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review

The role of p38 MAP kinase in cancer cell apoptosis

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Background. *Cellular behaviour in response to many extracellular stimuli is mediated through MAP kinase signalling pathways. p38 MAP kinase that is represented in mammals by four isoforms (p38α, p38β, p38γ and p38δ) is one of the four main subgroups of MAP kinases. Recent studies show that p38 activation is necessary for cancer cell death initiated by variety of anti-cancer agents. This finding connected cancer therapies previously considered to be mechanistically unrelated and raised the possibility of developing anti-cancer agents that lack the side effects caused by events upstream of p38 MAPK. Many of the details of p38 induced apoptosis still need to be elucidated. Since most of the past studies rely only on the cell culture models, all the results have to be verified using in vivo models. Also very little is known about the role of p38 mediated apoptosis on non-neoplastic cells in response to anti-cancer agents.*

Conclusion. *Although p38 activation of cancer cell apoptosis is a very complex process, recent studies indicate a good starting point for new strategies that would increase the efficiency and decrease the toxicity of proven therapies.*

Key words: tumor cells, cultured; apoptosis; MAP kinase; antineoplastic agents

Introduction

Many extracellular stimuli are converted into specific cellular responses through the activation of mitogen-activated protein kinase (MAPK) signalling pathways. MAPKs are serine/threonine protein kinases that can phoshorylate both cytoplasmic and nuclear targets.[1,2] Four distinct subgroups within the MAP kinase superfamily have been described: extracellular signal-regulated kinases (ERKs), c-jun N-terminal or stress-activated protein kinases (JNK/SAPK), ERK/big MAP kinase 1 (BMK1), and the p38 group of protein kinases.[3] The p38 group is in mammals represented by four isoforms (p38α, p38β, p38γ and p38δ) with overlapping but also distinct physiological roles.[4] Among them, p38α is the best characterized isoform. Recently, it was observed that retinoids, cisplantin and also other chemoterapeutic agents initiate cancer cell apoptosis through the activation of p38 MAP kinase. This finding connects cancer therapies previously considered to be mechanistically unrelated and raises the possibility of developing anti-cancer agents that

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lack the side effects caused by events upstream of p38 MAPK.[5] The potential therapeutic value of p38 and the availability of specific chemical inhibitors made these protein kinases the subject of intensive studies during the past years.[1]

The focus of this review will be to highlight the characteristics and components of the p38 pathway, its role in cancer cell apoptosis and to indicate possible implications for cancer therapy.

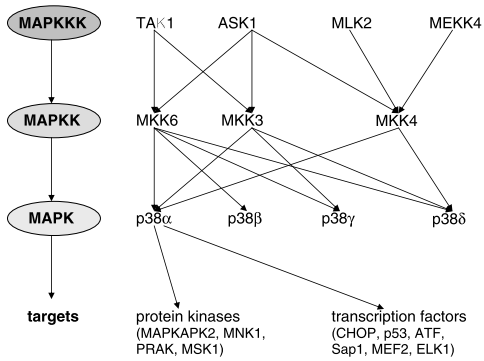

Figure 1. p38 MAP kinase signalling pathway (according to reference 3).

The p38 MAP kinase signalling pathway

p38 MAP cascade regulates a variety of cellular responses to environmental stress, pro-inflammatory cytokines, lipopolysaccharide (LPS) and other signals and was first described in 1994.[6-8] The cascade consists of three conserved kinase modules that include MAPK kinase, which activates MAPK kinase that in turn activates MAPK, in our case p38 (Figure 1). p38 MAPK responds to the signal by becoming rapidly activated by dual phosphorylation of the Thr-Gly-Tyr (TGY) motif.[9] Four isoforms of the p38 family have been identified in mammals: p38α (p38),[6-8] p38β,[10] p38γ[11] and p38δ,[12] which differ in their tissue expression and affinity for upstream activators and downstream effectors.[4] Among them, p38α and p38β show a relatively broad tissue expression in contrast to p38γ and p38δ that are differentially expressed depending on the tissue type.[13] A major contribution to the studies of p38α and p38β isoforms is the availability of specific inhibitors, developed principally using 2,4,5-triaryl imidazoles as a template.[14]

There are two main MAPKKs that are known to activate p38, MKK3[15] and MKK6.[16] While MKK6 is a common activator of all p38 isoforms, MKK3 is unable to activate p38β despite 80% homology between these two MKKs. In specific cell types also MKK4, an upstream kinase of JNK, can aid in the activation of p38α and p38δ. In addition to activation with upstream kinases there is also a MAPKK-independent mechanism of p38 activation involving TAB1, with no known biological context.[3]

The diverse range of MAPKKKs, upstream activators of MKKs, is responsible for susceptibility of p38 to such a wide range of extracellular stimuli. This MAP3K includes TAK1,[17] ASK1[18] DLK/MUK/ZPK, MLK2 and MEKK4.[19] The upstream of MAPKKs are also low molecular weight GTP-binding proteins from the Rho family and p21- activated kinases.[3]

The MAP kinase activation is often transient under physiological conditions, being downregulated by dephosphorylation of various members of the MAP kinase pathway. The proteins responsible for that are different dual-specificity phosphatases, all grouped in the MAP kinase phosphatase (MKP) family.[20]

Activated p38 MAP kinase regulates the activity of a wide range of protein kinases (MAPKAPK2, MNK1, PRAK, MSK1), transcription factors (CHOP, p53, ATF-1/2/6, Sap1, MEF2, ELK1 and others) and some other proteins, which then further regulate the activity of their targets. This complicated network of interacting proteins is in consequence responsible for different cell activities, like apoptosis, cell-cycle arrest, cytokine production, cell differentiation, cell senescence and tumour suppression.[3, 5]

The role of p38 in apoptosis in cancer cells

Apoptosis is an active form of cell death that plays an essential role in eliminating damaged cells or cells with defects in key-regulated processes such as growth.[21] Once this highly regulated process is triggered, the apoptotic program involves activation of a series of biochemical events that end with the release of proteins from the mitochondria into the cytoplasm and the nucleus.[22] Not surprisingly, several tumours emerge with mutations in genes conferring apoptosis resistance, allowing them to continue uncontrolled growth under, for normal cells, pro-apoptotic conditions.[23]

There are some evidence for pro-apoptotic and anti-apoptotic role of p38 MAPKs, depending on the cell type and the stimuli. Overexpression of the active form of the p38 activator MKK6 protects cardiac myocytes from β-adrenergic receptor-mediated apoptosis.[24] Similarly, the early activation of p38 is necessary and sufficient to protect Kym cells from tumour necrosis factor-α-mediated apoptosis,[25] and expression of p38β results in attenuated cell death induced by Fas ligand and UV light[26]. The activation of p38 may also protect through the down-regulation of the Fas receptor expression.[27]

Even more reports support the pro-apoptotic role of p38, for example, p38 is a mediator of apoptosis in neurons[28] and cardiac cells.[29] In other cell types, p38 activates apoptosis upon stimulation with tumour necrosis factor-α[30], transforming growth factor-β[31] or in response to oxidative stress.[32] The latter was also demonstrated in the case of TRAIL induced apoptosis mediated by reactive oxygen species (ROS)-activated p38 MAP kinase followed by the caspase activation in HeLa cells.[33] Cells treated with betulinic acid, a selective inhibitor of human melanoma, also induce apoptosis through the ROS mediated p38 activation.[34]

The mechanisms by which p38 contributes to an enhanced pro-apoptotic response include the phosphorylation and translocation of proteins from the Bcl-2 family, which leads to the release of cytochrome c from the mitochondria,[32, 35] the transforming growth factor-β-induced activation of caspase 8[36] as well as the regulation of membrane blebbing and nuclear condensation.[37] At the transcriptional level, expression of monoamine oxidase[28] or growth arrest and DNA damage (GADD)-inducible genes[38] have been shown to mediate pro-apoptotic effects of p38. The importance of p38 in apoptosis was also shown in the study of apoptotic response in different p38-deficient cells, like primary fibroblasts and immortalized cardiomyocytes and fibroblasts. All p38 deficient cells were more resistant to apoptosis induced by many different stimuli. The reduced apoptosis correlated with down-regulation of the proapoptotic proteins Fas and Bax as well as enhanced activity of the ERK survival pathway.[39]

This opposing effects on apoptosis observed for p38 probably reflect the multiple and complex activities of this signalling pathway, which acts on different targets at once and thus can yield distinct overall effects depending on the cellular context. Similar opposing effects were also found for the other stress-activated protein kinase JNK.[37]

p38, a convergence point in cancer therapy?

Recent studies show that the p38 MAP kinase activation is necessary for cancer cell death initiated by various anti-cancer agents. Retinoids like 13-*cis* retinoic acid or all-*trans* retionic acid (ATRA) initiate apoptosis in medulloblastoma cell lines by phosphorylating p38 MAPK through the induction of bone morphogenetic protein 2 (BMP2).[40] Another syntetic retinoid CD437 induces apoptosis in ovarian carcinoma cell culture also in p38 dependent way. The activated p38 phosphorylates the transcription factor MEF-2, which has a proposed role in mitochondrial depolar-

ization and apoptosis. In these cells ATRA does not induce p38 cascade, suggesting a distinct upstream mechanism from the one described for medulloblastoma.[41]

Four chemotherapeutic agents were shown to induce the p38 activation and mitotic cell-cycle arrest in HeLa human cervical carcinoma cells by depolymerizing microtubules, (nocodazole, vincristine and vinblastine) or stabilizing them (taxol). The extent of apoptosis in these cells is greater when induced by a direct activation of p38, because previously mentioned chemotherapeutics activate pro-apoptotic as well as pro-survival pathways in HeLa cells, which results in less apoptosis. The activated p38 induces cell death by stimulating translocation of Bax from the cytosol to the mitochondria. On the other hand, the p21-activated kinase (PAK) mediates cell survival by phosphorylating Bad, thereby inhibiting its pro-apoptotic function.[42]

The activation of p38 in several tumour cell lines was also observed after the treatment with cisplatin, an inorganic heavy metal coordination complex, and doxorubicin, a DNA intercalating agent.[43]

Some anti-cancer agents utilize two distinctive MAPK signalling pathways for killing cells. Phytosphingosine simultaneously downregulates the ERK survival pathway, which is critical for the death receptor independent activation of caspase-8, and activates p38 pathway, which is involved in the cell death pathway through the mitochondrial activation.[35] Another example is 2-methoxyestradiol (2-ME) apoptosis induction in prostate cancer cell line. A treatment with 2-ME leads to the p38 activation as well as JNK-mediated Bcl2 phosphorylation, which inactivates this anti-apoptotic protein.[44]

All these reports support the role of p38 MAPK as the key component for the cancer cell death after treating tumours with a variety of anti-cancer agents. This finding connects cancer therapies previously considered to be mechanistically unrelated and raises the possibility of developing anti-cancer agents with the lack of the side effects caused by events upstream of p38 MAPK.[5]

Still many of the details of p38 induced apoptosis need to be elucidated. Since most of the past studies rely only on the cell culture models, all the results have to be verified using in vivo models. Also very little is known about the role of p38 mediated apoptosis on non-neoplastic cells in response to anti-cancer agents. The issue is also the drug resistance, therefore, more has to be learned about how tumours protect themselves from the pro-apoptotic activation of p38 MAPK. The study made on 20 liver cancer specimens shows that both MKK6 and p38 protein levels are lover in hepatocellular carcinoma tumours than adjacent non-neoplastic liver. This reduction of p38 levels could represent an anti-apoptotic mechanism that provides growth advantage to tumour cells.[45]

Conclusions

Although the p38 activation of cancer cell apoptosis is a very complex process, recent studies indicate a good starting point for new strategies that would increase the efficiency and decrease the toxicity of proven therapies. One possible way to improve the efficiency would be by treating patients simultaneously with available p38-activating agents and antagonists of anti-apoptotic pathways, like PAK and ERK inhibitors. An alternative strategy would be to use combinations of p38-activating chemotherapeutics.

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Vloga p38 MAP kinaze pri apoptozi rakavih celic

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Izhodišča. Odzivanje celic na številne zunajcelične signale poteka preko aktivacije MAP kinaznih signalnih poti. Ena od štirih glavnih podskupin MAP kinaz je p38 MAP kinaza, ki je pri sesalcih prisotna v štirih izooblikah: p38α, p38β, p38γ in p38δ. Nedavne raziskave so pokazale, da je za aktivacijo apoptoze rakavih celic z različnimi kemoterapevtiki nujna aktivacija p38 kinaze. To spoznanje je v eni točki povezalo poti delovanja raznolikih kemoterapevtikov ter s tem nakazalo nove možnosti njihovega razvoja brez stranskih učinkov, ki jih sedaj povzročajo dogodki pred aktivacijo p38. Veliko podrobnosti o p38 posredovani apoptozi je potrebno še razjasniti. Dosedanja dognanja je potrebno preveriti v in *vivo* modelih, saj se ta sedaj nanašajo predvsem na celične kulture. Malo je znanega tudi o vlogi p38 pri apoptozi nerakavih celic po aktivaciji s kemoterapevtiki.

Zaključki. Čeprav je p38 posredovana aktivacija apoptoze rakavih celic zelo kompleksen proces, novejše študije ponujajo dober začetek za razvoj novih kemoterapevtikov s povečano učinkovitostjo in zmanjšano toksičnostjo.