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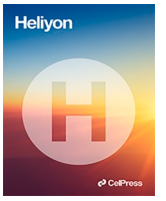

Research article

Cytokine profile of cerebrospinal fluid in pediatric patients with metastatic medulloblastoma

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ABSTRACT

Background: Medulloblastoma (MB) is a malignant pediatric central nervous system tumor that is prone to leptomeningeal metastasis. Currently, apart from magnetic resonance imaging and cerebrospinal fluid (CSF) cytology, there are no reliable biomarkers for MB progression. Cytokines are key proteins in signaling pathways in the tumor microenvironment and are closely related to tumor recurrence and progression. This study aimed to investigate the CSF cytokine profile in pediatric patients with MB to identify biomarkers of tumor progression and metastasis.

Methods: In total, 10 patients were recruited for this study. Five patients had nonmetastatic MB and five had metastatic MB. A cytokine antibody array was used to detect the expression of 120 cytokines in the CSF, and differentially expressed cytokines were screened by integrated bioinformatics analysis.

Results: Twenty-seven cytokines were upregulated in patients with MB compared to control individuals. Of these, eight were upregulated by $>$ 1.5-fold (CCL2, BMP-4, beta-NGF, FGF-7, IL-12p40, eotaxin-2, M-CSF, and NT-4). Twelve cytokines were differentially expressed between patients with metastatic MB and nonmetastatic (nine cytokines were upregulated and three were downregulated). Among them, NAP-2, MIP-1α, MIP-1β, IGFBP-1, IGFBP-2 and IGFBP-3 were upregulated by more than two-fold. Gene Ontology analysis revealed that the upregulated cytokines were enriched mainly in "epithelial cell proliferation" and "chemotaxis," and the Kyoto Encyclopedia of Genes and Genomes analysis indicated the enrichment of the "MAPK," "PI3K-Akt," and "Ras" signaling pathways.

Conclusions: The present study investigated cytokine profiles in the CSF of pediatric patients with MB. Our results suggest that these differentially expressed cytokines may serve as novel markers for detecting MB, especially for assessing the risk of progression and metastasis.

1. Introduction

Medulloblastoma (MB) is the most common embryonal tumor and accounts for approximately 20 % of pediatric central nervous system (CNS) tumors. Although the cure rate is approximately 70 %, the prognosis of patients with high-risk disease is poor [1]. MB

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readily spreads to other areas of the brain and spinal cord through the cerebrospinal fluid (CSF), especially in groups 3 and 4. Recurrence and metastasis are the main causes of death in children [2]. Currently, MB diagnosis relies on clinical symptoms and radiological findings, and the final diagnosis depends on histopathological examination. Due to the lack of markers for measurable residual disease (MRD), it remains challenging to use traditional imaging and CSF cytology to assess recurrence and progression [3]. Recently, the analyses of circulating tumor DNA, exosomes, circulating tumor cells, cytokines, and other components in plasma or other body fluids (including CSF) have become important for evaluating treatment response and metastasis [4]. CSF can be used as an important medium for liquid biopsy, as it interacts with tumor cells in the CNS more frequently than plasma [5]. Studies have shown that cell-free DNA in CSF can be used to detect MRD in patients with MB and predict treatment response [3]. Evidence suggests that certain mechanisms contribute to the ability of medulloblastomas to evade natural eradication by the immune system. Tumor-infiltrating immune cells play various roles in the tumor biology of medulloblastomas. Reports showed that patients with medulloblastoma have reduced systemic T-cell activity [6]. Natural killer (NK) cells have also been described as capable of ablating medulloblastoma cells through the activation of NKp46, NKp30, DNAM-1, and NKG2D receptors [7]. T helper-17 lymphocytes have also been found infiltrating medulloblastoma tumors [8]. In addition, tumor-infiltrating immune cells influence tumor growth through the secretion of cytokines, growth factors, and other metabolic determinants. Malignancies can result in cytokine storms (often triggered from inborn errors of immunity, cancer immunotherapy, or certain infections and carcinogens) [9,10], which are well-characterized in infection and inflammation but poorly characterized in cancer. A previous study demonstrated that aflatoxin B1-generated cell death triggers hepatocellular carcinoma tumor dormancy escape via a proinflammatory eicosanoid and cytokine storm. Resolution of cytokine storms prevent hepatocellular cancer progression initiated by carcinogens and inflammation [11]. The relationship between cytokine storms and tumor metastasis has been reported. Post-near-death cells acquire pro-metastatic states (PAMEs) and induce neighboring tumor cells to become PAME-induced migratory cells, forming distant metastases [12]. To date, the mechanism of cytokine storms in medulloblastoma has not been reported, and there are few studies on CSF cytokines detection in patients with MB. Additional systematic studies are still needed to evaluate the application value of CSF cytokines. Therefore, the aim of this study is to explore CSF cytokine profiles and identify novel cytokine biomarkers for predicting recurrence and metastasis in pediatric patients with MB.

2. Materials and methods

2.1. Patient demographics

CSF samples from 10 patients with MB (pathologically confirmed) who were admitted to the Pediatric Neurosurgery Department of Xinhua Hospital affiliated with the Shanghai Jiao Tong University School of Medicine were obtained. All the samples were collected through lumbar puncture before surgery. of which, four were male and six were female, and the mean age was 6.4 years (range: 2–10 years). Each tumor was histologically subtyped into classic (eight patients) and desmoplastic/extensive nodularity (two patients) based on the 2016 World Health Organization classification of CNS tumors [13]. For molecular subtyping, two patients had SHH (Sonic hedgehog), four had group 3, and four had group 4. All patients underwent standard posterior fossa surgical resection and gross total resection. The extent of resection was based on postoperative imaging and defined as gross total resection (no residual tumor) [14]. Brain and whole-spinal cord magnetic resonance imaging (MRI) were routinely performed during postoperative follow-up. The average follow-up time was 45.0 months. Five patients presented with CSF drop metastases either before surgery or during follow-up, and the other five patients had no metastases. CSF samples were centrifuged immediately after collection and stored at −80 °C. In addition, six patients diagnosed with arachnoid cysts in our department were selected as the normal control group. In this control group, there were three males and three females, and the mean age was 5.8 years (range: 2–10 years). This study was approved by the Institutional Review Board of Xinhua Hospital Affiliated with the Shanghai Jiao Tong University School of Medicine, and informed consent was obtained from the patients' parents. The basic clinical information of the study cohort is shown in Table 1.

Table 1
Summary of the study cohort's clinical data.

Patient No.	Gender	Age at diagnosis	Extent of resection	Histological subtype	Molecular subtype	Metastasis at initial diagnosis	Location of metastasis
1	Female	2	GTR	DNMB	SHH	No	/
2	Female	7	GTR	Classic	Group3	No	/
3	Female	7	GTR	Classic	Group3	No	/
4	Female	7	GTR	Classic	Group4	No	/
5	Female	10	GTR	Classic	Group4	No	/
6	Male	7	GTR	Classic	Group3	Yes	Intracranial, spine
7	Male	8	GTR	Classic	Group3	Yes	Intracranial, spine
8	Female	10	GTR	Classic	Group4	Yes	Intracranial, spine
9	Male	2	GTR	Classic	Group4	Yes	Intracranial, spine
10	Male	4	GTR	DNMB	SHH	Yes	Intracranial, spine

GTR, gross total resection; DNMB, desmoplastic/nodular medulloblastoma.

2.2. Antibody array processing

According to the manufacturer's instructions, 120 cytokines (Table 2) were measured using a semiquantitative human cytokine antibody array (G-Series Human Cytokine Antibody Array 2000, Raybiotech, Norcross GA, USA). Briefly, sample diluent (100 μL) was added into each well and incubated at room temperature for 30 min to block slides. The samples were decanted from each well, and then washed five times with wash buffer at room temperature with gentle shaking. Then, the detection antibody was reconstituted by adding sample diluent to the tube, and the detection antibody cocktail was added to each well and incubated at room temperature for 1–2 h. Samples were then incubated with Cy3 equivalent dye-conjugated streptavidin, and washed five times at room temperature with gentle shaking. Finally, the fluorescent signals were detected using a laser scanner (GenePix 4000B Microarray Scanner; Axon, Boston, MA, USA). The densities of the individual spots were measured using ImageJ software to determine the relative cytokine concentrations. With this analysis tool, signals are normalized using internal positive and negative controls included in the array. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were also conducted to determine the biological role of differentially expressed cytokines (DECs).

2.3. Identification of cytokine levels by ELISA

ELISA (Raybiotech) was used to detect the eight cytokines exhibiting the most significant differences (CCL2, BMP-4, beta-NGF, FGF-7, IL-12p40, eotaxin-2, M-CSF, and NT-4) according to the manufacturer's protocol. After dilution of the standards and sample loading, incubation, and washing steps, the absorbance of each well was measured at 450 nm using a microplate spectrophotometer. The experiments were repeated triplicated independently.

2.4. Statistical analysis

The protein microarray data were statistically analyzed using the IBM Statistical Package for Social Science Statistics 20 software. The measurement data are expressed as the mean ± standard deviation (mean ± standard error of the mean), and GraphPad Prism 5.0 or Excel 2007 software was used for data input and graphing. The statistical significance of the differences was calculated using Student's *t*-test or one-way analysis of variance. A P-value of <0.05 was considered statistically significant.

Table 2
Target cytokines in the semi-quantitative human antibody array.

No.	Cytokines	No.	Cytokines	No.	Cytokines	No.	Cytokines
1	6Ckine(CCL21)	31	MPIF-1(CCL23)	61	IGFBP-4	91	IL-1ra
2	Axl	32	MSP alpha/beta	62	IGFBP-6	92	IL-2
3	Betacellulin(BTC)	33	NAP-2(PPBP/CXCL7)	63	IGF-1	93	IL-4
4	CCL28(MEC)	34	Osteopontin(SPP1)	64	Insulin	94	IL-5
5	CTACK(CCL27)	35	PARC(CCL18)	65	M-CSF R	95	IL-6
6	CXCL16	36	CXCL4	66	NGFR(TNFRSF16)	96	IL-6 R
7	ENA-78(CXCL5)	37	CXCL12 alpha	67	NT-3	97	IL-7
8	Eotaxin-3(CCL26)	38	TARC(CCL17)	68	NT-4	98	IL-8
9	GCP-2(CXCL6)	39	TECK(CCL25)	69	Osteoprotegerin	99	IL-10
10	GRO	40	TSLP	70	PDGF-AA	100	IL-11
11	HCC-1(CCL14)	41	Amphiregulin	71	PLGF	101	IL-12p40
12	HCC-4(CCL16)	42	BDNF	72	SCF	102	IL-12p70
13	IL-9	43	bFGF	73	SCF R(CD117/c-kit)	103	IL-13
14	IL-17F	44	BMP-4	74	TGF alpha	104	IL-15
15	IL-18 BP alpha	45	BMP-5	75	TGF beta 1	105	IL-16
16	IL-28A	46	BMP-7	76	TGF beta 3	106	IL-17A
17	IL-29	47	beta-NGF	77	VEGF-A	107	MCP-1
18	IL-31	48	EGF	78	VEGFR2	108	M-CSF
19	IP-10(CXCL10)	49	EGFR	79	VEGFR3	109	MIG
20	I-TAC(CXCL11)	50	EG-VEGF(PK1)	80	VEGF-D	110	MIP-1a
21	LIF	51	FGF-4	81	BLC	111	MIP-1b
22	Light(TNFSF14)	52	FGF-7(KGF)	82	Eotaxin-1	112	MIP-1d
23	Lymphotactin(XCL1)	53	GDF-15	83	Eotaxin-2	113	PDGF-BB
24	MCP-2(CCL8)	54	GDNF	84	G-CSF	114	RANTES
25	MCP-3(MARC/CCL7)	55	Growth Hormone	85	GM-CSF	115	TIMP-1
26	MCP-4(CCL13)	56	HB-EGF	86	I-309	116	TIMP-2
27	MDC(CCL22)	57	HGF	87	ICAM-1	117	TNFa
28	MIF	58	IGFBP-1	88	IFNg	118	TNFb
29	MIP-3 alpha(CCL20)	59	IGFBP-2	89	IL-1a	119	TNF RI
30	MIP-3 beta(CCL19)	60	IGFBP-3	90	IL-1b	120	TNF RII

3. Results

3.1. CSF cytokine profile of MB patients as compared to control group

First, we compared the CSF cytokine data of all 10 MB patients with those of the control individuals. Based on the significance score ($P < 0.05$), 27 cytokines were upregulated in patients with MB, eight of which were upregulated by > 1.5-fold (CCL2, BMP-4, beta-NGF, FGF-7, IL-12p40, eotaxin-2, M-CSF, and NT-4) (Table 3). These cytokines were selected for validation by ELISA.

3.2. Differences in CSF cytokine expression between patients with metastatic and nonmetastatic MB

Compared with those in the nonmetastatic group, 12 cytokines were differentially expressed in the metastatic MB group (neutrophil-activating peptide 2 [NAP-2], macrophage inflammatory protein-1 [MIP-1α], MIP-1β, IGFBP-1, IGFBP-2, IGFBP-3, GDF-15, PDGF-BB, HGF, IL-12p40, amphiregulin, and Axl). Among these cytokines, IL-12p40, amphiregulin, and Axl were significantly downregulated, whereas the remaining nine cytokines were significantly upregulated (all $P < 0.05$) (Table 4). The cytokines that were increased by > 2-fold were NAP-2, MIP-1α, MIP-1β, IGFBP-1, IGFBP-2, and IGFBP-3. Fig. 1 shows the fluorescence signal intensities of the 12 cytokines in the two groups (each capture antibody was printed in quadruplicate horizontally, and the colored boxes indicate the different cytokines).

3.3. Integrated bioinformatics analysis

The cytokines that were differentially expressed between patients with MB patients and normal control individuals were subjected to unsupervised hierarchical clustering using Cluster 3.0 software, and 27 cytokines were significantly correlated ($P < 0.05$) (Fig. 2A). To elucidate the potential gene functional annotations and enriched pathways associated with the DECs, GO, and KEGG analyses were performed using the clusterProfiler (version 3.10.1) package. Herein, the upregulated cytokines were enriched mainly in "epithelial cell proliferation," "regulation of epithelial cell proliferation," "positive regulation of MAPK cascade," "taxis," and "chemotaxis" (biological process) (Fig. 2B). KEGG pathway analysis revealed that DECs were primarily involved in "cytokine–cytokine receptor interaction," "MAPK signaling pathway," "PI3K-Akt signaling pathway," and "Ras signaling pathway" (Fig. 2C).

3.4. ELISA verification

As previously shown, eight cytokines were upregulated by > 1.5-fold (CCL2, BMP-4, beta-NGF, FGF-7, IL-12p40, eotaxin-2, M-CSF, and NT-4) in patients with MB compared with control individuals. To reduce anomalies arising from individual deviation, ELISA was

Table 3
Protein microarray data of differential cytokines from medulloblastoma patients and controls.

Cytokines	Mean (Control,n = 6)	Mean (MB,n = 10)	Fold change (MB/Control)	*p* value
MCP-1(CCL2)	86.6	142.3	1.64	0.0463
BMP-4	77.2	115.4	1.50	0.0107
beta-NGF	102.6	157.3	1.53	0.0096
FGF-7(KGF)	109.1	163.5	1.50	0.0045
IL-12p40	114.7	180.1	1.57	0.0154
Eotaxin-2	113.6	170.1	1.50	0.0297
M-CSF	106.9	167.3	1.56	0.0011
NT-4	102.8	164.5	1.60	0.0100
I-309	88.9	125.7	1.41	0.0322
IFNg	114.7	148.6	1.30	0.0173
IL-1ra	82.7	110.0	1.33	0.0270
IL-16	139.7	186.1	1.33	0.0049
IL-28A	189.0	229.2	1.21	0.0001
IL-31	124.9	152.8	1.22	0.0015
MPIF-1(CCL23)	268.2	326.2	1.22	0.0071
TSLP	98.4	124.4	1.26	0.0001
Amphiregulin	128.4	179.6	1.40	0.0332
BDNF	146.0	206.7	1.42	0.0214
bFGF	115.2	161.1	1.40	0.0224
BMP-7	122.4	174.7	1.43	0.0280
IGF-1	96.8	143.5	1.48	0.0004
Insulin	114.3	145.4	1.27	0.0124
HB-EGF	87.2	122.0	1.40	0.0393
TGF alpha	88.3	109.6	1.24	0.0099
TGF beta 3	68.0	83.7	1.23	0.0030
VEGFR3	97.0	139.3	1.44	0.0092
VEGF-D	87.3	115.5	1.32	0.0023

MB: medulloblastoma.

Table 4
Protein microarray data of differential cytokines from metastasis and non-metastasis patients.

Cytokines	Mean (NMG,n = 5)	Mean (MG,n = 5)	Fold change (MG/NMG)	*p* value	Up/down (MG vs NMG)
NAP-2	405.4	4816.0	11.88	0.0397	Up
MIP-1a	154.1	1455.0	9.44	0.0484	Up
MIP-1b	363.8	1855.0	5.10	0.0453	Up
IGFBP-2	550.2	2718.6	4.94	0.0031	Up
IGFBP-3	119.2	420.6	3.53	0.0190	Up
IGFBP-1	358.1	912.7	2.55	0.0195	Up
GDF-15	242.8	423.9	1.75	0.0321	Up
PDGF-BB	119.8	209.8	1.75	0.0492	Up
HGF	139.7	208.3	1.50	0.0008	Up
IL-12p40	212.0	139.3	0.66	0.0325	Down
Amphiregulin	213.4	151.8	0.71	0.0345	Down
Axl	206.8	178.7	0.86	0.0082	Down

NMG: non-metastasis group; MG: metastasis group.

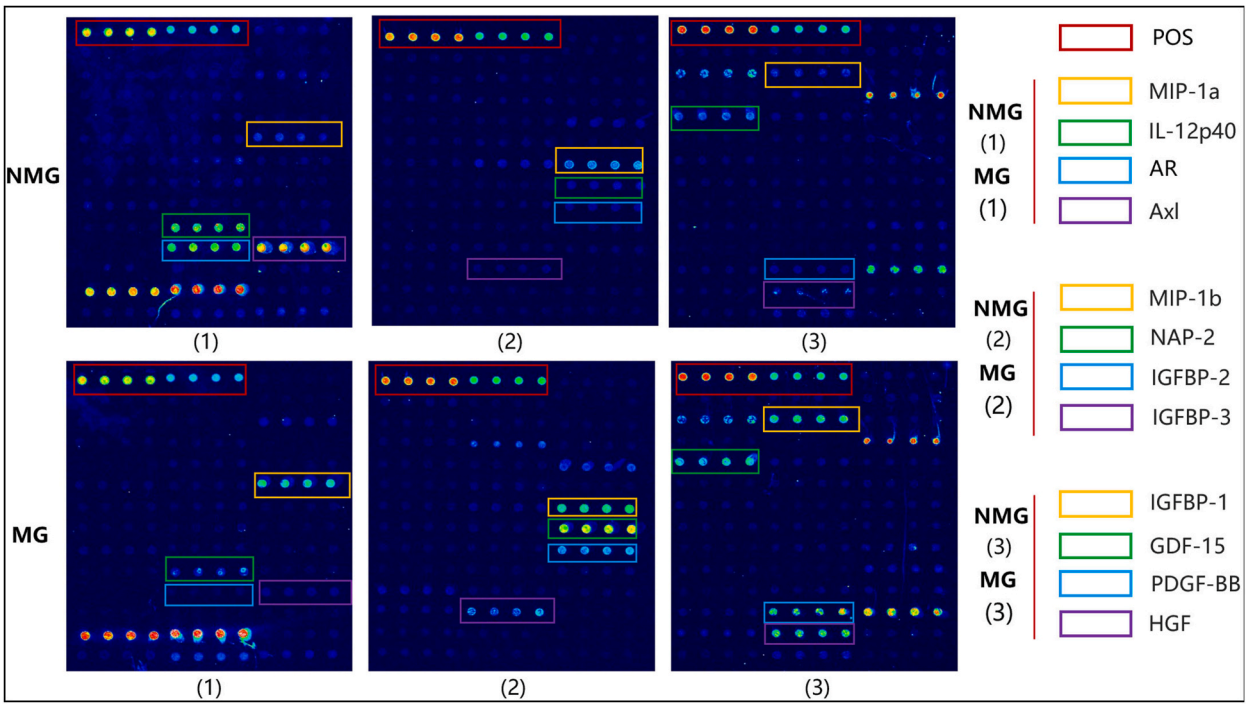

Fig. 1. Identification of CSF cytokines in MB patients in the metastasis and nonmetastasis groups via antibody arrays Each capture antibody was horizontally printed in quadruplicate. The colored boxes indicate the cytokines that were significantly different in the arrays. POS. Positive control; NMG: nonmetastatic group; MG: metastatic group; AR: amphiregulin.

performed to verify the results of the microarray analysis. The number of samples was increased to 30 MB and 20 control samples, and the results are shown in Fig. 3. Only five cytokines (CCL2, BMP-4, beta-NGF, FGF-7, and IL-12p40) were significantly different between patients and control samples, and the results were the identical to those of the microarray ($P < 0.05$). For the other three cytokines (eotaxin-2, M-CSF, and NT-4), ELISA showed increased expression in MB samples, but the difference was not significant ($P > 0.05$).

4. Discussion

MB originates in the cerebellum or brainstem and is prone to metastasis through the CSF. This tumor is highly malignant and has a poor prognosis. CSF drop metastases often occurs in the early stage, especially after multiple surgical treatments [15]. Currently, surgical resection combined with radiotherapy and chemotherapy is the preferred treatment option for MB [16]. Apart from MRI and CSF cytology, there are no reliable molecular biomarkers that reflect MB progression. Therefore, an independent method that can reliably monitor tumor treatment effects and detect early tumor recurrence before using advanced imaging technology is urgently needed.

Cytokines are synthesized by immune cells and certain nonimmune cells (endothelial, epidermal, or tumor cells). They regulate

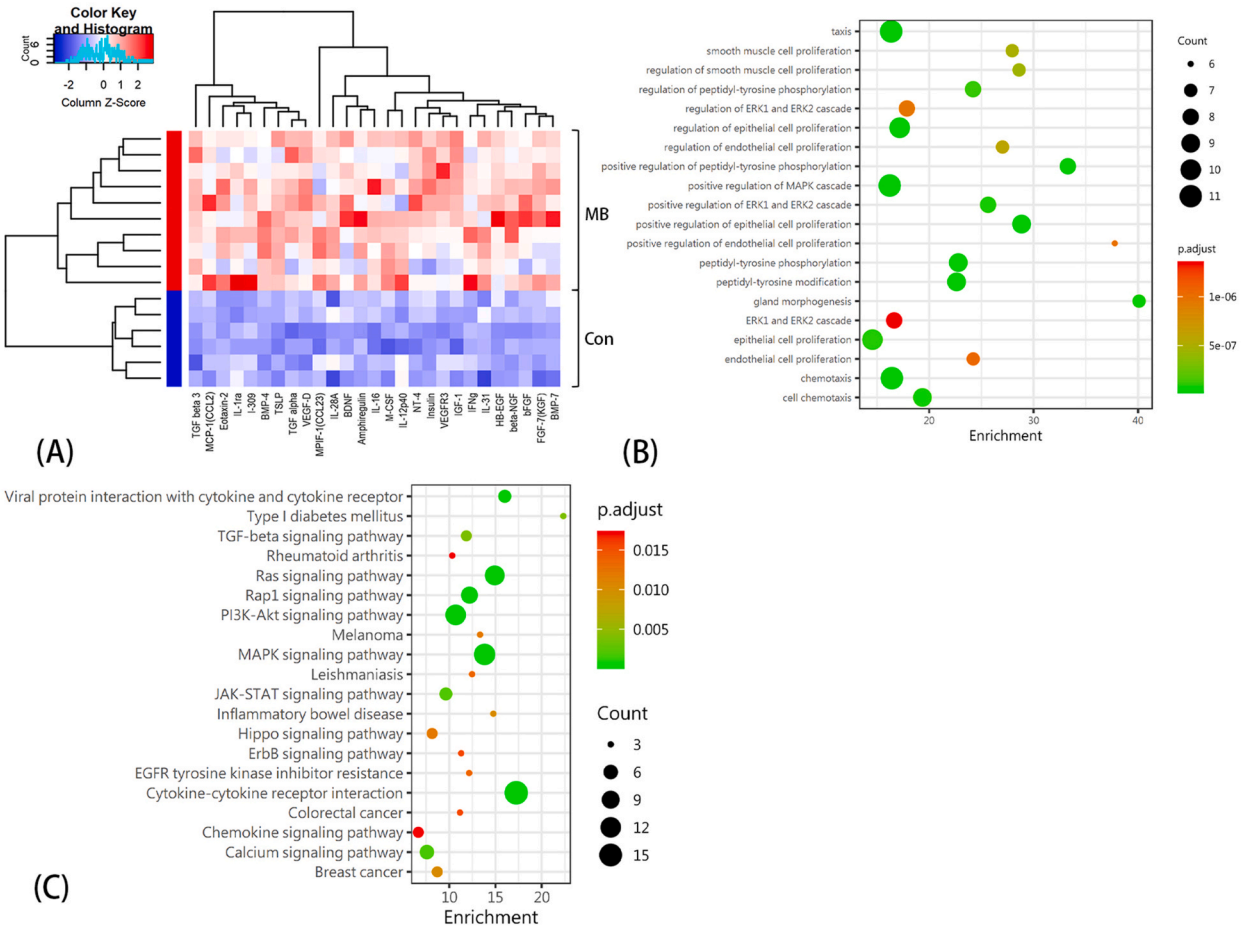

Fig. 2. Integrated bioinformatics analysis of differentially expressed cytokines. (A) Semiquantification of scanned antibody arrays. The levels were normalized to those of internal positive controls. Semiquantitative levels are represented in the heatmap. (B) and (C) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were also conducted to determine the biological role of differentially expressed cytokines in MB patients.

innate immunity, adaptive immunity, and cell growth by binding to corresponding receptors [17]. Changes in cytokine levels can regulate the tumor microenvironment (TME) and influence the proliferation and differentiation of immune and cancer cells [18]. Recently, the detection of CSF cytokines has gradually become a research hotspot in diagnosing CNS diseases, but such studies have focused mainly on inflammation and immune-related diseases [19,20]. There is currently only one previous study that investigated the expression of CSF cytokines in MB. Sharon et al. examined 43 cytokines (related to tumor angiogenesis) and reported that, compared with that in nonmetastatic MB, the expression level of CCL2 in the CSF was increased [21]. This is currently the only published study on the relationship between CSF cytokines and MB metastasis, and it offers insights into identifying CSF cytokine biomarkers for liquid biopsy of MB.

The present study analyzed the expression profiles of 120 cytokines in the CSF of patients with MB via an antibody array and revealed that 27 cytokines, including CCL2, BMP-4, beta-NGF, FGF-7, and IL-12p40, were markedly increased in patients with MB; these results was verified by ELISA. Integrated bioinformatics analysis revealed that the upregulated cytokines were mainly enriched in epithelial cell proliferation and chemotaxis, and were linked to the MAPK signaling pathway. In MB, overactivation of the MAPK/ERK pathway is often associated with tumor proliferation, invasion, metastasis, and drug resistance, and it represents another key molecular feature of group 4 MB [22]. The study revealed that CSF cytokines are related to the activation of the MAPK signaling pathway.

Cytokines are key mediators of cellular communication in the TME. Aberrant expression of inflammatory cytokines is a common downstream consequence of oncogenic changes in other nonmalignant cells. Cytokines are closely related to tumor development, progression, and metastasis [23]. Our study focused mainly on the relationship between CSF cytokines and MB metastasis. Compared with those in nonmetastatic patients, the levels of 12 cytokines, including chemokines (NAP-2, MIP-1α, and MIP-1β) and growth factors (IGFBP-1, IGFBP-2, and IGFBP-3), in patients with metastatic MB were increased; of these, six cytokines were upregulated by > 2-fold. These cytokines are discussed below.

NAP-2, also known as CXCL7, is a chemokine produced and stored in platelets, macrophages, monocytes, lymphocytes, and neutrophils. NAP-2 promotes inflammation, cell migration, proliferation, and angiogenesis; moreover, it is closely related to tumor

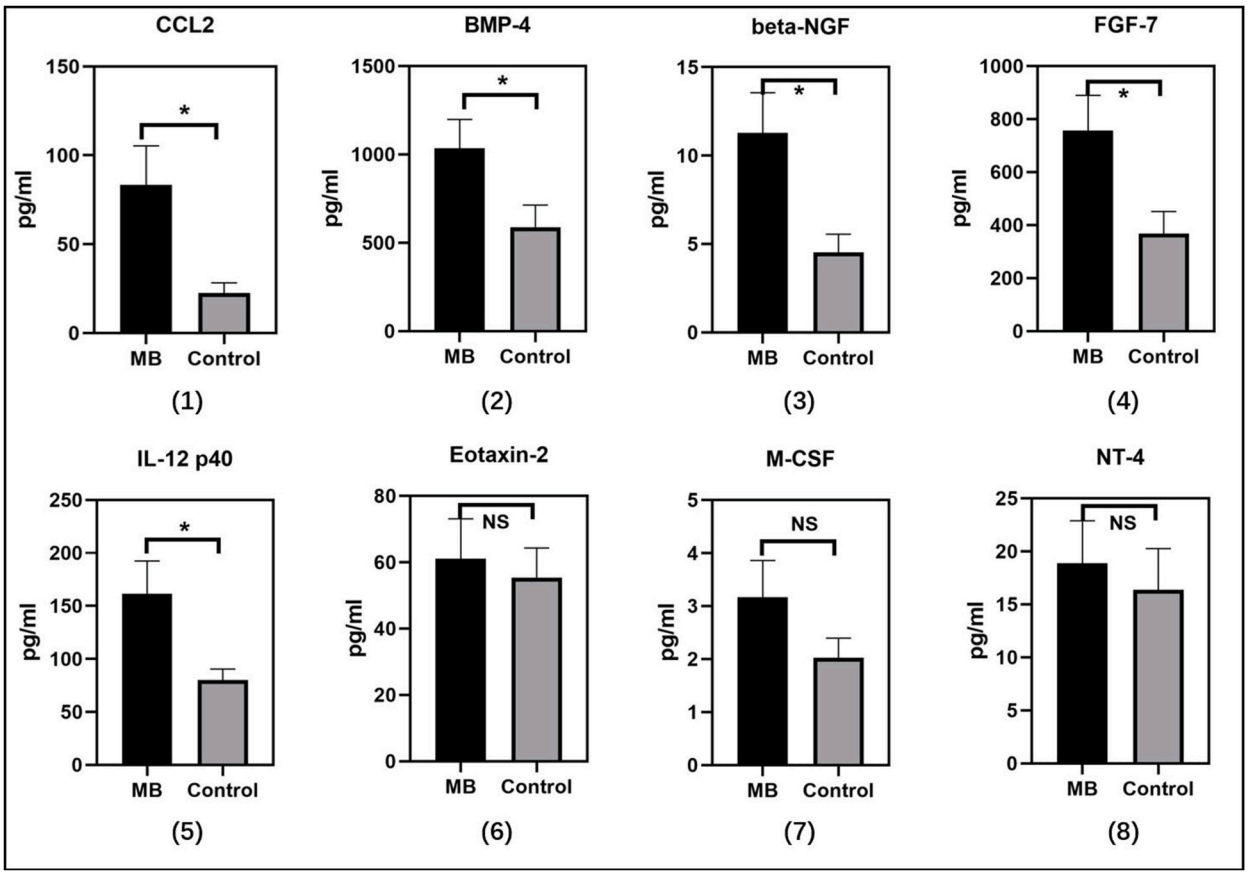

Fig. 3. Validation of differentially expressed cytokines in patients with MB and normal control individuals using an enzyme-linked immunosorbent assay (ELISA). Eight cytokines whose expression increased by more than 1.5 times were selected. Groups were compared using Student's *t*-test or one-way analysis of variance (ANOVA), and the P value for each protein was less than 0.05. MB: medulloblastoma.

development [24]. CXCL7 is a predictive marker of poor survival in patients with metastatic colorectal cancer [25]. IL-1β induces CXCL7 overexpression in renal cancer tissues, thereby leading to tumor cell proliferation and promotion of tumor growth. Blocking CXCL7 receptors (CXCR1 and CXCR2) can inhibit renal cancer endothelial cell proliferation and angiogenesis, thereby slowing tumor progression [26]. There have been some reports on CXCL7 in glioma. For instance, one study showed that the stem cell-like functions of glioma cells are regulated by FGL2 in the presence of macrophages, and the FGL2-CXCL7 paracrine signaling axis is critical for regulating this process [27]. However, there is no previous research on the relationship between MB and CXCL7. Our study revealed for the first time that CXCL7 is related to MB and may be involved in the mechanism of metastasis in patients with MB.

MIP-1 belongs to the CC chemokine family. The human MIP-1 family includes CCL3 (also known as MIP-1α) and CCL4 (also known as MIP-1β). MIP-1α is an important chemokine in humans that promotes the aggregation of T lymphocytes at inflammatory sites and can attract immune cells, such as NK cells and monocytes, to inflammatory sites [28]. Furthermore, MIP-1α is involved in the occurrence and development of various tumors [29]. Immunosuppression caused by glioma cells is considered a main factor leading to poor prognosis and reduced survival rate in patients with glioma. Immunosuppression fails to reduce tumor progression and interacts closely with glial cells to promote tumor progression [30]. Studies have shown that CCL3 is closely linked to glioma, with increased tumor-associated macrophage infiltration in the glioma TME. In addition, as the pathological grade of glioma increases, the number of antitumor macrophages (M1 macrophage phenotype) decreases. The CD163/CCL3 gene expression rate is greater in high-grade gliomas than in low-grade gliomas [31]. In addition, platelet-derived growth factor β is a potential inflammatory driver of high-grade glioma in children, and CCL3 is a key chemokine involved in this mechanism [32]. The relationship between CCL3 and MB has not been previously reported. Our study is the first to show that CCL3 in the CSF of patients with metastatic MB is significantly increased, suggesting that CCL3 could serve as an important marker for predicting metastasis.

CCL4 is the main chemotactic factor for T cells, NK cells, and macrophages. The chemokine CCL4 initiate inflammatory processes and participate in the migration of various cell types, affecting the proliferation, apoptosis, and inflammatory response of cancer cells [33]. Interaction between neurons and cancer cells can lead to tumor growth. For instance, retinal ganglion cells secrete heparin-binding cytokines, stimulating $CD8^+$ lymphocytes to secrete CCL4. This stimulates glioma-associated microglia/macrophages to secrete CCL5, which acts on the NF1-related optic nerve pathway to promote tumor progression [34]. As mentioned earlier, the TME of gliomas comprise immune (T cells and microglia) and nonimmune (neuronal) cells, which combine to form a "fertile soil" that

supports the initiation and progression of these tumors. In NF1-related low-grade gliomas, CCL5 secreted by microglia and bone marrow-derived myeloid cells was found to promote tumor growth. In the TME, neurons secrete the paracrine factor midkine, which stimulates the recruitment and activation of $CD8^+$ lymphocytes and secretion of CCL4. Thus, this induces the expression of CCL5 in microglia/myeloid cells, which triggers the cell cycle (proliferation signal) and inhibits cell apoptosis [35,36]. Our study is the first to confirm the relationship between CCL4 and MB. However, the underlying functional mechanism still requires further research.

Insulin-like growth factor (IGF) signaling plays an important role in mediating cell proliferation, differentiation, motility, development, and metabolism. IGFs differ from insulin in that they interact with six high-affinity IGF-binding proteins (IGFBPs) in the circulation and cellular environment. The IGF-IGFBP complex regulates complex, multilayered cell signaling cascades that induce cellular adaptations promoting survival, proliferation, and invasion in normal physiological processes and various cancer types. IGFBPs are emerging as biomarkers for various diseases and are involved in multiple cell signaling pathways that regulate tumor development. These molecules may also be useful prognostic biomarkers in various malignant tumors such as nasopharyngeal, ovarian, and pancreatic cancers [37–39]. IGFBPs have been confirmed to play an important role in CNS tumors [40]. IGFBP-2 overexpression is very common in high-grade gliomas, and has been established to be a driver of glioma development, as its expression has significant effects on glioma progression and survival [41]. Preoperative plasma IGFBP-2 levels are significantly greater in patients with high-grade gliomas than in patients with low-grade gliomas, and are significantly associated with recurrence and disease-free survival in patients with glioblastoma (GBM) [42]. IGFBP-2-driven tumors depend on the continued expression of IGFBP-2, and elimination of this oncogenic signaling pathway results in a significant slowing of tumor progression. Thus, inhibiting IGFBP-2 hinders the spread of tumor cells [41]. In addition, the relationship between IGFBP5 and gliomas has been confirmed. For instance, IGFBP5 binds to ROR1 to promote the formation of the ROR1/HER2 heterodimer, thereby inducing CREB-mediated expression of ETV5 and FBXW9 as well as promoting GBM stem cell-like cell invasion and tumorigenesis [43]. Studies have confirmed that IGFBP3 is also upregulated in gliomas and is associated with tumor histology and IDH1/2 mutations. Knockdown of IGFBP3 directly induces apoptosis and inhibits tumor growth [44]. To date, the relationship between IGFBPs and MB has been confirmed by several studies. For instance, the expression level of IGFBP-2 is significantly increased in MB, which promotes the proliferation and cell migration of SHH MB through STAT3 signaling. Furthermore, IGFBP-2 expression in MB indicates poor prognosis [45]. Analysis of the expression levels of IGFs and IGFBPs in CSF showed that in patients with MB, IGFBP-3 protein levels were significantly greater than those in control individuals. Furthermore, the elevated levels of IGFBP-3 proteolytic fragments are found in the CSF of patients with MB, which is consistent with the significantly elevated IGFBP-3 proteolytic activity in the CSF of these patients [46]. Additionally, IGFBP-3 expression is closely related to the molecular classification and clinical characteristics of MB [47]. The present study revealed that IGFBP-1, IGFBP-2, and IGFBP-3 were significantly increased in the CSF of patients with metastatic MB. These findings suggest that IGFBPs may play an important role in MB progression and metastasis as well as serve as novel prognostic markers for MB.

5. Conclusions

In summary, we conducted a systematic analysis of cytokines in the CSF of pediatric patients with MB, elucidated the CSF cytokine profile of patients with MB, and focused on the relationship between these cytokines and tumor metastasis. We expect that these cytokines may serve as novel biomarkers for MB. However, whether these CSF cytokines are specific and useful for evaluating patient prognosis as well as predicting recurrence and metastasis requires further research.

Data availability statement

The datasets used for the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Yufan Chen: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Heng Zhao:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Haibo Zhang:** Formal analysis, Data curation. **Baocheng Wang:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Jie Ma:** Validation, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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