Poster 380

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An untargeted proteomic study identifies proteins associated with post procedural myocardial infarction

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Introduction

Acute myocardial Infarction (AMI) accounts for 13% of deaths in Australia (ref 1). Early identification of myocardial ischemia/Infarction allows for timely interventions that have been shown to improve morbidity and mortality. These advances have resulted from an improved understanding of the mechanisms underlying myocardial ischemia/infarction. Disappointingly, current diagnostic tests are unable to identify the majority of those who will go on to have an AMI and even with optimal therapeutics there is residual risk that remains untreated. Continued investigation of the mechanisms involved in AMI are required.

Proteins mediate much of the cellular and extracellular signalling and are therefore pathophysiological targets. We have performed an untargeted proteomic study looking for differentially expressed proteins across the coronary circulation after an induced AMI following elective percutaneous coronary interventions (PCI). Proteins that are significantly altered may be markers of early ischemia or therapeutic targets.

Aims

Hypothesis:
Proteins are up or down regulated early following acute coronary syndromes and these proteins will act as markers of early ischemia or be targets for therapeutic intervention.

Aim:
1. Identify differentially expressed proteins across the coronary tree (Left Main-Coronary Sinus (LM-CS)) following elective PCI in relation to degree of troponin elevation.
2. Identify differentially expressed proteins pre PCI compared with post PCI from Coronary Sinus sampling

Methods

16 subjects undergoing elective PCI (stable , troponin negative) had simultaneous LM-CS plasma sampling pre and post PCI. Samples were collected into EDTA vacutainers, centrifuged at 4decC, 4000rpm for 10mins with plasma supernatant stored at -80deg. Protein was extracted via acetone method, reduced and alkylated, resuspended in urea prior to trypsin digestion. Peptides were dimethyl stable isotope labelled with isotopomers of formaldehyde and cyanoborohydride (ref 2). Paired samples were then combined and 2-dimensional liquid chromatography (strong cation exchange followed by online reverse phase chromatography) was performed. Mass Spectrometry was performed using an Orbitrap Elite. Troponin T was measured at 12 hours post PCI for peak level.

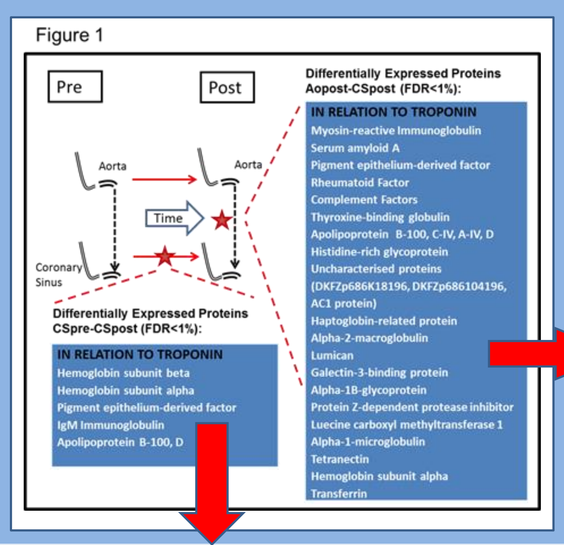
Protein identification was via MS/MS search (Uniprot (Swissprot and Trembl, July 2014)), and quantification with MaxQuant (v1.5.0.0). Both protein and peptide spectrum match FDR cut-offs were 1%. All other MaxQuant MS/MS search settings were left at their defaults.
Intensity ratios for labelled peptides between proximal (heavy labelled) and distal (light labelled) samples for the LM-CS analysis and pre-PCI-CS (heavy labelled) and post-PCI-CS (light labelled) samples were calculated (Figure 1).

Light labels were set to (Lys0, N-term0) and heavy labels set to (Lys4, N-term4). Statistical analysis was performed in R using the package limma. Normalized ratios were log base 2 transformed. All proteins were analysed together using the lmFit and eBayes functions in limma, which use empirical Bayes to increase statistical power. Intensity ratio data (response variable) were fit to a linear model that included a baseline effect (intercept) plus the measured Troponin level for each patient as an additional explanatory variable. Significance was assessed through Benjamini Hochberg adjusted p-values (t-tests) for the null hypotheses that the slope of the Troponin effect was 0. P-values of less than 0.05 were taken as significant.

Results

25 prospective proteins were associated with elevated troponin levels across the coronary circulation post procedure and 6 proteins were associated with elevated troponin levels when comparing pre to post PCI coronary sinus samples (Figure 1). Most proteins were involved in acute phase, coagulation or structural processes. These changes occurred very early (mean 34mins, range 12-120mins) after ischemia onset (as measured from initial ballooning).
Proteins of significant interest include Apolipoprotein B100, Apolipoprotein D, and Pigment epithelium-derived factor which were all differentially expressed across the coronary circulation (LM-CS) as well as pre-post PCI (CS sample).

Figure 1



Pre-PCI vs post-PCI: Significant protein changes using Troponin as a predictor

Protein	logFC	adj.P.Val
Hemoglobin subunit beta	-0.650	<0.001
Hemoglobin subunit alpha	-0.668	<0.001
Pigment epithelium-derived factor	0.251	0.003
IgM immunoglobulin	-0.467	0.007
Apolipoprotein B-100	-0.265	0.031
Apolipoprotein D	-0.246	0.031

LM-CS: Significant protein changes using Troponin as a predictor

Protein	logFC	Adj.P.Val
Myosin-reactive Immunoglobulin	0.149	0.014
Serum amyloid A	0.638	0.007
Pigment epithelium-derived factor	0.181	0.007
Rheumatoid Factor	-0.152	0.014
Thyroxine-binding globulin	0.092	0.014
Apolipoprotein B-100	-0.203	0.014
Histidine-rich glycoprotein	0.170	0.014
Haptoglobin-related protein	-0.165	0.017
Alpha-2-macroglobulin	0.141	0.017
Lumican	0.112	0.020
Apolipoprotein C-IV	-0.325	0.024
Galectin-3-binding protein	0.431	0.024
Alpha-1B-glycoprotein	0.064	0.026
Protein Z-dependent protease inhibitor	0.517	0.030
Luecine carboxyl methyltransferase 1	-0.217	0.030
Alpha-1-microglobulin	0.063	0.030
Apolipoprotein A-IV	0.127	0.030
Tetranectin	-0.087	0.037
DKFZp686K18196 (uncharacterised)	-1.701	0.038
Apolipoprotein D	-0.143	0.041
Hemoglobin subunit alpha	-0.494	0.041
Transferrin	0.048	0.050

Subject Characteristics (n=16)	
Age	66yrs (46-82)
Male	65%
Risk Factors	
T2DM	35.3%
FHx IHD	35.3%
Dyslipidaemia	64.7%
Hypertension	82.4%
Smoking	
Current	17.6%
Ex-Smoker	35.3%
Past History	
Prior AMI	35.3%
Medications	
Aspirin	88.2%
DAPT (pre)	47%
Statin	52.9%
ACE-I/ARB	52.9%
Beta-Blocker	47%
Baseline Examination	
BMI	28 kg/m^2
SBP	140 mmHg
DBP	82 mmHg
HR	72 bpm
AHA Lesion	
A	0%
B1	35%
B2	24%
C	41%

Conclusions

We describe the early post PCI trans-coronary and pre-post PCI CS protein profile that is associated with post procedural myocardial ischemia (PPMI).
We discuss 6 proteins that were identified as prospective markers based on a clinically significant level of fold change and statistical significance.

Apoliporotein B100 (ApoB100): log2FC -0.203, adj.p=0.014
ApoB100 is the primary Apo on LDL, IDL and VLDL lipoprotein particles. LDL chol is known to decrease after AMI in relation to troponin elevation. LDL particle sequestration across the coronary tree would explain the decrease in ApoB100 in the CS samples and the previously described decrease in LDL post AMI.

Histidine-Rich Glycoprotein (HRG): log2FC 0.170, adj.p=0.014
HRG binds to many proteins including heparin inhibiting the heparin/ATIII complex and is therefore procoagulant. Our finding that HRG is increased across the coronary tree in relation to troponin suggests inhibition of HRG may be a target to decrease PPMI.

Lumican: log2FC 0.112, adj.p=0.02
Lumican is one of the small leucine rich proteoglycan (SLRP) proteins . Lumican is found in higher concentration in atheroma prone arteries. In addition Lumican may be involved with intimal hyperplasia and has been implicated in the pathogenesis of aortic stenosis.

Haptoglobin related peptide (HRP): log2FC -0.165, adj.p=0.017
HRP is associated with ApoL1 containing HDL and is involved in the innate immune response. HRP and SAA are able to distinguish between atherothrombotic and cardioembolic strokes. A decrease across the coronary circulation in relation to PPMI requires further mechanistic investigation.

Pigment Epithelium Derived Factor (PDEF): log2FC 0.181, adj p=0.007
PDEF is a glycoprotein produced by adipose tissue and:
- Correlates with CIMT
- Is positively associated with CAD and metabolic syndrome and is elevated in diabetics
- Correlates with TG, CRP, LDL, ApoB and negatively with HDL levels.
- Binds to a phospholipase A2 receptor on cardiomyocytes leading to apoptosis.
- Is a potent inhibitor of angiogenesis
- Correlates negatively with post angioplasty neointimal hyperplasia (NIH).

We have demonstrated that PDEF is increased across the coronary circulation in relation to PPMI. Whether this is a reactive-protective or pathological effect remains unknown. We hypothesise that PDEF may inhibit angiogenesis and supress endothelial cell proliferation and this may explain the association with less NIH post angioplasty. Conversely PEDF may be a target of inhibition to prevent cardiomyocyte death.

Serum Amyloid A (SAA): log2FC 0.637, adj.p=0.007
SAA is a highly conserved acute phase protein. Post AMI up to 50% of ApoA1, the primary apolipoprotein of HDL is substituted for SAA. SAA decreases HDL clearance and impairs cholesterol uptake by HDL. SAA has previously been associated with CV risk. Our data confirms that SAA is produced from within the coronary circulation post coronary intervention.

Conclusion:
We have identified a number of proteins that are differentially expressed across the coronary circulation post elective PCI correlating with the degree of PPMI. These proteins are prospective markers of PPMI or may be prospective therapeutic targets .

References
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2. P Boersema et al, Multiplex peptide stable isotope dimethyl labelling for quantitative proteomics. Nature Protocols. 2009,4(4),pg484.

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