Proceeding

Proteomics detection of endothelial cell surface proteins following irradiation as potential targets for brain arteriovenous malformations molecular therapy

Abstract

Arteriovenous malformations (AVMs) in the brain are vascular neurological lesions consist of abnormal collection of arteries and veins. They are the most common cause of brain haemorrhage in children and young adults. Over 90% of large AVMs (>3cm) are not curable, or curable with unacceptable risks. Radiosurgery is the treatment option for lesions (< 3cm) in diameter and located in eloquent areas where surgery can cause neurological defecit. However vascular occlusion post radiosurgery can takes up to 3year to complete while patients remain at risk of haemorrhage. This study aims to develop a molecular therapy for human brain AVMs. Specifically we aim to identify unique protein targets of AVMs that are up-regulated post irradiation. These proteins will then be used to develop a ligand-directed vascular treatment that promotes rapid thrombosis in AVM vessels post radiosurgery.

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Introduction

AVMs consist of abnormal collection of arteries and veins. They are the most common cause of haemorrhagic stroke in children and young adults. Radiosurgery is the treatment option recommended for lesions less than 3cm in diameter and located in eloquent areas where surgery can cause neurological deficits. This study aims to identify endothelial protein targets of AVMs that are differentially expressed compared to normal vessels post radiosurgery, to develop a ligand directed vascular treatment that promotes rapid thrombosis in AVM vessels. This is the first time that proteomics approach has been used in AVMs study (Figure 1).

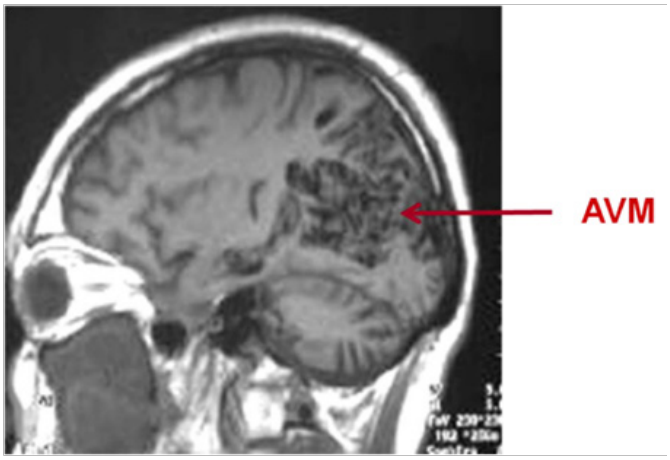
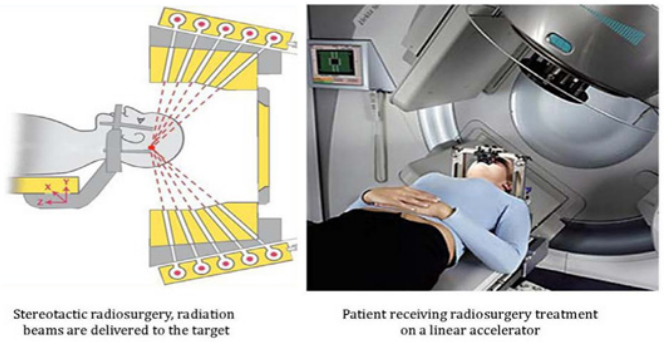


Figure 1

Objectives

- Assess membrane protein changes in the murine endothelial cell culture (bEnd.3) in response to radiation over time using proteomics analysis.
- Determine candidate proteins location with immunocytochemistry.
- Protein candidates will be used for a ligand-directed vascular targeting treatment to promote rapid thrombosis in AVM vessels post radiosurgery (Figure 2).



Stereotactic radiosurgery, radiation beams are delivered to the target

Patient receiving radiosurgery treatment on a linear accelerator

Figure 2

Methods

In vitro biotinylation and iTRAQ mass spectrometry was used to assess membrane protein changes in the murine endothelial cell

cultures (bEnd.3). Cells were irradiated with 25Gy and surface biotinylation was performed using NHS-LC-Biotin at 6h, 24h, 48h and 72hours post radiation. Biotinylated proteins were captured on streptavidin resin, digested with trypsin, and then labelled with iTRAQ 8-plex reagents kit (Applied Biosystems). Peptides were separated by SCX and analysed by nanoLC/ MS on Qstar Elite (AB Sciex) (Figure 3).

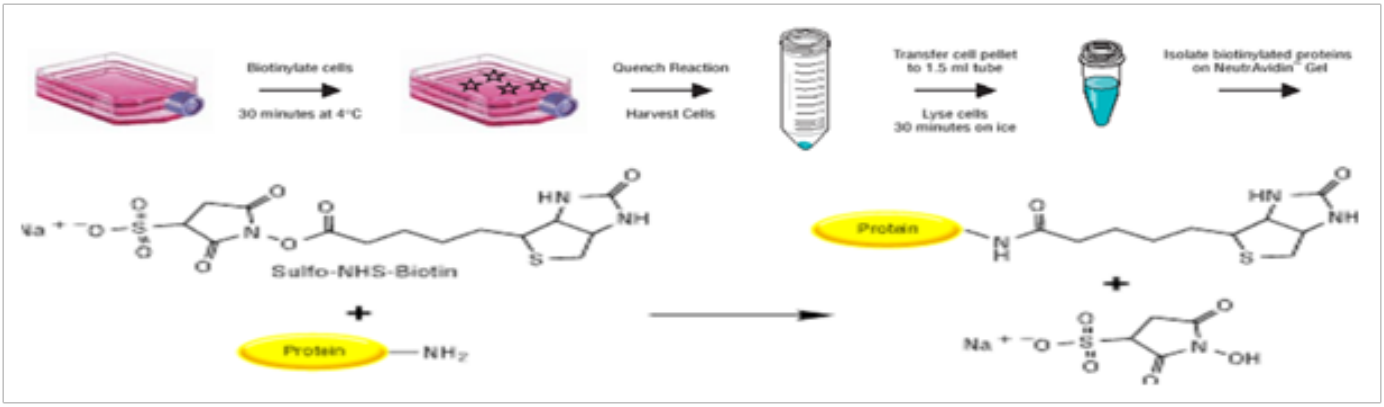


Figure 3 Immunocytochemistry was used to validate candidate proteins location and expression in bEnd.3 cells.

Immunocytochemistry was used to validate candidate proteins location and expression in bEnd.3 cells.

In vivo biotinylation of the rat model of AVM was also conducted. The rat was perfused with NHS-LC-biotin, the fistula was harvested, membrane proteins were extracted and captured on streptavidin resin, digested with trypsin and analysed by LC/MS.

Results

NanoLC ESI MS/MS data were submitted to Protein Pilot V4.0 (AB Sciex). iTRAQ MS identified on average 112 proteins from 1120 distinct peptides from 3 independent biological replicates. The detected protein threshold (unused ProtScore) was set as >1.3 (better than 95%confidence). 11 proteins were significantly differentially expressed in at least 2 out of 3 experiments. The most extensive changes were observed after 48h post radiation, e.g. cadherin 5 & CD109 (Table 1) (Figure 4–8).

Table 1 Mass spectrometry identified 120 proteins in the rat model of AVM, 32 of them were known as membrane proteins, e.g. in the table below

Annexin A2	CD99	Biglycan
ACO3	GP3	ATA1
Lumican	PAT3	PTPRC
CD36	AT1B1	Prolargin
VCAM2	PECAM	OME2
CAD13	Rab1B	Annexin A1

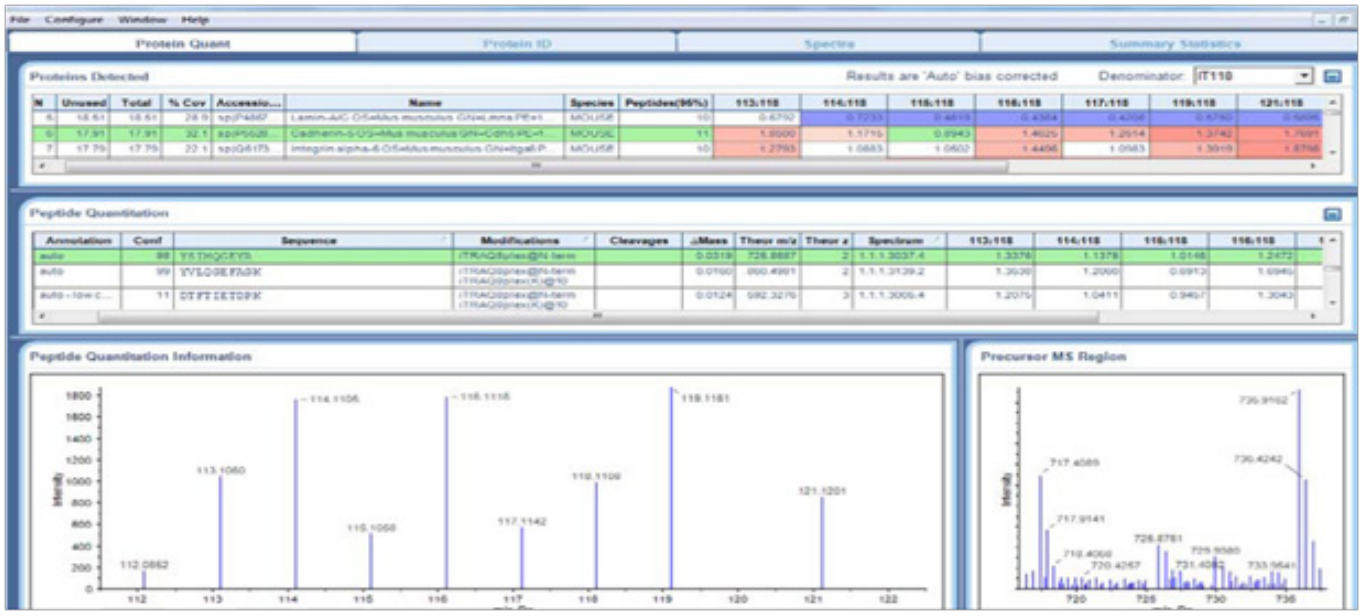


Figure 4 CADH5 protein; Expression level in C is higher than R sample at 48h post irradiation (117:118 ratio = C:R) increased 1.2614 fold.

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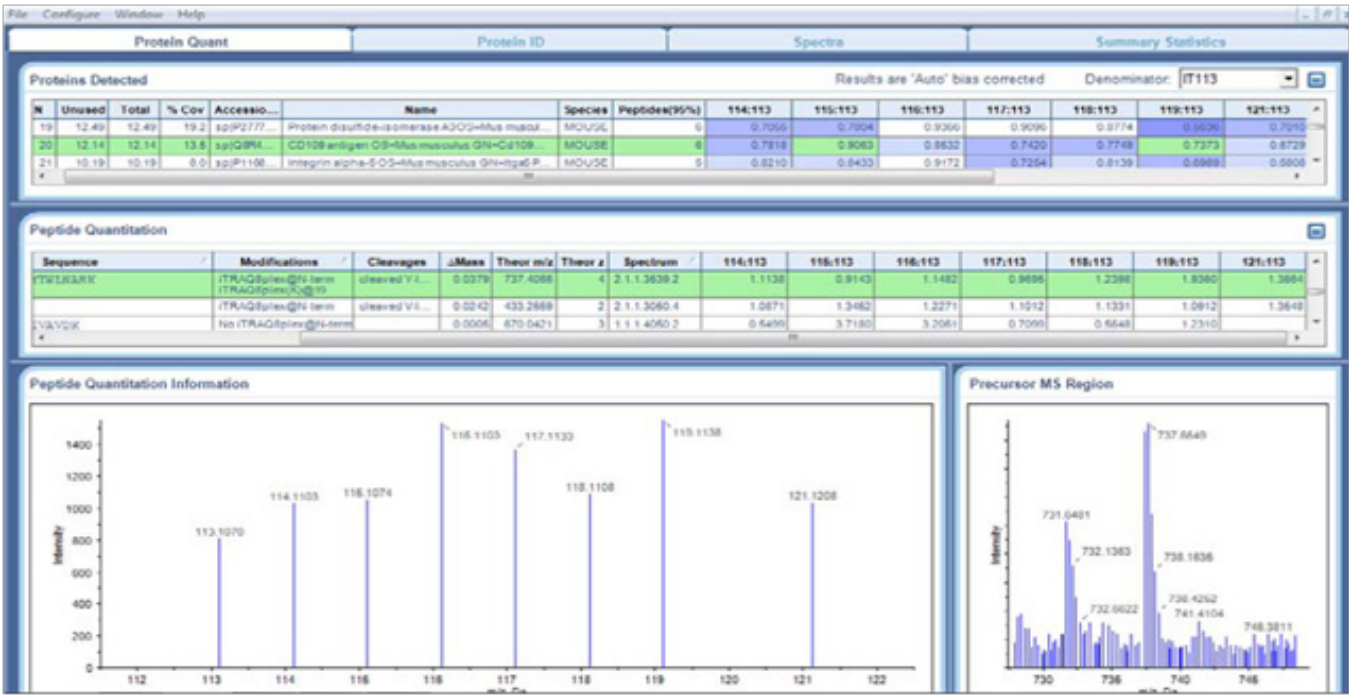

Figure 5 CD109 protein; Expression level in R is higher than C sample at 6h post irradiation (114 :113 ratio = C:R) decreased 0.7817 fold.

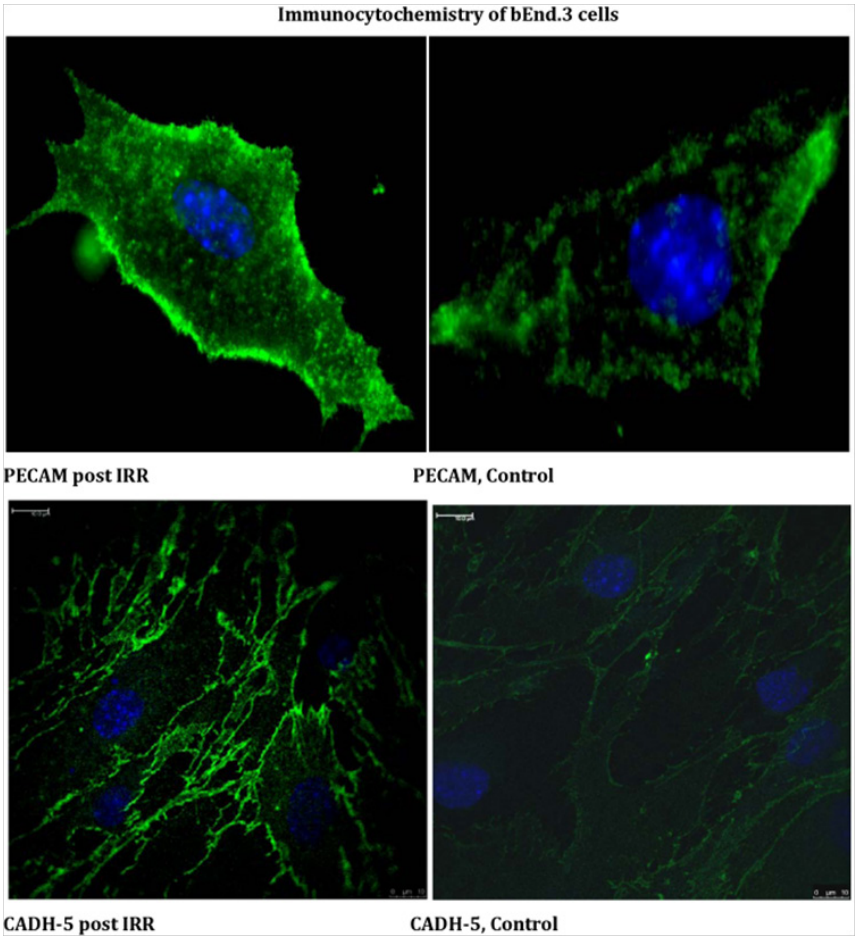

Figure 6 bEnd.3 cells stained for CADH5 and PECAM proteins. IRR, irradiated; C, controls.

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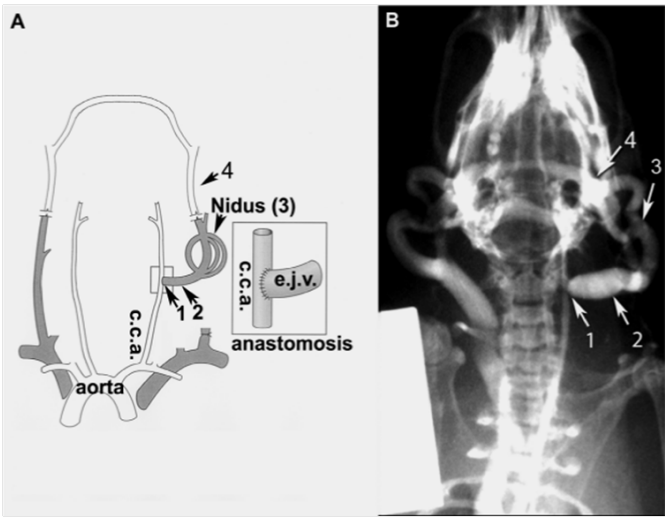

Figure 7 Rat AVM model closely resembles human AVMs, it has an arterial feeder, the nidus and adraining vein.

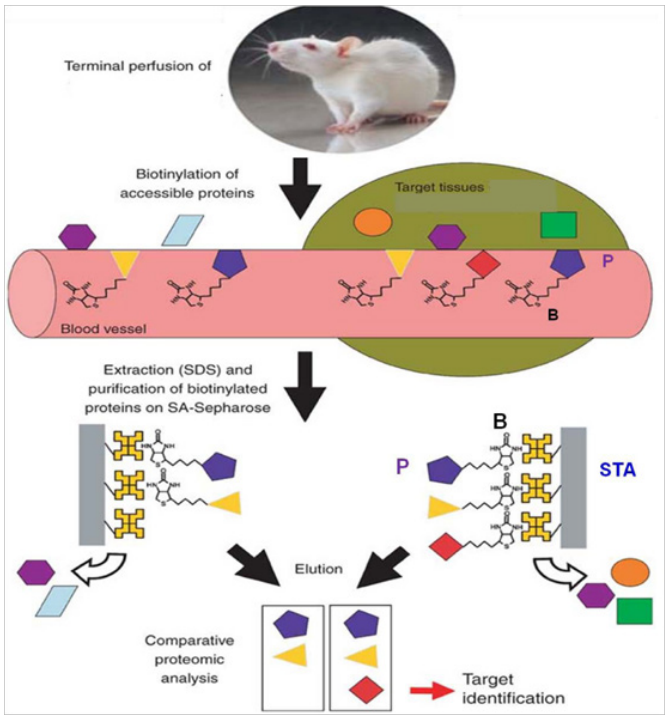

Figure 8 *In vivo* biotinylation perfusion of the rat model of AVM.

P: proteins; B, biotin; STA, streptavidin sepharose (Modified image from Rybak et al.[11])

Conclusion

Cell surface protein biotinylation and mass spectrometry of murine endothelial cells identified protein targets in response to irradiation.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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