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Radiation Induces Endothelial Surface Expression of Mitochondrial Protein PDC-E2: A Unique Candidate for Vascular Targeting in Cerebral Arteriovenous Malformations

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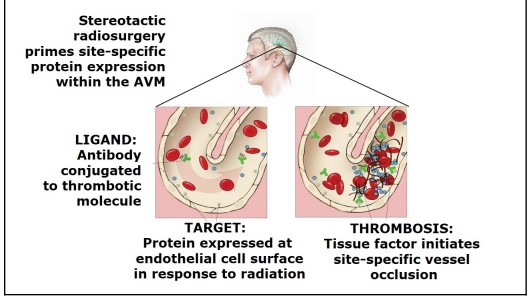
Introduction

One third of brain arteriovenous malformations (AVMs) are untreatable using current methods. Vascular targeting of AVMs with thrombotic antibody-conjugates to produce thrombosis and vessel occlusion is a promising biological treatment but requires identification of targets on the AVM endothelium that can discriminate AVM vessels from normal vessels. We hypothesize that stereotactic radiosurgery can prime site-specific protein expression within an AVM (Fig 1).

Fig 1 - Vascular targeting in AVMs: priming the AVM endothelium using radiosurgery



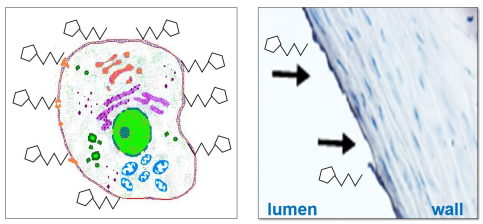
Our aim is to identify candidate proteins for vascular targeting induced by radiation on the endothelial cell surface.

Radiation-induced protein expression was examined *in vitro* and in an AVM animal model. Specific enrichment of surface proteins for proteomic analysis was achieved by biotin labelling (1).

Methods

A rat model arteriovenous fistula (AVF) was irradiated by Gamma Knife (20Gy) (2) or sham-irradiated. After 24h, animals were perfused with EZ-link Sulfo-NHS-LC Biotin to label surface-accessible proteins (Fig 2).

Fig 2 - Biotin binds surface proteins to allow downstream enrichment for proteomic analysis



Biotin-labelling in cell culture or by in vivo perfusion. EZ-link biotin cannot permeate the cell membrane, labelling only surface proteins.

Biotinylated AVFs were dissected, pooled (n=4/group) and protein extracted. Cultured brain microvascular endothelial cells were irradiated by LINAC (20Gy, n=3) or sham-irradiated (n=3), then biotin-labelled and extracted at day 6.

Labelled proteins were enriched on streptavidin-sepharose beads before comparative proteomics using LC-MS/MS and SWATH acquisition label-free mass spectrometry. Expression was validated by immunocytochemistry and immunohistochemistry.

Results

Proteomic Analysis

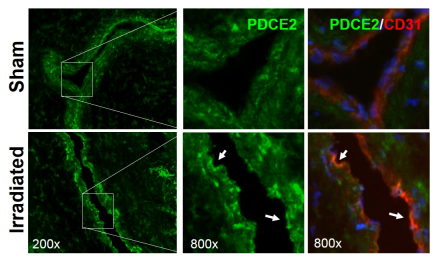
In the AVM model, comparative proteomics identified 56 surface-associated proteins up-regulated in irradiated versus control AVMs (greater than 1.5-fold). *In vitro*, 104 proteins were elevated in irradiated versus control cell extracts (greater than 1.3-fold, $P<0.05$).

The mitochondrial protein PDC-E2 (pyruvate dehydrogenase complex, subunit E2) increased in the labelled fractions of both irradiated AVMs (1.7-fold) and irradiated cells (2.2-fold, $P<0.01$).

Immunohistochemistry

Ex vivo AVM staining demonstrated PDC-E2 expression throughout the vessel wall. Stereotactic radiosurgery increased staining at the endothelium in a heterogeneous manner (Fig 3).

Fig 3 - Validation of endothelial PDCE2 expression: Rat AVM



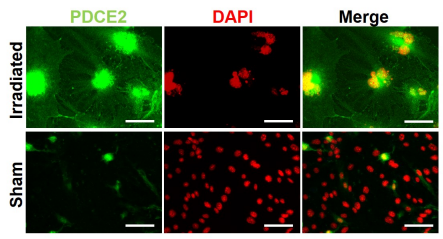
Immunohistochemical staining of PDCE2 (green) in rat AVM. PDCE2 co-localizes with CD31 endothelial marker (red) after irradiation (arrows).

Results

Immunocytochemistry

Immunocytochemistry of irradiated brain endothelial cells demonstrated that radiation induced cellular enlargement and flattening concomitant with increased cell surface expression of PDC-E2 (Fig 4).

Fig 4 - Validation of PDCE2 surface translocation in cultured endothelial cells



Immunocytochemical staining of PDCE2 (green) at the cell surface in non-permeabilized mouse brain endothelial cells after irradiation.

Conclusions

Using biotin labelling we discovered the intracellular protein, PDC-E2, is translocated to the endothelial cell surface in response to radiation.

The abnormal radiation-stimulated externalization of mitochondrial PDC-E2 may provide a unique candidate for vascular targeting in AVMs.

References

(1) Rybak *et al.* (2005) Nat Meth 2:4:291
(2) Yassari *et al.* (2004) Acta Neurchir 146:5:495