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Laboratory Studies

p38MAPK activation and DUSP10 expression in meningiomas

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ABSTRACT

The mitogen activated protein kinase (MAPK) p38MAPK has been implicated in regulation of cell proliferation and apoptosis. However, expression, activation and regulation has not been studied in meningiomas, to our knowledge. p38MAPK is regulated, in part, by dual specificity phosphatases (DUSP) that inactivate signaling by dephosphorylation. DUSP10 is also a likely participant in regulating meningioma proliferation. Five fetal and an adult human leptomeninges and 37 meningioma cultures (MC) were evaluated for DUSP10 as well as phosphorylation of its substrates p38MAPK and p44/42MAPK by western blot and DUSP10 expression by polymerase chain reaction. Platelet derived growth factor-BB (PDGF-BB), transforming growth factor B1 (TGFB1) and cerebrospinal fluid effects on DUSP10 and signaling were also studied *in vitro*. DUSP10 and phospho-p38MAPK and phospho-p44/42MAPK were detected in all six leptomeninges. DUSP10 was detected in 13 of 17 World Health Organization grade I, 11 of 14 grade II and four of six grade III meningiomas. Phospho-p38MAPK was detected in nine of 17 grade I, two of six grade II, and four of six grade III meningiomas. In the majority of meningiomas DUSP10 expression correlated inversely with phosphorylation of p38MAPK. PDGF-BB increased DUSP10 in MC2 and MC4 and weakly in MC3. TGFB1 increased phosphorylation of p38MAPK and caspase 3 activation. Thus p38MAPK and DUSP10 likely participate in the pathogenesis of meningiomas.

1. Introduction

The mitogen activated protein kinase (MAPK) transmit growth factor receptor/tyrosine kinase signals that promote cell proliferation and alter apoptosis in many forms of neoplasia [1–3]. Of these p44/42MAPK is the most extensively studied in meningiomas and transduces signals from a number of growth factor receptor tyrosine kinases [4–7]. The effects of activated p38MAPK on meningioma formation have not been specifically studied but, in other tissues, acts as a tumor suppressor by promoting apoptosis and oncogene-induced senescence [8,9]. Nonetheless, many growth factors, such as transforming growth factor B1 (TGFB1) and platelet derived growth factor-BB (PDGF-BB), that appear to have autocrine [10,11] and paracrine/cerebrospinal fluid [12–16] effects on meningioma formation, signal, in part, via p38MAPK [1–3,8,9].

Activation of MAPK requires dual phosphorylation of threonine and tyrosine residues to activate the specific MAPK. p38MAPK signaling is terminated, in part, by dual specificity phosphatases (DUSP) that inactivate signaling by dephosphorylation of these residues on MAPK [17–20]. The DUSP family includes 10 phosphatases that regulate MAPK. DUSP1, 2, 4 and 5 are mitogen and stress activated DUSP. DUSP 6, 7, and 9 act on cytoplasmic p44/42MAPK. DUSP 8 and 10 act on cytoplasmic and nuclear MAPK and are more specific as p38MAPK phosphatases [17–23].

Due to their potential growth regulatory properties, these phosphatases may be targets for new therapies [8,9]. Such therapies are desperately needed because no consistently effective chemotherapy has been identified for meningiomas, particularly inoperable or recurrent tumors [24,25].

2. Materials and methods

2.1. Human leptomeningeal and meningioma tissue

Tissues were collected at the University of Rochester Medical Center or the Cooperative Human Tissue Network (Philadelphia, PA, USA), with Institutional Review Board approval and World Health Organization (WHO) grading [26] (Table 1–3). 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 tissue as described previously [15] (Table 1–3).

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Table 1
Meningioma tissue used for western blots

Subtype	N	Mean age	Sex
Leptomeninges, fetal	5		U
Leptomeninges, adult	1		
WHO grade I	17	57 years	14F
Meningothelial	7 (41%)		
Transitional	7 (41%)		
Fibrous	3 (18%)		
WHO grade II	14	50 years	10F
Meningothelial	6 (43%)		
Transitional	3 (21%)		
Fibrous	4 (29%)		
Microcystic	1 (7%)		
WHO grade III	6	61 years	4F
Anaplastic	3 (50%)		
Transitional	1 (17%)		
Fibrous	1 (17%)		
Papillary	1 (17%)		

F = female, U = unknown, 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	1F
WHO grade I	7	65 years	4F
Meningothelial	5 (71%)		
Transitional	2 (29%)		
WHO grade II	11	60 years	6F
Meningothelial	3 (%)		
Transitional	1 (%)		
Fibrous	4 (%)		
Secretory	1 (%)		
Microcystic	1 (%)		
WHO grade III	3	64 years	2F
Anaplastic	2 (67%)		
Papillary	1 (33%)		

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

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 [27]. Only cerebrospinal fluid with no inflammation from patients with no neurological disease was used.

2.4. Western blot analysis

Western blots were prepared as described previously loading 35 μg protein [16]. Detection was achieved with Clarity Western ECL substrate (BioRad Laboratories, Hercules, CA, USA) and Chemidoc software. Blots were evaluated using antibodies to DUSP10/MKP5 (1:1000), and phospho-p38MAPK phosphorylated at Thr180/Tyr182 and phospho-p44/42MAPK phosphorylated at threonine 202 and tyrosine 204 (1:400) or GAPDH (1:1000) (all from Cell Signaling Technology, Beverly, MA, USA). The majority of western blots were repeated. Band intensity was normalized relative to GAPDH using the Image Lab for Chemidoc (Bio Rad Laboratories).

2.5. Analysis of DUSP10 RNA

RNA was isolated as described previously [16]. Reverse transcription was performed as described previously [16] using 20 base DNA primers DUSP10L and DUSP10R from Integrated DNA Technologies, Coralville, IO, USA.

Table 3
Sources of leptomeninges and meningiomas used for primary cultures

LC/MC	Age/Sex	Location	Classification/WHO grade	Merlin
LC1	20 weeks	Convexity	Leptomeninges	ND
LC2	20 weeks	Convexity	Leptomeninges	ND
LC3	22 weeks	Convexity	Leptomeninges	ND
MC1	65/F	Clivus	Meningothelial/I	+
MC2	51/F	Convexity	Meningothelial/I	ND
MC3	51/F	Right frontal	Transitional/II	ND
MC4	43/NA	T4 spinal	Transitional/II	ND
MC5	74/F	Frontal	Meningothelial/II	+
MC6	57/F	Left frontal	Transitional/II	+

F = female, LC = leptomeninges culture, MC = meningioma culture, NA = not available, ND = not done, WHO = World Health Organization, + = present.

2.6. PDGF-BB and cerebrospinal fluid effects

Primary cultures from three WHO grade I and five WHO grade II meningiomas (MC) were serum deprived overnight then treated then treated with Dulbecco's modified Eagle's medium (DMEM), or DMEM with PDGF-BB (10 ng/ml) or cerebrospinal fluid for 72 hours. Cells from four WHO grade II meningiomas were also treated as above for 24 hours.

2.7. TGFB1 effects

Cells from four WHO grade II meningiomas were serum deprived overnight, treated with DMEM, or DMEM with TGFB1 (10 ng/ml), PDGF-BB (10 ng/ml) or PDGF-BB (10 ng/ml) and TGFB1 (10 ng/ml) for 4 hours, then evaluated by western blot. Poly (ADP ribose) polymerase 1 (PARP) fragmentation is an indirect, semi-quantitative measurement of caspase 3 activation and apoptosis. Cleavage of the 117-kDa PARP into the 85 kDa product was assessed by western blot with antibody to PARP (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and blotting procedures as detailed previously [16].

3. Results

3.1. DUSP10, phospho-p38MAPK and phospho-p44/42MAPK in human leptomeningeal and meningioma tissue

DUSP10 was detected in all five fetal and the adult leptomeninges tested, 13 of 17 WHO grade I and 11 of 14 grade II meningiomas and four of six grade III meningiomas (Fig. 1 and data not shown).

Phospho-p38MAPK was detected in all six leptomeninges, nine of 17 grade I and two of six grade II meningiomas tested and four of six grade III meningiomas.

Phospho-p44/42MAPK was detected in all six leptomeninges, all 17 grade I, all 14 grade II, and all six grade III meningiomas tested (Fig. 1 and data not shown).

Two of six leptomeninges with high DUSP10 had low phospho-p38MAPK. None with low DUSP10 had high phospho-p38MAPK. Three of six with low DUSP10 also had low phospho-p38MAPK.

In grade I tumors, three with moderate DUSP10 had low phospho-p38MAPK, and one with low DUSP10 had high phopspho-p38MAPK.

In grade II tumors, one with high DUSP10 had low phospho-p38MAPK, while five with low DUSP10 had low phospho-p38MAPK.

In grade III tumors, one with DUSP10 had low phospho-p38MAPK, and one with low DUSP10 had high phopspho-p38MAPK.