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MRM-based validation assay for serum proteomic alterations in meningioma patients

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Introduction

Meningiomas are tumors that arise from the outer layering of the brain namely the dura, arachnoid and pia mater. These tumors account for nearly 30% of all primary brain tumors and are majorly treated via surgical resection. WHO classifies meningiomas into three types namely the benign (MGI), atypical (MGII) and anaplastic (MGIII) [1] [2].Recently there are reports of aggravated recurrence rates and diagnostic ambiguity within the grades and certain molecular signatures have been ascribed to such manifestation. [3]

However apart from the conventional modalities of treatment there are no molecular markers that can be used for diagnosis and prognosis of these tumors. In this study we attempt to validate differentially expressed proteins in meningioma patients and assess its utility in context to meningioma pathobiology.

Workflow and key findings of the discovery phase

In the discovery phase, global tissue proteomic profiling of the samples was done using QTOF and Q-Exactive mass spectrometric platform which yielded 157 proteins (1% FDR), among which where found to be differentially expressed across various grades on meningioma. [4]
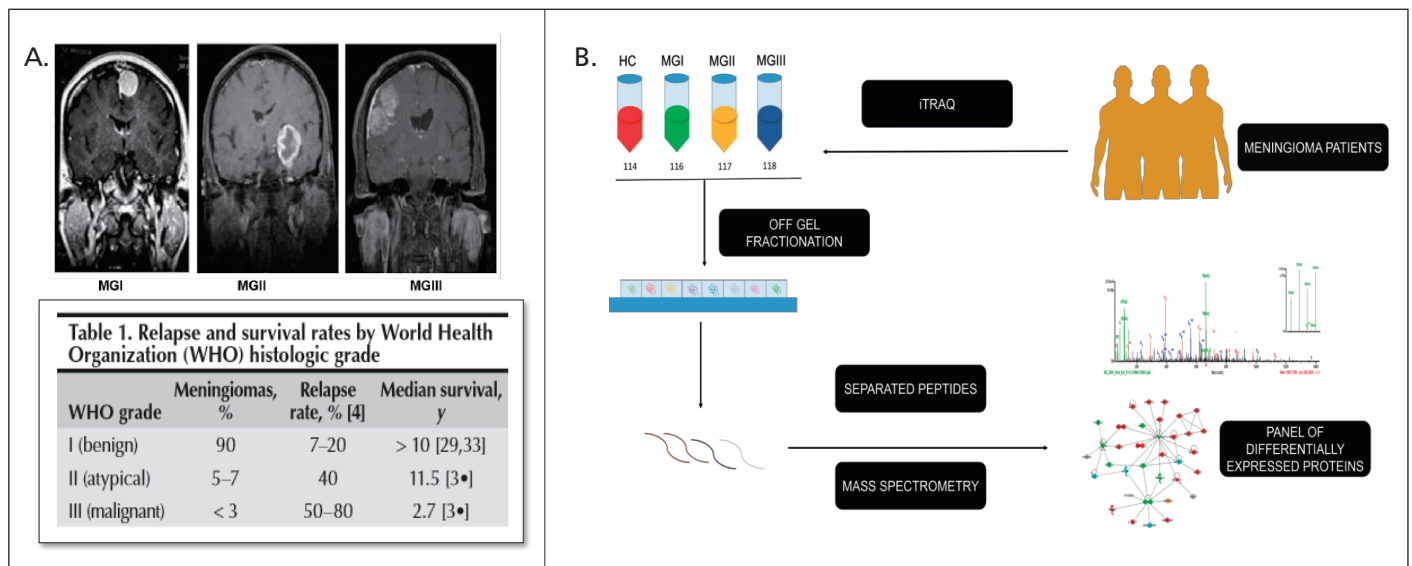

Figure 1A. Different grades of meningioma and survival rates. ((Norden *et al. Curr Neurol Neurosci Rep*. 2009)
              B. Schematic depicting the discovery based workflow for global serum proteomic profiling in meningiomas [5]

Panel of protein selected from discovery phase

Global proteomics study on meningioma patient sera using iTRAQ and label free approaches enabled identification of around a total of 157 proteins being significant with 1% FDR. *In silico*. analysis using bioinformatics tools revealed dysregulation in several components of the lipid metabolism, coagulation cascade. Components like Apolipoprotein B-100, Ceruloplasmin, Vitamin D Binding protein, Angiotensinogen was found to be differentially expressed in different grades of meningiomas

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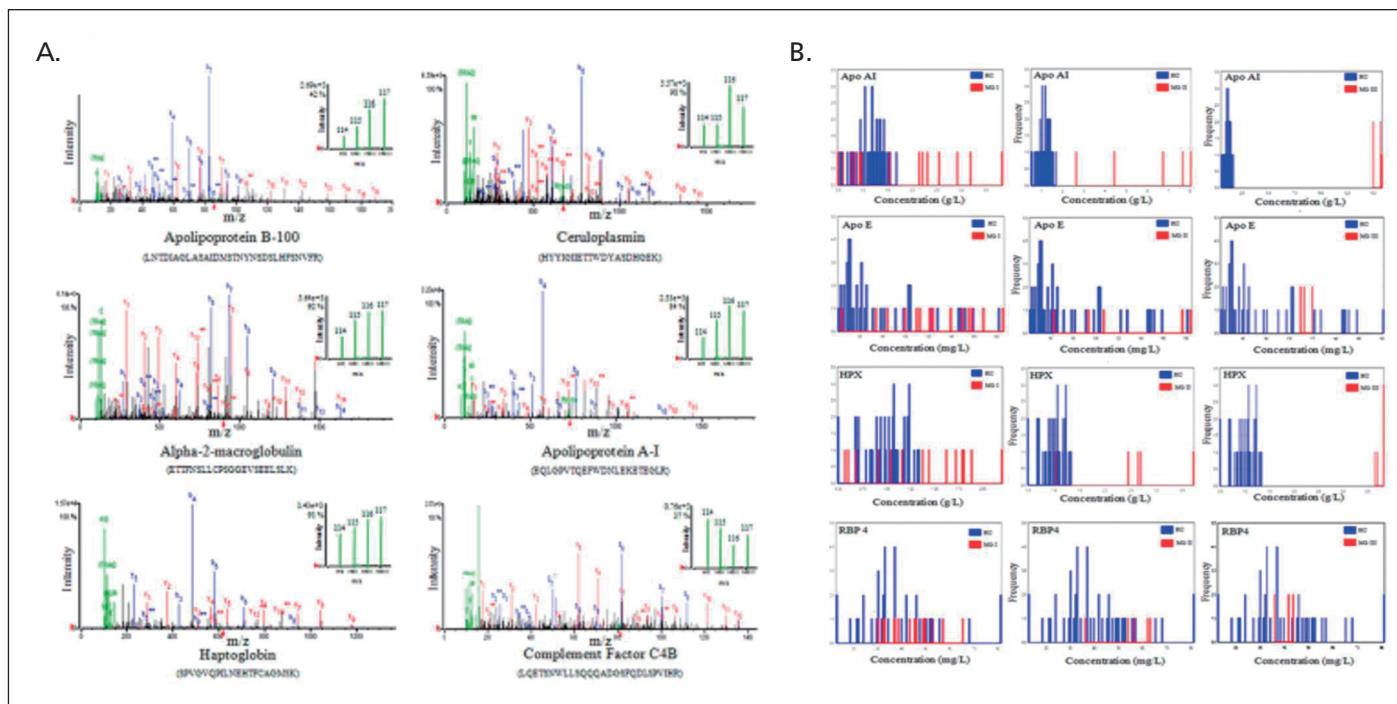

Figure 2. Panel of differentially expressed proteins in meningiomas that were selected from discovery phase:
A: iTRAQ based MS/MS revealed differential expression of the above mentioned peptides.
B: Conc. of key proteins in patient vs Healthy sera as measured by ELISA. [5] (*Adapted from Sharma et al., 2014*)

Methods and Materials

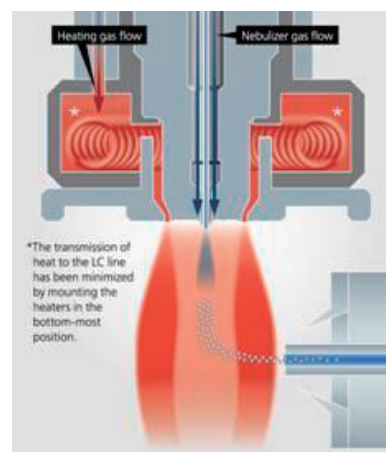Figure 3. LCMS-8050 triple quadrupole mass spectrometer by Shimadzu

Figure 4. Heated ESI probe

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LCMS-8050 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 3), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 4) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

LC/MS/MS analysis

MRM based assays were performed on individual patients for relative quantitation of key dysregulated proteins. Quantitation of 9 proteins were performed from meningioma patient sera using LCMS-8050. Details of LC/MS/MS conditions are given in Table 1.
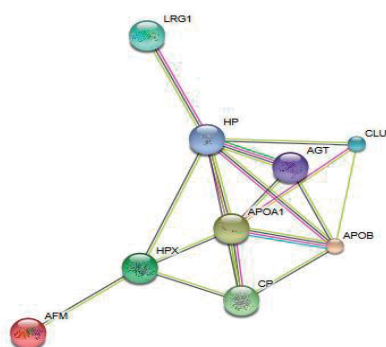
Table 1. LC/MS/MS conditions

Column	: Shim-pack XR-ODS II (75 mm L x 3 mm I.D.; 2.2 µm)
Guard column	: Phenomenex SecurityGuard ULTRA cartridge
Mobile phase	: A: 0.1 % formic acid in water
	B: 0.1 % formic acid in acetonitrile
Gradient program (B %)	: 0.01–1.5 min → 3 (%); 1.5–30.0 min → 3-50 (%); 30.0–31.0 min → 50-95 (%); 31.0–35.0 min → 95 (%); 35.0–36.0 min → 95-3 (%); 36.0–40.0 min → 3 (%)
Flow rate	: 0.4 mL/min
Oven temperature	: 40 °C
Injection volume	: 4 µL
MS interface	: Electro Spray Ionization (ESI)

Results

MRM based assays on meningioma patient sera was done to monitor transitions of 10 peptides that were found to be differentially expressed via global quantitative proteomic approaches. Quantifiable peptides for Apolipoprotein

B-100 that were monitored in patient sera include TGISPLALIK.G, TEVIPPLIENR.Q and IAELSATAQEIIK and were found to be differentially expressed in various grades of meningiomas in individual patients.

Figure 5. Schematic depicting the interaction of the proteins that were taken forward for validation using STRING DB®

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The FASTA sequences were used to generate transitions for precursor ions using Skyline software and method files were created. Further optimization of LC peaks, gradient and dwell time were carried out to acquire the data. Precursor ions: +2,+3 charge and Product ions: +1,+2 charge were taken into consideration and then the transition lists were imported into LabSolutions software (Shimadzu).Peptides for several of the targets identified in discovery phase were monitored including Apo B, Apo A1, Ceruloplasmin and Angiotensinogen in meningioma sera. Angiotensinogen peptides have been implicated in several cancers and they are involved in a plethora of functions including vasoconstrictors, cell growth factors thereby promoting tumorigenesis. Its role, in particular, to meningiomas has not been deciphered [7]. Peptides that were monitored include DPTFIPAPIQAK, ALQDQLVLVAAK and SLDFTELDVAAEK and levels in patient sera were found to be higher. Furthermore, many of the peptides monitored were also found to interact thus, pointing out an overall dysregulation in the network. [Figure 5,6] and [Table 2].
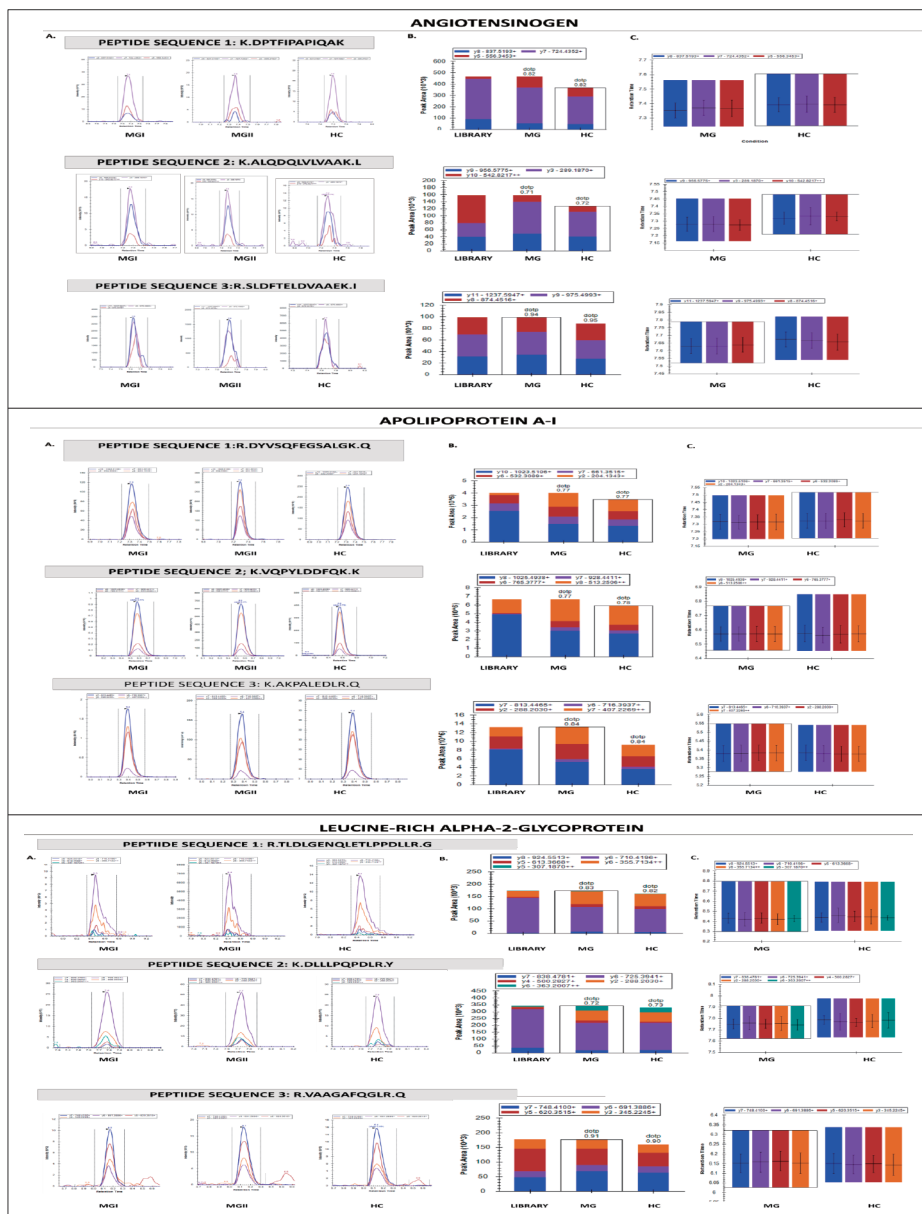

Figure 6. Overall schematic of peptides measured via TQ mass spectrometry analysed by Skyline.
A: Transitions monitored; B: Matching the peptides with the Library (NIST); C: Retention Times Monitored.

Table 2. The proteins and the corresponding peptides which were monitored in different grades of meningiomas from patient sera

Protein	Peptides Monitored	RT
Haptoglobin	R.VGYVSGWGR.N [277, 285]	6.2
	K.YVMLPVADQDQCIR.H [297, 310]	7.1
	K.VTSIQDWVQK.T [391, 400]	6.7
Afamin	K.LPNNVLQEK.I [88, 96]	5.8
	R.AIPVTQYLK.A [205, 213]	6.8
	K.TNFAFR.R [500, 505]	6.0
Angiotensinogen	K.DPTFIPAPIQAK.T [63, 74]	7.4
	K.ALQDQLVLVAAK.L [82, 93]	7.3
	R.SLDFTELDVAAEK.I [237, 249]	7.6
Apo A1	R.DYVSQFEGSALGK.Q [51, 63]	7.3
	K.VQPYLDDFQK.K [120, 129]	6.6
	K.AKPALEDLR.Q [230, 238]	5.4
Hemopexin	K.NFPSPVDAAFR.Q [91, 101]	7.2
	K.SGAQATWTELPWPHEK.V [386, 401]	7.5
	K.VDGALCMEK.S [402, 410]	5.5
Apo B	R.TGISPLALIK.G [219, 228]	7.8
	K.TEVIPPLIENR.Q [949, 959]	7.2
	K.IAELSATAQEIIK.S [4465, 4477]	7.1
Leucine-rich alpha-2-glycoprotein	R.TLDLGENQLETLPPDLLR.G [191, 208]	8.4
	K.DLLLPQPDLR.Y [229, 238]	7.7
	R.VAAGAFQGLR.Q [250, 259]	6.1
Ceruloplasmin	K.ALYLQYTDETFR.T [69, 80]	7.3
	R.IYHSHIDAPK.D [177, 186]	4.5
	K.DIFTGLIGPMK.I [547, 557]	8.6
Clusterin	R.ASSIIDELFQDR.F [182, 193]	8.6
	R.ELDESLQVAER.L [325, 335]	6.2
	K.FMETVAEK.A [429, 436]	5.5

Conclusion

- The current study attempts to establish a method to screen potential protein biomarkers from patient sera for analytically distinguishing and confirming various grades of meningioma patients. Since there are no studies on MRM based validation of potential markers for meningioma, this can help in constructing a panel to get insight into the tumor pathobiology and recurrence rates.
- Several peptides that were found to be differentially expressed in the discovery phase were detected using the MRM assay. These peptides when screened in larger cohort with synthetically labelled peptides would enable detection of absolute levels in the patient samples and substantiate the clinical relevance of these findings.
- The MRM based assays along with parallel proteomic profiling and histopathological studies can lead to accurate determination of grades of meningioma thereby limiting diagnostic dilemma.

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Acknowledgements

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