CSF proteomics identifies IGF signaling and angiogenesis as hydrocephalus therapeutic targets

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ABSTRACT
We propose that the composition of the CSF provides specific information on the 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 proposition was generated in a recent study by Yang et al. (2019) by comparative proteomics of CSF collected from the brain of healthy and *Mpdz* knockout (KO) mice. These mice phenocopy the severe hydrocephalus linked to human loss-of-function mutants of *MPDZ*, the human ortholog. The overall protein concentration was 2.5-fold higher in the CSF of *Mpdz* KO versus healthy mice. The vast majority of the significantly overabundant proteins was present in the CSF of KO mice. Their functional classifications suggested that they are not a haphazard collection but rather epitomize a concerted response of the brain to ventriculomegaly. 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: CSF proteomics could be used to identify biomarkers of case-specific injuries of ventriculomegaly in fetuses or neonates; once the overabundance of these biomarkers is detected, the IGF and angiotensin/PEDF signaling pathways could be exploited to reduce CSF production as a non-invasive therapy, replacing or aiding current invasive treatments of hydrocephalus.

Key words: hydrocephalus, CSF, proteomics, choroid plexus, biomarkers

BACKGROUND

Hydrocephalus is the most common cause of pediatric surgical intervention [1], occurring about once per 1000 births in the US [2]. All current treatments are invasive. They involve either CSF drainage by ventriculo-peritoneal shunt (VPS), ventriculo-atrial shunt, and lumbo-peritoneal shunt, or endoscopic third ventriculostomy (ETV). These procedures fail and require revision in 12%-30% of shunting cases [3] and in 24%-45% of ETV cases [4]. Surgery in young patients such as neonates harboring congenital hydrocephalus is particularly prone to revision [2]. Shunting entails increased rates of mortality and morbidity due to intraparenchymal hematoma during implantation and device failure or infection [2, 5, 6]. The hospitalization required for these procedures costs annually more than 2 billion dollars [7]. CSF drainage from the brain ventricles to the peritoneum, which is still the prevalent treatment for hydrocephalus, requires multiple revisions during the patients' lifetimes at an average interval of 14.1 years [8]. Ultimately, surgical treatment is palliative and does not address the underlying cause of hydrocephalus. 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 [9]. 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 response 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 following analysis, based on our published comparative proteomics data [10], reveals previously unappreciated information gained from comparative proteomic analysis between the CSF of healthy and hydrocephalus-harboring *Mpdz* KO mice. This mouse model phenocopies closely the severe hydrocephalus linked to loss-of-function mutations in the human ortholog of *Mpdz*. The comparative proteomics data identified the nature of the damage inflicted by ventriculomegaly on the parenchyma and detects autoregulatory pathways activated by the brain to counter this damage. We propose that the apparent stimulation of these pathways in response to ventriculomegaly calls for their exploitation for therapeutic purposes.

DISCUSSION

Comparative proteomics of CSF from healthy and hydrocephalic mice.

Out of the total 313 proteins identified in 3 samples of CSF from either wild type (WT) or *Mpdz* KO mice, 23 were more than 2-fold 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 several functional groups (from top to bottom in Table 1): extracellular matrix (ECM), complement factors, lipoproteins, cytokine binding proteins, and serine protease 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 [10]. The proteins were scanned against the literature to examine their potential association with central nervous system (CNS) pathological conditions. Based on the matches, the interpretation of the overabundance of each protein was classified into two group: either as an indicator of damage or as a response of the parenchyma to the trauma of severe hydrocephalus. Proteins were included in the first group if they were associated with injuries that occur in the parenchyma under the condition of hydrocephalus. Proteins were categorized as part of the brain's response to this condition if they were constituents of autoregulatory pathways that would potentially counter ventriculomegaly.

Biomarkers of brain injury
The overabundant ECM proteins, complement factors and apolipoproteins (Table 1), were associated with mechanisms of ventriculomegaly-inflicted injuries. ECM1 was linked to neuroinflammation, whereby it ameliorated the demyelination caused by T helper cells [11]. Plasma fibronectin, the soluble isoform detected in our previous study [10], supported neuronal survival and reduced brain injury following cerebral ischemia [12]. Extracellular gelsolin ameliorated inflammation [13] and had a neuroprotective effect during stroke [14]. Vitronectin activated microglia [15], the primary immune effector cell of the CNS.

The complement system had a neuroprotective role in CNS neurodegenerative diseases [16]. Specifically, complement C4-B was produced by microglial cells in response to ischemia and inflammation [17]. Complement regulator H was produced by neurons and had a protective function in neuroinflammation [18]. Properdin (complement factor P), activated microglia and contributed to inflammation in response to ischemia [19].

Apolipoprotein E (ApoE), which was produced by the choroid plexus [20], protected the brain against injury and promotes neuronal survival [20, 21]. ApoD had a similar neuroprotective role in CNS degeneration [22].

Collectively, the overabundances of the aforementioned proteins reveal that severe hydrocephalus entailed a set of injuries to the parenchyma that included neuroinflammation and demyelination, and impaired perfusion. Activation of CNS immune defense by microglial cells accompanied these processes and possibly aggravates inflammation. This injury pattern conforms to the empirical picture of neonatal hydrocephalus [23].

Biomarkers of autoregulatory pathways
The IGF-binding proteins (IGFBPs) are expressed in the CNS, including the choroid plexus [24]. Specifically, IGFPB2 and IGFBP4, which were overabundant in the CSF of *Mpdz* KO mice [10], are produced by astrocytes in response to brain injury [25]. While the differential functions of each IGFPB are not fully understood, they are thought to stabilize IGF, extend it half-life [26], and modulate its activity [27]. α-2-HS-glycoprotein, which binds to and modulates the signaling of the IGF receptor [28], α-fetoprotein, a cytokine-binding [29] plasma component, and sulfhydryl oxidase 1 participate in IGF uptake and transport by IGFPBs [30]. IGF and the IGFPBs were produced in choroid plexus epithelial cells [31, 32]. The secretion of IGF into the CSF was increased in response to injury [33]. Remarkably, all the five proteins in this group (Table 1) regulate multiple facets of IGF signaling.

Two of the overabundant proteins are the serine protease inhibitors angiotensinogen and PEDF (Table 1). Angiotensinogen is the precursor of several cleaved derivatives and peptides. One of the latter, angiotensin-2, reduced blood flow to the choroid plexus [34] and CSF production [35]. However, its cleaved derivatives were antiangiogenic [36]. This activity was shared by PEDF [37], which was secreted by ependymal cells in the subventricular zone (SVZ) [38] . It appears, therefore, that serine protease inhibitors may reduce CSF production by pruning the dense capillary network that surrounds the choroid plexus vessels [39].

CONCLUSIONS
Few previous studies had analyzed the diagnostic potential of the CSF. Developmental defects originating from attenuated cycling of germinal matrix cells in the brains of hydrocephalus-harboring rats had been attributed to alteration in the composition of the CSF relative to healthy subjects, but the putative difference between the CSF of health and hydrocephalic rats had not been identified [40]. Comparison of select proteins in the CSF of patients with idiopathic normal pressure hydrocephalus before VPS implantation and of healthy subject, was used to diagnose the damage to the patients'

parenchyma. Subsequently, the concentrations of the same proteins after VPS shunting were used to gauge the if the shunt ameliorated the damage [41]. We are not aware, however, of previous non-biased and exhaustive CSF proteomics for diagnostic or therapeutic purpose.

Potential diagnostic implications

The interpretation of the overabundance of ECM proteins, complement factors, and apolipoproteins as biomarkers of specific brain injuries inflicted by ventriculomegaly is supported by previous studies [23]. Their individual or collective overabundances could be potentially identified and measured in the CSF collected during VP shunting or ETV of patients with congenital hydrocephalus. 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.

Potential therapeutic implications

The combined overabundance of all the five cytokine-binding proteins provides unambiguous evidence that IGF signaling is a major autoregulatory pathway employed by the brain to counteract the injury caused by ventriculomegaly. Similarly, the concurrence of the overabundances of angiotensinogen and PEDF is a strong indicator that suppression of angiogenesis is employed as a negative feedback loop to counter ventriculomegaly by reduction of CSF production. 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.

List of abbreviations
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.

Authors' contributions
AH initiated the proteomics data analysis; all authors contributed to the writing of the manuscript.

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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[10]. 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[10]).

Protein Function	Protein Name	Gene ID	LFQ KO/WT Ratio	*P*-value
ECM Proteins	Extracellular matrix protein 1	Ecm1	11.691	0.0012
	Fibronectin	Fn1	10.589	0.015
	Gelsolin	Gsn	4.415	0.0018
	Vitronectin	Vtn	3.125	0.026
Complement Factors	Complement C4-B	C4b	5.121	0.0091
	Complement factor H	Cfh	3.194	0.042
	Properdin	Cfp	2.791	0.042
	Fibrinogen β chain	Fgb	48.03	0.027
Apolipoproteins	Apolipoprotein D	ApoD	13.377	0.0003
	Apolipoprotein E	ApoE	53.085	0.011
Cytokine Binding Proteins	α-fetoprotein	Afp	7.195	0.008
	α-2-HS-glycoprotein	Ahsg	5.664	0.024
	Insulin-like growth factor-binding protein 2	Igfbp2	18.437	0.0005
	Insulin-like growth factor-binding protein 4	Igfbp4	20.381	0.0003
	Sulfhydryl oxidase 1	Qsox1	4.754	0.035
Serine protease Inhibitors	Angiotensinogen	Agt	8.229	0.003
	Pigment epithelium-derived factor	Serpinf1	11.238	0.0009