratory chain by illumination would also increase production of superoxide anions $O_2^{\bullet -}$. It has been shown that the total cellular production of $O_2^{\bullet -}$ depends primarily on the metabolic state of the mitochondria. Other redox chains in cells can also be activated by LLLT. In phagocytic cells irradiation initiates a nonmitochondrial respiratory burst (production of reactive oxygen species, especially superoxide anion) through activation of NADPH-oxidase located in the plasma membrane of these cells [15]. The irradiation effects on phagocytic cells depend on the physiological status of the host organism as well as on radiation parameters.

It is now known that under physiological conditions the activity of cytochrome c oxidase is also regulated by nitric oxide (NO). This regulation occurs via reversible inhibition of mitochondrial respiration. It was hypothesized that laser irradiation and activation of electron flow in the molecule of cytochrome c oxidase could reverse the partial inhibition of the catalytic center by NO and in this way increase the respiration rate ("NO hypothesis") [16]. Recent experimental results on the modification of irradiation effects with donors of NO do not exclude this hypothesis. Note also that under pathological conditions the concentration of NO is increased (mainly due to the activation of macrophages producing NO). This circumstance also increases the probability that the respiration activity of various cells will be inhibited by NO. Under these conditions, light activation of cell respiration may have a beneficial effect.

Several important regulation pathways are mediated through the cellular redox state. This may involve redox-sensitive transcription factors or cellular signaling homeostatic cascades from cytoplasm via cell membrane to nucleus. It is proposed that LLLT produces a shift in overall cell redox potential in the direction of greater oxidation. The overall redox state of a cell represents the net balance between stable and unstable reducing and oxidizing equivalents in dynamic equilibrium and is determined by three couples: NAD/NADH, NADP/NADPH, and GSH/GSSG (GSH = glutathione). It is believed now that extracellular stimuli elicit cellular responses such as proliferation, differentiation, and even apoptosis through the pathways of cellular signaling. Modulation of the cellular redox state affects gene expression through cellular signaling (and induction of transcription factors. There are at least two well-defined transcription factors — nuclear factor kappa B (NF-kB) and activator protein (AP)-1 that have been identified as being regulated by the intracellular redox state [17-19].

As a rule, oxidants stimulate cellular signaling systems, and reductants generally suppress the upstream signaling cascades, resulting in suppression of transcription factors. It is believed now that redox-based regulation of gene expression appears to represent a fundamental mechanism in cell biology. It is important to emphasize that in spite of some similar or even identical steps in cellular signaling, the final cellular responses to irradiation can differ due to the existence of different modes of regulation of transcription factors. The magnitudes of the LLLT-effects are likely to be dependent on the initial redox status of a cell. The cellular response is weak or absent when the overall redox potential of a cell is optimal or near optimal for the particular growth conditions. The cellular response is stronger when the redox potential of the target cell is initially shifted to a more reduced state (and intracellular pH is lowered). This explains why the degrees of cellular responses can differ markedly in different experiments and why they are sometimes nonexistent.

4. Experiments in Isolated Mitochondria

Since the respiratory chain and cytochrome c oxidase are located in mitochondria, several groups have tested the effect of LLLT on preparations of isolated mitochondria. The most popular system to study is the effects of HeNe laser illumination of mitochondria isolated from rat liver. Increased proton electrochemical potential and ATP synthesis was found [20]. Increased RNA and protein synthesis was demonstrated after 5 J/cm^2 [21]. Pastore et al [22] found increased activity of cytochrome c oxidase and an increase in polarographically measured oxygen uptake after 2 J/cm^2 of HeNe. A major stimulation in the proton pumping activity, about 55% increase of <-- H+/e- ratio was found in illuminated mitochondria. Yu et al [10] used 660 nm laser at a power density of 10 mW/cm^2 and showed increased oxygen consumption (0.6 J/cm^2 and 1.2 J/cm^2), increased phosphate potential, and energy charge (1.8 J/cm^2 and 2.4 J/cm^2) and enhanced activities of NADH: ubiquinone oxidoreductase, ubiquinol: ferricytochrome C oxidoreductase and ferrocytochrome C: oxygen oxidoreductase (0.6 J/cm^2, 1.2 J/cm^2, 2.4 J/cm^2 and 4.8 J/cm^2).

5. Cell Types Responding to LLLT

There is evidence that multiple mammalian and microbial cell types can respond to LLLT. Much of Karu's work has used Escherichia coli (a Gram-negative aerobic bacterium) [23] and HeLa cells [24], a human cervical carcinoma cell line. However for the clinical applications of LLLT to be validated it is much more important to study the effects of LLLT on non-malignant cell types likely to be usefully stimulated to remediate some disease or injury. For wound healing type studies, these cells are likely to be endothelial cells [25], fibroblasts [26], keratinocytes [27] and possibly some classes of leukocytes such as macrophages [28] and neutrophils [29]. For pain relief and nerve regrowth studies these cells will be neurons [30-32] and glial cells [33]. For anti-inflammatory and anti-edema applications the cell types will be macrophages [28], mast-cells [34], neutrophils [35], lymphocytes [36], etc. There is literature evidence for in vitro LLLT effects for most of these cell types.

6. Animal Studies

It is probable that applications of LLLT in animal models will be more effective if carried out on models that have some intrinsic disease state. Although there have been several reports that processes such as wound healing are accelerated by LLLT in normal rodents [3, 37], an alternative approach is to inhibit healing by inducing some specific disease state. This has been done in the case of diabetes, a disease known to significantly depress wound healing in patients. LLLT significantly improves wound healing in both diabetic rats [38, 39] and diabetic mice [40, 41]. LLLT was also effective in X-radiation impaired wound healing in mice [42]. Another report [43] found a greater effect of LLLT in stimulating wound healing in malnourished compared to normally fed rats. Other animal models employed to study LLLT effects in tissue repair include bone fracture healing in rats [44], regenerating rat facial and sciatic nerves after crush injury or transection [45].

7. Clinical Applications for LLLT

LLLT is used by physical therapists (to treat a wide variety of acute and chronic musculoskeletal aches and pains), by dentists (to treat inflamed oral tissues and to heal diverse ulcerations), by dermatologists (to treat edema, non-healing ulcers, burns, and dermatitis), by rheumatologists (to relieve pain and treat chronic inflammations and autoimmune diseases), and by other specialists, as well as general practitioners. Laser therapy is also widely used in veterinary medicine (especially in racehorse-training centers) and in sports-medicine and rehabilitation clinics (to reduce swelling and hematoma, relieve pain, improve mobility, and treat acute soft-tissue injuries). Lasers and LEDs are applied directly to the respective areas (e.g., wounds, sites of injuries) or to various points on the body (acupuncture points, muscle-trigger points). The methods for delivering the therapeutic light are diverse. The field is characterized by a variety of methodologies and uses of various light sources (lasers, LEDs) with different parameters (wavelength, output power, continuous-wave or pulsed operation modes, pulse parameters, polarization state etc).

In 2002 MicroLight Corp received 510K FDA clearance for the ML 830-nm diode laser for treatment of carpal tunnel syndrome. There were several controlled trials reporting significant improvement in pain and some improvement in objective outcome measures [46-48]. Since then several light sources have been approved as equivalent to an infra-red heating lamp for treating a wide range of musculoskeletal disorders with no supporting clinical studies.

8. Light Sources for LLLT

There exists a bewildering variety of light sources employed as therapeutic devices, possible wavelengths they can emit, and maximal output power used in LLLT. For many years HeNe lasers (632.8-nm) were the preferred light source. Light emitting semiconductor diodes (GaAlAs, AlGaInP, InGaAsP etc) are used in both diode lasers and LEDs; the difference is whether the device contains the resonator (as the laser does) or not (LED). These diodes are available in a wide range of wavelengths from 630-nm to 980-nm. In recent years, longer wavelengths (~800 to 900 nm) and higher output powers (to 100 mW) have been preferred in therapeutic devices. One of the most topical and widely discussed issues in the low-power-laser-therapy clinical community is whether the coherence and polarization of laser radiation have additional benefits as compared with monochromatic light from a conventional light source or LED with the same wavelength and intensity. One theory that could explain the extra positive benefit that many practitioners insist is provided by laser over non-coherent light, is the action of laser speckle. Speckle is more pronounced in long-coherence length lasers such as HeNe. Laser speckle provides a rapidly alternating pattern of varying energy density with a spatial dimension of approximately 1 micron. The theory proposes that this dimension is on the same order of magnitude as the size of mitochondria inside the cell, and could explain the extra stimulation provided by a laser. There does not seem to be any scientific explanation for claims that pulse structure (pulse length and repetition rate) and/or polarization state of the light are important or even crucial variables in LLLT.

9. LLLT for Hair Regrowth

Since the first pioneering publication of Mester reported stimulation of hair growth in mice, there have been virtually no follow-up studies on LLLT stimulation for hair growth in animal models. Mester's study involved delivering 1 J of pulsed light (1 millisecond pulse duration) into a 1 cm^2 spot from a ruby laser at 694-nm to the depilated abdominal area of black C57 and white Balb/c mice every week for up to 11 weeks. Before each successive treatment the skin was again depilated. Increased hair growth in the irradiated spot growth was observed in all black animals between the 5th and 7th treatment. This reaction continued to the 9th treatment and it was characteristic of the hair growth intensity that in places that were completely bare at the time of the respective irradiation, hair growth as dense as on other body parts was observed only 4 – 6 days after the irradiation. On the other hand, it was found after the 9th irradiation that hair growth stopped in the irradiated locations only. Instead, a peripheral, ring-shaped hair growth was observed around the irradiated area. This ring-shaped hair growth first appeared in the animal on which the central growth stimulation was first observed. The peripheral growth appeared in all treated black mice between the 7th and 9th irradiation with the intensity varying from mouse to mouse. In white mice no effect on hair growth was detected up to the 8th irradiation. The central growth described for black mice only began to form after the 8th irradiation. Further irradiation caused the hair growth just described in some of the mice, but the peripheral hair growth characteristic of the 2nd phase was already appearing in some as well. The hair growth of the control animals developed as follows: The depilated skin grew hair slowly and diffusely. However, on half of the control animals (both among black and white mice), no further hair growth whatsoever was observed. At the same time, a diffuse hair growth appeared on some animals, but in other animals an uncharacteristic, sometimes diagonal strip appeared.

Despite the fact that LLLT devices are widely marketed and used for hair regrowth, there have been only a few literature reports containing some observations of LLLT-induced hair growth in patients, and amelioration or treatment of any type of alopecia. A Japanese group reported [49] on the use of Super Lizer (a linear polarized light source providing 1.8W of 600 – 1600-nm light) to treat alopecia areata. Three-minute sessions every one or two weeks produced significant hair growth compared to non-treated lesions in 47% of patients. A Spanish group has reported [50, 51] on the use of HeNe laser for both alopecia androgenic and areata. A report from Finland [52] compared three different light sources used for male-pattern baldness (HeNe laser, InGaAl diode laser at 670-nm and non-coherent 635-nm LED and measured blood flow in the scalp.

Recent work has uncovered some biological mechanisms involved in the regulation of hair growth that could be good candidates to explain the stimulating effects of LLLT. Peters et al [53] found that nerve growth factor (NGF) promotes proliferation via its high affinity receptor (TrkA). and identified NGF and p75 as important hair growth terminators. By rtPCR we found, that NGF/proNGF mRNA levels peak during early anagen in murine back skin while NGF/proNGF protein levels peak during catagen, indicating high turnover in early anagen and protein accumulation in catagen. By immunohistochemistry, NGF and TrkA were found in the proliferating compartments of the epidermis and hair follicle throughout the cycle. Commercial 7S NGF, which contains both NGF and proNGF, promotes anagen development in organ-cultured early anagen mouse skin, while it promotes catagen development in late anagen skin. Therefore the data suggest an anagen-promoting/-supporting role for NGF/TrkA.

Another report from this group [54] studied the expression and function of p75 neurotrophin receptor (p75NTR), which is implicated in apoptosis control in spontaneous catagen development in murine skin. They found that p75NTR alone was strongly expressed in TUNEL+/Bcl2- keratinocytes of the regressing outer root sheath, but both p75NTR and TrkB and/or TrkC were expressed by the nonregressing TUNEL-/Bcl2+ secondary hair germ keratinocytes. There was significant catagen retardation in p75NTR knockout mice as compared to wild-type controls. Instead, transgenic mice-overexpressing NGF (promoter: K14) showed substantial acceleration of catagen.

Schwartz et al [55] reported in 2002 that helium/neon laser irradiation (3J/cm^2) augmented the level of NGF mRNA fivefold and increased NGF release to the medium of myotubes cultured in vitro. This correlated with a transient elevation of intracellular calcium in the myotubes. Yu and coworkers found a significant increase in nerve growth factor release from cultured human keratinocytes [27]. Therefore it is postulated that LLLT may influence hair regrowth via the NGF/p75NTR signaling system.

Zcharia and colleagues [56] identified the endoglycosidase, heparanase, as an Important regulator of murine hair growth. Degradation of the extracellular matrix barrier formed by heparan sulfate by heparanase enables cell movement through extracellular barriers and releases growth factors from extracellular matrix depots, making them bioavailable. This allows follicular stem cell progeny migration and reconstitution of the lower part of the follicle, which is a prerequisite for hair shaft formation. Heparanase contributed to the ability of the bulge-derived keratinocytes to migrate through the extracellular matrix barrier in vitro. In heparanase-overexpressing transgenic mice, increased levels of heparanase enhanced active hair growth and enabled faster hair recovery after chemotherapy-induced alopecia.

Thymosin beta4 (TB4) is a 43-amino acid polypeptide that is an important mediator of cell migration and differentiation, also promotes angiogenesis and wound healing [57]. Philp et al [58] reported that TB4 stimulated hair growth in normal rats and mice. A specific subset of hair follicular keratinocytes in mouse skin expressed TB4 in a highly coordinated manner during the hair growth cycle. These keratinocytes originated in the hair follicle bulge region, a niche for skin stem cells. Rat vibrissa follicle clonogenic keratinocytes, closely related, if not identical, to the bulge-residing stem cells, were isolated and their migration and differentiation increased in the presence of nanomolar concentrations of TB4. Expression and secretion of the extracellular matrix-degrading enzyme matrix metalloproteinase-2 were increased by TB4. Thus, TB4 accelerated hair growth, in part, due to its effect on critical events in the active phase of the hair follicle cycle, including promoting the migration of stem cells and their immediate progeny to the base of the follicle, differentiation, and extracellular matrix remodeling.

A recent report [59] identified the transforming growth factor-beta family member activin is a potent regulator of skin morphogenesis, repair and hair growth. Mice overexpressing the secreted activin antagonist follistatin, however, have the reduced hair growth. Mice expressing a dominant-negative activin receptor IB mutant (dnActRIB) in keratinocytes had unaltered architecture of adult skin, but delays were observed in postnatal pelage hair follicle morphogenesis and in the first catagen-telogen transformation of hair follicles.

As yet there are no reports of LLLT affecting heparanase, TB4, or activin expression levels in tissue culture or in mouse skin, but these molecules are good candidates for further study to explain the hair growth-induction by LLLT.

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