Medical Hypothesis

Extracting hydrocephalus diagnostic and therapeutic information from CSF proteomics

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Abstract

We hypothesize that the composition of the CSF provides specific information on damage sustained by the hydrocephalus-harboring brain and on its autoregulatory responses to the intracranial pressure (ICP) exerted on the parenchyma. The data analyzed to form this hypothesis was generated in a previous study by comparative proteomics of CSF collected from the brain of healthy and *Mpdz* knockout (KO) mice[1]. These mice phenocopy the severe hydrocephalus linked to human loss-of-function mutants of the same gene[2, 3]. The overall protein concentration was 2.5-fold higher in the CSF of *Mpdz* KO versus healthy mice. Practically all the 313 proteins identified by mass spectroscopy were overabundant in the CSF of the KO mice. Proteins that were more than 2-fold overabundant in the CSF of hydrocephalic mice were classified into seven functional groups. The overabundance of extracellular matrix (ECM) proteins, complement factors, and apolipoproteins indicated that the hydrocephalic brain underwent ischemia, inflammation, and demyelination. The overabundance of cytokine-binding proteins was linked uniquely to the activation of insulin-like growth factor (IGF) signaling. The overabundance of angiotensinogen and pigment epithelium-derived factor (PEDF) indicated the activity of negative-feedback mechanisms to reduce CSF production by the choroid plexus. These observations raise intriguing propositions: the composition of the CSF could be used as biomarker of case-specific injuries of ventriculomegaly in fetuses or neonates; once the overabundance of these biomarkers is detected, the IGF and angiotensin signaling pathways could be exploited to reduce CSF production as a non-invasive therapy, replacing or aiding current invasive treatments of hydrocephalus.

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Glossary
Apo = apolipoprotein; CNS = central nervous system; ECM = extracellular matrix; ETV = endoscopic third ventriculostomy; ICP = intracranial pressure; IGF = insulin-like growth factor; IGFBP = IGF-binding protein; KO = knockout; LFQ = label-free quantification; Mpdz = multi PDZ; PDZ = PSD-95 (= postsynaptic density 95), Dlg (= discs large), ZO-1 (= zonula occludens-1); PEDF = pigment epithelium-derived factor; SVZ = subventricular zone; VP = ventriculoperitoneal; WT = wild type.

Hydrocephalus is the most common cause of pediatric surgical intervention[4], occurring about once per 1000 births in the US[5]. The currently available treatments, either ventriculoperitoneal (VP) shunting or endoscopic third ventriculostomy (ETV), fail in 12%-30%[6] or in 24%-45%[7] of cases, respectively. The hospitalization required for these procedures costs annually more than 2 billion dollars[8]. CSF drainage from the brain ventricles to the peritoneum, which is still the prevalent treatment for hydrocephalus, requires multiple revisions during the patients' lifetime at an average interval of 14.1 years[9]. These exigencies provide the rationale for the implementation of non-invasive approaches in place of or in support of invasive treatments. To date, no effective non-invasive treatment has been reported.

Shunt implantation facilitates sampling and analysis of the CSF for early detection of infection[10]. It could conceivably be analyzed also for the evaluation of the health of the parenchyma. If suitable markers are identified, their overabundance in the CSF of patients relative to the CSF of healthy subjects could potentially serve as biomarkers of case-specific injuries. Furthermore, the composition of the CSF could provide information on the responses of the brain to the elevated ICP exerted on the parenchyma as a result of ventriculomegaly. Delineation of the molecular pathways employed by the brain to this end could be used in principle to guide future pharmacological approaches, including the identification of new druggable targets.

The information gained from comparing the compositions of the CSF of hydrocephalic versus healthy subjects by proteomic analysis had been mined previously for studying the response of the brain to ventriculomegaly[11]. Similarly, such comparison had been used to identify biomarkers for facilitating the diagnosis and characterizing the pathophysiology of hydrocephalus[12].

The following analysis, based on our published data[1], reveals previously unappreciated information gained from comparative proteomic analysis between the CSF of healthy and hydrocephalus-harboring *Mpdz* KO mice. This information identifies the nature of the damage inflicted by ventriculomegaly on the parenchyma and detects autoregulatory pathways activated by the brain to counter this damage. These pathways could be exploited for therapeutic purposes.

Methods
The animals, CSF collection, identification of its constituent proteins and measurement of their concentrations by mass spectroscopy were described in Yang et al., 2019[1]. Access to the mass spectroscopy data set is provided in the same publication.

Results and Discussion
Out of the total 313 proteins identified in 3 samples of CSF from either wild type or *Mpdz* KO mice, 23 were at least twice overabundant in the CSF of the latter mice in a statistically significant manner (i.e. P, the probability of the null hypothesis, was ≤ 0.05) (Table 1). Based on their known attributes, we classified them into a relatively small number of functional groups (from top to bottom in Table 1): extracellular matrix (ECM), complement factors, lipoproteins, immune system proteins, cytokine binding proteins, enzymes and enzyme binding proteins, and peptidase inhibitors. Given the combination of these functional groups, the overabundance of the above 23 proteins was interpreted as a concerted

reaction of the parenchyma to ventriculomegaly rather than a haphazard collection[1]. The proteins were scanned against the literature to examine their potential association with central nervous system (CNS) pathological conditions. Based on the matches, the overabundance of each protein was classified into two groups: either as an indicator of damage or as a response of the parenchyma to the trauma of severe hydrocephalus. The inclusion of proteins in the causal group was informed by the known attributes of the pathophysiology of congenital hydrocephalus. Proteins were categorized as forming the brain's response if they were known as constituents of autoregulatory pathways that would potentially counter ventriculomegaly.

Despite the multiple etiologies of congenital hydrocephalus, i.e. 'obstructive' versus 'communicating', the pathophysiology of this condition is shaped primarily by ventriculomegaly and the ensuing elevation of ICP. The damage to the parenchyma is manifested as neuroinflammation and demyelination, one of the secondary injury mechanisms attributed to ventriculomegaly[13]. The compression exerted on the parenchyma by increased ICP is accompanied by cerebrovascular injury, resulting in ischemia and hypoxia[14].

Biomarkers of brain injury

There is evidence to associate most of the overabundant ECM proteins, as well as complement factors and apolipoproteins, with mechanisms of ventriculomegaly-inflicted injuries (Table 2). ECM1 had been linked to neuroinflammation, whereby it ameliorated the demyelination caused by T helper cells[15]. Plasma fibronectin, the soluble isoform detected in our previous study[1], supported neuronal survival and reduced brain injury following cerebral ischemia[16]. Extracellular gelsolin ameliorated inflammation[17] and had a neuroprotective effect during stroke[18]. Vitronectin activated microglia[19], the primary immune effector cells of the CNS.

The complement system has a neuroprotective role in CNS neurodegenerative diseases[20]. Specifically, complement C4-B is produced by microglial cells in response to ischemia and inflammation[21]. Complement regulator H is produced by neurons and has a protective function in neuroinflammation[22]. Properdin, named alternatively complement factor P, activates microglia and contributes to inflammation in response to ischemia[23].

Apolipoprotein E (ApoE), which is produced by the choroid plexus[24], protects the brain against injury and promotes neuronal survival[24, 25]. ApoD has a similar neuroprotective role in CNS degeneration[26].

Collectively, the overabundance of the aforementioned ECM proteins, complement factors, and apolipoproteins reveal that severe hydrocephalus entails a set of injuries to the parenchyma that includes neuroinflammation and demyelination, impaired perfusion. Activation of CNS immune defense by microglial cells accompanies these processes and possibly aggravates inflammation. This injury pattern conforms to the empirical picture of neonatal hydrocephalus[14].

Biomarkers of activate autoregulatory pathways

The IGF-binding proteins (IGFBPs) are expressed in the CNS, including the choroid plexus[27]. Specifically, IGFPB2 and IGFBP4, which were overabundant in the CSF of *Mpdz* KO mice[1], are produced by astrocytes in response to brain injury[28]. While the differential functions of each IGFPB are not fully understood, they are thought to stabilize IGF, extend it half-life[29], and modulate its activity[30]. α-2-HS-glycoprotein, which binds to and modulates the signaling of the IGF receptor[31], α-fetoprotein, a cytokine-binding[32] plasma component, and sulfhydryl oxidase 1 participate in IGF uptake and transport by IGFPBs[33]. Remarkably, all the 5 proteins in this group (Table 3) regulate multiple facets of IGF signaling. IGF is produced in choroid plexus epithelial cells[34]. Its secretion into the CSF is increased in response to injury[35].

The two overabundant endopeptidase inhibitors, angiotensinogen and PEDF (Table 4), are biomarkers of pathways that would evidently remedy the detrimental effects of ventriculomegaly. Angiotensinogen is a precursor of angiotensin-2, a potent vasoconstrictor that reduces blood flow to the choroid plexus[36] and CSF production[37], thus countering the rise of ICP and the expansion of the ventricles. PEDF, which is secreted by ependymal cells in the subventricular zone (SVZ)[38] would potentially have a similar negative effect on CSF production because of its anti-angiogenic activity[39]. Additionally, PEDF promotes self-renewal of neural stem cells in the SVZ niche[38], thus possibly facilitating the replacement of neurons damaged as a result of the deformation of the parenchyma by the elevated ICP.

Potential diagnostic and therapeutic implications

The hypothetical interpretation of the overabundance of ECM proteins, complement factors, and apolipoproteins of as biomarkers of specific brain injuries inflicted by ventriculomegaly is supported by previous studies[14]. Their individual or collective overabundance could be potentially identified and measured in fetal CSF collected during corrective surgery for myelomeningocele or spina bifida, in the CSF of neonates collected during VP shunting or ETV, or in CSF drawn by lumbar puncture for other diagnostic purposes. Beyond verifying the occurrence of hydrocephalus, measuring the overabundance of individual biomarkers would highlight case-specific attributes, such as the presence of demyelination revealed by the overabundance of ECM1, or of an aggressive immune response flagged by the overabundances of vitronectin and complement factors.

The combined overabundance of 5 cytokine-binding proteins provides unambiguous evidence that IGF signaling is a major autoregulatory pathway employed by the brain to counteract the damage caused by ventriculomegaly. Similarly, the concurrence of the overabundances of angiotensinogen and PEDF is a strong indicator that reduction of CSF production is employed by the brain as a negative feedback loop to counter ventriculomegaly and increased ICP. Each of these pathways could potentially be exploited for non-invasive therapies in place of or as adjuvants to the current invasive approaches. Finally, the marked overabundances of ApoE and ApoD suggest that these apolipoproteins may be used directly for the neuroprotection of the parenchyma.

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Table 1: List of proteins that were at least twice overabundant in the CSF of *Mpdz* KO mice relative to the CSF of healthy mice with a null hypothesis probability (*P*) of ≤ 0.05[1]. The alternative clear and gray shadings separate the functional groups with which each protein is associated (from top to bottom): ECM proteins, complement factors, apolipoproteins, immune system proteins, cytokine binding proteins, enzymes and enzyme-binding proteins, and endopeptidase inhibitors. The ratios of label-free quantification (LFQ) intensities provide the KO to wild type (WT) relative abundance of each protein (modified from Yang et al., 2019[1]).

Protein name	Gene ID	MW (kDa)	Average LFQ intensities ($\times 10^3$)		LFQ KO/WT ratio	*P*-value
			***Mpdz*$^{+/+}$**	***Mpdz*$^{-/-}$**		
Extracellular matrix protein 1	Ecm1	48.356	9.247	108.1	11.691	0.0012
Fibronectin	Fn1	272.53	594.234	6292.53	10.589	0.015
Gelsolin	Gsn	85.94	2186.3	9651.9	4.415	0.0018
Vitronectin	Vtn	54.84	409.711	1280.37	3.125	0.026
Complement C4-B	C4b	192.91	586938	3005.67	5.121	0.0091
Complement factor H	Cfh	139.14	3049.323	9739.17	3.194	0.042
Properdin	Cfp	50.32	101.668	283.75	2.791	0.042
Fibrinogen β chain	Fgb	54.75	45.285	2175.04	48.03	0.027
Apolipoprotein D	ApoD	21.53	45.159	604.07	13.377	0.0003
Apolipoprotein E	ApoE	35.87	955.539	50725	53.085	0.011
β-2-microglobulin	B2m	13.78	425.157	1419.5	3.339	0.0074
Macrophage colony-stimulating factor 1 receptor	Csf1r	109.18	22.71	141.25	6.22	0.01
α-fetoprotein	Afp	67.34	78.845	567.28	7.195	0.008
α-2-HS-glycoprotein	Ahsg	37.32	7831.367	44357.3	5.664	0.024
Insulin-like growth factor-binding protein 2	Igfbp2	32.846	62.722	1156.4	18.437	0.0005
Insulin-like growth factor-binding protein 4	Igfbp4	27.81	2.61	53257	20.381	0.0003
Sulfhydryl oxidase 1	Qsox1	63.34	60.672	288.44	4.754	0.035
α-2-macroglobulin-P	A2m	164.35	125.699	4668.7	37.142	0.0007
Lysozyme C-2	Lyz2	16.69	49.978	2118.97	42.398	0.017
Angiotensinogen	Agt	51.99	106.615	877.37	8.229	0.003
Pigment epithelium-derived factor	Serpinf1	46.23	36.352	408.52	11.238	0.0009

Table 2: ECM, complement factor, and apolipoproteins, and the type of injury indicated by each protein.

Protein name	Gene ID	LFQ KO/WT ratio	Indicated injury	References
Extracellular matrix protein 1	Ecm1	11.691	Neuroinflammation, demyelination	15
Fibronectin	Fn1	10.589	Neuronal survival	16
Gelsolin	Gsn	4.415	Amelioration of inflammation and neuroprotection	17, 18
Vitronectin	Vtn	3.125	Activation of microglia	19
Complement C4-B	C4b	5.121	Response to ischemia and inflammation	21
Complement factor H	Cfh	3.194	Protective function in neuroinflammation	22
Properdin	Cfp	2.791	Activation of microglia	23
Apolipoprotein D	ApoD	48.03	Neuroprotection and survival	24, 25
Apolipoprotein E	ApoE	13.377	Neuroprotection	26

Table 3: Cytokine-binding proteins and their roles in IGF signaling.

Protein name	Gene ID	LFQ KO/WT ratio	Role in IGF signaling	References
Insulin-like growth factor-binding protein 2	Igfbp2	18.437	Neuronal survival	27-30
Insulin-like growth factor-binding protein 4	Igfbp4	20.381	Amelioration of inflammation and neuroprotection	27-30
α-2-HS-glycoprotein	Ahsg	5.664	Neuroinflammation, demyelination	31
α-fetoprotein	Afp	7.195	Activation of microglia	32
Sulfhydryl oxidase 1	Qsox1	4.754	IGF uptake and transport by IGFPBs	33

Table 4: Endopeptidases and their roles in negative-feedback autoregulation of ventriculomegaly.

Protein name	Gene ID	LFQ KO/WT ratio	Negative-feedback autoregulation	References
Angiotensinogen	Agt	8.229	Vasoconstriction; reduction of blood flow to the choroid plexus	36, 37
Pigment epithelium-derived factor	Serpinf1	11.238	Anti-angiogenesis, neural stem cell self-renewal	38, 39