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CONTEMPORARY VIEWS AT THE PROBLEMS OF HEART REGENERATION

Summary. *Contemporary knowledges of genetic and epigenetic results while cells reprogramming, heart development and cardiomyocytes differentiation develops rapidly. Apoptosis of cardiomyocytes is the main process in pathogenesis of a number of heart diseases, including ischemic heart disease and heart failure. Ensuring the survival of heart cells through blocking apoptosis is an important strategy of improving heart functioning. The combination of rapid genome editing and highly effective chemical methods of heart cells differentiation enables to define the effectors of various stages of heart differentiation and cardiomyocytes proliferation.*
Key words: *cardiomyocytes, apoptosis, regeneration.*

Acute myocardial infarction causing ischemic heart disease is not only one of the most devastating diseases, but also the main cause of death in the whole world.

Myocardial infarction, as a rule is accompanied by cell death and a great loss of cardiomyocytes. Stem cells are capable of preventing heavy outcomes of acute and chronic affections in various experimental models and clinical tests on people. Strategies including the use of stem cells appeared among prospective approaches to sustaining and improving intracardial regeneration [30] through the protection of own cardiomyocytes or myocardial regeneration increase. Mesenchymal stem cells (MSC) received from the marrow bone improve cardiac function, reduce the area of infarction and enhance the myocard regeneration in post infarction period [1, 22, 24, 26].

The aim of our work is to show real place of the mesenchymal stem cells, microRNA, genome editing and numerous chemical methods in heart differentiation and cardiomyocytes proliferation.

Apoptosis of cardiomyocytes is the main process in pathogenesis of a number of heart diseases, including ischemic heart disease and heart failure. Ensuring the survival of heart cells through blocking apoptosis is an important strategy of improving heart functioning. An important factor protecting MSC from apoptosis is CTRP3 (C1q - protein 3 related tumor necrotic factor related protein-3). CTRP3 is the leading component in adipokine family and has wide functions not only regarding adipokine secretion and metabolism, but also in inflammation, cell proliferation, differentiating, heart protection [10, 12, 15, 16, 32]. The increase of CTRP3 level protects cardiomyocytes from apoptosis during myocardial infarction due to its ablity to influence cell survival, and can remarkably enhance the survival of MSC through increase of Bcl-2/Bax ration and the potential of mitochondrial membrane, as well as by means of inhibiting the release of cytochrome C and activation of caspase 3.

Apoptosis in the heart is a necessary mechanism for normal remodeling and morphogenesis. It also plays an important role in the onset of heart failure during trauma caused by ischemia/reperfusion and myocardial infarction. Apoptosis is observed in heart cells exposed to various harmful agents both in laboratory conditions and intact heart in normal conditions. Thus, apoptosis of myocytes is both induced in response to ischemia and during human tissues reperfusion.

Mitochondrial signal pathway of apoptosis is realized as a result of release of apoptogenic proteins from mitochondria intermembrane space into the cell cytoplasm. The release of apoptogenic proteins is realized in two ways: due to the rupture of mitochondrial membrane or through opening of highly permeable canals on the outer membrane of mitochondria.

The key event of the mitochondrial apoptosis pathway is the increase of mitochondrial outer membrane permeabilization (MOMP). Apoptotic proteins Bcl-2 Bax and Bak play an essential role in MOMP increase. They fit into the mitochondrial outer membrane and get oligomerizated. At that the entity of the mitochondrial outer membrane is disturbed [5]. When MOMP rises, soluble proteins, involved in apoptosis, release from the mitochondrial intramembrane space into cytosol: cytochorm c - protein with molecular mass 15 kDa; procaspases -2, -3 and -9; protein AIF (apoptosis inducing factor) - flavoprotein with molecular mass 57 kDa.

The rupture of mitochondrial outer membrane is explained by the increase of mitochondrial matrix volume. This process is often referred to the mitochondrial membrane pores opening, which leads to the decline in membrane potential and high-amplitude swelling of mitochondria following the osmotic misbalance. The pores with diameter of 2,6-2,9 nm are able to permeate low-molecular substances with the mass up to 1,5 kDa. Pores opening triggers the following factors: non-organic phosphate; caspases; SH-reagents; cells devastation by regenerated glutathione; formation of active oxygen forms; disintegration of oxidative phosphorylation with protonophore compositions; increase of Ca2+content in cytoplasm; action of ceramide; depletion of mitochondrial pool AIF and others [8].

Cytochrome c in cell cytoplasm is involved in apoptosome formation alongside with protein APAF-1 (Apoptosis Protease Activating Factor-1). Prior to this, APAF-1 undergoes conformation changes following the reaction with AIF energy consumption. It is supposed, that transformed APAF-1 acquires the ability to bind cytochrome c, and CARD-domain gets access to APAF-1 for procaspase 9. It results in oligomerization 7 of subunits of the transformed protein APAF-1 with involvement of cytochrome c and procaspase-9 [20]. It leads

to the formation of apoptosome, that activates caspase -9. Mature caspase-9 binds and activates procaspase -3 followed by the formation of effector caspase-3. Flavoprotein AIF released from the mitochondrial intramembrane space is an apoptosis effector, acting independent of caspases [18].

Caspases are formed due to the procaspase activation (molecular mass 32-56 kDa), in the content of there are three domains: regulatory N-final domain (pro-domain), bigger (17-21 kDaa) and smaller (10-13 kDa) subunits [23]. Activation takes places through proteolytic processing: all three domains disintegrate, releasing prodomain, while the left bigger and smaller subunits associate forming heterodimer. Two heterodimers further form tetramer which is complete caspase with two catalytic areas.

Caspase activation can be regulated directly or indirectly by the proteins family Bcl-2. Proteins family Bcl-2 are the main regulators of mitochondrial apoptosis pathway. They have the decisive role in changing the mitochondrial outer membrane permeabilization (MOMP). In the family Bcl-2 we differentiate between proapoptotic and anti-apoptotic proteins. On the basis of structural and functional differences they differentiate three subgroups of proteins family Bcl-2 [33]: Antiapoptotic Bcl-2 proteins, containing 4 BH-domains(BH1-4): Bcl-2, Bcl-xL, Bcl-W, Mcl-1, A1, Boo/Diva; Proapoptotic Bcl-2 proteins, containing 3 BH-domains (BH123): Bax, Bak, Bok/Mtd; Bcl-2 proteins, containing only BH3-domain, which can act as boosters or repressors of apoptosis: Bid, Bad, Bim, Bmf, Bik, Hrk, Blk, Nip3, BNip3/ Nix,Puma, Noxa.

Apoptotic proteins Bcl-2 - Bax and Bak also play an essential role in enhancing MOMP. They fit into the mitochondria outer membrane and oligomerize, disturbing the entity of the mitochondria outer membrane [30]. Bax and Bak-proteins functioning depends on their prior boost by proteins Bid and Bim, for instance, which are referred to the subgroup BH3 proteins. On the other hand, Bax and Bak boosting and functioning can be blocked by anti-apoptotic proteins of family Bcl-2: Bcl-2, Bcl-xL, Mcl-1 and others. In their turn, anti-apoptotic proteins may also be blocked by depressing proteins (for.ex., Bad), belonging to subgroup of BH3 proteins. Finally, the combined regulation of MOMP as well as of apoptosis is achieved, through the interaction of apoptotic, anti-apoptotic and BH3 boosting and depressing proteins. Regulation of function of BH3 proteins is realized on the level of transcription, molecule stability, while interacting with other proteins and other modification.

It is also stated, that Bid proteins is a connective link between receptor-dependent and mitochondrial pathways of apoptosis. Initiating caspase-8 boosted through the receptors of cell death is able to boost Bid-protein, which further participates boosting proteins Bax and Bak, which in their turn trigger mitochondrial pathway of apoptosis.

As far as protein p53 is concerned, in normal cells it generally exists in non-active latent form. Its activation occurs in response to DNA damage by ultraviolet or gamma-radiation, oncogenes hyperexpression, viral infection, oxidative stress, hypo- or hyperthermia and others [13]. Activated protein p53 coordinates the process of DNA reparation and regulates the transcription of a number of apoptosis boosting genes in case of irreversible DNA damages or cell cycle regulation failures. Besides, there is evidence that p53 participates in triggering apoptosis by stimulating death receptors, through interaction with apoptosis promoter Bax, by boosting p53-dependent apoptosis modulator PUMA (p53-upregulated modulator of apoptosis), which blocks the action of Bcl-2. The increase of p53 level in response to DNA damage causes apoptosis.

Suffice it to mention, that H9c2 ventricular myoblasts undergo apoptosis at ischemia which is prevented by phorbol-12-myristate-13-acetate (PMA). PMA protective effect is related to the decrease of proapoptotic protein Bax and increase of anti-apoptotic protein Bcl-XL. PMA inhibits apoptosis by sustaining all levels of IAP-proteins expression. Besides, exosomes, containing cardiac predecessor cells (CPC) protect H9C2 from oxidative stress by inhibiting caspase 3/7, activated in vitro. In natural conditions CPC-exosomes at acute myocardial ischemia/reperfusion block cardiomyocytes apoptosis approximately by 53% in comparison with PBS ($p<0,05$).

Morphologic changes occurring in the endocardial tissue after the damage initially influence the majority of cells of endocardium ventricle, but remain localized at damage area during regeneration and sustain distinct morphology and profile of genes expression.

There is evidence in favour of the hypothesis, that the molecular mechanism with which help epicardium and endocardium cells initiate heart regeneration and promote cardiomyocytes proliferation is the RA (retinoic acid) production in the damages heart tissue. RA thanks to retinal dehydrogenase (RALDHs) and two-stage metabolic pathwas, moves through changes in a more polar cytochrome P450 enzymes metabolite and transforms the signals by forming heterodimers with retinoid X-receptors, formed between nuclear hormonal receptors.

Suffice it to mention the essential role of micro RNA in realizing cardiac regeneration. MicroRNA are tiny (about 21-23 nucleotides long) endogenic non-coordinating RNK areas functioning as repressors of translation gene [17]. MicroRNA is encoded in genome both in exon and intron gene areas. Irrespective of their genome location, microRNA transcription is initiated by RNA polymerase II, which results in Pri-micro RNA generation. Pri-micro RNA are processed into pre-micro RNA with the help of RNA processing complex, received Drosha and DGCR8 and exported from the nucleus Exportine 5. In cytosol. Pre-micro RNA undergo the second stage of processing by cytoplasmic endonuclease Dicer, which forms mature microRNA duplexes [31]. Further, one duplex micro RNA thread enters the RISC (RNA-induced silencing complex -RISC), which uses micro RNA to identify and inhibit/silence its target-genes [11, 18].

The impacts of microRNA on cardiomyogenesis are remarkable, since the single microRNA can be directed at

several signaling pathways simultaneously (multiple microRNA targets) [29]. Thus, for instance, microRNA-1 and microRNA-133 (loci in genome on chromosomes 18 and 20) are regulated by myogenic transcription factors, including OCP, MEF2C and Nkx2-5 [25], and are key regulator of myocytes differentiation [9]. Their loss leads to cardiac failure, defective morphogenesis, electric conductivity failure and cardiomyocytes proliferation. Micro RNA-133 enhances the effect of micro RNA-1 by depressing specific genes of myogenic predecessors, inhibits signaling pathway of Apaf and caspase-3,-9, reduces fibrosis, thus simplifying cardiomyocytes maturation.

Conclusions and prospects for further research

1. The combination of rapid genome editing into hiPSCs using CRISPRs and highly effective chemical methods of heart cells differentiation enables to define the effectors of various stages of heart proliferation. Besides, the possibility to generate billions of hiPSC-CMs quickly and effectively using suspension according the valid protocols contributes to the solution of the unsolved issues related to engraftment of hiPSC-CMs in the heart. Among them are direct reprogramming in natural conditions; effectiveness increase of this therapy; possibility to apply these methods to human heart regeneration. Finally, it is also necessary to achieve understanding the mechanism of mature CPCs' contribution to regeneration function.

2. Clinical research with the use of MSC has shown documented evidence in favour of safety and eligibility of patients with acute myocardial infarction and chronic ischemic heart disease. Apart from it, the implanted MSC participate in the regeneration process of myocardial tissues by differentiating into cardiomyocytes and endothelial cells, or through releasing biologically proangiogenic and cardioprotective factors.

3. MicroRNAs analogically can induce reprogramming of fibroblasts into cardiomyocytes and can be delivered to cardiac tissue without integrating viruses, thus promoting security in a clinical context. The issue which still requires study is how to transport these microRNA safely and effectively to the damage area and the cell choice for performing corresponding functions.

Our knowledge of genetic and epigenetic results while cells reprogramming, heart development and cardiomyocytes differentiation develops rapidly. The combination of rapid genome editing and highly effective chemical methods of heart cells differentiation enables to define the effectors of various stages of heart differentiation and cardiomyocytes proliferation.

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СУЧАСНІ ПОГЛЯДИ НА ПРОБЛЕМИ РЕГЕНЕРАЦІЇ СЕРЦЯ

Резюме. *Сучасні дані щодо генетичних та епігенетичних механізмах перепрограмування клітин, розвитку, диференціації та регенерації кардіоміоцитів швидко розвиваються. Апоптоз кардіоміоцитів є основним процесом в патогенезі ряду захворювань серця, зокрема ішемічної хвороби серця та серцевої недостатності. Забезпечення збереження серцевих клітин шляхом блокування апоптозу є важливою часткою стратегії для поліпшення функції серця. Поєднання швидкого редагування геному, комбінованої регуляції апоптозу та використання високоефективних хімічних методів диференціації клітин серця дозволяє окреслити ефектори різноманітних стадій серцевої диференціації та проліферації кардіоміоцитів.*

Ключові слова: *кардіоміоцити, апоптоз, регенерація.*

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СОВРЕМЕННЫЕ ВЗГЛЯДЫ НА ПРОБЛЕМЫ РЕГЕНЕРАЦИИ СЕРДЦА

Резюме. *Современные данные о генетических и эпигенетических механизмах перепрограммирования клеток, развития, дифференциации и регенерации кардиомиоцитов быстро развиваются. Апоптоз кардиомиоцитов является основным процессом в патогенезе ряда заболеваний сердца, в том числе ишемической болезни сердца и сердечной недостаточности. Обеспечение выживания сердечных клеток путем блокирования апоптоза является важной частью стратегии для улучшения функции сердца. Сочетание быстрого редактирования генома, комбинированной регуляции апоптоза и использования высокоэффективных химических методов дифференциации клеток сердца позволяет обозначить эффекторы различных стадий сердечной дифференциации и пролиферации кардиомиоцитов.*

Ключевые слова: *кардиомиоциты, апоптоз, регенерация.*

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