Roles of p38 MAPKs in invasion and metastasis

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Abstract

Cells from primary tumours need to go through several steps to become fully metastatic. During this process, cancer cells acquire the ability to invade, migrate across the surrounding tissue, enter into the circulation and colonize distant organs. In the present paper, we review recent progress in understanding how the p38 MAPK (mitogen-activated protein kinase) signalling pathway participates in the different steps of metastasis. Experimental evidence suggests that tumour cells need to modulate p38 MAPK activity levels to successfully metastasize.

Introduction

Metastasis is responsible for most of the mortality in cancer patients. The mechanisms that control the multistage process required for malignant cells to spread and colonize distant organs remain poorly characterized. During metastatic dissemination, cells from the primary tumour follow an orderly sequence of steps to acquire the properties required for successful metastasis [1]. First, cancer cells need to be able to migrate and to invade the surrounding local tissue, which usually involves a process referred to as EMT (epithelial–mesenchymal transition) [2]. Then, cells should enter blood and lymphatic vessels, which requires degradation of the ECM (extracellular matrix), and should be able to survive in the circulation until they reach a proper site to colonize. In most cases, there is a temporal gap between the initial organ infiltration and the formation of a new tumour in a distant site, referred to as metastatic latency. During this latency, tumour cells may enter a dormancy stage [3].

The process of metastasis involves interactions of the tumour cells with the surrounding environment and with immune cells, which may facilitate the initial dissemination as well as eventual niche colonization [4]. These interactions involve secreted signalling molecules such as TGFβ (transforming growth factor β), VEGF (vascular endothelial growth factor) and TNFα (tumour necrosis factor α), as well as cytokines such as IL (interleukin)–6 (interleukin-6).

The p38 MAPK (mitogen-activated protein kinase) signalling pathway plays important roles in the ability of cells to integrate external cues and elaborate appropriate responses. Four p38 MAPK family members are known, of which p38α is ubiquitously expressed usually at high levels, and p38β is thought to be expressed at lower levels but can potentially perform overlapping roles with p38α, whereas p38γ and p38δ have more restricted expression patterns [5]. The regulation and functions of p38 MAPKs in tumour development have been reviewed in [6,7]. In the present paper, we focus on the roles of p38 MAPKs in different aspects of invasion and metastasis (Figure 1).

EMT

Cells from the initial tumour that are able to invade the surrounding tissue and disperse to produce metastasis usually undergo EMT that provides epithelial cell plasticity (Figure 1). This process plays an important role in developmental programmes and is regulated by transcription factors that repress E-cadherin (epithelial cadherin) expression, such as Twist1, Snail and Slug [2].

Several reports have associated p38 MAPK signalling with the regulation of EMT. For example, p38 MAPKs have been implicated in the phosphorylation of Twist1 on Ser178, which enhances Twist1 protein stability and potentiates its ability to induce EMT and invasiveness in mammary epithelial cells [8]. In addition, reverse-phase protein microarrays have correlated Snail expression with phosphorylated p38 MAPK in primary ovarian tumours [9].

p38 MAPKs have been proposed to regulate mouse gastrulation, where EMT is necessary for the migration of mesoderm from the primitive streak. In particular, using the p38α and p38β chemical inhibitor SB203580, p38 MAPKs have been implicated in the down-regulation of E-cadherin protein expression downstream from NIK (Nck-interacting kinase) and independently of the Snail transcription factor and FGF (fibroblast growth factor) signalling during gastrulation [10].

Increased levels of ROS (reactive oxygen species) can also regulate EMT, and p38 MAPKs are known to respond to ROS accumulation [11]. In the SGP2 breast cancer cell line,
Figure 1 | Roles of p38 MAPKs in metastasis

The activation of p38 MAPKs has been reported to contribute to the EMT of cells in the primary tumour, to the acquisition of invasion and migrating capabilities, and to the extravasation of migrating tumour cells. Pre-metastatic niche formation and the recruitment of myeloid BMDCs are also positively regulated by p38 MAPKs. In contrast, p38 MAPK inhibition has been correlated with the resistance to anoikis, which allows circulating cancer cells to survive. Tumour cell dormancy has been associated with high p38 MAPK activity in combination with low activity of the ERK1/2 pathway. Candidate regulatory molecules implicated in these processes downstream of p38 MAPKs are indicated in Table 1.

MMP (matrix metalloproteinase)-3 triggers Rac1b activation, increasing the cellular levels of ROS, which in turn induce Snail expression and EMT [12]. On the basis of the use of the inhibitor SB203580, p38 MAPKs have been implicated in EMT induced by depletion of RKIP (Raf kinase inhibitor protein), which could be mediated by ROS production and GSK3β (glycogen synthase kinase 3β) phosphorylation [13].

p38 MAPKs have been implicated in TGFβ-induced EMT and lung metastasis of breast cancer cells [14]. In addition, EGF (epidermal growth factor) facilitates p38 MAPK activation and metastatic progression in post-EMT populations of breast cancer cells [14]. Physical interaction of the cytokine-activated TGFβ receptor with the E3 ubiquitin ligase TRAF6 (TNF-receptor-associated factor 6) has been shown to allow the ubiquitination and activation of TAK1 (TGFβ-activated kinase 1), which in turn triggers activation of the JNK (c-Jun N-terminal kinase) and p38 MAPK pathways. Importantly, depletion of TRAF6 does not affect canonical (Smad-mediated) TGFβ signalling in mammary epithelial cells, but prevents TGFβ from activating JNK and p38 MAPK, as well as from stimulating EMT [15,16].

LOXs (lysyl oxidases) are copper-dependent amine oxidases that can be induced by TGFβ and cross-link collagen and elastin in the ECM, enhancing integrin-mediated signalling as well as breast cancer cell invasion and metastasis. Overexpression of constitutively active LOX increases basal p38 MAPK activation, whereas interfering with the expression or the activity of LOX inhibits p38 MAPK activation by TGFβ in malignant mammary epithelial cells, suppressing EMT and invasion without affecting the TGFβ canonical pathway mediated by Smad2/3 activation [17]. This may be related to the requirement of hydrogen peroxide as a mediator of TGFβ-induced activation of p38 MAPKs.

EMT has been also related to the inflammatory response. For example, the cytokine IL-6 has been proposed to promote EMT in the MCF-7 human breast cancer cell line [18], and Snail can induce IL-6 expression in keratinocytes [19]. Additionally, p38α has been shown to regulate the production of IL-6 [20], and can mediate the IL-6 triggered transcriptional activation of STAT3 (signal transducer and activator of transcription 3) in human hepatoma cells [21].

Hypoxia is another important inducer of EMT and p38α can activate HIF-1α (hypoxia-inducible factor 1α) through the stabilization of its α-subunit [22]. HIF-1α is a potent transcriptional regulator of growth factors and cytokines such as VEGF and TGFβ. In addition, HIF-1α can directly activate the expression of the EMT transcription factors Snail and Twist [23].

In contrast with the above results, which are all consistent with a role for p38 MAPK signalling in EMT induction at
different levels, p38α has also been proposed to promote E-cadherin expression in human peritoneal mesothelial cells [24]. On the basis of experiments using chemical inhibitors, dominant-negative mutants and shRNAs (short hairpin RNAs), the authors conclude that p38α maintains E-cadherin expression, interfering with mesothelial EMT, by suppressing the phosphorylation of TAK1 as well as nuclear translocation and transcriptional activity of p65 NF-κB (nuclear factor κB).

Invasion
To enter the blood and lymphatic circulation and to infiltrate distant organs, cancer cells must invade the surrounding tissues. Various mechanisms that confer invasiveness, such as cellular motility and basement membrane degradation, have been proposed to mediate cancer cell entry into the circulation stream [1]. Next, we describe how these processes can be regulated by p38 MAPKs (Table 1).

Activation of p38α can trigger cell migration and cytoskeletal remodelling in tumour cells by increasing the phosphorylation of HSP27 (heat-shock protein 27) [25], which induces its release from F-actin (filamentous actin) caps [26], or by activating the protein kinase LIMK1 (LIM domain kinase 1), which in turn phosphorylates and inactivates the cytoskeletal protein cofilin [27].

MMPs are key proteins implicated in ECM remodelling and degradation by metastatic cells [28]. The expression of MMP-1, MMP-2, MMP-9 and MMP-13 has been shown to be mediated by p38α in bladder (HTB5 and HTB9), breast (MDA-MB231), liver (SK-Hep1 and SNU-387), skin keratinocytes (UT-SCC7) and prostate (PC3 and PC3-M) cell lines derived from human tumours [29–33]. In these cell lines, inhibition of p38α, using SB203580, siRNAs (small interfering RNAs) or dominant-negative mutants, results in decreased cell invasion [29–33]. Using SB203580, p38 MAPKs have been also implicated in the negative regulation of Maspin tumour-suppressor by the thrombin receptor PAR-1 (protease-activated receptor 1), which is thought to be important for the acquisition of metastatic properties by melanoma cells. In particular, Maspin inhibition has been proposed to mediate the PAR-1-induced expression of MMP-2 and VEGF [34]. Moreover, p38α can modulate uPA (urokinase-type plasminogen activator) [35], which is a critical regulator of invasion, intravasation and metastasis [36]. The metastatic potential of human breast (MDA-MB231) and lung (H460 and A549) cancer cell lines has been reported to rely on uPA induction by p38 MAPK on the basis of the effect of chemical inhibitors [37,38].

The use of cancer cell lines in various assays supports further the implication of p38 MAPKs in cell invasion. For example, p38α activity is required for the invasive capacity of pancreatic, hepatocellular and head and neck squamous carcinoma cell lines on the basis of the use of SB203580 and dominant-negative mutants [39–41]. Moreover, glioma invasiveness is promoted by MKK (MAPK kinase) 3-induced activation of p38α in cell-based assays in vitro and in rat brain slices ex vivo [42]. The p38 MAPK inhibitor SB203580 also impairs Ras-induced activation of FOXM1 (forkhead box M1), a forkhead/winged-helix transcription factor required for in vitro invasion and anchorage-independent growth, which is up-regulated in a broad variety of cancers [43].

In addition to p38α and maybe p38β, other p38 MAPK family members have been proposed to regulate cancer cell invasion. For example, expression of p38δ dominant-negative mutants reduces head and neck squamous cell carcinoma invasion, which correlates with reduced expression of MMP-1 and MMP-13 [41]. Moreover, dominant-negative mutants of p38γ can interfere with K-Ras-induced invasion, which might be explained by the co-operation between p38γ and c-Jun in transcriptional activation of MMP-9 [44]. Furthermore, shRNA-mediated knockdown of p38γ markedly decreases migration and invasion of 4T1 breast cancer cells in vitro, as well as the ability of these cells to produce mammary gland tumours and lung metastasis in nude mice [45]. In contrast, contradictory results have been reported on the implication of the p38 MAPK activator MKK6 in metastasis regulation, depending on the experimental model used [46,47].

Table 1 | p38 MAPK-regulated molecules involved in metastasis

<table>
<thead>
<tr>
<th>Step</th>
<th>p38 MAPK status</th>
<th>Molecules involved</th>
<th>Process regulated</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration/invasion</td>
<td>Active</td>
<td>Snail, Twist1, IL-6, HIF-1α, MLC, HSP27, LOX, MMPs, uPA, FOXM1, VEGF</td>
<td>EMT, Inflammation, Hypoxia, Cytoskeletal remodelling, ECM degradation, Invasion, Angiogenesis</td>
<td>[3,60,20,22,17,25,52,29,33,35,37,38,43,57]</td>
</tr>
<tr>
<td>Extravasation</td>
<td>Active</td>
<td>E-selectin, VCAM-1, ICAM-1</td>
<td>Trans-endothelial migration, Anoikis</td>
<td>[54,49]</td>
</tr>
<tr>
<td>Circulating cells</td>
<td>Inhibited</td>
<td>Bax</td>
<td>BMDC recruitment</td>
<td>[55,56,59]</td>
</tr>
<tr>
<td>Pre-metastatic niche</td>
<td>Active</td>
<td>S100A8, S100A9, CXCL12/CXCR4, HIF-1α, LOX</td>
<td>MLC, HSP27, LOX, S100A8, S100A9</td>
<td>[24,26,52]</td>
</tr>
</tbody>
</table>

References:
[1] Dormancy Active p53, c-Jun, FOXM1 G0/G1 arrest
[2] MAPK-regulated molecules involved in metastasis
[3] p38 MAPK status
[6] Reference(s)
[8] The Authors Journal compilation ©2012 Biochemical Society
Anoikis resistance
Tumour cells in the circulatory system require an anchorage-independent survival mechanism to be able to disseminate and colonize distant organs (Figure 1). Chemical inhibitors of p38α and p38β have been shown to induce anoikis resistance in human breast cancer cells and murine mammary epithelial cells [48–50]. The underlying mechanism may be related to the activation of mitochondrial Bax by p38 MAPK, leading to cytochrome c release from the mitochondria [49]. Interestingly, the induction of anoikis by p38 MAPK has been proposed as a possible anticancer therapy in head and neck squamous cell carcinoma treated with tetrathiomolybdate [31]. Recently, a combination of chemical inhibitors and genetically modified cells has been used to show that p38α induces anoikis during mammary morphogenesis by increasing transcription of the death-promoting protein BimEL, which seems to involve the transcription factors ATF-2 (activating transcription factor 2) and c-Jun [51].

Extravasation
Tumour cell extravasation can be also regulated by p38 MAPKs. Adhesion of colon cancer cells to E-selectin-expressing endothelial cells leads to the activation of p38 MAPKs in both tumour and endothelial cells [25,52]. This activation induces stress fibre formation, which results in endothelial remodelling due to actin-polymerization-mediated phosphorylation of MLC (myosin light chain) allowing extravasation [52]. Intriguingly, intravenous injection of LLC (Lewis lung carcinoma) or B16 melanoma cells has been reported to generate fewer lung metastases in p38α heterozygous mice than in wild-type mice, which correlates with reduced expression of E- and P-selectins in endothelial cells and platelets of the p38α heterozygous mice [53]. The IL-17-induced expression of endothelial markers such as E-selectin, VCAM-1 (vascular cell adhesion molecule 1) and ICAM-1 (intercellular adhesion molecule 1) is also impaired by the chemical inhibitors SB203580 and BIRB796, suggesting a role for p38 MAPKs in stimulating the trans-endothelial migration of neutrophils as well as the transmigration of colon carcinoma cells into the lung [54].

Organ colonization
The ability of circulating cancer cells to colonize an organ depends on cell autonomous functions as well as on the barriers imposed by the microenvironment at the metastatic site, which together influence the specificity for different tissues. Extracellular factors produced by the primary tumour have been shown to prime certain tissues for tumour cell engraftment due to the production of local chemokines. For example, LLC or B16 tumour cells implanted intradermally into nude mice release VEGF, TGFβ and TNFα, which in turn lead to the expression of the inflammatory chemoattractants S100A8 and S100A9 in lungs. The S100A8 and S100A9 produced by the lung induce p38 MAPK-mediated migration of both Mac1⁺-myeloid cells and tumour cells [55]. On the basis of the effect of SB203580, the p38 MAPK pathway has been also implicated in lung metastasis in mice via recruitment of myeloid BMDCs (bone-marrow-derived cells) mediated both by the chemokine receptor CXCR4 (CXC chemokine receptor 4) and by VEGFR1 (VEGF receptor 1) [56].

Activation of angiogenesis is also very important for the establishment of metastatic tumours. Experiments using chemical inhibitors indicate that induction of VEGF by p38 MAPKs can regulate endothelial cell migration and angiogenesis [57]. Moreover, activation of p38 MAPKs by expression of constitutively active MKK6 has been shown to induce endothelial cell migration [58].

Tissue hypoxia together with expression of LOX has been also implicated in the recruitment of BMDCs to the pre-metastatic niche [59] and, as mentioned above, p38 MAPKs could potentially be involved in both events.

Metastatic dormancy
In some cases, there is a significant lag until tumour cells can colonize a distant organ. The time between primary tumour diagnosis and clinically detectable metastatic relapse is defined as metastatic latency [1]. Disseminated tumour cells are usually responsible for the distant metastasis. When tumour cells are not able to colonize, they can enter a state of proliferative dormancy, which involves a G2/M cell-cycle arrest (Figure 1). Alternatively, disseminated tumour cells can form micrometastasis unable to expand due to an equilibrium between cell death and cell proliferation rates. Using human head and neck carcinoma cells as a model, the ratio of ERK1/2 (extracellular-signal-regulated kinase 1/2) activity to p38 MAPK activity has been correlated with the ability of tumour cells to proliferate or to enter a state of dormancy. According to this model, external signals derived from fibronectin are transduced by the interaction of uPA receptor, α5β1 integrin, FAK (focal adhesion kinase) and EGF receptor, resulting in ERK1/2 activation and the inactivation of p38 MAPK due to repression of Cdc42 (cell division cycle 42). Under these conditions, the dormancy stage is abolished and proliferation of disseminated tumour cells occurs. In contrast, the loss of any of the surface receptors (uPA receptor, α5β1 integrin or EGF receptor) results in impaired external signalling and low ERK1/2 compared with high Cdc42/p38 MAPK activity, leading to dormancy [3]. Previous studies have correlated the induction of dormancy by p38α with up-regulation of p53 and down-regulation of c-Jun and FOXM1 [3,60].

Conclusions
The implication of p38 MAPKs in the different steps required for cancer cells to form metastatic tumours is supported by an increased number of reports (Figure 1 and Table 1). However, we are still lacking in vivo results to understand how p38 MAPK signalling is able to modulate metastatic processes. Important questions to be addressed in the future include: (i) interplay among different p38 MAPK family members and cross-talk with other signalling pathways and miRNAs (microRNAs) implicated in the metastatic process;
(ii) detailed characterization of the function of the p38 MAPK pathway in tumour microenvironment components that affect the metastatic process, such as macrophages or fibroblasts; (iii) implication of p38 MAPKs in the ability of metastatic cells to colonize different niches; and (iv) in vivo evidence for a role for this pathway in dormancy regulation. The generation of genetically modified mice to modulate the pathway in a time- and tissue-specific manner should provide useful tools to address these questions and elucidate in vivo functions. Most importantly, the knowledge generated may be used to develop new therapeutic approaches.

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