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The Role of Nitric Oxide in Stem/Progenitor Cells

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Abstract

The research on Nitric Oxide (NO) and stem cells are the focus in recent years. However, seldom do people conclude the function, mechanism and clinical value of NO in various stem cells including Embryonic Stem Cells (ESCs), Endothelial Progenitor Cells (EPCs), Mesenchymal Stem Cells (MSCs) and Neural Stem Cells (NSCs). In the present review, we evaluate the recent studies on NO in different stem cells and display the latest progresses of NO therapy for tumor, cardiovascular, neurologic and immune system diseases by stem cells.

Introduction

NO, which was first discovered as Endothelium-Derived Relaxing Factors (EDRF) in cardiovascular system, has been established as a diffusible universal messenger that mediates cell-cell communication throughout the body and regulates different physiological and pathological processes in many tissues [1-7]. It works mainly through activation of its target receptor, the enzyme soluble Guanylate Cyclase (sGC), which, when activated, produces the second messenger cyclic-Guanosine Monophosphate (cGMP). Interestingly, a functional NO-cGMP signaling system that involves in development and early differentiation of Embryonic Stem Cells (ESCs) can be evolutionarily conserved between vertebrates and invertebrates [8]. In addition, NO, as a short-lived free radical gas is synthesized from L-arginine by a family of enzymes known as NO synthases (NOS) [9]. Three NOS isozymes encoded by three separate genes, including the Ca21/calmodulin-dependent and constitutively expressed neuronal NOS (nNOS), endothelial NOS (eNOS) enzymes, and a calmodulin-independent cytokine-inducible NOS (iNOS) enzyme found in various cell types [10]. A small amount of NO, produced by the constitutive NOS in response to increase in intracellular calcium, play a crucial role in numerous physiological functions, including neurotransmission [11], vascular tone [12] and platelet aggregation [13], whereas the large amounts, generated by iNOS, are implicated in pathological functions such as cytotoxicity of activated macrophages [14]. Recently, experimental evidence has been presented that not only can stem cells produce NOS, but its production, exogenous and endogenous NO, can also affect the proliferation, mobilization, and differentiation of different stem cells.

Role of NO in ESCs

ESCs are pluripotent stem cells derived from the inner cell mass of the blastocyst, an early-stage embryo. Krumenacker et al [15-17] have examined the expression of various subunits of sGC alpha (1), alpha (2), beta (1), beta (2), NOS, MLC2 (cardiac marker) and a cardiac-specific transcription factor (Nkx2.5) in human Embryonic Stem (hES) cells (H-9 cells) and differentiated cells subjected to differentiation in cell suspension using Embryoid Body (EB) formation. Their results clearly demonstrate the role of NO signaling components in differentiation events or physiological processes of human ES or ES cell-derived cardio myocytes. In addition, cGMP analysis in undifferentiated stem cells revealed a lack of stimulation with NO donors. Differentiated cells however, acquired the ability to be stimulated by NO donors. Although 3-(4-amino-5-cyclopropylpyrimidin-2-yl)-1-(2-fluorobenzyl)-

1H-pyrazolo [3,4-b] pyridine (BAY 41-2272) alone was able to stimulate cGMP accumulation, the combination of NO donors and BAY 41-2272 stimulated cGMP levels more than either of the agents separately. These studies demonstrate that cGMP-mediated NO signaling plays an important role in the differentiation of ES cells into myocardial cells. Additionally, they also found nNOS and eNOS are detected in undifferentiated mouse ES cells while iNOS were very low or undetectable. However, although analysis of sGC activity in cell lysates derived from undifferentiated ES cells revealed that NO could not stimulate cGMP, lysates from differentiated EB outgrowths produced abundant cGMP levels after NO stimulation. Furthermore, purification of ES-cell derived Cardio Myocytes (CM) revealed that mRNA expression of all the NOS isoforms was very low to absent while sGCα1 and β1 subunit mRNAs were abundant and sGC-mediated cGMP production was apparent in this population of cells. These data suggest that cGMP-mediated NO signaling may play a minor role, if any, in undifferentiated ES cells but could be involved in the early differentiation events or physiological processes of ES cells or ES cell-derived lineages. Moreover, Huang et al. [18] suggests that NOS elements are present in Endothelial Cells (ECs) but inactive until later stages of differentiation, during which NOS inhibition reduces expression of EC markers and impairs angiogenic function. Further researches has been reported by Mora-Castilla S et al. [19] who indicate that exposure to 0.5 mM DETA-NO induces early differentiation events of cells with acquisition of epithelial morphology and expression of markers of definitive endoderm, such as FoxA2, Gata4, Hfn1-beta and Sox 17.

Role of NO in EPC

EPCs are a controversial and hypothetical population of rare cells believed to circulate in the blood with the ability to differentiate into endothelial cells, the cells that make up the lining of blood vessels. The process by which blood vessels are born *de novo* from endothelial progenitor cells is known as vasculogenesis which involves in NO that can stimulate endothelial cell proliferation, survival and motility, and enhances matrix invasion and tubulogenesis with the help of the pro-angiogenic activity of Growth Factors such as VEGF, transforming growth factor-β and FGF [20-23]. Downstream mediators of NO, including cAMP- and cGMP-dependent protein kinases [PKA (Protein Kinase A) and PKG (Protein Kinase G)], Rho GTPases and ROS (Reactive Oxygen Species), are likely to play a part. Recent studies have showed that the activity of Rho GTPases, key regulators of endothelial cell motility and angiogenesis is modulated by altering the metabolism of Asymmetric Dimethyl Arginine (ADMA) which is a cardiovascular risk factor, an endogenous inhibitor of NOS, increased when abnormal angiogenesis in cardiovascular disorder happened, and is metabolized by Dimethylarginine Dimethyl Amino Hydrolases (DDAHs) *in vivo* and *in vitro*. Fiedler et al. [24] believed that the ADMA/DDAH pathway is to regulate angiogenesis by influencing NO bioavailability [25]. Consistent with the role of NO in the ADMA/DDAH pathway, DDAH I gene deletion in mice leads to inhibition of angiogenic responses, similar to that seen in eNOS-knockout mice [22,26-28]. Additionally, NO also modulates gene expression of factors that promote angiogenesis, such as αvβ3 integrin, and suppresses the production of antiangiogenic factors such as angiostatin, the degradation product of plasminogen [20]. And Shen et al. [29] indicate that suppressed NO production from EPCs was involved in the glycation end products (AGE)-induced apoptosis, which is in part mediated by Mitogen-Activated Protein Kinases (MAPKs) signaling.

Role of NO in MSC

MSCs are multipotent stem cells that can differentiate into a variety of cell types include osteoblasts, chondrocytes and adipocytes. Tatsumi R et al. [30] demonstrated that the quiescent satellite cells of resident myogenic stem cells which are from MSCs are activated to enter the cell proliferation cycle, divide, differentiate, and fuse with the adjacent muscle fiber, and are responsible for regeneration and work-induced hypertrophy of muscle fibers by activation mechanism which is a cascade of events including calcium ion influx, calcium-calmodulin formation, NO synthase activation, NO radical production when muscle is injured, exercised, overused or mechanically stretched. Therefore, MSCs have received special attention for cardiomyoplasty. Rebelatto et al. [31] report an investigation of the effects of two NO agents (SNAP and DEA/NO), able to activate both cGMP-dependent and independent pathways, on the cardio myogenic potential of bone marrow-derived mesenchymal stem cells (BM-MSCs) and Adipose Tissue-Derived Stem Cells (ADSCs). They found that untreated (control) ADSCs and BM-MSCs expressed some muscle markers and NO-derived intermediates induce an increased expression of some cardiac function genes in BM-MSCs and ADSCs. And NO agents considerably increased the pro-angiogenic potential mostly of BM-MSCs as determined by VEGF mRNA levels. Additionally, Mookerjee et al. [32] investigated the signaling mechanisms by Human gene-2 (H2) relaxin which can inhibit renal my fibroblast differentiation by interfering with TGF-beta1/Smad2 signaling regulates myofibroblast differentiation *in vitro* by examining its effects on mixed populations of fibroblasts and myofibroblasts propagated from injured rat kidneys. Inhibition of nNOS, NO, and cGMP significantly blocked the inhibitory effects of relaxin on alpha-SMA and Smad2 phosphorylation, while the NO inhibitor, L-Nitro Arginine Methyl Ester (hydrochloride) (L-NAME) significantly blocked the inhibitory actions of relaxin on collagen concentration *in vivo*. Moreover, Bironaite et al. [33] clearly demonstrate sustained activation of MAPKs which actively participate in the regulation of cell survival and of proapoptotic signals in myogenic stem cells after exposure to the NO inducer, NOC-18. Inhibition of MAPKs phosphorylation by specific inhibitors revealed the anti-apoptotic role of MAPKs in myogenic stem cells. On the other hand, Kraft DCE et al. [34] indicate that Pulsating fluid flow (PFF) stimulated NO production within 5 min

by human dental Pulp-Derived Mesenchymal Stem Cells (PDSCs) portraying mature (PDSC-mature) but not by PDSC immature. The rapid stimulation of NO production by PFF in PDSCs is probably a result of the activity of eNOS, but not iNOS, since unlike iNOS, eNOS is constitutively expressed in bone cells and dental pulp cells. Additionally, NO produced by eNOS is primarily regulated by Ca^{2+} fluxes and subsequent binding of calmodulin, and eNOS only produces NO for minutes after stimulation [35-37] demonstrate that NO-induced osteogenic differentiation through Heme Oxygenase-1 (HO-1) may be an important mediator of periodontal regeneration or bone tissue engineering.

Role of NO in NSCs

NSCs can be propagated for extended periods of time and differentiated into both neuronal and glia cells. Tegenge et al. [38] indicate that NO plays a role in the development of the human nervous system. They used a model of human Neuronal Precursor Cells (NPCs) from a well-characterized teratocarcinoma cell line (NT2). Their results from the differentiating NT2 model neurons point towards a vital role of the NO/cGMP/PKG signaling cascade as positive regulator of cell migration in the developing human brain. And Yoneyama et al. [39] also suggest that NO and endogenous ROS are essential for the proliferation of embryonic NSCs and NPCs. However, NO can also cause apoptosis of Neural Progenitor Cells (NPCs). Hung et al. [40] studied the role of p53 in the NO-induced apoptosis was examined in an *in vitro* model of NPCs. Their results suggest a central role of p53 in the NO-induced apoptotic pathway in NPCs, which may hence provide new insights into the regulation of cell death in NPCs that respond to overproduction of NO in injured brain.

Current NO Research on Various Diseases by Stem Cells

Gene Therapy Techniques for Pulmonary Hypertension

NO synthesized by eNOS is important in regulating vascular resistance and in vascular remodeling in the lung. NO deficiency due to endothelial dysfunction plays an important role in the pathogenesis of Pulmonary Hypertension (PH) which is a serious, often fatal disease characterized by remodeling of the pulmonary vascular bed, increase pulmonary arterial pressure, and right heart failure. Deng et al. [41] describe the use of two gene transfer techniques, i.e., adenoviral gene transfers of eNOS and eNOS gene-modified rat marrow stromal cells, for eNOS gene delivery to the lung of laboratory animals for the treatment of PH. Therefore, local eNOS gene delivery to the lung is a promising approach for the treatment of PH and Adenoviral-mediated *in vivo* gene therapy and adult stem cell-based *ex vivo* gene therapy are two attractive current gene therapies for the treatment of cardiovascular and pulmonary diseases.

Gene Therapy Techniques for Fibro Sarcoma

Emerging evidence suggests that MSC are able to migrate to sites of tissue injury and have immunosuppressive properties that may be useful in targeted gene therapy for sustained specific tissue engraftment. Xiang et al. [42] observed that xenogenic MSC selectively migrated to the tumor site, proliferated and expressed the exogenous gene in subcutaneous fibro sarcoma transplants and no MSC distribution was detected in other organs, such as the liver, spleen, colon and kidney. They further showed that the FGF2/FGFR pathways may play a role in the directional movement of MSC to the Rif-1 fibro sarcoma and they performed *in vitro* co-culture and *in vivo* tumor growth analysis, showing that MSC did not affect the proliferation of Rif-1 cells and fibro sarcoma growth compared with an untreated control group. Finally, they demonstrated that the xenogenic MSC stably expressing iNOS protein transferred by a lentivirus-based system had a significant inhibitory effect on the growth of Rif-1 tumors compared with MSC alone and the non-treatment control group. Therefore, iNOS delivered by genetically modified iNOS-MSC showed a significant anti-tumor effect both *in vitro* and *in vivo*. MSC may be used as a target gene delivery vehicle for the treatment of fibro sarcoma and other tumors.

Perivascular NO Involved in Stem-Like Character in PDGF-Induced Glioma Cells

eNOS expression is elevated in human glioblastomas and correlated with increased tumor growth and aggressive character. Charles et al. [43] investigated the potential role of NO activity in the Perivascular Niche (PVN) using a genetic engineered mouse model of PDGF-induced gliomas. eNOS expression is highly elevated in tumor vascular endothelium adjacent to perivascular glioma cells expressing Nestin, Notch, and the NO receptor, sGC. In addition, the NO/cGMP/PKG pathway drives Notch signaling in PDGF-induced gliomas *in vitro*, and induces the side population phenotype in primary glioma cell cultures. NO also increases neurosphere forming capacity of PDGF-driven glioma primary cultures, and enhances their tumorigenic capacity *in vivo*. Loss of NO activity in these tumors suppresses Notch signaling *in vivo* and prolongs survival of mice. This mechanism is conserved in human PDGFR amplified gliomas. The NO/cGMP/PKG pathway's promotion of stem cell-like character in the tumor PVN may identify therapeutic targets for this subset of gliomas.

NO Involved in The Therapy for Cardiovascular Disease

Recent studies have reported a marked impairment in the number and functions of EPCs in patients with Coronary Artery Disease (CAD). LiN [44] found that eNOS in the host myocardium

promotes MSC migration to the ischemic myocardium and improves cardiac function through cGMP-dependent increases in cell-derived factor-1alpha (SDF-1alpha) expression. Furthermore, Kaur et al. [45] conclude that eNOS gene transfection is a valuable approach to augment angiogenic properties of *ex vivo* expanded EPCs and eNOS-modified EPCs may offer significant advantages than EPCs alone in terms of their clinical use in patients with myocardial ischemia. Moreover, Spallotta et al. [46] found that NO-treated ES injected into the cardiac left ventricle selectively localized in the ischemic hind limb and contributed to the regeneration of muscular and vascular structures. These findings establish a key role for NO in therapy of cardiovascular diseases.

NO Involved in The Immunosuppression

MSCs hold great promise for treating immune disorders because of their immunoregulatory capacity and the mechanism of MSC-mediated immunosuppression varies among different species. Immunosuppression by human- or monkey-derived MSCs is mediated by Indole amine 2,3-Dioxygenase (IDO), whereas mouse MSCs utilize NO, under the same culture conditions. When the expression of IDO and iNOS were examined in human and mouse MSCs after stimulation with their respective inflammatory cytokines, Ren et al. [47] found that human MSCs expressed extremely high levels of IDO, and very low levels of iNOS, whereas mouse MSCs expressed abundant iNOS and very little IDO. However, immunosuppression by human MSCs was not intrinsic, but was induced by inflammatory cytokines and was chemokine-dependent, as it is in mouse. Further studies have reported that NSCs may exert direct anti-inflammatory activity. This action has been attributed, in part, to T-cell suppression. Wang et al. [48] indicate that NSCs appear to suppress T-cells, at least in part, by NO and Prostaglandin E2 (PGE2) production which, in turn, would account for the well-documented reduction of central nervous system immunopathology by transplanted NSCs. These findings provide critical information about the immunosuppression of MSCs and for better application of MSCs in treating immune disorders.

Conclusions

In summary, the downstream mediators of NO and NO itself are likely to exert the function of modulation in the process of EPCs differentiation. Although scarcely can NO influence the undifferentiated ESCs, it can be dramatically involved in the early differentiation events or physiological processes of ES cells or ES cell-derived lineages. Nevertheless, overproduction of NO may induce the apoptosis of NPCs. Therefore, NO plays an important role in physiological and pathological processes of stem cells and we can utilize these characters in the treatment of various diseases by various methods like gene transfer techniques, stem cell transplantation et al.

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