병태생리학적 조건 하에서 배아줄기세포의 기능조절

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Functional Regulation of Embryonic Stem Cells under Pathophysiological Conditions

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〈요 약〉

These studies investigated how well-known cell regulatory factors and pathophysiological conditions regulate ES cell functions. We examined effects of dopamine and ATP on DNA synthesis and cell prolifeation of mouse embryonic stem (ES) cells were examined. Dopamine inhibited DNA synthesis in both a dose- and time-dependent manner. Moreover, the inhibitory effect of dopamine on DNA synthesis resulted from the increases of cyclic adenosine 3, 5-monophosphate (cAMP), protein kinase C (PKC), $[Ca^{2+}]_i$, p44/42 mitogen activated protein kinases (MAPKs), p38 MAPK, stress-activated protein kinase/Jun-N-terminal kinase (SAPK/JNK) phosphorylation, and NF-κB via D1 and D2 receptor. On the other hand, ATP stimulates mouse ES cell proliferation, which was confirmed by the increases of DNA synthesis and cell cycle regulatory proteins, through PKC, PI3K/Akt, and MAPKs via the P2 purinoceptors. In next steps, we observed whether physiological and pathophysiological states such as hypoxia and high glucose influence the stem cell functions. Hypoxia increased the 2-DG uptake (GLUT-1 protein expression level) and DNA synthesis while the undifferentiated state of ES cells and cell viability were not affected by the hypoxic condition (1-48 hours). Thus, we suggest there might be a parallel relationship between the expression of GLUT1 and DNA synthesis, which is mediated by the Ca^{2+}/PKC, MAPK, and the HIF-1 signal pathways in mouse ES cells. High glucose level also showed to have growth promoting effect on mouse embryonic stem cells. In this study, DNA synthesis and cell cycle regulatory protein levels were increased via the PI3-K/Akt and MAPKs pathways. In conclusion, these studies suggest how physiological and pathophsyiological conditions regulate ES cell functions and what kinds of signal molecules are involved in these processing.

Embryonic stem cells are defined as cells that have capacity to self-renewal and to generate multiple differentiated cell types[1, 2]. Stem cells appear to have the capacity to sense various growth factors and external pathophysiological conditions and to express many of the downstream signaling components involved in the transduction of these signals. Thus, an investigation of factors and molecular networks needed for the functions of ES cells is warranted. Here,

we found out exogenous factors (growth factors and pathophysiological conditions) participating in the regulation of ES cell functions and analyzed the underlying mechanisms for the regulatory activities in mouse ES cells.

Effect of neurotransmitters on self-renewal of ES cells

Neurotransmitters such as dopamine and ATP are potent signaling molecules that play important biological roles in different cell types. Monoamines, such as serotonin or dopamine, appear in the embryo before cell differentiation, and may have functions other than neurotransmission during embryogenesis such as differentiation and growth[3, 4]. At the cellular level, the actions of dopamine are mediated via the activation of specific receptors, which are classified into D1-like and D2-like receptor subtypes[5]. In particular, activation of the dopamine D1-like receptor reduces G1- to S-phase entry[6], whereas the activation of the D2-like receptor promotes G1- to S-phase entry[7]. These effects usually involve the activation (phosphorylation) of a class of intracellular proteins known as mitogen-activated protein kinases (MAPKs). In our results, dopamine inhibited DNA synthesis and the protein levels of cell cycle regulatory proteins. Moreover, both D1-like and D2-like receptors were expressed in mouse ES cells. D1-like receptor stimulated Gs protein coupled signal molecules such as adenylate cyclase, cAMP, and protein kinase A (PKA). On the other hand, D2-like receptor activated Gq protein-induced signal pathways such as PLC/PKC/Ca^{2+}. Subsequently, these signals induced MAPKs and NF-kB activation. Finally, all of dopamine-activated signal molecules inhibited DNA synthesis by regulating the cyclin dependent kinases (inhibitors) via cAMP, Ca^{2+}, PLC/PKC, MAPKs, and NF-κB pathway in mouse ES cells (Fig. 1)[8]. Extracellular ATP is also an important signaling molecule in many embryonic cell types that increases the intracellular Ca^{2+} concentration, which can regulate cell proliferation, migration, and differentiation[9, 10].

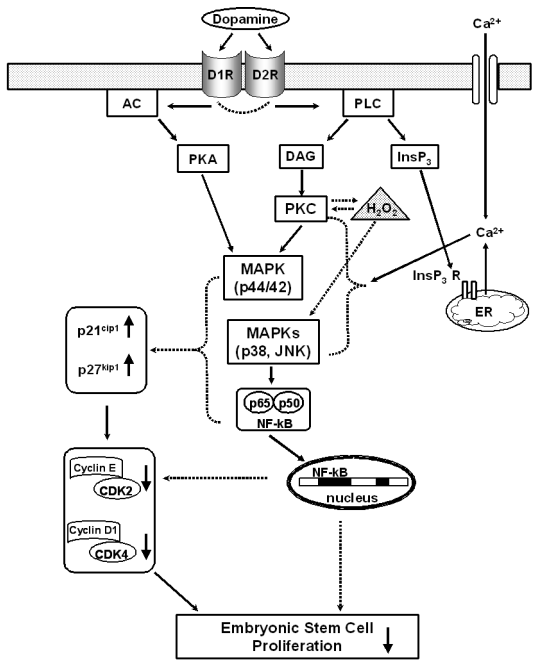

Fig. 1. *The hypothesized model for the signal pathways involved in dopamine-induced inhibition of ES cell proliferation. Dopamine activates D1 and D2 receptor, which stimulate either adenylyl cyclase to induce PKA activation or PLC to generate IP_3 and DAG. In turn, DAG activates PKC, which induces p44/42 MAPK activation, continuously inducing inhibition of cell cycle progression. PKC also increases H_2O_2 formation, subsequently leading to the activation of p38 MAPK and JNK, which stimulates NF-kB activation. In another pathway, IP_3 stimulates the release of Ca^{2+} from an intracellular Ca^{2+} pool and to sustain the spiking activity, Ca^{2+} influx from an extracellular medium is required. In turn, Ca^{2+} activates PKC and MAPKs leading to the inhibition of cell cycle progression. D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; AC, adenylyl cyclase; PKA, protein kinase A; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; NF-kB, nuclear factor-kappa binding; $InsP_3$, 1,4,5-inositol-triphosphate; $InsP_3R$, $InsP_3$ receptor; ER, endoplasmic reticulum; CDK, cyclin-dependent kinase. The solid line is the proposed pathway and the dashed line is suspected pathway.*

Therefore, extracellular ATP has the potential to regulate many important processes in embryonic development. These effects are mediated by nucleotide receptors known as P2 plasma membrane receptors (P2Rs), which are grouped into two main subfamilies (P2YRs and P2XRs) according to their molecular structure[11, 12]. Moreover, it was reported that the purinergic ATP receptors are expressed in the early stages of embryonic development, which indicates that these receptors play a role in embryogenesis[13, 14]. In our study, the treatment of ATP increased DNA synthesis and cell cycle regulatory proteins on contrary of dopamine effects. Moreover, RT-PCR analysis revealed $P2X_3$, $P2X_4$, $P2Y_1$, and $P2Y_2$ expression in mouse ES cells. Subsequently, ATP increased the level of intracellular cAMP, inositol phosphates, and translocation of PKC α, β, and from the cytosol to the membrane compartment. ATP and its agonists also increased $[Ca^{2+}]_i$ and activated PI3-K/Akt as well as p44/42 MAPKs. Finally, ATP stimulates mouse ES cell proliferation through PKC, PI3K/Akt, and MAPKs via the P2 purinoceptors (Fig. 2)[15].

Effect of pathopysiological conditions on ES cell functions

Preimplantation embryos develop in vivo under conditions of low oxygen. Uterine oxygen concentration decreases to around 3-5% at the time of implantation in the hamster and rabbit[16]. Consistent with this, lowering the oxygen concentration in the gaseous phase during embryo culture, from atmospheric levels to more physiological levels, has been associated with improved embryo development, in terms of blastocyst development rate and embryo cell number, in a number of species[17]. The primary transcriptional regulators of both cellular and systemic hypoxic adaptation in mammals are hypoxia-inducible factors (HIF), which consists of an α- and β-subunit[18-20]. HIFs regulates the expression of at least 180 genes involved in metabolism, cell survival, erythropoiesis, and vascular remodeling by binding cis-acting hypoxia response elements located in the enhancers and/or promoters of these genes[21]. Moreover, GLUT1 gene expression and glucose transporter are also stimulated in a variety of cells under hypoxic conditions, a response that is mediated by the transcription factor HIF-1[22, 23]. In our study, hypoxic exposure increased the 2-DG uptake and GLUT-1 protein expression level while the undifferentiated state of ES cells and cell viability were not affected

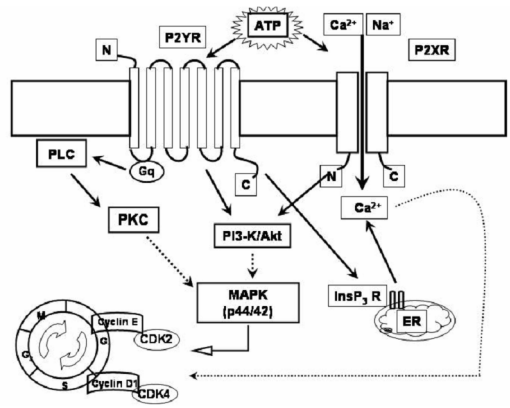

Fig. 2. *The hypothesized model for the signal pathways involved in ATP-induced increase of ES cell proliferation. ATP activates P2X and P2Y receptor, which stimulate either Ca^{2+} influx or Gq protein to activate PLC. PLC then activates PKC, which induces p44/42 MAPK activation, continuously inducing increase of cell cycle progression or generates IP3 to stimulate the release of Ca^{2+} from an intracellular Ca^{2+} pool and to sustain the spiking activity. In turn, P2X and P2Y receptor also activates PI3-K/Akt pathways, subsequently, stimulates p44/42 MAPK activation leading to the increase of cell cycle progression. P2XR, P2X purinoceptor; P2YR, P2Y purinoceptor; Gq, G-protein alpha (q) subunit; PLC, phospholipase C; IP_3, 1,4,5-inositol-triphosphate; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PI3K, phosphatidyl inositol 3-kinase; $InsP_3R$, $InsP_3$ receptor; ER, endoplasmic reticulum; CDK, cyclin-dependent kinase. The solid line is the proposed pathway and the dashed line is suspected pathway.*

by the hypoxic condition (1-12 hours). Moreover, [^{3}H] thymidine incorporation was significantly increased at 12 hours of hypoxic exposure. Hypoxia increased the Ca^{2+} uptake and PKC β_I, ε, and ζ translocation from the cytosol to the membrane fraction. Moreover, hypoxia increased the level of p44/42 MAPKs phosphorylation and hypoxia inducible factor-1 (HIF-1α) in a time-dependent manner. Finally, under hypoxic exposure, there might be a parallel relationship between the expression of GLUT1 and DNA synthesis, which is mediated by the Ca^{2+}/PKC, MAPK, and the HIF-1 signal pathways in mouse ES cells (Fig. 3).

Several studies have reported a correlation between the development of preimplantation diabetic embryopathy and hyperglycemia, and its subsequent biochemical events[24, 25] but there is some controversy as to its precise mechanism. Glucose is the major source of energy for most mammalian cells and is particularly important during fetal development when the cells are rapidly dividing and differentiating. Recently, a high glucose concentration itself has been reported to have diverse effects on gene expression, insulin secretion, neurotransmitter release, and apoptosis[26-29]. Excessive glucose levels can also be transported intracellularly and be metabolized to change the redox potential, increase the level of sorbitol production via aldose reductase, or to alter the signal transduction pathways, such as PI3-K, Akt, and MAPKs[30], which are involved in cell proliferation and differentiation. In our study, high glucose level significantly increased [^{3}H] thymidine incorporation, BrdU incorporation, the number of cells, [^{3}H]leucine, and [^{3}H]proline incorporation in a time-(>3 hr) and dose-(>25 mM) dependent manner. Moreover, high glucose level increased the cellular reactive oxygen species (ROS), Akt, and p44/42 MAPKs phosphorylation. Finally, high glucose level stimulates mouse ES

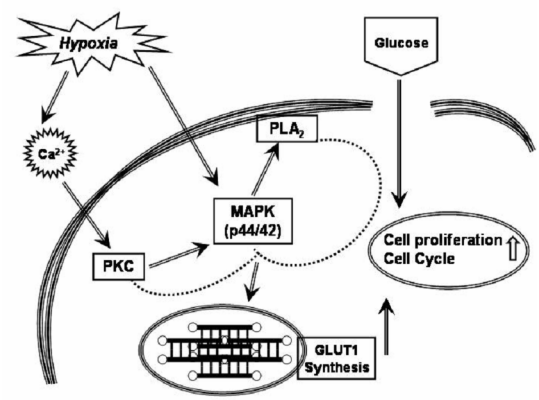

Fig. 3. *The hypothesized model for the signal pathways involved in hypoxia-induced ES cell functions. Hypoxia activates Ca^{2+} influx inducing PKC and p44/42 MAPKs activation. Subsequently, p44/42 MAPKs activates $cPLA_2$, continuously inducing increase of GLUT1 synthesis and 2-DG uptake. In turn, increased glucose levels stimulates ES cell proliferation. PKC, protein kinase C; MAPK, mitogen-activated protein kinase; $cPLA_2$, cytosolic phospholipase A_2. The solid line is the proposed pathway and the dashed line is suspected pathway.*

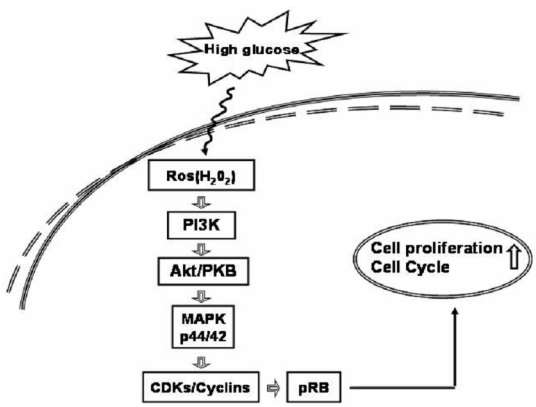

Fig. 4. *The hypothesized model for the signal pathways involved in high glucose-induced ES cell proliferation. High glucose level increases ROS generation and then PI3K/Akt activation, continuously inducing p44/42 MAPKs activation. Subsequently, p44/42 MAPKs activates increases the levels of cell cycle regulatory proteins and cell cycle progression. ROS, reactive oxygen species; PI3K, phosphatidyl inositol 3-kinase; PKB, protein kinase B; MAPK, mitogen-activated protein kinase; CDK, cyclin-dependent kinase; pRB, phosphorylation of retinoblastoma protein.*

cell proliferation via the PI_3-kinase/ Akt and p44/42 MAPKs pathways (Fig. 4)[31].

All these researches on stem cells can provide new insights into stem cell development and new keys as to how to maintain and regulate stem cell functions, as well as translational support into therapeutic outcomes.

Acknowledgements

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