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Proteomic Profiling of Fractionated Post-myocardial Infarction Plasma Identifies MMP-9 Dependent Markers

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SUMMARY

Following myocardial infarction (MI), matrix metalloproteinase-9 (MMP-9) levels increase; MMP-9 deletion improves post-MI remodeling of the left ventricle (LV). MI was induced in wild type (WT) and MMP-9 null mice. Plasma was collected on day 0 (no MI) and day 1 post-MI. Plasma proteins were fractionated and the lowest and highest molecular weight fractions were separated by 1-D SDS-PAGE, and analyzed by HPLC-ESI-MS/MS. We identified over 100 proteins in each genotype. Proteins commonly associated with acute inflammation, such as serum amyloid A (SAA), neutrophil galectin associated lipocalin (NGAL), and α-2 macroglobulin (α2M), were substantially increased post-MI in both genotypes. α2M increased only in the null samples, suggesting that this protein is MMP-9 associated.

INTRODUCTION

Acute myocardial infarction remains a leading cause of morbidity and mortality worldwide. Heart failure is the result of adverse remodeling of the collagenous scar that replaces the damaged myocardium after MI. Markers of LV remodeling can be either identified in the circulation (e.g. serum or plasma) or detected in the heart by imaging technologies or biopsy. Post-MI, specific matrix metalloproteinases (MMPs) increase and mediate left ventricular remodeling. MMP-9 has been reported as a prognostic indicator of cardiac dysfunction in MI patients.[1,2] Animal models show that MMP-9 levels increase early post-MI,[3,4] and MMP-9 deletion has previously been shown to improve remodeling of the LV post-MI. Plasma has a complex proteome with a wide range of protein concentrations. We hypothesized that the analysis of plasma proteins post-MI in WT and MMP-9 null mice will identify prospective markers of early MI that are MMP-9 dependent. Accordingly, plasma proteins were fractionated by molecular weight to permit proteomic analysis of plasma proteins post-MI in wild type and MMP-9 null mice.

METHODS

Animals and Surgery

- Male C57BL/6J WT and MMP-9 null mice (4 – 8 months old).
- n = 3/genotype at day 0 (no MI, controls) and day 1 post-MI.
- MI induced by ligation of left anterior descending coronary artery.
- Blood collected at day 0 and day 1 post-MI, and plasma isolated.
- Hearts removed, and separated into right and left ventricles.
- LV stained with 1% 2,3,5-triphenyltetrazolium chloride (TTC), cut into 3 transverse sections, and photographed for measurement of the infarct area.

Plasma Fractionation

- Total protein quantification by Quick Start Bradford Protein Assay (Bio-Rad).
- Plasma fractionation by GelFree™ 8100 Fractionation System (Protein Discovery):
  - 500 µg total protein
  - Proteins reduced for 10 min at 50°C with 1x acetate sample buffer (Protein Discovery, Inc.) and 0.053 M dithiothreitol (DTT).
  - Proteins alkylated with 15 mM iodoacetamide in the dark for 10 min.
  - Proteins loaded into an 8% Tris acetate cartridge.
  - Running buffer: MES [0.05 M MES, 0.05 M Tris, 0.1% SDS pH 7.9].
  - 12 protein fractions collected (F1 to F12).
  - Protein fractions visualized by SDS-PAGE using a 12% Bis-Tris gel.
  - F1 (lowest molecular weight) further separated on a 10-20% Tricine/peptide gel.

Mass Spectrometry

- F1 & F12 analyzed by HPLC-ESI-MS/MS (Orbitrap Velos) after in-gel tryptic digestion.
- On-line HPLC separation: Eksigent/AB Sciex NanoLC-Ultra 2-D HPLC system.
- Determination of protein and peptide identity probabilities: Scaffold (version 3; Proteome Software).
- Thresholds for acceptance of peptide and protein: 95% and 99.9%, respectively.
- Proteins of interest further analyzed by immunoblotting.

RESULTS

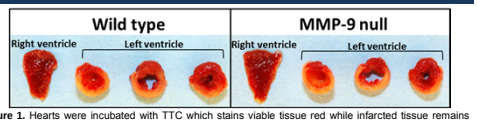
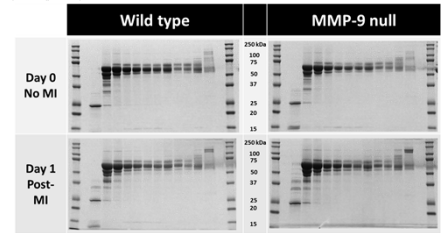


Figure 1. Hearts were incubated with TTC which stains viable tissue red while infarcted tissue remains white. The LV was cut into 3 transverse sections for quantification of the infarcted tissue; Image J analysis was used to measure the infarct area. Infarct areas were similar between WT (52±8%) and MMP-9 null (54±2%) mice (p=0.85).



Fractionation program #3. *During this step, running buffer was replaced with fresh running buffer.

Step	1	2	3	4	5	6	7	8	9	10	11	12	13
Voltage (V)	50	50	100	100	80	80	80	80	80	80	80	100	100
Fraction time (min)	16	44	7	5	3	3	3	3	5	5	5	10	20
Fraction #	---	1*	2	3	4	5	6	7	8	9*	10	11	12

Figure 2. Each plasma sample was separated into 12 fractions with a GelFree™ 8100 Fractionation System, using an 8% acetate cartridge and analyzed on 12% Bis-Tris gels. The figure shows a representative gel from each group.

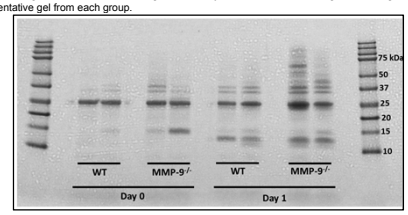


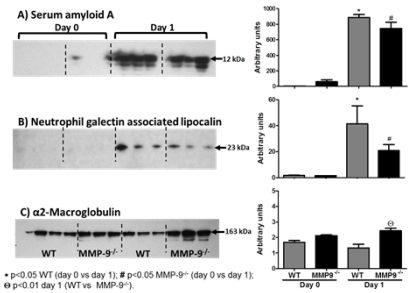
Figure 3. F1 (n=2/group) proteins were further separated on a 10-20% Tricine/peptide gel. The gel lane for each replicate was divided into six slices. Each slice was separately destained and dehydrated and the proteins digested *in situ* with trypsin. The digests were analyzed by HPLC-ESI-MS/MS.

RESULTS

Table 1. Proteins identified only in post- MI plasma samples.

WT	MMP-9 null
14-3-3 zeta	14-3-3 protein gamma
alpha-1-microglobulin/bikunin precursor	adiponectin precursor
alpha-2-macroglobulin	alpha-1 protease inhibitor 2
antithrombin-III precursor	alpha-1-acid glycoprotein 1 precursor
apolipoprotein A-I preproprotein and -IV precursor	apolipoprotein A-IV precursor
apolipoprotein B and -E	apolipoprotein B and -E
carbonic anhydrase 1	alpha-1-antitrypsin 1-5 precursor
carbonic anhydrase 2	anti-idiotypic 4C8 immunoglobulin light chain
chain L, Fab Fragment mouse anti- Human Fas ab Hfe7a	antithrombin-III precursor
clusterin	ceruloplasmin isoform b
cytochrome c, somatic	cytochrome c, somatic
fetuin	complement factor I precursor
fibronectin 1	extracellular matrix protein 1 precursor
glycogen phosphorylase, muscle form	fetuin and fetuin-B isoform
heat shock 70 kDa protein 4	fibrinogen beta chain precursor
H2-Q10 protein	ficolin-1 precursor
LDHa protein	glycogen phosphorylase, muscle form
L-lactate dehydrogenase B chain	hemopexin precursor
malate dehydrogenase 1, NAD (soluble)	hepatocyte growth factor activator
microtubule-actin crosslinking factor 1	kininogen-1 isoform 2
neutrophil gelatinase-associated lipocalin precursor	LDHa protein
phosphoglycerate mutase 2	leucine-rich alpha-2-glycoprotein
proteasome (prosome, macropain) subunit, beta type 6	liver carboxylesterase N
proteasome subunit alpha type-4	L-lactate dehydrogenase B chain
protein S100-A9	malate dehydrogenase, cytoplasmic
serine (or cysteine) peptidase inhibitor,clade A IC	neutrophil gelatinase-associated lipocalin precursor
serine protease inhibitor A3M and A3N precursor	parotid secretory protein
serum amyloid A (AA at 131)	proteasome subunit alpha type-3
serum paraoxonase	proteoglycan 4 isoform 1
triosephosphate isomerase	serine protease inhibitor A3M, A3K and A3N
vinculin	serine (or cysteine) peptidase inhibitor, clade A and clade F
vitronectin	serum amyloid A (AA at 131)
	vitamin D-binding protein
	vitronectin
	zinc finger protein 608

RESULTS



* p<0.05 WT (day 0 vs day 1); # p<0.05 MMP-9$^{-/-}$ (day 0 vs day 1); Θ p<0.01 day 1 (WT vs MMP-9$^{-/-}$).

Figure 4. Immunoblots for: A) SAA. Protein levels increased significantly post-MI for both genotypes (p<0.001). B) NGAL. NGAL was observed only post-MI in both genotypes (p<0.05). C) α2M. MMP-9 null mice showed higher levels of α2M at day 1 post-MI compared to the WT mice counterparts. Densitometry was used to quantify protein levels in all immunoblots; n=3/group.

CONCLUSIONS

- By performing plasma fractionation prior to proteomics analysis, we were able to reduce the presence of high abundant proteins, such as albumin, and enrich samples for the detection of lower abundance proteins.
- We compared plasma samples from WT and MMP-9 null mice post-MI, and identified α2M as a prospective MI marker that may be MMP-9 dependent. Although the association between MMP-9 and α2M has been previously reported, this is the first time that α2M has been found to be associated with MMP-9 in the myocardial infarction setting.

ACKNOWLEDGEMENTS

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