REVIEW ARTICLE

Regulation between nitric oxide and MAPK signal transduction in mammals*

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Abstract Nitric oxide (NO) is an important biological messenger in the regulation of tissue homeostasis. It exhibits a wide range of effects during physiological and pathophysiological processes. Typical beneficial properties of NO include the regulation of vascular tone, the protection of cells against apoptosis, the modulation of immune responses, and the killing of microbial pathogens. On the other hand, NO may cause severe vasodilation and myocardial depression during bacterial sepsis or act as a cytotoxic and tissue-damaging molecule in autoimmune diseases. Mitogen-activated protein kinase (MAPK) is a family of serine/threonine protein kinases that are widely distributed in mammalian cells. MAPK cascade plays pivotal roles in gene expression, cell proliferation, differentiation, neuronal survival and programmed cell death under a variety of experimental conditions. MAPKs transduce the signal for the cellular response to extracellular stresses or stimuli. The relation between them, however, has never been reviewed. Based on our researches and other reports in the field, we review their reciprocal regulatory functions.

Keywords: MAPK, nitric oxide, nitric oxide synthase, signal transduction.

1 Nitric oxide

Nitric oxide (NO) is a short-lived and highly reactive gaseous free radical and widely exists in mammalian organs and tissues. It often exists in intracellular and extracellular fluids as a binding form and functions as both the first messenger and the second messenger. NO has been recognized as an important physiological mediator and is involved in numerous biological actions in the vascular, central nervous, cardiovascular, respiratory, endocrine and immune system[1]. NO also regulates various reproductive processes, such as sexual behavior, steriodogenesis, folliculogenesis and follicle survival, ovulation and atresia, fertilization, implantation, embryo development and pregnancy[2—4]. We also find that NO plays an important role in mouse[5,6] and porcine oocyte meiotic resumption[7]. In male, NO is involved in spermatogenesis, testosterone secretion and testis vasodilation. In addition, NO also serves as a key signal molecule in pathological process, like toxication, cerebral ischemia injury, endocrine disorder, platelet aggregation, inflammation, programmed cell death (apoptosis) and tumorogenesis[8]. Recently, it has been found that the action of NO has dualism in various cell types[9,10].

NO is produced when NADPH-dependent NO synthase (NOS) catalyzes L-arginine (L-Arg) and other cofactors to L-citrullin, so NOS controls the generation of NO. Three isoforms of NOS have been isolated, including neuronal (NOS1/bNOS/nNOS), endothelial (NOS2/eNOS), and macrophage NOS (NOS3). nNOS consists of 1430—1434 amino acid residues, and its molecular weight is 160—161 kD. iNOS has 1140—1150 amino acid residues and its molecular weight is around 130 kD. eNOS contains 1153—1205 amino acid residues and its molecular weight is about 130 kD. nNOS and eNOS are Ca^{2+}/calmodulin-dependent and they are also called constitutive NOS (cNOS) while macrophage NOS is Ca^{2+}/calmodulin-independent and also called inducible NOS (iNOS). Both cNOS synthesize small amounts of NO while iNOS expression results in a sustained synthesis of over long periods and produces 100—1000-fold larger amounts of NO. NOS is localized in the variety

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of organs and tissues, but the expression pattern differs much at local organs or tissues. For instance, nNOS is localized in brain and periphery neurons but not in mammalian ovaries. Even in a certain specific place, certain NOS is expressed differently at existence and expression amount, depending on physiological status or developmental stages[11—13]. Physiologically, iNOS is not expressed or only weakly expressed, and it is produced only in response to a stimulus like bacterial lipopolysaccharide (LPS), cytokines (interferon gamma, tumor necrosis factor alpha, interleukin-1 beta) and hormones[14]. Strong expression of iNOS is found in inflammatory and tumor tissues.

Intracellular NO signaling pathway is quite complex and flexible. NO exerts functions by activating cyclooxygenase enzyme (COX) or protein kinase C (PKC). It can also play a role through P53/Bax pathway. But NOS/NO/cGMP/PKG pathway has been believed to be most important in a variety of cell types. NOS oxidizes L-Arg to L-citrullin and NO, and NO then binds soluble guanylate cyclase (sGC), which catalyzes transmit GTP to cGMP. cGMP then activates cGMP-dependent protein kinase (PKG), which exerts many specific biological actions. In many types of GC, only sGC is the target of NO, and NO recognizes and binds Fe^{2+} of haemachrome coenzyme in sGC, so the conformation of sGC is changed. This change enables sGC to catalyze GTP to cGMP by cyclization. By now, this pathway has been proved in neurons[15], vascular endothelial cells[16], hepatocytes[17], ovarian granulosa cells of rat[18] and swine[19], mouse implantation embryos[8], and etc. Our previous studies found that NO can also function through NO/cAMP[20]. The biological functions of NO will lose when it binds superoxide ions, haemoglobin, and other proteins containing haemachrome.

2 Mitogen-activated protein kinase

Mitogen-activated protein kinase (MAPK) exists in cytoplasm and nucleolus of various cell types and so does its substrates. The best known physiological substrates of MAPK are the p90 ribosomal S6 kinase (p90rsk) encoded by *rsk* gene. In unstimulated cells, MAPK mainly distributes in the cytoplasm and a little in nucleolus. In response to stimulus, MAPK quickly migrates to nucleulus, resulting in the change of certain genes expression. MAPK monomer is about 40 to 50 kD, so in theory, it can pass through the nuclear pore by free diffusion. However, this ability is limited and active nulear translocation controls final distribution of MAPK[21]. In mammalian cells, the phosphorylation of extracellular signal-regulated protein kinase 2 (ERK2), a type of MAPK, can induce itself to form dimer. But ERK2 which is phosphorylated but not demerized cannot enter nucleulus, indicating that ERK2 phosphorylation and dimerization are necessary for its translocation from cytoplasm to nucleulus[22].

MAPK cascade is one of the most important signaling systems in mammal (also in plant), and it regulates many pathophysiological processes, such as cell proliferation, growth, development, differentiation, apoptosis, and inflammation. For example, MAPK plays a pivotal role in mammalian oocyte meiotic maturation[23—32]. We also found that the activation of MAPK is essential for the transition from metaphase I (MI) to metaphase II (MII), pronucleus formation after fertilization, and first meiotic resumption of porcine oocyte[25, 33]. In male, MAPK regulates spermatogenesis. In mouse testis, the expression of both p38 MAPK mRNA and its protein changes at different age, suggesting the regulation of MAPK on spermatogonium proliferation and differentiation. Disruption of MAPK signal transduction influences the physiological functions of various systems, organs and tissues seriously and results in tumorogenesis, myocardial hypertrophy, Parkinson's disease and other diseases.

In MAPK family, the name is a bit confusing. They are sometimes named after their own substrates, like microtuble-associated protein 2 kinase (MAP2K). Some have their names according to extracellular signal types, like extracellular-signal regulated kinases (ERKs). Some others are described as their molecular weight with a superscript of gene name, like $p42^{mapk}$ and $p44^{ERK1}$.

MAPK consists of 5 subfamilies altogether, namely ERK1/2 which is also termed p42/p44 MAPK, ERK3/4, ERK5 which is also called big MAPK kinase 1 (BMK1), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and the p38 MAPK[34,35]. Every subfamily represents a signal transduction pathway. JNK and p38 MAPK pathways can be activated by ultraviolet radiation, osmotic change, cytokines, inflammatory stimuli, and other stimulation, so they are also called stress-regulated MAPK pathways. ERK pathways are the proto-

type and extensively investigated in mammals. ERK is mainly involved in cytokine- or hormone-mediated signal transduction. Recently, MAPK structure has been identified basically, including primary, secondary, supersecondary and advanced structures. The difference of MAPK structure tells that different ERKs belong to different MAPK subfamilies, and that ERK1/2 is more different from ERK3/4 than from other subfamilies. In all ERKs, ERK1/2 exist most widely and therefore are studied more intensively.

3 Relationship between NOS/NO/cGMP pathway and MAPK signal transduction

NO and MAPK are simultaneously involved in many physiological and pathophysiological processes, such as inflammation, tumorogenesis and apoptotic cell death in various mammalian cells. Therefore, some questions arise. Is there any relationship between them? If so, what is the specific relation? In recent years, many researchers have begun to investigate this issue since Ding et al.'s first report in 1994.

3.1 Change of NO level and MAPK activity in a pathophysiological process

The recognition to the possible relationship between NO/cGMP pathway and MAPK signal transduction began with the fact that both NO production and MAPK activity are changed in a pathophysiological process. Ding et al. investigated the age-associated change of immune response in mice. They found that IFNγ-induced release of NO in macrophages was 50% lower in old mice than in young mice, and that IFNγ-induced tyrosine phosphorylation of MAPK also declined. Consequently, aged mice were not as capable of killing intracellular microorganisms and lysis of tumor cells as young ones[37]. Such work first implied the possible link between them.

Zhang et al. found that rat spinal cord injury caused the increased iNOS expression by reverse transcription PCR, p38 MAPK activity by Western blotting, and neuronal apoptosis by flow cytometry. Their results suggested a certain link between NOS expression and MAPK activity. Based on these, they injected antisense oligodeoxynucleotides of iNOS into the subarachnoid space of spinal cord injured rat, and found that iNOS mRNA expression decreased, p38 MAPK expression reduced, and the neuronal apoptosis was alleviated. This implied that iNOS antisense oligodeoxynucleotides could inhibit iNOS expression and neuronal apoptosis following spinal cord injury might be related to p38 MAPK signal transduction pathway. Most recently, Mishra et al. reported that hypoxia induced the activation of ERK and JNK in cerebral cortical nuclei of new born piglet, which was mediated by NO[38].

Regulatory relation between NO and MAPK signaling interested more researchers and more and more related reports have appeared since 1996 with the evidence accumulation.

3.2 Regulation of NO on MAPK signal transduction

Lander et al. found that exdogenous NO, generated from NOS, activated ERK, and this effect could be blocked by the farnesyl transferase inhibitors. Critical signaling kinases, such as ERK, p38 MAPK, and JNK, were activated by NO-related species and thus participated in NO signal transduction. JNK was 100-fold more sensitive to the species than p38 MAPK and JNK and the activation of JNK and p38 MAPK was more rapid than ERK activation. NO related chemical species activated ERK, p38 MAPK, and JNK in human Jurkat T cells[39]. These results indicated the regulatory effect of NO on MAPK signaling. Ingram et al. also reported the relation of them in rat mesangial cells. They found that, however, NO inhibited MAPK activation by cGMP[40].

From then on, more studies have been undertaken to investigate the regulatory function of NO pathway on MAPK signal transduction in other cells. MAPK plays a critical role in cardiac myocytes hypertrophy mediated by many factors[41, 42], and NO inhibited the process obviously[43]. Lu et al. examined the crosstalk between MAPK and NO in myocardial hypertrophy of male rat using an established Goldblatt renovascular hypertensive model. They found that L-Arg, an NO precursor, significantly attenuated the activity of MAPK, increased protein expression of eNOS and MAPK phosphatase-1 (MKP-1) and potentiated production of NO in the cardiac tissue, and these effects could be inhibited by L-NAME, an NOS inhibitor. These results suggested that MKP-1 plays an important role in the NO-induced inhibition of myocardial hypertrophy in the cardiac tissue[44]. Rakhit et al. also studied the MAPK signaling in NO-induced cardioprotection against simulated ischemia-reoxygenation injury in rat cardiac myocytes. Protein kinase C (PKC)-mediated regulation of MAPK may

play a role in the protection afforded by ischemic preconditioning while NO can influence MAPK activation via interaction with PKC or farnesylation of low-molecular-weight G proteins. The mechanism of NO-induced cardioprotection was a PKC-independent process. They found that neonatal rat cardiomyocytes treated for 90 min with SNAP, an NO donor, were protected from the damage caused by 6 h of simulated ischemia and 24 h reoxygenation under normal culture conditions. NO-induced protection was blocked by the G protein inhibitor. They studied the time course of p42/44 and p38 MAPK dual-phosphorylation hourly during simulated ischemia using phosphospecific antibodies. p38 MAPK was phosphorylated during simulated ischemia and the peak phosphorylation was significantly delayed by SNAP pretreatment. The p38 inhibitor gave the protection against the injury. Thus the delay in peak p38 activation may contribute to, rather than be the effect of, NO-induced cardioprotection. The main isoform present in these cells and thought to be responsible for the observed phenomenon is the alpha isoform of MAPK, not beta[45].

As noted above, both NO and MAPK signal pathways are closely related to immune system functions, and it is well known that the heavy metals like mercury can damage mammalian immunity seriously. Therefore, Kim et al. investigated the correlation in mercury-induced immunity reduction. They found that mercury suppressed NO synthesis by inhibition of the NF-kappa B pathway and modulated cytokine expression by p38 MAPK activation in macrophage cells[46].

In central nervous system, oxidative and nitrosative stress is increasingly associated with the pathology of neurodegeneration and aging. The molecular mechanisms underlying oxidative/nitrosative stress-induced neuronal damage are emerging and appear to involve a mode of death in which MAPK signaling pathways are strongly implicated. Thus, attention is turning towards the modulation of intracellular signaling as a therapeutic approach against neurodegeneration. Both endogenous and dietary agents have been suggested as potent modulators of intracellular signal transduction, e.g. NO[47]. Fiebich et al. found that parthenolide, an iNOS synthesis and NO generation inhibitor, suppressed p42/44 MAPK activity in rat primary microglial cells, which indicated that it could be used as a drug for central nervous system diseases, like multiple sclerosis and hemicrania[48].

Large amouts of NO produced by microglial cells in brain can cause many pathological changes of central nervous system, such as neurodegeneration and multiple sclerosis. Tranilast (TNL), an anti-allergic compound, suppresses the activation of monocytes. LPS is from gram positive bacteria, and induces the activation of MAPK, such as ERK1/2. LPS also induces iNOS mRNA expression and NO production[49], which were inhibited by TNL. TNL did not modulate LPS-stimulated nuclear factor-kappa B reporter gene activity and phosphorylation of inhibitory kappaB (IkappaB), indicating that NF-kappaB is not involved in the TNL-mediated suppression of LPS-induced iNOS expression. TNL also inhibited LPS-induced phosphorylation of ERK2. TNL abolished translocation of PKC delta to the nucleus and suppressed the phosphorylation of the PKC delta substrate. TNL suppressed microglial iNOS induction by LPS via inhibition of a signalling pathway involving PKC delta and ERK2[50].

The regulatory effects of NO on MAPK signal transduction have also been proven on other aspects. For instance, NO can induce hepatic preconditioning by activating p38 MAPK through a guanylate cyclase/PKG-mediated pathway in rat hepatocytes[51]. NO-cGMP-PKG pathway plays an important role in the activation of ERK1/2 declustering in rat cerebellar Purkinje cells[52]. Cytokine-stimulated iNOS expression in human kidney epithelial cells involves activation of p38 MAPK.

3.3 MAPK regulates nitric oxide pathway

As described above, many reports demonstrate that NO regulates MAPK signal transduction pathway. The other evidence shows that MAPK influences NOS expression and NO production. The first report was found in 1996. Both adult rat ventricular myocytes and cardiac microvascular endothelial were found to express iNOS following exposure to soluble inflammatory mediators. However, iNOS gene expression was regulated differently in response to specific cytokines in each cell type. Singh et al. examined the specific signal transduction pathways that could regulate iNOS mRNA levels, including activation of ERK1/2. Although IL-1 beta treatment increased ERK1/ERK2 activities in both cell types, IFNγ activated these MAPKs only in myocytes. The farnesyl transferase inhibitor blocked activation of

ERK1/ERK2 and induction of iNOS by IFNγ and IL-1 beta in myocytes. IL-1 beta and IFNγ-induced iNOS gene expression in myocytes was also down-regulated by both PKC desensitization and IFNγ in cardiac muscle cells. The MAPK kinase inhibitor PD98059 blocked activation of ERK1/ERK2 and down-regulated IL-1 beta-mediated iNOS induction, whereas activation of ERK2 in the absence of cytokines by okadaic acid, an inhibitor of phosphoserine protein phosphatases, also induced iNOS mRNA. ERK1/ERK2 activation appeared to be necessary for the induction of iNOS by IL-1 beta and IFNγ in cardiac myocytes and cardiac microvascular endothelial cells. These overlapping yet distinct cellular responses to specific cytokines may serve to target iNOS gene expression to specific cells or regions within the heart and also provide for rapid escalation of NO production if required for host defense[53].

Thereafter, there are increasing reports studying the regulation of MAPK signaling on NOS expression. The results, however, are not well consistent. Some reporters found p38 MAPK up-regulated LPS-induced iNOS expression in astrocyte and macrophages. SB 203580, a specific inhibitor of p38 MAPK, inhibted iNOS expression[54—56]. But other researchers found that SB 203580 did not affect iNOS expression induced by LPS in mouse macrophages[57, 58].

Xu and Malave investigated the role of MAPK in iNOS expression by using the specific MAPK inhibitors. First the induction of NO by LPS, TNF alpha, IFNγ, alone or their combination, was studied in C6 glioma cells. Administration of LPS, TNF alpha or IFNγ alone had no detectable stimulatory effect on the production of nitric oxide (NO). However, combination of the three factors elicited a significant elevation of NO level in C6 cell culture medium. Subsequently pretreatment of C6 cells with a specific inhibitor of p38 MAPK, SB202190, resulted in a dose-dependent inhibition of NO production and iNOS expression, but PD98059, an inhibitor of p42/p44 MAPK activation, had no effect. These results suggested that p38 MAPK mediated iNOS expression in C6 glioma cells, but p42/p44 MAPK was not involved in this process[59]. Cartwright et al. found that inhibition of p42/44 MAPK reduced iNOS expression in human trophoblast.

Su et al. investigated the communication between LPS-induced MAPK activation and NO signal pathway in mouse peritoneum suppressive macrophages (MPSM). They found that iNOS mRNA and iNOS protein expression decreased and NO production reduced when ERK1/2 and p38 MAPK activities were inhibited, indicating that NO-mediated macrophage immunity was regulated by p38 MAPK and ERK1/2. LPS is involved in gene expression regulation of many cytokines such as TNF alpha and IL by activating tyrosine kinase, ERK1/2 and p38 MAPK, which then stimulates transcriptional factors. ERK1/2 and p38 MAPK were quickly phosphorylated in MPSM induced by LPS, which was inhibited by SB 203580 and PD 98059, specific inhibitors for p38 MAPK and ERK1/2 respectively. Both inhibitors suppressed iNOS expression and then reduced NO production by blocking iNOS mRNA expression (LPS-induced NO production of macrophage is iNOS-dependent). After being induced by LPS, MPSM becomes modulated cells which do not inhibit cell immune response, increase T and B lymphocytes and NK cells activity, and, meanwhile, maintain or even increase anti-tumor activity. Such transition is called immunomodulation of macrophage. They found that the immunomodulation was linked to MAPK signal transduction pathway[61]. They also found that LPS-induced modulated macrophages produced much more NO. Two inhibitors suppressed NO production alone or concomitantly. The evidence implied that NO production of immunomodulated macrophages was mediated by p38 MAPK and ERK1/2 pathways[61].

Kan et al. then examined the role of p38 MAPK in LPS-induced expression of iNOS and NO production in human umbilical artery endothelial cells. They found an obvious enhancement of p38 MAPK activity in endothelial cells in response to LPS stimulation. SB 203580 inhibited iNOS mRNA and protein expression. These implied that p38 MAPK played an important role in iNOS expression and NO production, and inhibition of the signal transduction pathway was an effective approach to reducing the production of iNOS and other cytokines for the treatment of septic shock.

Almost at the same time, they investigated the role of p38 MAPK in iNOS expression of mouse lung tissues induced by LPS for exploring its function in gene regulation by an endotoxic shock model. They found that normal lung tissues only had a low level expression of iNOS, and that LPS treatment increased NO level in plasma, and iNOS mRNA and

protein expression were time-dependent and dose-dependent. LPS stimulation also enhanced p38 MAPK activity. SB 203580 inhibited LPS-induced NO production in plasma and the expression of iNOS protein and mRNA. The increase of iNOS expression was also found in multiple organs and the most apparent one was in the lung during endotoxic shock. These findings supported that p38 MAPK was involved in the signal transduction of iNOS expression after LPS stimulation and indicated that lung may act as an initial organ in the pathogenesis of multiple organ dysfunction syndrome.

All the studies mentioned above demonstrate that the regulatory functions between NO pathway and MAPK signal transduction might differ much, depending on the cell types, inducers, cytokines and subfamilies of MAPK.

It is clear that NO is an important biological messenger in the regulation of tissue homeostasis and pathophysiological processes in mammals, including human beings. Slomiany et al. investigated the effect of NO on gastric mucus glycoprotein (mucin) synthesis, apoptotic processes, and the involvement of ERK and p38 MAPK[64,65]. Exposure of gastric mucosal cells to NO donor led to a dose-dependent decrease in mucin synthesis, accompanied by a marked increase in caspase-3 activity and apoptosis. Inhibition of ERK with PD98059 accelerated the NO-induced decrease in mucin synthesis, and caused further enhancement in caspase-3 activity and apoptosis. Blockade of p38 kinase with SB203580 produced reversal in the NO-induced reduction in mucin synthesis, and substantially countered the induced increase in caspase-3 activity and apoptosis. Moreover, caspase-3 inhibitor not only blocked the NO-induced increase in caspase-3 activity but also produced an increase in mucin synthesis. Thus, the detrimental influence of NO on mucin synthesis is closely linked to caspase-3 activation and apoptosis, and involves ERK and p38 kinase participation. Activation of p38 kinase leads to the upregulation of proapoptotic signal, while ERK activation stimulates the anti-apoptotic pathway. LPS of porphyromonas gingivalis, a Gram-negative periodontapathic bacterium, also upregulated iNOS expression as a key detrimental culprit affecting salivary mucin synthesis[64,65].

Guo et al. studied the transcriptional regulation of iNOS gene by p38 MAPK in human embryonic kidney 293 cells and found that LPS-induced transcription and activation of iNOS gene p38 MAPK was involved in the transcription regulation of iNOS gene[66]. It is well known that growth hormone and prolactin secreting GH3 cells express nNOS and produce NO. Secondo et al. found that prolactin receptor activation up-regulated the expression of both nNOS alpha and nNOS beta proteins via a protein tyrosine kinase and MAPK signaling transduction components. This action may represent the molecular mechanism by which prolactin (PRL) exerts the "short-loop" feedback on its own secretion[67]. Most recently, Chio et al. found that PKA activation in macrophages stimulated p38 MAPK, which contributes to the induction of iNOS genes[68].

In summary, NO signal system and MAPK cascade transduction pathway correlate closely with each other in the variety of mammalian cells. The regulation between them is complicated and far from clear. It is not sensible, at least now, to understand the relation in a single way that NO mediates MAPK pathway, and vice versa. Virtually, their mediatory actions are reciprocal in many physiological and pathophysiological processes. Taking other signaling bypasses into account, colossal but intact regulatory net-

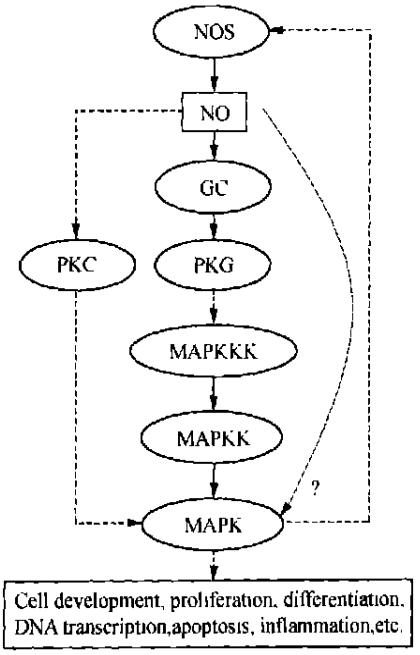

Fig. 1. Regulatory sketch map for nitric oxide and MAP kinase signaling pathway. Solid arrows denote the known and direct regulation; dashed arrows represent indirect regulation; question mark means unknown actions. NO, nitric oxide; NOS, nitric oxide synthase; GC, guanylate cyclase; PKG, cGMP-dependent protein kinase; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; PKC, protein kinase C.

work finally forms to meet the various demands to respond to numerous stimuli under different circumstances. Fig. 1 illustrates the regulatory relation between NO and MAPK signaling pathway.

4 Perspectives

Although many reports have so far tried to elucidate the relationship between NO and MAPK signal pathway, many problems still remain unresolved, even with the studies themselves. First of all, present experimental results are not well identical. In another word, it still lacks more reasonable explanations for the differences. This needs more and further researches. Secondly, most of studies on the issue only stay at the cellular level and few investigators study it at molecular level. For instance, how does p38 MAPK regulate iNOS gene transcriptional factor? And is p38 MAPK related to the transcriptional factor binding sites? This would be the direction of further work and fortunately, more and more investigators begin to focus on the mechanisms. The elucidation of genome information of mammals including human helps a lot. Thirdly, previous studies mainly concentrate on nervous system, cardiovascular system, and immune system, but little work is on others. Take reproductive system for example, both NO system and MAPK signal transduction pathway have been extensively studied, and proven to be closely involved in reproductive activities. However, few researchers report their link in reproductive system. Finally, there is still a shortage of work to examine the reciprocal regulation in a process.

It is of the great importance to elucidate the regulatory relation between NO and MAPK signal pathway, which will not only help to understand the physiological processes on the whole, but also help to recognize the discipline of pathological events. Most importantly, such research can lead to the finding of new therapies and treatment alternatives for some difficult diseases. Some drugs might be developed for disfunction by stimuli, neurodegeneration, immune disruption, endocrine disorder, inflammation, cell death, shock, and tumor. This direction and the promising future will interest more scientists and these potential applications must benefit mammals, including, of course, human beings ourselves.

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