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PHOTOBIOMODULATION-MEDIATED PATHWAY DIAGNOSTICS

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Cellular pathways are ordinarily diagnosed with pathway inhibitors, related gene regulation, or fluorescent protein markers. They are also suggested to be diagnosed with pathway activation modulation of photobiomodulation (PBM) in this paper. A PBM on a biosystem function depends on whether the biosystem is in its function-specific homeostasis (FSH). An FSH, a negative feedback response for the function to be performed perfectly, is maintained by its FSH-essential subfunctions and its FSH-non-essential subfunctions (FNSs). A function in its FSH or far from its FSH is called a normal or dysfunctional function. A direct PBM may self-adaptatively modulate a dysfunctional function until it is normal so that it can be used to discover the optimum pathways for an FSH to be established. An indirect PBM may self-adaptatively modulate a dysfunctional FNS of a normal function until the FNS is normal, and the normal function is then upgraded so that it can be used to discover the redundant pathways for a normal function to be upgraded.

Keywords: Signal transduction pathway; photobiomodulation; homeostasis; redundancy.

1. Introduction

The optical techniques in diagnosis may be primarily classified as the ones in tissue diagnosis[1] and the ones in pathway diagnosis.[2] The interaction of light within tissue has been used to recognize disease since the mid-1800s. The recent developments of small light sources, detectors, and fiber optic probes provide opportunities to quantitatively measure these interactions, which yield information for diagnosis at the biochemical, structural, or (patho) physiological level within intact tissues.[1] Classically, the pathway has been studied using a combination of electron microscopic, biochemical, and genetic approaches. In the last 30 years, with the arrival of molecular biology and epitope tagging, fluorescence microscopy has become more important than before. Moreover, with the common availability of fluorescent proteins and confocal microscopes in the last 20 years, live cell imaging[2] and even live body imaging[3] have become major experimental approaches. Low-level laser irradiation or monochromatic light (LLL) has been widely used to diagnose

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biosystems, but its photobiomodulation (PBM) on the diagnostic biosystems was not always considered.[4] PBM disturbs the general optical diagnosis, but it can be also used to diagnose pathways for a dysfunctional function or subfunction to become normal in combining with biochemical and genetic approaches.[5,6] This PBM-mediated pathway diagnostics will be discussed in this paper.

2. Negative Feedback

Negative feedback is common in biological processes and can increase a system's stability to internal and external perturbations.[7] It is one of the three most important governing principles in cellular and molecular responses.[8] Romero *et al.*[9] found the dead iguanas were typified by a reduced efficacy of negative feedback (i.e., poorer post-stress suppression of corticosterone release) compared with surviving iguanas, which suggested a greater ability to terminate a stress response conferred for a survival advantage during starvation.

Homeostasis is one well-known negative feedback response of a biosystem to maintain its internal constancy.[10] However, the internal constancy cannot exactly be deeply studied, therefore homeostasis has been extended to function-specific homeostasis (FSH) in our laboratory.[6,11,12] An FSH is a negative-feedback response of a biosystem to maintain the function-specific conditions inside the biosystem so that the function is perfectly performed. A function far from its FSH is called a dysfunctional function, which corresponds to deficient *yang* or *yin* in TCM. A function in its FSH is recognized as a normal function, which corresponds to *yin-ping-yang-mi* in TCM. The process of improving from dysfunctional to normal is called functional normalization. Each normal function of a biosystem is maintained by its FSH. A cell has proliferation-specific homeostasis (PlSH), differentiation-specific homeostasis (DiSH), and so on. It has been found that C2C12 myoblasts are in their PlSH in mitogenic (10%) concentration of fetus bovine serum (FBS) and mitogenic (22.5 mM) concentration of glucose which is called normal glucose (nG) so that its corresponding PlSH is called PlSH in nG (nPlSH).[13]

The quality of an FSH, functional fitness[14] includes function complexity and function stability.[6,11] The higher the functional fitness of a normal function, the higher the resistance of the normal function to disturbance. The 5-year survival for cancer patients after diagnosis and treatment is strongly dependent on the tumor type. Prostate cancer patients have a $>99\%$ chance of survival five years after diagnosis, and pancreatic patients have $<6\%$ chance of survival past five years. Each cancer type has its own molecular signaling network. Breitkreutz *et al.*[15] found cancers that have a more complex molecular pathway are more refractory than those with less complex molecular pathway.

An FSH-specific stress (FSS) is a stress to disrupt the FSH. It is also a function of a biosystem, and there is an FSS-specific homeostasis (FSSH).[6] After the existing FSH is disrupted, there are many possible kinds of would-be FSH (wFSH) that would be established. Among them, the wFSH of the highest functional fitness, mFSH, would be established by the FSS in its FSSH so that the normal FSS is called a successful stress, such as under self-limiting/limited conditions,[16,17] but the other kinds of wFSH, wFSHa, wFSHb, ... , wFSHz, would be established by the FSS far from FSSH so that the dysfunctional FSS is called a chronic stress such as under the delayed self-limiting/limited conditions.

A complicated biosystem is just a network of functions.[7] Let $\mathbf{Q}$ be the functional fitness of a normal function. A biosystem might simultaneously have many kinds of normal functions, $\{\mathrm{FSH}_i, i = 1, 2, \ldots, n\}$, and then has $\{\mathbf{Q}_i, i = 1, 2, \ldots, n\}$. Let $\mathbf{Q}\max = \max\{\mathbf{Q}_i, i = 1, 2, \ldots, n\}$. Let Fmax and FmaxSH denote the corresponding normal function and its homeostasis, respectively. For cells in 10% FBS, normal proliferation and its PlSH are Fmax and FmaxSH, respectively. There are many subfunctions to maintain a normal function/Fmax, FSH/FmaxSH-essential subfunctions (FESs/Fmax ESs), and FSH/FmaxSH-non-essential subfunction (FNSs/FmaxNSs).[6,11] For a normal function/Fmax, all the FESs/FmaxESs should be in their respective FES/FmaxES-specific homeostasis (FESH/FmaxESH), but some FNSs/FmaxNSs may be allowed to be far from their respective FNS/FmaxNS-specific homeostasis (FNSH/FmaxNSH). Obviously, the more normal the FNSs/FmaxNSs, the higher the functional fitness of the normal function/Fmax. The response of a biosystem to an FSS disrupting an FESH/FmaxESH or FNSH/FmaxNSH is defined as an extraordinary or ordinary stress of the function.[6]

Obviously, there are ordinary stresses for a normal function/Fmax, and its successful ordinary stress can upgrade FSH/FmaxSH. The successful ordinary stress might be mediated by redundant genes/pathways.[6] Genetic redundancy means that

two or more genes are performing the same function and that inactivation of one of these genes has little or no effect on the biological phenotype.[14] The two or more genes and their corresponding pathways are redundant genes and redundant pathways.

3. Therapeutic Diagnosis

A PBM is to stimulate or inhibit biological functions by laser irradiation or monochromatic light. To prevent irreversible cell damage, the light used in a PBM is always low intensity light (LIL) with approximately $10\,mW/cm^2$. Moderate intensity light (MIL) in the range of $0.1-1.0\,W/cm^2$ can also be used for PBM if the irradiation time is not long enough to damage the organelles or cells. However, LIL and MIL with short irradiation times are two kinds of LLL. A PBM on a function/FSS of a biosystem depends on whether the biosystem is in its FSH/FSSH. The self-adaptative PBM of LLL has been classified as direct PBM (dPBM) and indirect PBM (iPBM).[6,18] A dPBM cannot modulate a function/FSS in its FSH/FSSH, but it may self-adaptatively modulate a chronic stress until the stress is successful. An iPBM may self-adaptatively modulate an ordinary stress until the FNSH is established and the FSH is then upgraded. The PBM can be used to diagnose the pathways for a chronic stress to establish an FSH/FSSH or for an FSH to be upgraded.

A dPBM may self-adaptatively modulate a chronic stress until the stress is successful[6,18] so that it can be used to discover the optimum pathways for an FSH to be established. An iPBM may self-adaptatively modulate a chronic ordinary stress until the stress is successful[6,18] so that it can be used to discover the redundant pathways for an FSH to be established.

Proliferative cells in 10% FBS is always in its PlSH, but its differentiation is far from its DiSH. The differentiation-produced proteins may enhance the PlSH through redundant pathways. An iPBM may self-adaptatively modulate the dysfunctional differentiation until the DiSH as a FNSH of a PlSH is established and the PlSH is then upgraded[6,18] so that the enhanced effects can be used to diagnose the redundant pathways for a PlSH to be upgraded.

3.1. *Interleukin*-8

A highly metastatic human melanoma cell line A2058 was maintained in 10% FBS. Then, the cells were irradiated with 0, 0.5, 1.0 and $2.0\,J/cm^2$ He-Ne laser radiation and incubated for 1−5 days, respectively. Hu *et al.*[19] found that LIL at 1.0 and $2.0\,J/cm^2$ promoted normal proliferation three days after He-Ne laser treatment. They further found LIL at $1.0\,J/cm^2$ immediately induced an increase in mitochondrial membrane potential, adenosine-5'-triphosphate, and 3'-5'-cyclic adenosine monophosphate via enhanced cytochrome *c* oxidase activity, promoted phosphorylation of Jun N-terminal kinase/activator protein-1 (AP-1) expressions and increased expression of interleukin (IL)-8 and transforming growth factor (TGF)-beta 1.

IL-8 is a multifunctional cytokine that can stimulate the proliferation of melanoma cells and keratinocytes in both an autocrine and a paracrine fashion.[20] Inflammatory processes are implicated in the development and progression of cancer.[21] Zell *et al.*[22] found that regular nonsteroidal anti-inflammatory drugs (NSAIDs) use (1−3 times/week, 4−6 times/week or daily) versus none was associated with decreased colorectal cancer-specific mortality among patients in the lowest meat consumption tertile, but not among patients in the higher meat intake tertiles. The inflammatory cytokines IL-1-beta and tumor necrosis factor-alpha (TNF-alpha) upregulated IL-8 expression, in a time- and concentration-dependent manner, in three metastatic melanoma variants, SBC-2 (non-metastatic), A375P (low metastatic), and A375SM (high metastatic), by increased transcription of the IL-8 gene, leading to increased levels of IL-8 mRNA and protein production.[23] Fortunately, Singh *et al.*[23] found that the upregulation of IL-8 expression in melanoma cells might be rapidly and specifically inhibited by interferon (IFN)-alpha or IFN-beta, independent of *de novo* protein synthesis, perhaps due to a transient modification of a pre-existing factor(s). It should be pointed that IFN-alpha and IFN-beta did not inhibit steady-state IL-8 production.[23]

It has been widely reported that IL-8 is over-expressed in ovarian cyst fluid, ascites, serum and tumor tissue from ovarian cancer (OVCA) patients, and elevated IL-8 expression correlates with a poor final outcome and chemosensitivity. Wang Y. *et al.*[24] showed that both exogenous (a relatively short period of treatment with recombination IL-8) and endogenous IL-8 (by transfecting with plasmid encoding for sense IL-8) induce cisplatin and paclitaxel resistance in non-IL-8-expressing A2780 cells,

while deleting of endogenous IL-8 expression in IL-8-overexpressing SKOV-3 cells (by transfecting with plasmid encoding for antisense IL-8) promotes the sensitivity of these cells to anticancer drugs. IL-8-mediated resistance of OVCA cells exhibits decreased proteolytic activation of caspase-3. Meanwhile, further study demonstrates that the chemoresistance caused by IL-8 is associated with an increased expression of both multidrug resistance-related genes (MDR1) and apoptosis inhibitory proteins (Bcl-2, Bcl-xL, and XIAP), as well as activation of PI3 K/Akt and Ras/MEK/ERK signaling. Therefore, modulation of IL-8 expression or its related signaling pathway may be a promising strategy of treatment for drug-resistant OVCA.

Human melanoma cell line A2058 is highly metastatic. TGF-beta 1, the most abundant isoform of the TGF-beta family, had no effects on the monolayer growth of highly metastatic cells either in submitogenic (0.5%) or mitogenic (10%) concentrations of FBS.[25] TGF-beta 1 signaling in tumor cells has been implicated in tumor angiogenesis and metastasis by regulating matrix proteolysis through TGF-beta-activated protein kinase 1.[26] As a fact, TGF-beta 1 is elevated in the plasma of melanoma patients, especially those with metastatic lesions.[27] The above iPBM indicated the metastasis is a property of tumor cells themselves.

Tumor cells *in vivo* are always in their respective PlSH. The above iPBM indicated that melanoma cell proliferation in its PlSH might be upgraded by IL-8. In other words, IFN-alpha or IFN-beta might inhibit the IL-8 enhancement on melanoma growth, but might not inhibit melanoma growth itself, which is supported by Singh *et al.*[23] This might be the limitation of anti-inflammation treatment of cancers.

3.2. *Insulin-like growth factor-1 and forkhead box O family 3a*

Our iPBM on nPlSH of C2C12 myoblasts found that the redundant pathways maintaining its FSH should be completely activated.[28] The myoblasts in nPlSH were irradiated with red light at 640 nm from a light-emitting diode array (RLED 640) at 0.035, 0.067, 0.098, 0.194, 0.330, 0.530, 0.558, or 0.885 mW/cm^2 for 15 min once a day for six days, respectively. It has been found that RLED 640 at 0.035 J/cm^2 promoted the proliferation in its PlSH from Day 4 on. The other doses of RLED 640 promoted the proliferation from Day 5 on. RLED 640 at 0.035 mW/cm^2 at Day 3 did not affect the mRNA of sirtuin 1, manganese superoxide dismutase (MnSOD) and p27, but increased the messenger ribonucleic acid (mRNA) of insulin-like growth factor (IGF)-1 and decreased the mRNA of Bcl-2 interacting mediator of cell death (Bim) and forkhead box O family (FOXO) 3a. IGF-1 inhibits the mRNA of Bim.[29,30] FOXO3a inhibits breast cancer cell proliferation.[31] As the redundant pathways, IGF-1 was completely activated and FOXO3a was completely inhibited so that the nPlSH was upgraded with the aid of RLED 640.

The concentration of high glucose (hG) is higher than the one of nG. Our dPBM on hG-induced chronic ordinary stress of C2C12 myoblasts found that the redundant pathway maintaining its FSH should be also completely activated.[13] HG disrupted the PlSH and induced an ordinary stress. HG increased IGF-1 mRNA expression, but its proliferation was lower than the one in the nPlSH. RLED 640 promoted IGF-1 mRNA expression, and both its proliferation and its FOXO3a mRNA were the same as the ones in nPlSH, respectively. In other words, the redundant pathway, IGF-1, was completely activated so that the PlSH in hG (hPlSH) was established with the aid of RLED 640. Active efflux of xenobiotics is a major mechanism of cell adaptation to environmental stress. Glucose transporters (GLUTs) are important in hG-induced response. Asada *et al.*[32] demonstrated that the effect of IGF-I on the expressions of GLUTs was almost the same as that of insulin. GLUT 2 and its mRNA in the liver were elevated, whereas GLUT 4 and its mRNA in the heart were decreased in streptozotocin-diabetic rats. A two-week treatment with recombinant IGF-I mostly restored the expression of GLUT 2 and GLUT 4 to normal rat level. Short-term treatment using IGF-1 improves life expectancy of the delta-sarcoglycan deficient hamster.[33]

The above PBM indicated that IGF-1 might promote the myoblast proliferation in hG to establish the hPlSH in which the FOXO3a is the same as in the nPlSH, and it might also promote myoblast proliferation in its nPlSH might be upgraded by IGF-1 upregulation and FOXO3a downregulation. Penna *et al.*[34] have hyperexpressed IGF-1 by gene transfer in the tibialis muscle of the C26 hosts. In healthy animals, they found that IGF-1 overexpression markedly increased both fiber and muscle size. As a positive control, IGF-1 was also overexpressed in the muscle of aged mice. In IGF-1

hyperexpressing muscles, the fiber cross-sectional area definitely increased in both young and aged animals, while, by contrast, loss of muscle mass or reduction of fiber size in mice bearing the C26 tumor were not modified. It might be because the conserved AMPK/FOXO3a energy sensor pathway is still inducible in human cancer cells in response to metabolic stress.[35] Okamoto *et al.*[36] subjected male C57BL/6 mice to hindlimb immobilization, which induced similar percentage decreases in muscle mass in the soleus and plantaris muscles. After a 3-day period of atrophy, they found a total FOXO3a protein level had increased in both muscles, while phosphorylated FOXO3a protein had decreased in the plantaris muscle, but not in the soleus muscle. As a fact, Senf *et al.*[37] have shown that heat shock protein 70 (Hsp70) overexpression prevented disuse muscle fiber atrophy through inhibition of FOXO3a.

3.3. *Platelet-derived growth factor-C*

Stock cultures of a fibroblast cell line NIH3T3 from wild-type mice were grown in 10% FBS. The cells were irradiated with RLED 627 at 25 mW/cm^2 for 160 s twice, first at subculture and 24 h later. Komine *et al.*[38] found RLED 627 promoted the proliferation in its PlSH 24 h after the second irradiation. In the irradiated group, they further found that the expression of platelet-derived growth factor (PDGF)-C had significantly increased, but the expression of PDGF-A, PDGF-B, basic fibroblast growth factor (bFGF), TGF-beta, PDGF-alpha receptor, and TGF-beta receptor did not change. Although strong activation of the extracellular signal-regulated kinase (ERK) pathway was observed in the irradiated group, its activation was completely suppressed by the PDGF receptor inhibitor. In other words, RLED 627 promoted the proliferation in its PlSH by increasing autocrine production of PDGF-C and activating the ERK pathway through phosphorylation of the PDGF receptor. PDGF-C is one of four members in the PDGF family of growth factors, which are known mitogens and survival factors for cells of mesenchymal origin.[39] PDGF-C has a unique two-domain, structure consisting of an N-terminal CUB and a conserved C-terminal growth factor domain that are separated by a hinge region. PDGF-C is secreted as a latent dimeric factor (PDGF-CC), which undergoes extracellular removal of the CUB domains to become a PDGF receptor alpha agonist. Jinnin *et al.*[40] have examined the effect of recombinant PDGF-CC on the expression of fibrogenic/fibrolytic genes such as type I collagen, fibronectin (FN), matrix metalloproteinases (MMPs), and their inhibitors (TIMPs) in normal human dermal fibroblasts *in vitro*. PDGF elevated the levels of MMP-1 or TIMP-1 protein as well as mRNA, whereas this cytokine had no influence on the expression of type I collagen, FN, or TIMP-2. PDGF-CC also increased the levels of MMP-1 catalytic activity in the cultured media and mRNA expression, which paralleled the levels of promoter activation. Additionally, PDGF-CC induced the mitogenic and migratory activity of human dermal fibroblasts in a dose-dependent manner. Their results suggest that PDGF-C plays a role in the tissue remodeling.

Normal human fibroblasts of HS27 newborn foreskin in 5% FBS was irradiated with RLED 628 at 11.46 mW/cm^2 for three days, and their gene expression was then assessed with the cDNA microarray technique to find the mechanism of proliferation promotion.[41] The gene expression profiles revealed that 111 genes were regulated by RLED 628 and can be grouped into 10 functional categories. Among the upregulation genes, nicotinamide adenine dinucleotide (NADH) dehydrogenase (ubiquinone) 1β subcomplex, 2 (8 kDa, AGGG) is one of the peptides of mitochondria respiratory complex I that transfer electrons from NADH to the respiratory chain.[42] The upregulation gene increased the ratio of NADH oxide form (NAD^+) and NADH, NAD^+/NADH, and then sirtuin 1 activity,[43] which might promote differentiation. In other words, RLED 628 promote 5% FBS-induced ordinary stress to establish DiSH-aided PlSH in 5% FBS, which was supported by the downregulation of heat shock 70 kDa protein 1A and stress-induced phosphoprotein 1 and the upregulation of Janus kinase (JAK) binding protein. The DiSH was supported by the upregulation of PDGF-C, which is further supported by the above discussion. In a summary, the proliferation of normal human fibroblasts of HS27 newborn foreskin in 10% FBS is in its PlSH in 10% FBS; 5% FBS disrupted the PlSH and induced an ordinary stress, but the PlSH at 5% FBS is established with the aid of RLED 628 through PDGF-C pathway.

The above PBM indicated that PDGF-C might promote the proliferation in 5% FBS to establish the PlSH in 5% FBS, and fibroblast proliferation

in its PlSH in 10% FBS might be upgraded by PDGF-C. Fredriksson *et al.*[39] have studied how the PDGF-C/tPA (tissue plasminogen activator) axis is regulated in primary fibroblasts and found that PDGF-C expression is induced by TGF-beta 1 treatment. Jinnin *et al.*[40] found that PDGF-C may contribute to fibrosis. A mouse model in which PDGF-C is overexpressed (Pdgf-c Tg) resulted in hepatic fibrosis, steatosis, and eventually, hepatocellular carcinoma development, and peretinoin, a member of the acyclic retinoid family, inhibited the signaling pathways of fibrogenesis, angiogenesis, and Wnt/beta-catenin in PDGF-C transgenic mice through inhibiting PDGF-C-activated transformation of hepatic stellate cells into myofibroblasts.[44] As a fact, PDGF-C also plays a role in cardiac fibrosis,[45] renal interstitial fibrosis[46] and bleomycin-induced lung fibrosis.[47] Peretinoin might be used to treat cardiac fibrosis, renal interstitial fibrosis and bleomycin-induced lung fibrosis. It should be further verified.

3.4. *Vascular endothelial growth factor and transforming growth factor beta* 1

Khanna *et al.*[48] have studied the effects of He-Ne laser irradiation at 1.67 mW/cm^2 on fetal human cardiomyocytes in 10% FBS. The cells were irradiated with LIL for 0, 5, 10, 15 and 20 min. Cells treated with LIL had increased in cell number at 18 h after treatment compared with control. Stimulating effects on proliferation were most pronounced significantly after irradiation of 15 and 20 min. Increased expression of vascular endothelial growth factor (VEGF) and TGF-beta 1 was observed with 10-, 15- and 20-min exposures. Wong *et al.*[49] have examined the effects of intense pulsed light (IPL) on normal human dermal fibroblasts grown in contracted collagen lattices in 10% FBS. A dose-dependent increase in viable cells was demonstrated after the IPL irradiation. There was no significant change in mRNA levels of collagen I and fibronectin. Upregulated expression of collagen III and TGF-beta 1 in dermal fibroblasts was verified.

Kipshidze *et al.*[50] have further studied the effects of the laser irradiation on smooth muscle cells (SMC) and fibroblasts in 2% FBS. The cells were irradiated with LIL for 0, 1, 3, 5, 10, 15, 20, 30, 40 and 60 min. They found the LIL promoted the proliferation by promoting VEGF secretion. Gavish *et al.*[51] have studied the effects of the laser irradiation at 780 nm and 2 mW/cm^2 on smooth muscle cells (SMC) in 2% FBS, and found the LIL promoted the proliferation by promoting collagen synthesis, which might be realized by promoting TGF-beta 1 secretion. As a fact, Gavish *et al.*[51] found TGF-beta 1 indeed promoted collagen syntheisis. ERK-dependent production of TGF-beta1 plays a crucial role in the soy-peptide−induced proliferation of human-adipose-tissue−derived mesenchymal stem cells under serum-free conditions.[52]

The roles of VEGF and TGF-beta 1 in the proliferation of many cells were further supported. *Ganoderma lucidum* is a popular medicinal mushroom that has been used as a home remedy for the general promotion of health and longevity in East Asia. The dried powder of *G. lucidum*, which was recommended as a cancer chemotherapy agent in traditional Chinese medicine, is currently popularly used worldwide in the form of dietary supplements. Stanley *et al.*[53] found that *G. lucidum* inhibits an early event in angiogenesis: the capillary morphogenesis of the human aortic endothelial cells. These effects are caused by the inhibition of constitutively active AP-1 in prostate cancer cells, resulting in the downregulation of the secretion of VEGF and TGF-beta 1 from PC-3 cells. Their results suggest that *G. lucidum* inhibits prostate-cancer-dependent angiogenesis by modulating MAPK and Akt signaling and could have potential therapeutic use for the treatment of prostate cancer.

Bone marrow mesenchymal stem cells (BMSCs) have been shown to ameliorate diabetes in animal models. In recent reports, BMSCs injected into the circulation of diabetic animals have been shown to partially/totally reverse the diabetic phenotypes and improve glucose control, but with very poor direct β-cell differentiation. Milanesi *et al.*[54] have tested the ability of human BMSCs (hBMSCs) genetically modified to transiently express VEGF to reverse diabetes and whether these cells were differentiated into β-cells or mediated recovery through alternative mechanisms. They found that hBMSCs expressing VEGF reversed hyperglycemia in more than half of the diabetic mice and induced overall improved survival and weight maintenance in all mice. They also found that the sustained reversion of diabetes mediated by hBMSCs-VEGF was secondary to endogenous β-cell regeneration and correlated with activation of the insulin/IGF receptor signaling pathway involved in maintaining β-cell mass and function.

3.5. *Insulin-like growth factor*-1 *and transforming growth factor beta* 1

Fibrosis is one of the largest groups of diseases, but there is no effective therapy. Delineation of the central role of TGF-beta and identification of the specific cellular receptors, kinases, and other mediators involved in the fibrotic process have provided a sound basis for development of effective therapies. The inhibition of signaling pathways activated by TGF-beta represents a novel therapeutic approach for the fibrotic disorders.[55] However, TGF-beta-activated kinase 1 (TAK1) is an important upstream regulator of skeletal muscle cell differentiation.[56]

Our dPBM on fibrosis with low-intensity gallium aluminum arsenide 635 nm laser irradiation (LIGL) found that IGF-1 and TGF-beta 1 should be coordinately modulated.[57] A total of 96 male Sprague-Dawley rats were randomly divided into three groups: control, contusion, and LIGL groups. LIGL at 17.5 mW/cm^2 was administered to the gastrocnemius contusion for 30 min each time once a day for 10 days. It has been found that LIGL promoted IGF-1 mRNA expression on the 1st, 2nd, 3rd and 7th days, but inhibited the one on the 14th and 21st days, respectively. LIGL increased IGF-1 level on the 2nd, 3rd and 7th days, but decreased the one on the 14th and 21st days, respectively. LIGL decreased TGF-beta 1 level on the 3rd and 28th days, but increased the one on the 7th and 14th days, respectively. Obviously, the LIGL self-adaptatively and coordinately modulated IGF-1 and TGF-beta 1 so that it completely inhibited fibrosis by promoting the regeneration of muscle.

The above work has been supported by the promotion of autologous conditioned serum (ACS) injection on muscle injury[58] as fibroblast growth factor-2 (FGF-2) plays a role similar to IGF-1 in muscular growth.[59] Mice were subjected to an experimental contusion injury to their gastrocnemius muscle; one group received local injections of ACS at 2 h, 24 h and 48 h after injury, a control group received saline injections. The histology results showed that satellite cell activation at 30/48 h post-injury was accelerated and the diameter of the regenerating myofibers was increased compared to the controls within the first week after injury. Enzyme-linked immunosorbent assay results on the ACS have shown that the elevations in FGF-2 (460%) and TGF-beta 1 (82%) could be partly responsible for the accelerating effects on regeneration due to proliferative and chemotactic properties.[58]

4. Less is More

That less is more has been shown in the politics of innovation by some industrialists such as Steve Jobs and in the art of scientific research by some scientists such as Albert Einstein. It is also a biological phenomenon. The mechanisms of recognition involve reductions of activity in the medial temporal lobe. Gonsalves *et al.*[60] provided some of the strongest evidence to date demonstrating that the reduced medial temporal activity is correlated with stronger recognition of items in humans. The human brain's capacity for cognitive function is thought to depend on the coordinated activity in sparsely connected, complex networks organized over many scales of space and time. Bassett *et al.*[61] found the information processing performance of a network can be enhanced by a sparse or low-cost configuration with disproportionately high efficiency. Less is more has been also maintained by negative feedback in biological systems. Sparse coding presents practical advantages for sensory representations and memory storage. In the insect olfactory system, the representation of general odors is dense in the antennal lobes but sparse in the mushroom bodies, only one synapse downstream. Papadopoulou *et al.*[62] have found the existence of a normalizing negative-feedback loop within the mushroom body to maintain sparse output over a wide range of input conditions.

A human has approximately 4000 enzyme systems, 1000 chemical mediators, 2,000,000 possible single nucleotide polymorphisms (SNPs), approximately 250 nutrients known to be important in human health, and several thousand endogenous and exogenous xenobiotics.[63] However, a function in its FSH has sparse kinds of FESH,[6,11] and the best pathways for a function to establish its FSH are then also sparse as discussed in this paper. Melanoma cell proliferation in its PlSH might be upgraded by IL-8. IGF-1 might promote the myoblast proliferation in hG to establish the hPlSH in which the FOXO3a is the same as in the nPlSH, and myoblast proliferation in its nPlSH might be upgraded by IGF-1 upregulation and FOXO3a downregulation. PDGF-C might promote the proliferation in 5% FBS to establish the PlSH in 5% FBS, and fibroblast proliferation in its PlSH in 10%

FBS might be upgraded by PDGF-C. IGF-1/FGF2 and TGF-beta 1 are coordinately modulated in promoting the regeneration of muscle.

5. Discussion

Functional medicine, a modern edition of TCM,[64] is the best approach in dealing with common chronic diseases.[63] The functional medicine approach to diagnosis demands not only that we determine what disease the patient is suffering from and what the patient's underlying physiological dysfunctions are but also which pathways are the best pathways for the patient to recover completely. FSH should be one of the key concepts of functional medicine. As discussed in this paper, self-adaptative PBM of LLL should be the best candidate to diagnose which pathways are the best pathways for the patient to recover completely. We have summarized five cases that all uses red lights to diagnose the pathway. More generally, laser irradiation at green, red, or infrared wavelengths at a range of dosage parameters can cause significant changes in the cellular gene expression and release of these mediators such as endothelin-1 (ET-1), bFGF, hepatocyte growth factor (HGF), IFN-γ, IGF-1, IL-1α, IL-1β, IL-6, IL-8, IL-10, keratinocyte growth factor (KGF), melanocyte stimulating factor (MSH), monocyte chemotactic protein-1 (MCP-1), nerve growth factor (NGF), PDGF, stem cell factor (SCF), TGF-β, TNF-α and VEGF.[65]

A diagnosis is always passive and is used to diagnose what has already occurred. For example, long-term drug treatment induced drug resistance, and the biological assessment may then be used to find which redundant pathways have been fully activated. However, the PBM-mediated diagnosis is active. It can be used to diagnose what a biosystem becomes and how the change takes place. A dPBM may further promote the activation of a partially activated pathway until it is fully activated, and the biological assessment may then be used to find the pathway. An iPBM may induce drug resistance by activating the redundant pathway fully, and the biological assessment may then be used to find the redundant pathway.

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