On Mechanisms Underlying Regeneration and Reparation Processes in Tissues

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Studies of the possibility of regulating the regenerative and reparative processes in pathologically modified tissues are reviewed. A ready cell system providing the realization of reparative and regenerative processes in all organs (cell sources of regeneration) exists in all organisms. The authors suggest that active bioregulators presented in this paper are involved in the mechanisms of tissue regeneration by modulating the cell sources of regeneration.

Key Words: *cell sources of regeneration; bioregulators*

The possibility of regulating the regenerative and reparative processes in pathologically modified tissues attracts special attention of scientists in recent years. A natural course of pathological process development in epithelial mesenchymal tissues results in the formation of a connective tissue cicatrix or a glial cicatrix in the nervous tissues. The cicatricial tissue impairs the structure of the adjacent tissue and partially (sometimes significantly) modifies its function. For example, glial cicatrix formed after brain stroke or brain surgery impairs or even blocks nerve pulse conduction in this area. One more example is the so-called adhesion disease. Its main cause is excessive growth of connective tissue elements after surgical intervention. Hence, the idea of using stem cells seems to be particularly attractive [7,9].

Numerous studies were devoted to the development of methods for the use of fetal stem cells (pluripotent cells capable of differentiation into cells of virtually any phenotype). Being injected to humans, they restore the structure of the pathologically modified tissue at the site of injury (surgery). However, using this approach one should remember that the genetic system of cells is modified during ontogeny and, hence, injection of fetal cells to adults can have unpredictable consequences, greatly delayed in time. One of these potential negative aftereffects is fetal cell asynchronism in comparison with the recipient organism. The fate of these cells is unpredictable during a long time.

As for studies of tissue regeneration after injury, we should like to emphasize the fact that all organisms have ready cell system providing realization of reparative and regenerative processes in all organs. Due to the existence of this system, regeneration of the entire organism after loss of its significant components is possible in some invertebrates. Some lower vertebrates (particularly at the larval stage) are capable of restoring lost limbs and some organs [1,12,13]. This reparation capacity is practically lost in warm-blooded animals (including mammals), despite the fact that they have cell sources of regeneration (CSR) in their tissues. During regeneration, the formation of a complete set of differentiated cells in the tissue is realized via two main mechanisms: stimulation of CSR1 (multipotent stem cells) and CSR2 (cell dedifferentiation with subsequent redifferentiation into the needed cell types) [1,12,13].

This review presents the role of a group of protein bioregulators (BR) detected in various tissues of vertebrate animals in both mechanisms of regeneration [2,5,9,10,15] (Fig. 1).

Multipotent stem cells in CSR1 are capable of asymmetrical mitosis, resulting in the formation of

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two cells. One remains a stem cell, while the other starts differentiation and forms the population of the so-called progenitor cells, which, in turn, are capable of further division and differentiation (Fig. 1, *a*) [12]. Hence, the progenitor cells are the sources of all cells intrinsic of a certain tissue. The work of CSR1 at the cell level is now actively studied. The progenitor cell differentiation route is followed up, the fate of CSR1 in during ontogeny is studied, *etc.* However, the nature and routes of signals transmitted to CSR1 and initiating division and subsequent differentiation of progenitor cells are little studied. The results of our studies of the new group of BR suggest that these proteins are involved in transmission of information signal to CSR1 [5,6]. This hypothesis is supported by experimentally proven facts:

1) BR of this group modulate the main processes constantly coursing in tissues and determining their viability and functioning [2,5,9,10,15];

2) BR stimulate reparative and regenerative processes in pathologically modified (damaged) tissues [3,9,10,15]. It was shown that BR isolated from bovine cornea stimulate proliferation and migration of epithelial cells, support adhesion interactions between the corneal epithelium, stroma, and endothelium, and promote wound healing in this tissue [10]. BR isolated from the retina protect cells of the retinal inner layers [2]. BR isolated from pigmented epithelium of mammalian eye stabilize cell adhesion and proliferation in this tissue and protect Muller and bipolar cells in CSR (CSR1 and CSR2) of triton retina [2]. BR isolated from the lens of the eye prevent cataractogenesis induced by destructive chemicals or injury to this ocular structure [9].

As we hyopthesized, these BR are components of the "minor matrix", a supramolecular structure of the extracellular space reacting with the extracellular matrix and cell plasma membrane [6]. The minor matrix is primarily involved in the maintenance of a certain status of adhesion cell-cell interactions playing the principal role in the regulation of the main biological processes, such as cell proliferation, differentiation, apoptosis, work of the main enzyme systems in tissues, *etc.* We also hypothesized that the minor matrix is involved in the transmission of information signal in the tissue [6]. Now we put forward a hypothesis according to which the minor matrix is involved in the regulatory transduction. Specifically, we discuss the role of BR of this group in CSR functioning during tissue regeneration.

The counts of dying cells and cells regenerating due to division of adjacent cells or CSR activation

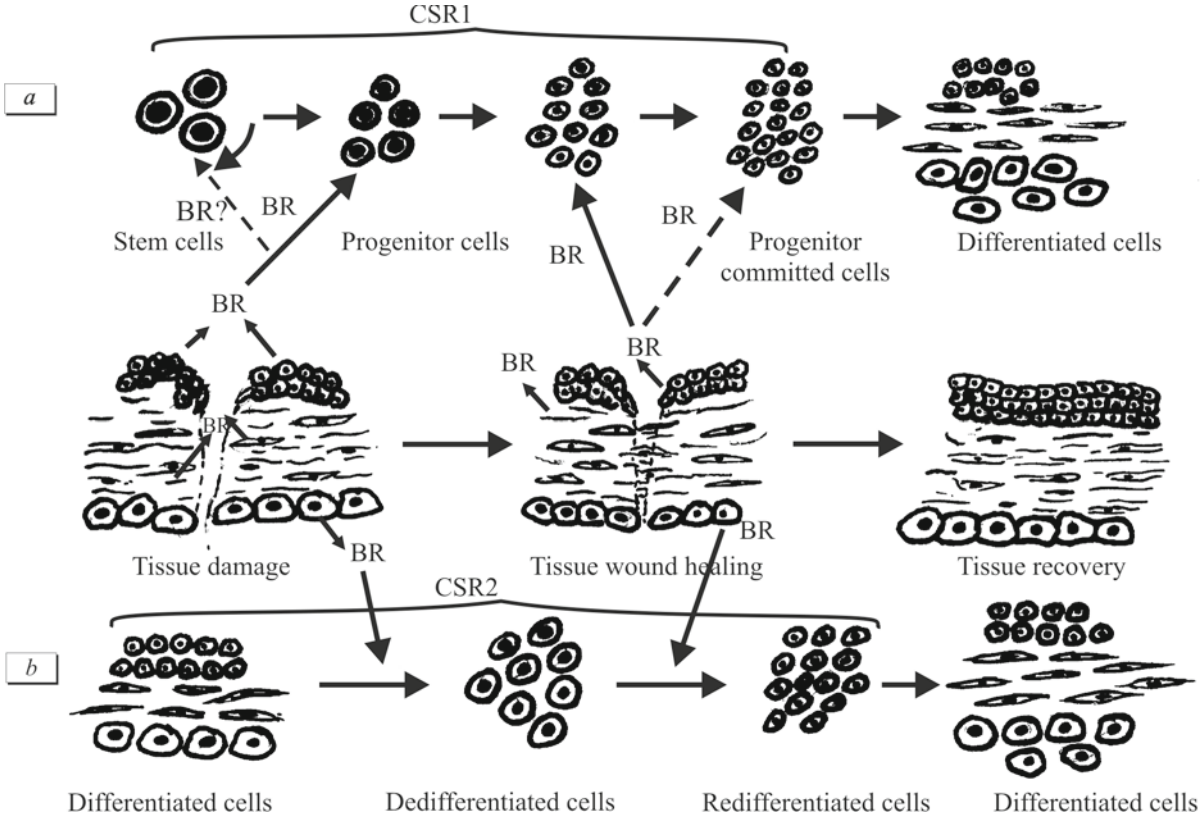

Fig. 1. Mechanism underlying the effects of BR, active in ultralow doses, on CSR. *a*) stimulation of CSR1. CSR1 includes multipotent stem cells; *b*) stimulation of CSR2 based on cell dedifferentiation and subsequent redifferentiation into the needed cell types.

are equilibrated in constantly regenerating tissues [4]. When the tissue is exposed to a negative factor causing overall cell death, this exposure leads to disorders in cooperative interactions between the cells in the tissue, organization of the macromolecular structures of extracellular space (intracellular matrix, minor matrix, cell-cell contact ultrastructures), and the development of inflammation because of accumulation of tissue detritus. The duration of intervals needed for these processes plays the key role in the selection of the approach to subsequent tissue regeneration with the formation of a cicatrix or with complete recovery of the histostructure [1,13]. Immune system cells involved in the acute phase of inflammation can have dual effects on the regenerative and reparative processes in the tissue. For example, macrophages involved in phagocytosis of tissue detritus secrete collagenases destroying collagen at the site of injury, promote restoration of epithelial tissues, and thus stimulate regeneration [13]. On the other hand, macrophages promote angiogenesis and release cytokines: transforming growth factor-β and platelet growth factor stimulating fibroblast proliferation and migration, which, in turn, promotes the formation of cicatricial tissue [13].

Hence, for complete regeneration of the tissue structure at the site of the defect it is essential that dead cell material is removed, inflammation arrested, and cell division and differentiation processes are then controlled so that tissue structure reparation is possible without development of the cicatrix. The key point in the regulation of the last stage of regeneration is, on the one hand, regulation of proliferation of connective tissue elements rapidly filling the defect with violation of the normal tissue morphology, and on the other hand, stimulation of cell proliferation and differentiation essential for complete recovery of the histostructure. In most cases (particularly in injuries to the deep layers of tissues), the course of events conforms to variant 1 of tissue regeneration (with the formation of a connective tissue cicatrix). Variant 2 of complete recovery of tissue structure has no time to develop, because the processes leading to the formation of differentiated cells essential for restoration of the normal histostructure are much slower than cicatrix formation.

Secretion of BR is stimulated after tissue damage (irrespective of the nature of the destructive agent) for stimulation of CSR. However, this stimulation can be rapidly leveled, for example, because of the development of inflammatory reaction (macrophage and leukocyte migration and production of numerous cytokines by these cells), which can arrest hypersecretion of BR and create the information block situation for CSR. It can be expected that creation of conditions for constant delivery of BR to CSR (for example, by exogenous injection of these proteins) will lead to reparation of the histostructure corresponding to the morphology of normally functioning tissue.

Studies of the location of this group of BR have shown that these proteins are present in the extracellular space of tissues determining the integrity of the corresponding histostructure of the organ. For example, BR isolated from the cornea is located in the extracellular space of the epithelium and endothelium (the interface layers of the cornea). Our studies showed that corneal BR also stimulates CSR located in the limbic compartment and basal layer of the triton eye cornea [10]. BR isolated from the lens of the eye is located in the extracellular space of the epithelium, in fact, the lenticular CSR, because this epithelium is responsible for the maintenance of its shape and structure [9]. The fact that BR in all cases are located in areas of direct contacts of the neighboring cells, is worthy of note; in other words, their location completely corresponds to the function of adhesion molecules. It seems that BR are additionally released by cells in response to damage in order to maintain spatial organization of cell-cell adhesion interactions (the main condition of regulation of the main biological processes) [14]. It is known that impairment of cell adhesion is the first step in the development of any pathological process, irrespective of its nature, while retention of cooperative relations between the cells provides the improvement of cell viability [11,14]. As we have mentioned above, one of approaches to tissue regeneration is cell dedifferentiation with subsequent redifferentiation into cells needed for restoration of the tissue structure. Presumably, BR, by improving cell viability, promote the dedifferentiation-redifferentiation processes (Fig. 1, *b*).

Are the data obtained in studies of characteristics of these proteins confirm the hypothesis on the involvement of BR from this group in tissue regeneration? We think that BR are candidate factors essential for complete regeneration of tissue. BR are a complex consisting of a regulatory peptide and modulator proteins modulating its activity. This complex is resistant to many physicochemical factors, including temperature changes from -70 to +100°C, pH shifts, effects of various enzymes, *etc.* [15]. BR are active when used in ultralow doses. This means that just few molecules of BR are sufficient for stimulation of CSR. In addition, BR activity is characterized by tissue, but not species specificity. Many regulatory factors, for example, adhesion molecules and cytokines exhibit these properties [8,11]. BR molecules exhibit a pronounced trend to the formation of high molecular associations and are present in solutions as nanoparticles 50-200 nm in size [9,10,15]. Presumably, the involvement of BR of this group in transmission of a regulatory signal can be due to the nanosize status of these protein associations

forming the minor matrix structure in the extracellular space of the tissue.

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