BRAIN GENE EXPRESSION SIGNATURES FROM CEREBROSPINAL FLUID EXOSOME RNA PROFILING

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1. BACKGROUND

VIIP and IIH

- Visual symptoms reported in astronauts returning from long duration missions in low Earth orbit are thought to be related to fluid shifts within the body due to microgravity exposure, leading to increased intracranial pressure (ICP) and visual impairment and intracranial pressure (VIIP) syndrome.
- Idiopathic intracranial hypertension (IIH) is a condition characterized by increased ICP without clinical, laboratory, or radiologic evidence of an intracranial space-occupying lesion, meningeal inflammation or venous outflow obstruction.
- While the described VIIP syndrome focuses on ocular symptoms, spaceflight has been also associated with a number of other performance and neurologic signs, such as headaches, cognitive changes, vertigo, nausea, sleep/circadian disruption and mood alterations, which, albeit likely multifactorial, can also result from elevation of ICP.
- We hypothesize that these various symptoms are caused by disturbances in the neurophysiology of the brain structures and correlated with molecular markers in the cerebrospinal fluid (CSF) as indicators of neurophysiological changes.
- The purpose of this study is to investigate changes in brain gene expression via exosome analysis in patients suffering from ICP elevation of varied severity and to evaluate which of these biomarkers can also be detected in plasma.

Exosomes

- Exosomes are 30-200 nm microvesicles that are actively released from cells into all biofluids such as blood, urine, and CSF. They carry a highly rich source of intact protein and RNA cargo (Figure 1).
- Exosomes have been isolated from CSF and measured for brain associated genes.
- Exosomes are likely important in the cellular communication and homeostasis of the brain milieu (Figure 2)

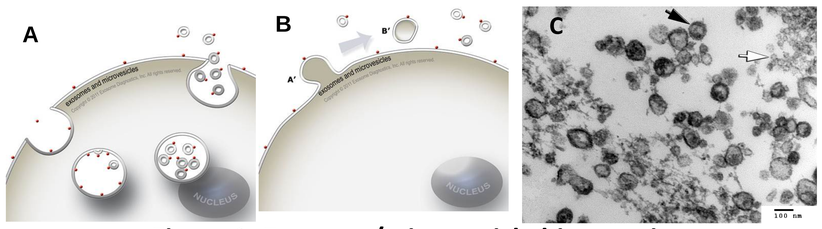


Figure 1. Exosome/microvesicle biogenesis

Exosomes and other vesicles can be released by (A) multivesicular body pathway or through (B) direct budding at the plasma membrane. (C) Transmission electron microscopy of microvesciles.

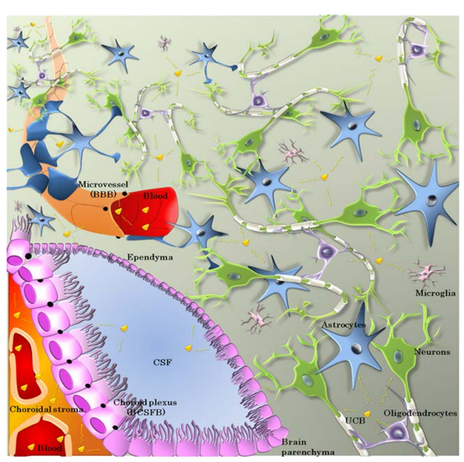
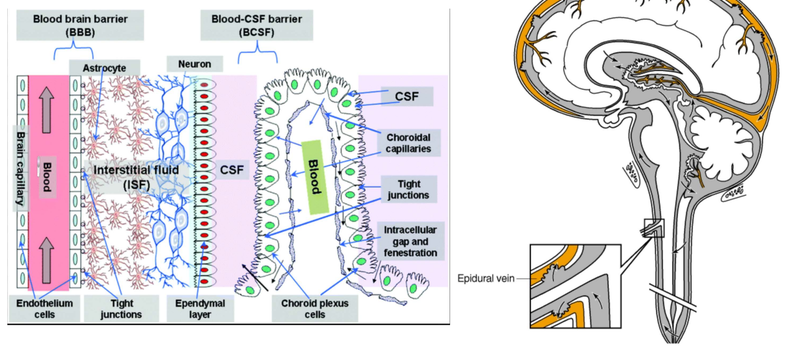


Figure 2. Neural cells release microvesicles with several known or suggested functions. (affecting synaptic plasticity, for example) Microglia modulate neurotransmission via shedding microvesicles. Astrocyte-derived exosomes carry neuroprotective cargo and could contribute to neuronal survival.

The CSF-blood barrier present in the ependymal and choroid plexus, as well as in the CSF drainage system via the arachnoidal granulations, offer a path through which biomarkers present in the CSF may be also represented in the plasma. Therefore, this study seeks also to determine whether plasma could eventually be used as test sample given the less invasive nature of the collection.



3. Data and Results

Patients with suspected or known IIH are being recruited under a BCM and JSC IRB approved protocol (Drs Zanello and Bershad). To date, we have collected CSF and serum from 18 subjects (Table 1).
The study population is a pool of neurological patients at BCM requiring clinically indicated lumbar puncture (LP). LP is performed in the lateral decubitus position, legs slightly extended. Once the needle is in the lumbar thecal sac, ICP is measured via manometer for 5 minutes. Then, CSF is drained as per the normal clinical procedure and 5 ml are collected for analysis. Those undergoing LP, who end up not having elevated ICP (≤18 cmH20) AND no inflammatory findings, will serve as "control" (normal ICP) subjects.

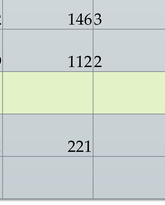
2. Materials and Methods

CSF and plasma from 20 subjects → Exosome isolation from 2ml CSF per patient using membrane affinity spin columns (Figure 4) → RNA profiling on OpenArray® Human Inflammation Panel

Baylor College of Medicine

Exosome Diagnostics CLIA facility

Figure 3. Workflow diagram



Spin column

- Bind vesicles to membrane & wash
- QIAzol lysis and release of RNA
- Phenol/Chloroform extraction
- Ethanol conditioning
- Bind to RNeasy column and wash
- Elute RNA

Figure 4. Exosome RNA extraction

Spin column to purify exosomes and the extraction workflow

Subject ID#	Gender Male=1 Female=2	Age (Years)	Weight (Lbs)	Weight (kg)	Height (m)	BMI (kg/m2)	Average ICP (cmH20)	Average ICP (mmHg)	RNFL (right) micrometer	RNFL (left) micrometer	Frisén grade right eye	Frisén grade left eye	Signs and symptoms (right eye)	Signs and symptoms (left eye)	IIH
001	2	40	225	102.3	1.6	38.7	42.7	31.4	83	85	1	1	enlarged blind spot	enlarged blind spot	Yes
002	2	28	213	96.8	1.6	37.8	19.7	14.5	94	99	1	1	scotoma	scotoma	No
003	2	51	242	110.0	1.6	41.6	19.2	14.1	284	400	2/3	4	constricted visual field	constricted visual field	No
004	2	32	160	72.7	1.6	29.3	28.3	20.8	158	135	1	1	visual field defect	visual field defect	Yes
005	2	30	171	77.7	1.7	28.5	25.5	18.8	155	199	1	1	scotoma	scotoma	Yes
006	2	32	180	81.8	1.6	32.0	32.5	23.9	114	109	0/1	0/1	visual field defect	visual field defect	Yes
007	2	28	211	95.9	1.8	30.3	25.5	18.8							Yes
008	1	33	205	93.2	1.8	30.3	26.6	19.6	123	112	2	1	scotoma	none	Yes
009	2	28	155	70.5	1.5	30.3	30.5	22.4	94	95	0/1	0/1	visual field defect	visual field defect	Yes
010	2	26	253	115.0	1.6	43.5	ND	ND	159	168	1	1	scotoma	scotoma	ND
011	2	18	243	110.5	1.7	39.3	29.6	21.8							Yes
012	2	43	130	59.1	1.6	23.1	15.9	11.7	96	98	0	0	visual field defect	visual field defect	No
013	2	29	307	139.5	1.6	54.5	36.3	26.7	343	576	3	4	enlarged blind spot	enlarged blind spot	Yes
014	2	21	247	112.3	1.7	41.2	35.8	26.3	215	263	1/2	1/2	scotoma	scotoma	Yes
015	2	45	204	92.7	1.8	28.5	22.8	16.7	89	85	0/1	0/1	scotoma	scotoma	No
016	2	47	150	68.1	1.6	25.8	31.7	23.3	212	146	3	2	scotoma	scotoma	Yes
017	1	32	252	114.5	1.8	36.2	24.3	17.9	119	112	2	2	scotoma	scotoma	No
018	2	51	154	70.0	1.7	24.9	15.0	11.0							No
019	2	32	195	88.6	1.6	35.2	31.8	23.4	131	221	1	2	nasal sector defect	nasal sector defect	Yes
020															

Table 1. CSF samples from 18 patients (out of 20 targeted) with elevated ICP and control (green rows)

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