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REVIEW

MAPK signal pathways in the regulation of cell proliferation in mammalian cells

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ABSTRACT

MAPK families play an important role in complex cellular programs like proliferation, differentiation, development, transformation, and apoptosis. At least three MAPK families have been characterized: extracellular signal-regulated kinase (ERK), Jun kinase (JNK/SAPK) and p38 MAPK. The above effects are fulfilled by regulation of cell cycle engine and other cell proliferation related proteins. In this paper we discussed their functions and cooperation with other signal pathways in regulation of cell proliferation.

Key words: *MAPK, ERK, JNK/SPAK, p38, cell proliferation, cross-talk.*

INTRODUCTION

Mitogen-activated protein kinase (MAPK) cascades have been shown to play a key role in transduction extracellular signals to cellular responses. In mammalian cells, three MAPK families have been clearly characterized: namely classical MAPK (also known as ERK), C-Jun N-terminal kinse/ stress-activated protein kinase (JNK/SAPK) and p38 kinase. MAP kinases lie within protein kinase cascades. Each cascade consists of no fewer than three enzymes that are activated in series: a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAP kinase (MAPK). Currenly, at least 14 MAPKKKs, 7 MAPKKs, and 12 MAPKs have been identified in mammalian cells[1] (Tab 1).

MAPK pathways relay, amplify and integrate signals from a diverse range of stimuli and elicit an appropriate physiological response including cellular proliferation, differentiation, development, inflammatory responses and apoptosis in mammalian cells.

Tab 1. Compontents of MAPK pathways in mammalian cells

MAPKKK	MAPKK	MAPK
Raf-1, A-Raf, B-Raf, Mos,	MEK1(MKK1), MEK2(MKK2),	ERK1, ERK2, p38a, p38b,
TAK1, MUK, SPRK, MST,	MEK5, MKK3, MKK4, MKK6,	p38g, p38 d, JNK1, JNK2,
MEKK1, MEKK2, MEKK3,	MKK7	JNK3, ERK3, ERK4, ERK5
MEKK4, Tpl-2, ASK		

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MAPK pathway in the regulation of cell proliferation

The regulation of cell proliferation in multicellular organism is a complex process, which is primarily regulated by external growth factors provided by surrounding cells. The MAPK pathways involving a series of protein kinase cascades play a critical role in regulation of cell proliferation (Fig 1).

ERK pathway

ERK has been the best characteried MAPK and the Raf-MEK-ERK pathway represents one of the best characteried MAPK signaling pathway.

The stimulation of tyrosine kinase receptors (RTKs) provokes the activation of MAPKs in a multistep process. For example, the essential linkers from epidermal growth factor receptors to MAP kinase include adaptor protein Grb2, a guanine nucleotide exchange protein, such as Sos, a small GTP binding protein, $p21^{ras}$, a cascade of protein kinase defined sequentially as MAPKKK (represented by c-Raf-1), and MAPKK such as MEK1 and MEK2. MEKs ultimately phosphorylate p44 MAPK and p42 MAPK, also known as ERK1 and ERK2 respectively, thereby increasing their enzymatic activity[2]. Then the activated ERKs translocate to the nucleus and transactivate transcription factors, changing gene expression to promote growth, differentiation or mitosis.

G protein-coupled receptors (GPCRs) can also lead to activation of MAPKs mediated by stimulation of a large number of complex cascades. One

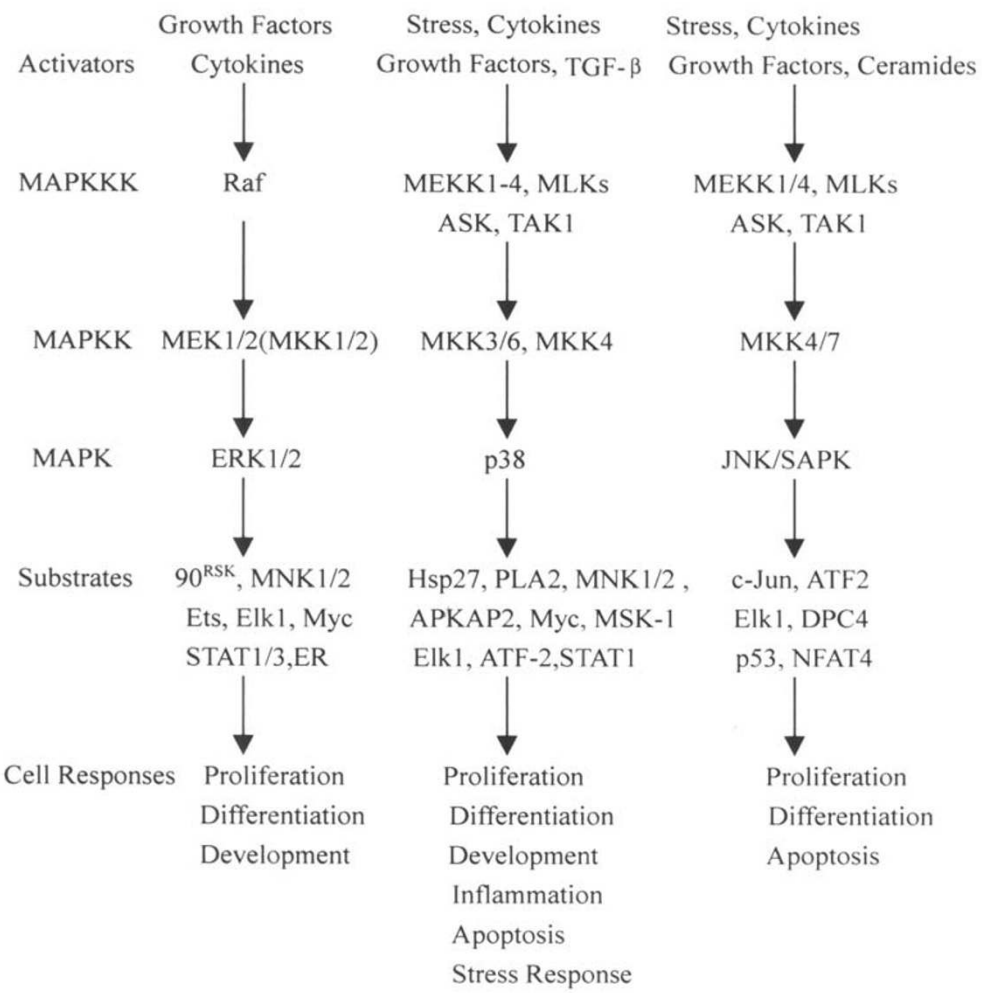

Fig 1. Major MAP kinase cascades in mammalian cells

novel mechanism is that GPCRs stimulation can lead to tyrosine phosphorylation of RTK, such as the EGFR, which ultimately results in ERK activation [3]. Instead of RTKs, the integrin-based scaffold and β-arrestin scaffold also involved in GPCRs stimulated MAPK cascades. Several cytokine receptors activate the ERK pathway through the activation of JAK (JAK1, 2, 3 and Tyk2). JAK can phosphorylate Shc leading to activation of the ERK1/2 pathway[4]. Several cytoplasmic proteins have been shown to be substrate for ERK1/2 including RSK (90KDa ribosomal S6 kinase, p90rsk, also known as MAPKAP-K1), cytosolic phospholipase A2 and several microtubule-associated proteins (MAP), including MAP-1, MAP-2, MAP-4 and Tau[5],[6]. It was suggested that ERK1/2 may involve in controlling MTOC担 function[7]. The MTOC controls the assembly of the cytosolic microtubules in interphase cells and the mitotic spindle of dividing cells. ERK1/2 can activate the C-terminal kinase of RSK, leading to activation of the N-terminal kinase. The substrates of RSK include transcription factors like CREB, ER α, IkB α/NF κB, c-Fos and glycogen synthase kinase 3 (GSK 3). So RSK can regulate gene expression via association and phosphorylation of transcriptional regulators. RSK is implicated in cell cycle regulation by inactivation of the Myt1 protein kinase leading to activation of the cyclin-dependent kinase $p34^{cdc2}$ in xenopus laevis oocytes[8]. RSK can also phosphorylates the Ras GTP/GDP-exchange factor, Sos leading to feedback inhibition of the Ras-ERK pathway.

ERK can translocate to nucleus and phosphorylate different transcription factors, including the ternary complex factor (TCF) Elk-1, serum response factor accessory protein Sap-1a, Ets1, c-Myc, Tal etc. One of Ras-induced cellular responses is transcriptional activation of multiple genes, such as the immediate early gene c-fos. So the ERK pathway can link G_0/G_1 mitogenic signals to the immediate early response.

The classical ERK family (p42/44 MAPK) is known to be an intracellular checkpoint for cellular mitogenesis. In cultured cell lines, mitogenic stimulation by growth factors correlated with stimulation of p42/44 MAP kinase. In Chinese hamster lung fibroblasts and ovary cells a biphasic activation of MAPK at G_1 was correlated with the ability to enter S phase[9]. Interfering with components of the ERK signaling pathway with dominant negative mutants or antisense constructs for raf-1 or ERK1 shows significant inhibition of cell proliferation. On the contrary, stimulating ERK1 activity results in enhanced cell proliferation[6],[10]. It was demonstrated that in PC-12 cells transient Ras/Raf signal induces cell proliferation whereas a sustained activation causes these cells to differentiate and slowly ceased the cell cycle[11]. These data demonstrated that the ERK cascade plays a pivotal role in the control of cell cycle progression.

One link between cell cycle progression and growth factor signaling is provided by Cyclin D1, whose gene is induced as a secondary response gene following mitogenic stimulation. It was reported that dominant-negative mutants of MEK inhibit proliferation of NIH-3T3 cells, and a constitutively active MEK has been shown to induce cellular transformation or proliferation[12]. Activated Ras or MEK proteins were shown to induce the expression of reporter genes driven by the *cyclinD1* promoter[13]. Terada et al demonstrated that the *cyclinD1* promoter contains two potential sites targeted by the activity of Ras/Raf function. cyclinD1 promoter's activity increased significantly when a constitutive activated form of MKK1(S222E) was expressed and inhibited by the MKK1 inhibitor PD98059[14]. The c-Jun response element might be important for the expression of the CyclinD1 protein and the Ets responsive element might be a mediator for the normal growth factor response[15]. Given the dependence of CyclinD1/Cdk4 function on Rb, the Ras function in mid to late G1 is Rb-dependent[16]. Besides regulation the expression of *cyclinD1*, Raf-MEK-ERK cascade can also regulate the posttranslational regulation of the assemly of CyclinD-Cdk4/6 complexes. The complexes then phosphorylate the Rb protein causing the activation of E2F transcription factors that regulate the transcription of genes required for G1/S transition. So Raf-MEK-ERK cascade is responsible for the regulation of the G_1/S progression.

Cell proliferation is controlled by Cdk2 which in association with CyclinE and CyclinA regulates G_1/S transition and S phase progression. Cdk2 activation is dependent on its localization in the nucleus. Blanchard et al reported that nuclear translocation of Cdk2 and the resulting G_1/S transtion of IL-2

dependent Kit 225 T cell is directly associated with the physical interaction of Cdk2 with MAPK and dependent on MAPK activity[17].

In mammalian cells the Cdks are dephosphorylated and activated by Cdc25 phosphatases. So the Cdc25s play a crucial role in the regulation of cell cycle. All three Cdc25 (Cdc25A, B, C) phosphatases exist in complexes together with the c-Raf-1 kinase. Cdc25A is directly phosphorylated and activated by the c-Raf-1 kinase. c-Raf-1 kinase is also involved in the regulation of cdc25A expression via c-Myc induction[18]. Ras/Raf signaling is involved in the induction of c-myc expression. The c-Myc protein is a DNA binding protein which is involved in transcriptional control of gene expression and has been shown to be essential for cell proliferation. Coexpression of Ras with Myc allows the generation of Cyclin E-dependent kinase activity and the induction of S phase[19]. Recent data show that high level of c-Myc protein prevent the association of p27kip1 with Cyclin E/Cdk2 complexes. The c-Myc protein drives the $p27^{kip1}$ protein out of Cdk2/CyclinE complexes, which then facilitates the phosphorylation of p27 and thereby marks the protein for ubiquitination and degradation[15]. The $p27^{kip1}$ protein is repressed by Ras/Raf signaling. The $p27^{kip1}$ can bind CyclinE-Cdk2 to form a complex and inhibit the activity of CyclinE-Cdk2, block the G_1/S transition. The $p27^{kip1}$ mRNA level does not change between arrested and proliferating cells. The rate of translation and degradation through ubiquitin dependent pathway make the differences in the protein level. The ERKs can phosphorylate the $p27^{kip1}$ protein that could be a trigger for the enforced degradation of the $p27^{kip1}$ protein by the ubiquitin-proteasome pathway. We ourselves also found that CKI p15INK4b can delay G1/S transition of human melanoma cells by inhibiting the cell cycleengine molecule and increasing the expression of $p27^{kip1}$ which correlates with reduced activity of ERK1 and ERK2. The ERKs play a central role in the control of the level of $p27^{kip1}$. ERK can effect the progression of the cell cycle by phosphorylation and degradation of the $p27^{kip1}$ protein (in press).

MAP kinase (MAPK) also involved in Oocytes maturation. Oocytes are released from their prophase I arrest, usually by hormonal stimulation, only to again halt at metaphase II, where they await fertilization. Mos protein, a MAPKKK is a key regulator of Oocytes maturation process. It encoded serine/threonine protein kinase, which can phosphorylate and activate MEK1. Mos plays a key cell cycle-regulatory role during meiosis. The Mos protein is required for the activation and stabilization of M phase-promoting factor MPF, the master of cell cycle switch, through a pathway that involves the mitogen-activated protein kinase (MAPK) cascade. Upon expression in somatic cells, Mos causes cell cycle perturbation resulting in cytotoxicity and neoplastic transformation. All the known biological activities of Mos are mediated by activation of the MAPK pathway[20],[21].

JNK pathway

JNK signal transduction pathway is implicated in multiple physiological processes. There are three genes that encode JNK (α, β, and γ) with 12 possible isoforms derived from alternative splicing products[22]. Several MAPKKKs have been reported to activate the JNK signaling pathway. These include members of the MEKK group, the mixed lineage protein kinase group, the ASK group, TAKI and Tpl2 [23]. JNK can bind the NH2-termianl activation domain of c-Jun and phosphorylate c-Jun on Ser-63 and Ser-73. Transactivation of c-Jun leads to increased expression of genes with AP-1 sites in their promoters, for example c-jun gene itself. So it initiates a positive feedback loop. The substrates that have been identified for JNK include c-Jun, ATF-2 (activating transcription factor 2), Elk-1, p53, DPC4, Sap-1a and NFAT4[1]. Because these factors can positively regulates the c-fos promotor, their activation resulting increased expression of c-Fos protein, further increasing AP-1 level. Interestingly, JNK also phosphorytates JunB, JunD and the Ets-related transcription factor PEA3[24],[25].

Pedram et al reported that through a novel ERK to JNK cross-activation and subsequent JNK action, the important events for VEGF-induced G1/S progression and cell proliferation are enhanced[26]. ERKs can activate JNK kinases. VEGF-induced ERK was necessary and sufficient for rapid JNK activation and that both MAP kinases mediated the cell proliferation effects of VEGF. They found that JNK is the final mediator for ERK to stimulate cell proliferation. The role of ERK is mainly to induce

the activation of JNK when activated by an endothelial cell (EC) growth factor such as VEGF. The identified role of JNK and the importance of ERK/JNK cross-activation is specifically seen for the stimulation of important G_1 cell cycle events that lead to progression to S phase (DNA synthesis)[26]. It is likely that the cross-talk between members of the MAP kinase family contributes to the decision by a cell to divide or terminally differentiate.

JNKs activation is associated with transformation in many oncogene and growth factor-mediated pathway. The transactivation of c-Jun might play an important role in this process. JNKs can transduce signals for differentiation in the hematopoietic system, and possibly involve in embryonic development. JNK pathway has been implicated in both apoptosis and survival signaling. It has been reported that UV-induced apoptosis in fibroblasts requires JNK for cytochrome C release from the motochondria[27]. But the mechanism is unclear.

p38 pathway

The mammalian p38 MAPK families are activated by cellular stress including UV irradiation, heat shock, high osmotic stress, lipopolysaccharide, protein synthesis inhibitors, proinflammatory cytokines (such as IL-1 and TNF-α) and certain mitogens. At least four isoforms of p38, known as p38 α, p38 β, p38 γ and p38 δ have been identified[28], which can all be phosphorylated by the MAPK kinase MKK6 (SKK3). Other MAKKs can phosphorylate some p38 isoforms. MKK3 can activate p38 α, p38 γ and p38 δ and MKK4 can activate p38 α.

It was demonstrated that p38 δ is a necessary component for IFN signaling where it directs the phosphorylation and activation of cytosolic phospholipase A2. IFN α or γactivation of p38 MAPK also results in the phosphorylation of the transcription factor Stat1 on Ser727[29]. p38 can phosphorylate the transcription factor ATF-2, Sap-1a and GADD153 (growth arrest and DNA damage transcription factor 153)[30]. p38 can regulate NF-κB-dependent transcription after its translocation in to nucleus. Certain p38 isoforms also activate non-transcription factor targets such as the mitogen- activated protein kinase-activated protein kinase (MAPKAPKs, -2, -3 and -5) and the related protein MNK1.

p38 MAPK appears to play a major role in apoptosis, differentiation, survival, proliferation, development, inflammation and other Stress responses. p38 activity is required in Cdc42-induced cell cycle arrest at G_1/S. This inhibitory role may be mediated by the inhibition of cyclinD1 expression. Activated p38 can cause mitotic arrest in somatic cell cycles at the spindle assembly check point[31],[32]. Recently it was reported that p38 involved in various vertebrate cell differentiation processes such as adipocytes, cardiomyocytes, chondroblasts, erythroblasts, myoblasts and neurons[33].

The TGF-β-activating kinase (TAK)-1 is a novel MAPKKK. It is reported to participate in the signal transduction of TGF-β and the phosphorylation of the p38 kinase and / or JNK pathway. Transfection of p38 kinase and p38 kinase kinase, MKK3/6 caused inhibition of mitogen-induced cyclinD1 expression. Thus the TAK1-MKK6-p38 kinase pathway can negatively regulate cyclinD1 expression and cell cycle progression. On the other hand, the MKK1-p44/p42 pathway can up-regulate cyclinD1 promoter activity [14]. The contour balance of p42/44 MAPK and p38 may play a crucial role in the regulation of cell cycle.

Besides the MAPK pathways recited above, other MAPK families have been identified. One of them is BMK1 (Big mitogen-activated protein kinase, also known as ERK5), a recently identified member of the mammalian MAPK family. It is reported that BMK1 can be activated by growth factors, oxidative stress and hyperosmolar conditions. MEK5, which is activated by MEKK 3 is a specific upstream kinase of BMK1. Expression of a dominant negative form of BMK1 blocks EGF-induced cell proliferation and prevent cells from entering the S phase[34].

The MAPK pathways in the signaling networks in regulation of cell proliferation

Signaling network is increasingly important for our understanding of cell prolifeation. Cross-talk can take place at many levels from the membrane to the nucleus. It involves components that are in common pathways, as well as positive and negative feedback signals. The MAPK pathways are tightly regulated by and cross-communicates with other signaling pathways (Fig 2).

One of the best-characterized signal pathways that regulates the activation of MAPKs is cAMP. cAMP

play an opposite role in the regulation of MAPKs depending on cell and receptor type. The small G proteins such as Rap1, Rac and Cdc42 play a key role in this decision. cAMP inhibits the growth of fibroblasts cells, smooth muscle cells, and adipocytes at least in part, by blocking the binding of Raf-1 to Ras, thus blocking the MAPK pathway[35]. On the contrary, in PC12 cells, cAMP induces activation of MAPK through PKA-induced activation Rap1. The activated Rap1 is both a selective activator of B-Raf and an inhibitor of Raf-1. In most cells where Raf-1 is the predominant Raf isoform, cAMP inhibits the MAPK pathway[36].

PKC isoforms can directly regulate Raf-1 activity. Phorbol esters and the macro-cyclic lactone bryostatin1 can activate PKC and have been shown to activate Raf-1 and MAP kinase in many cell types. Exposure of a variety of leukemic cell lines to phorbol esters results in a PKC/MAP kinase-dependent differentiation response consisting of increased p21cip expression and cell cycle arrest. Schonwasser et al revealed that phorbol esters treatment of quiescent 3T3 cells activates ERK via MEK and stimulate DNA synthesis. Using transient transfection of six PKC isotypes (α, $\beta 1$, γ, ξ, η, and ζ) mutants into Cos-7 cells, they further demonstrated that PKC can control MAPK activation and furthermore that the mechanism of activation shows some isotype specificity. cPKC- α and nPKC- η are potent activators of c-Raf-1[37]. It was shown PKC activation

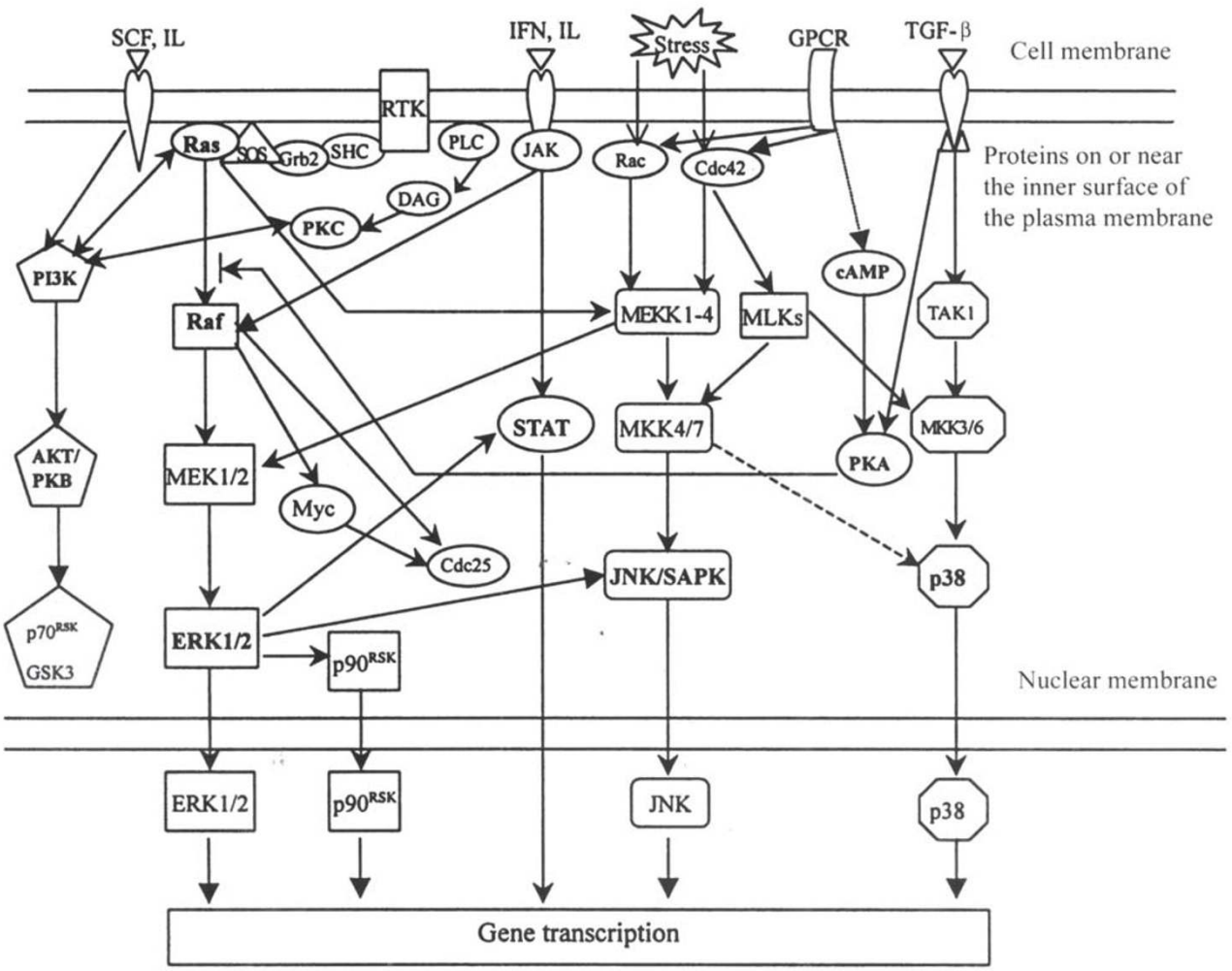

Fig 2. MAPK pathways in the signaling network in mammalian cells

induced dephosphorylation of site in C-terminal of c-Jun and increased of AP-1 binding activity by enhanced phosphatase or inhibited c-Jun protein kinase. In addition, c-Jun is positively regulated by phosphorylation of its N-terminal activation domain by MAPK, resulting a rapid and significant increased in the activity of AP-1[38]. We ourselves also found that the TPA (PKC activator) promoted G_1/S progression of synchronized HeLa cells and the MAPK activity increased. On the contrary, the G_1/S progression of HeLa cells was inhibited by GF-109203X (PKC inhibitor) treatment. The PKC inhibition correlated with decreased activity of MAPK in HeLa cells[39]. In addition, we observed that the expression of antisense PKC ζ results in the decrease of growth rate and inhibition of transition from G_1 to S phase in human keratinocyte Colo16 cells. The level and the activity of ERK1 in Colo16 cells expressing antisense PKC ζ were decreased compared with parent cells and control cells.These results shown that these two signaling pathway cooperated to regulate the progression from G_1 to S phase.

It is well known that TGF-β signal pathway play a growth inhibitory effect on the cells. This involves cross talk among signal pathways. TGF-β signal actives two independent pathways, the TAK1(TGF-β-activated kinase 1) -mediated and the Smad-mediated pathways. In the TAK1 pathway TGF-β activates the TAK1-MKK6-p38 kinase cascade leading to the phosphorylation of ATF-2, and ATF-2 associates with Smad4 in response to TGF-β. Therefore, Smad complexes and phosphorylated ATF-2 may interact in a nucleoprotein complex that associates with DNA and activates transcription of TGF-β-responsive genes[40]. It is possible that other MAP kinase-related pathways such as JNK/SAPK and classical MAP kinse pathways are involved in the transcriptional activation through phosphorylation of ATF-2 and ATF-2-related transcriptional factors. The data from Shaochun Yan et al shown that in mouse C3H10T1/2 cells, TGF-β1 first decreases and later potentiates the levels of EGF-activated MEK1/MAPK and PKB. They demonstrated that MAPK pathway plays a major in EGF-induced DNA synthesis, the activation of PI3K-PKB pathway play a minor role[41]. Furthermore, TGF-b1 may active PKA to inhibit the EGF-activated MEK1-MAPK pathway[42].

Recent evidence suggests that a significant amount of cross-talk occurs between the PI3K and MAPK pathways. PI3K may be able to interact with the Ras GDP/GTP exchange protein in a GTP-dependent fashion. Ras has been shown to function either upstream or downstream of PI3K depending upon the particular stimulus. Activated P13Ks can phosphorylate and activate the downstream targets p70ribosomal S6 kinase, PKB/Akt and NF-κB. In this paper it is reproted that PI3K has been implicated in MEKK1 activation as well as MEK1/ERK activation[43]. Logan et al demonstrated that a dominant negative form of PI 3-kinase as well as the inhibitor wortmannin bloks EGF-induced JNK activation dramatically. In addition, a membrane-targeted, constitutively active PI 3-kinase was shown to produce in vivo products and to activate JNK, while a kinase-mutated form of this protein showed no activation. On the basis of these experiments, they propose that PI 3-kinase activity plays a role in EGF-induced JNK activation[44]. It has been demonastrated that Rac can be activated by a region of Sos in a Ras- and PI3K-dependent manner[45]. Rac1 and Cdc42 have been implicated in the activation of CyclinD1 promoter activity, JNK and p70S6K [46-48]. It was suggested that the Raf/MEK/MAPK pathways cooperate with PI3K and Rac1 signaling events to induce DNA synthesis[49],[50]. But some data shown that in C2C12 cells activation of PI3K-PKB/Akt pathway inhibited the activation of ERK. Akt interacted with Raf and phosphorylated this protein in its regulatory domain in vivo. The phosphorylation of Raf by Akt inhibited the activation of the Raf-MEK-ERK signaling pathway and shifted the cellular response from cell cycle arrest to proliferation in MCF-7 cells[51].

Cytokine receptors without intrinsic kinase activity can transmit their regulatory signals primarily by JAK kinase family. JAK kinase can phosphorylate STAT molecules on their tyrosine residues. The activated and dimerized STAT translocate to nuclear and ultimately bind DNA and regulate gene expression[52]. It was demonstrated that several STATs such as STAT1a, STAT3 and STAT4 are phosphorylated, on a conserved serine residue. This serine residue is a target of the serine/threonine kinase ERK. The phosphorylation on serine residue is required for these STATs to maximally transactivate gene expression. It was reported that treatment of human aortic endothelial cells with re-

combinant hepatocyte growth factor (rHGF) resulted in a significant increase in DNA synthesis and phosphorylation of ERK by rHGF. Interestingly, treatment with rHGF significantly increased the phosphorylation of STAT3 and significantly increased the promoter activity of c-fos. Whereas PD98059 (MAPKK inhibitor) completely attenuated the phosphorylation of STAT3 and the activation of the c-fos promoter induced by rHGF. The cell proliferation induced by rHGF was decreased significantly. These data demonstrated that HGF stimulated cell proliferation through the ERK-STAT3 pathway in human aortic endothelial cells[53].

Conclusion

In summary, the MAP kinase signal transduction pathways play an important role in regulation of proliferation in mammalian cells in a manner inextricable from other signal transduction system by sharing substrate and cross-cascade interaction. Furthermore, to explore the complex overlapping mechanism is important. It is known that the regulation of cell cycle is critical for the normal proliferation and development of multicellular organisms. Loss of control ultimately leads to cancer. So to investigate the mechanism of cell cycle is very important. Leland Hartwell, Paul Nurse and Tim Hunt have been awarded the 2001 Nobel Prize for their contributions to reveal the mysteries of the cell cycle[54]. Recently the numerous reports indicated the MAP Kinase pathways were involved in many pathological conditions, including cancer and other diseases. It seems that MAP kinase signaling pathways represent a potential target for therapeutic intervention. Therefore, a better understanding of the relationship between MAP kinase signal transduction system and the regulation of cell proliferation is essential for the rational design of novel pharmacotherapeutic approaches.

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