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Laboratory Studies

MKP-3 regulates PDGF-BB effects and MAPK activation in meningioma cells

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ABSTRACT

Autocrine platelet derived growth factor-BB (PDGF-BB) and cerebrospinal fluid, which also contains PDGF, stimulate proliferation of leptomeningeal and meningioma cells, in part, by activation of the Raf-1-MEK-1-MAPK pathway. The negative regulators of this activation are not known. However, PDGF receptors and p44/42 MAPK are regulated, in part, by mitogen activated kinase phosphatase 3 (MKP-3) and Src homology carboxyl terminus protein (SHP-2). Six fetal and one adult human leptomeninges specimens and 22 meningiomas were evaluated for MKP-3, SHP-2, and phospho-SHP-2 as well as activation/phosphorylation of MEK1/2, p44/42 MAPK, Akt and signal transducer and activator of transcription 3 (STAT3) by western blot and MKP3 expression by polymerase chain reaction. PDGF-BB and cerebrospinal fluid effects on these phosphatases and signaling were also studied *in vitro*. MKP-3 and phospho-p44/42 MAPK were detected in all or six of seven leptomeninges, respectively. MKP-3 was detected in six of eight World Health Organization grade I and II meningiomas. Three of four grade I and five of five grade II with no or low MKP-3 had high levels of phospho-p44/42MAPK. MKP3 was not detected in four of six grade III meningiomas. These had high levels of phospho-p44/42MAPK. SHP2 was found in all leptomeninges and meningiomas while phospho-SHP-2 was found in 11 to 33% of grade I–III meningiomas. Reduced MKP-3 may facilitate PDGF-BB autocrine and paracrine mitogenic effects in a subpopulation of higher grade meningiomas by increasing phospho-p44/42 MAPK.

1. Introduction

In previous studies, we and others have found that platelet derived growth factor-BB (PDGF-BB) stimulates proliferation of leptomeningeal and World Health Organization (WHO) grade I and II meningioma cells, in part, by activation of the mitogen activated protein kinase (Raf-1-MEK-1-p44/42 MAPK) pathway [1–3]. PDGF-BB is an autocrine and paracrine mitogen of meningioma cells [4–7] that has also been detected in the cerebrospinal fluid that bathes meningiomas [8]. Moreover, recently we have found that cerebrospinal fluid consistently stimulates leptomeningeal and meningioma proliferation, in part, by altering activation of the Raf-1-MEK-1-p44/42 MAPK pathway [1,2,8]. Nonetheless, the negative regulators limiting PDGF-BB and the mitogenic effects of cerebrospinal fluid on normal leptomeningeal cells and slower growing lower grade meningiomas has not been studied to our knowledge. Because the Raf-1-MEK-1-p44/42 MAPK pathway is tightly regulated by several protein tyrosine phosphatases [9–13], we have hypothesized that complex interactions with phosphatases block PDGF-BB and/or the mitogenic effects of cerebrospinal fluid under physiological circumstances, constraining the growth rate of the lower grade tumors. However, the identity and contributions of these phosphatases are not known. One possible candidate is MAPK phosphatase 3 (MKP-3), a phosphatase relatively specific for p44/42 MAPK (ERK 1 and 2). MKP3 has complex effects on the Raf-1-MEK-1-p44/42 MAPK pathway but inhibits p44/42 MAPK [14]. The Src homology carboxyl terminus protein (SHP-2) also regulates p44/42 MAPK phosphorylation [9–13]. SHP-2 has complex effects on Raf-1-MEK-1-MAPK activation and may increase or reduce activation of the pathway depending on the circumstances [9–11].

MKP-3 is part of a large family of dual specificity MAPK phosphatases. In contrast to other members, MKP-3 or dual specificity MAPK phosphatase 6 (DUSP6) is a cytoplasmic phosphatase that specifically binds to p44/42 MAPK (ERK) and dephosphorylates

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threonine and tyrosine residues autophosphorylated by PDGF and other receptor activation. This results in inhibition of p44/42 MAPK pathway activation [14–17]. SHP-2, another protein tyrosine phosphatase, modulates PDGF-BB effects after PDGF's binding to and activation of PDGF receptors. PDGF receptor autophosphorylation of its tyrosine residues results in recruitment of SHP-2 to SH2 binding domains on the receptor resulting in catalytic activation of SHP-2. The phosphatase activity dephosphorylates substrates resulting in inactivation of the Raf-1-MEK-1-p44/42 MAPK pathway [11,13]. In addition, SHP-2 also reduces growth factor activation of phosphoinositide 3-kinase (PI3K) by dephosphorylating Gab1 p85 binding sites [13,15,18–21]. Due to their potential growth regulatory properties, these phosphatases may be targets for new therapies since sustained activation of the p44/42 MAPK pathway promotes cell proliferation, migration and inhibits apoptosis [18,20]. Such therapies are desperately needed because no consistently effective chemotherapy has been identified for meningiomas, particularly inoperable or recurrent tumors [22].

2. Materials and methods

2.1. Human leptomeningeal and meningioma tissue

Tissues used were collected at University of Rochester Medical Center or obtained from the Cooperative Human Tissue Network (Philadelphia, PA, USA), with Institutional Review Board approval and WHO grading [23] (Table 1–3). Because the NF-2 gene product merlin protein inhibits MAPK, we screened for and only included cases with detectable merlin. All of the grade II and III meningiomas had detectable merlin.

2.2. Human leptomeningeal and meningioma cell cultures

Primary leptomeningeal cultures were established from two 20 week and one 22 week human fetuses and meningioma cell cultures were established from three WHO grade I meningothelial (MC1–MC3) and two grade II meningiomas (MC4, MC5) as described previously [1,2,8].

2.3. Human cerebrospinal fluid from patients without neurologic disease

Remnant, discarded lumbar cerebrospinal fluid was obtained with Institutional Review Board approval from samples collected at the University of Rochester Medical Center as described previously [8]. Only cerebrospinal fluid with no inflammation from patients without neurological disease was used.

Table 1
Meningioma tissue used for western blots

Subtype	N	Mean age, years	Sex
Leptomeninges, fetal	6		U
Leptomeninges, adult	1		
WHO grade I	8	55	6 F
Meningothelial	3 (37.5%)		
Transitional	3 (37.5%)		
Fibrous	2 (25%)		
WHO grade II	8	56	8 F
Meningothelial	8(50%)		
Transitional	1(12.5%)		
Fibrous	2(25%)		
Microcystic	1(12.5%)		
WHO grade III	6	61	4 F
Anaplastic	3 (50%)		
Transitional	1 (17%)		
Fibrous	1 (17%)		
Papillary	1 (17%)		

F = female, U = undetermined, WHO = World Health Organization.

Table 2
Meningioma tissue used for polymerase chain reaction

Subtype	N	Mean age	Sex
Leptomeninges	3		
Fetal	2	18 weeks	U
Adult	1	67 years	1 F
WHO grade I	9	61 years	7 F
Meningothelial	6 (66%)		
Transitional	3 (33%)		
WHO grade II	11	60 years	6 F
Meningothelial	3 (33%)		
Transitional	1 (9%)		
Fibrous	4 (37%)		
Secretory	1 (9%)		
Microcystic	1 (9%)		
WHO grade III	4	64 years	3F
Anaplastic	3 (75%)		
Papillary	1 (25%)		

F = female, U = undetermined, WHO = World Health Organization.

Table 3
Sources of leptomeninges and meningiomas used for primary cultures

Lepto/Menin	Age/Sex	Location	Classification	Merlin
LC1	20 wk/U	Convexity	Leptomeninges	n.d.
LC2	20 wk/U	Convexity	Leptomeninges	n.d.
LC3	22 wk/U	Convexity	Leptomeninges	n.d.
MC1	65/F	Clivus	Meningothelial I	+
MC2	84/M	R frontal	Meningothelial I	n.d.
MC3	51/F	Convexity	Meningothelial I	n.d.
MC4	80/M	L frontal	Mixed II	+

F = female, LC = leptomeningeal culture, M = male, MC = meningothelial culture, n.d. = not done, R = right, U = undetermined, wk = weeks.

2.4. Western blot analysis of MKP-3, SHP-2, phospho-SHP-2, phospho-MEK-1/2, phospho-MAPK 44/42, phospho-Akt and phospho-STAT3 protein in human leptomeningeal and meningioma tissue

Western blots were prepared as described previously [8]. Detection was achieved with Clarity Western ECL substrate and Chemidoc software (both BioRad Laboratories, Hercules, CA, USA).

2.5. Analysis of MKP-3 RNA in leptomeningeal tissue and meningioma tumors

RNA was isolated from two fetal and one adult leptomeninges specimens, nine WHO grade I, 11 WHO grade II and four WHO grade III meningiomas as described previously [24]. Reverse transcription was performed as described previously [24] using established primers [25].

2.6. Comparison of PDGF-BB and cerebrospinal fluid effects on MKP-3 levels and phosphorylation of SHP-2 and pathways and DNA synthesis

Primary cultures from three WHO grade I and one WHO grade II meningiomas were serum deprived overnight then treated and characterized as described previously [8]. To correlate with cell proliferation, MC1 and MC2 were also treated as above and analyzed with CYQUANT cell proliferation assay (Life Technologies, Carlsbad, CA, USA) [8].