Editorial

Paracrine Mechanisms, Signaling and Epigenetics in Repairing Damaged Tissue

Paracrine Factors and Stem Cells

Paracrine mechanisms, such as cell-produced growth factors and cytokines, which go into the blood stream or act locally by signaling to target cells through biochemical and epigenetic mechanisms, play a fundamental role in tissues homeostasis and repair upon damage in our bodies. This applies many disturbances/processes including cardiovascular diseases, metabolic diseases and stem cells biology. Research on the temporal and spatial organization of signaling cues driven by paracrine factors is expanding rapidly, mirrored by an increase in the number of filed patents.

It is commonly considered that tissue regeneration is synonymous of stem cells. The outstanding proliferative and differentiation capacities of stem cells hold the potential of an unlimited supply of functioning adult cells to repair damaged tissues. A key issue in designing rational stem cell-based therapy approaches for diseases is the understanding of the exact mechanisms whereby each stem cell or progenitor-cell type can affect the performance of the organ. Stem cells differ from other cell types by two unique features: first, they are self-renewing, even after long inactive periods; they can become tissue- or organ-specific functionally specialized cells with the proper physiological or experimental setting (milieu of autocrine/paracrine growth and signaling factors). Second, stem cells can be pluripotent or multipotent. Pluripotent stem cells can differentiate theoretically to any cell type in the body whereas multipotent stem cells give rise to a restricted profile of cell types. Stem cells can be derived from three sources: embryonic, non-embryonic (somatic or adult), or induced pluripotent stem cells. Embryonic stem cells (ESCs) are derived from embryos that are 5 to 8 days old in humans, or from a blastocyst stage in mammals; howeve, they can be isolated even from earlier developmental stages. ESCs extracted in any of these stages are pluripotent. Non-embryonic stem cells (somatic and adult) are found in small quantities in most adult tissues in the body. Examples include the above mentioned CSC, hematopoietic stem cells (HSC), endothelial progenitor cells (EPC), mesenchymal stem cells (MSC) and bone marrow-derived stem cells (BMSC), which are widely studied for cellular transplantation therapies and [1, 2]. Adult stem cells can also be found in placentas (placental stem cells, PSC) and umbilical cords: they have though a more limited proliferative capacity. Between 2006 and 2007, the team of scientists led by Shinya Yamanaka, identified conditions that both in mouse and in humans allow somatic adult cells to be reprogrammed genetically to assume a stem cell-like condition (iPSCs [3-5]): accordingly, iPSCs can be defined as stems cell artificially derived from a non-pluripotent cell by inducing forced expression of specific key pluripotency genes (originally this was achieved by retroviral transfection of master regulators of transcription: Oct-3/4, SOX2, c-Myc, and Klf4). In other terms, iPSCs are adult cells that have been genetically rewired to an embryonic stem cell-like state by expressing genes and factors that define the properties of embryonic/pluripotent stem cells.

The potential of exploiting "cocktails" of paracrine factors opens exceptional new avenues for therapy, because it would imply not to use invasive cell or tissue transplantation for organ repair, but therapeutic injections and solutions administration would act at the signaling and epigenetic level in target cells. The aim of this special issue is to review recent patents that have been generated, including search strategies and major trends in scientific and patent production, in the field of paracrine signaling and epigenetics mechanisms in repairing damaged tissue. Here below, some examples of paracrine and epigenetic factors for regenerative therapies in human diseases have reviewed and discussed in depth by the outstanding authors of this special issue.

microRNAs as Inducers of iPSCs

Although, iPSCs generation for interventions to repair tissues is an exciting tool by Yamanaka and colleagues, it is inefficient. Developing alternative methodologies other than using the previously defined four factors (Oct-3/4, SOX2, c-Myc, and Klf4) have been sought for. Several of these new inventions adopted a novel strategy of using a single microRNA to induce iPSC generation [6-8], which presented the first evidence of using a small microRNA, miR-302, to reprogram human skin cells into a pluripotent state. MicroRNAs are a class of small non-protein-coding RNAs that function to suppress the translation of their respective target genes through complementary binding and formation of RNA-induced silencing complexes in the 3′-untranslated regions (3′-UTRs) of the targeted messenger mRNA. Since this kind of miRNA-target interaction does not require a perfect match in complementarity, miRNAs can silence multiple target genes at the same time. MiR-302 is expressed copiously in iPSCs but is absent in all differentiated normal tissue cells, and can be exploited for iPSCs generation. miR-302 functions as a gene silencer capable of down-regulating multiple key epigenetic regulators, including lysine-specific histone demethylases 1 and 2 (namely AOF2/1, LSD1/2, or KDM1/1B), DNA (cytosine-5-)-methyltransferase 1 (DNMT1), and methyl-CpG binding proteins 1 and 2 (MECP1/2). Silencing of these important epigenetic regulators induces global DNA demethylation, the first sign of nuclear reprogramming to form iPSCs [9-11]. These findings suggest that miR-302 induces global demethylation to remove transcriptional blocks and hence activates gene expression, particularly of Oct4 and Nanog, for the initiation of reprogramming to form iPSCs.

Regulation of Myogenic Events by Epigenetic and Paracrine Mechanisms

The decline in functional performance and restriction of adaptability represents the hallmark of skeletal muscle pathologies. The characteristic loss in muscle mass, coupled with a decrease in strength and force output, has been associated with a selective activation of apoptotic pathways and a general reduction in survival mechanisms. Tissue remodeling is therefore an important physiological process that allows skeletal muscle to respond to environmental demands and to undergo adaptive changes in response to a variety of stimuli [12]. Muscle ageing is characterized by a decline in functional performance and restriction of adaptability, due to progressive loss of muscle tissue coupled with a decrease in strength and force output. Together with selective activation of apoptotic pathways, a hallmark of age-related muscle loss or sarcopenia is the progressive incapacity of regeneration machinery to replace damaged muscle. Modulation of extracellular agonists, receptors, protein kinases, intermediate molecules, transcription factors and tissue-specific gene expression collectively compromise the functionality of skeletal muscle tissue, leading to muscle degeneration and persistent protein degradation through activation of proteolytic systems. A better understanding of the mechanisms underlying the pathogenesis of muscle wasting associated with different diseases has been the objective of numerous studies and represents an important first step for the development of therapeutic approaches. In particular, the role of paracrine, signaling and epigenetic mechanisms involved in skeletal muscle regeneration is increasingly recognized. Muscle tissue is comprised of myofibres, supported by muscle satellite cells that lie between the myofibre membrane and the basement membrane. Following injury, myogenesis requires the activation of quiescent satellite cells, their commitment to the myogenic lineage and ongoing proliferation, followed by cell cycle withdrawal and fusion to form myotubes, which then mature to form myofibres. A highly specific gene expression profile characterizes and drives myogenesis, defined largely by the myogenic regulatory factors (MRFs) [13]. Key epigenetic (histone deacetylases, DNA methylation, microRNA etc.), signaling (Notch, Wnt, JNK, NF-kB etc.) and paracrine (HGF, IGFs, TNFalpha, nitric oxide etc.) mechanisms regulate the temporal MRF and related gene expression profile, and thus the process of myogenesis. Interventional methodologies on these myogenic players have been protected by numerous patents [14-18].

Growth Factors and Cardiac Endogenous Repair

The mammalian heart has a limited capability of physiological cardiomyocyte turnover during adult life to substitute damaged cells. While this regenerative mechanism has been preserved throughout mammalian evolution, it is insufficient to counteract more extensive tissue loss, which results in scar formation at the expense of cardiac function. Recently, regenerative medicine studies investigated the efficiency of stem cells to regenerate the heart via cell therapy, while pre-conditioning the hostile environment of the injured cardiac tissue by administration of cell survival and anti-inflammatory molecules. Indeed, post-infarct combinatorial therapies using cells and factors (including growth factors, chemokines and cytokines) increased cardiac function recovery and tissue regeneration. In addition, the influence of other factors and molecules capable of inducing adult cardiomyocytes to re-enter cell cycle were explored to overcome the intrinsic cell cycle block or the loss of mitogenic stimuli in the postnatal heart. Nevertheless, the field has yet to solve significant obstacles including the incomplete differentiation of stem cells and the paucity of tissue-specific stem cells. Different population of stem cells have been involved in regenerative processes, ehich are recently patented and employed in clinical trials: BMSC, Islet1 cardiovascular progenitors, c-KIT CSC, iPSCs [19, 20]. A new challenge is the attempt of joining efforts between the regenerative potential of stem cells and paracrine factors such as growth factors (IGF, HGF, FGF etc.) to improve heart repair upon damage; clinical trials and preclinical studies in this direction are encouraging and several protocols have been patented [21-25].

Driving Mesenchymal Stem Cells Towards Cardiac Stem Cells

Knowing that the myocardial plasticity of a stem cell could be modulated or enhanced over time, it has led scientists to develop and assess various chemical agents, selective or pleiotropic, aimed at programming in vitro the potential of cardiac differentiation of adult stem cells *in vivo*. Some chemicals have a large property to evoke an effective response similar to what could be achieved by manipulating gene expression.

For instance, the survival, growth and function of the cardiomyocytes can be modulated *in vitro* and *in vivo* by natural or synthetic chemical compounds [26]. In this regard, it has been shown that hyaluronan mixed esters with retinoic and butyric acid (HBR) act as novel modulator of cardiomyocytes by eliciting *de novo* cardiomyogenesis *in vivo* without any side effects [27, 28]. The rationale for the synthesis of these novel glycoconjugates has been described [27, 28]. HBR enhanced the transcription of vasculogenic, proliferative and pro-survival factors, such as EGF, HGF, KDR, AKT, and pim-1. The gene transcription in cardiomyocytes, endothelial cells and stem/progenitor cells was due to long-term acetylation of histone type 4. In particular, chromatin immune precipitation and transcriptional analyses showed that HBR increased the transcription of the cardiogenic gene Nkx-2.5 through the binding of Smad4, a well known cardiac signal transducer, to its own consensus Smad site in human MSCs [29]. It was also shown *in vitro* that HBR can redirect the gene profile of human MSCs to cardiovascular feature in a dose-dependent manner [30]. Human MSCs, mostly those of placental origin, after exposure to HBR appear to be epigenetically more sensitive to the extracellular environment and more oriented towards a more effective path to cardiovascular differentiation. Epigenetic activation of MSCs with the HBR is so stable and strong as to limit significantly the post-ischemic myocardial remodeling in swine heart, which is ontogenetically more similar to the human heart, and with a plasticity lower than rodent heart [31]. In addition to HBR, other patented examples of synthetic inducers of stem cell-derived

cardiac cells include sulfonyl hydrazones, derivatives of dihydropyrydines, benzimidazoles, phenothiazines, tamoxifen, macrocyclic compounds, HDAC inhibitors [8, 20, 30, 32].

Regenerators of Pancreatic Islets

Diabetes, currently considered one of the most common noncommunicable diseases in the world, causing 4.6 million deaths only in 2011, owns this giant rise in incidences because of the development of obesity, due to shifting dietary habits and increasing sedentary lifestyles of humans. One of the major strategies of this disease is to preserving and/or regenerating the functional pancreatic insulin-producing β-cell mass. Top paracrine or ligand-mediated activated signaling molecules and candidate targets discussed in this Issue, for which an intense patent activity occurs, are the G-protein coupled receptor 119 (GPR119), GPR119, also termed glucose-dependent insulinotrophic receptor, is a Gαs-coupled receptor that is predominantly expressed in pancreatic β-cells; GIP a 42 amino acid incretin hormone that is produced by the endocrine K-cells of the gut and released into the circulation in response to food intake [33]; BACE-2, a protease homologous to the BACE-1 protein, an enzyme involved in the amyloid formation of Alzheimer disease (AD) [34, 35]. Neurturin is a neurotrophic factor that preserves and/or increases cell mass most likely through a paracrine effect resulting in improved blood glucose [36].

This thematic issue tempts to provide some new insights into the potential therapeutic roles of paracrine and epigenetic signaling pathways to repair damaged tissues, and to review the pertinent patent activity.

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