

Free radicals react with redox sensitive cysteines and form oxidative post translational modifications (Ox-PTM) which alters protein function. The effect of redox signalling on Jag1 has not yet been investigated. However, we have identified Jag1 as a redox target in ECs. Additionally, this signalling regulates expression of proteins involved in adhesion, the cell cycle, and the extracellular matrix (ECM). An Iodo-TMT redox proteomics screen was performed to identify Ox-PTMs in ECs involved in vascular signalling mechanisms. A group of proteins involved in angiogenesis was identified including Jag1, one of the canonical Notch ligands. The patterning of blood vessel sprouts during angiogenesis is regulated by Notch-Jag interactions. Quantitative proteomics was performed to determine changes in protein expression between WT and a redox-insensitive variant of Jag1. This 'redox dead' (RD) variant was generated through cysteine to serine substitutions. Human umbilical vein ECs (HUVEC) transiently expressing these Jag1 variants were treated with H₂O₂ or VEGF. Jag1RD downregulated proteins related to adhesion and the ECM while upregulating cell cycle proteins. Using the histone ruler method, protein content per cell was estimated. This showed lower protein in cells expressing Jag1RD compared to Jag1WT, correlating with an increased cell cycle rate. Results show that Jag1 redox modifications alter important EC processes which may be Notch-independent.

14 **METFORMIN DOES NOT REVERSE THE EFFECT OF PHARMACOLOGICAL INHIBITION OF PROTEIN PHOSPHATASE 2A ON VE-CADHERIN IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS**

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Background Several neurological diseases and CNS complications accompanying diabetes mellitus are associated with increased permeability of the blood brain barrier. Dysregulation of kinase and phosphatase signalling may underpin breakdown of the blood brain barrier. Therefore, the aim of the current study was to investigate the role of protein phosphatase 2A (PP2A) in modulating VE-cadherin expression and its modulation by metformin.

Methods Human brain microvascular endothelial cells were exposed to okadaic acid (10nM; OA) alone or in combination with metformin (0.1 or 15mM). VE-cadherin and PP2Ac abundance and expression were determined using Western blot and PCR. PP2Ac activity were determined using a phosphatase assay. Proteomic analysis (microarray chip) was undertaken in collaboration with Sciomics. Data were analysed by One-way ANOVA or T-test/F-test ($P < 0.05$ indicates significance).

Results OA (1-20nM) caused a dose dependent decrease in VE-cadherin abundance while metformin had no effect (0.1-15mM). OA (10nM) decreased VE-cadherin abundance while increasing mRNA expression. This effect was not altered by metformin (15 mM). OA and metformin did not alter PPP2Ac expression. Although OA (10nM) decreased PP2Ac activity, this was unchanged in combination with metformin (15mM). Furthermore, metformin (1mM) did not alter PP2Ac abundance. Following proteomic analysis OA differentially regulated 103 proteins. Of these, cathepsin and polyubiquitin were differentially regulated.

Conclusion OA decreased PP2A activity and VE-cadherin abundance independently of altering transcriptional regulation which may be mediated through lysosomal degradation and increased ubiquitination. Furthermore, metformin did not alter PP2A activity which is consistent with its inability to offset OA-mediated attenuation of VE-cadherin.

15 **INVESTIGATION OF ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTOR AS AN IMAGING TARGET FOR MYOCARDIAL INFARCT HEALING**

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PET imaging of myocardial healing following myocardial infarction (MI) could have prognostic value and aid novel therapeutic development. Stimulation of peripheral $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChR) is known to promote many aspects of myocardial healing, including inhibition of inflammation and promotion of angiogenesis. We hypothesised that $\alpha 7$ nAChR could serve as a marker of myocardial healing with early expression due to angiogenic activity. Therefore, we set out to assess the temporal expression of $\alpha 7$ nAChR in rat MI tissue using the $\alpha 7$ nAChR-specific radiotracer NS14490, in addition to histology and proteomic profiling.

Adult male Sprague-Dawley rats underwent coronary artery ligation (30 mins) followed by reperfusion to induce MI (n=4-5), or sham-surgery (n=4-6), before culling and collecting their hearts at d2, d7, d14 and d28. $\alpha 7$ nAChR was detected by autoradiography using [³H]NS14490 in wax processed tissue, with serial sections used for histology. d2 and d28 fresh frozen infarct tissue was used for proteomics.

Proteomics and histology (CD68+ve macrophage and collagen) demonstrate early dominance of inflammation followed by later extracellular matrix (ECM) deposition. $\alpha 7$ nAChR imaging revealed that expression starts at d14 and peaks at d28 post-injury, almost exclusively within the infarct territory. $\alpha 7$ nAChR expression strongly correlated with total collagen levels.

This expression pattern does not support a role for $\alpha 7$ nAChR in early angiogenesis in this model, but is consistent with a role in regulation of ECM deposition after resolution of inflammation. Further investigation is required to ascertain how the level of $\alpha 7$ nAChR measured by NS14490 relates to myocardial repair and long term functional outcome.

16 **VASCULAR REDOX SIGNALLING AND PROTEIN OXIDATION: FOCUS ON PALMITOYLATION OF NADPH OXIDASE 5 (NOX5) AS A POTENTIAL POSTTRANSLATIONAL REGULATORY PROCESS**

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NOX5 is a significant source of reactive oxygen species (ROS), which controls vascular tone and blood pressure,