Protein Expression Analyses in Bone Marrow Stem Cell Mediated Restoration of Myocardium After Ischemic Insult.

Kate Lee

William Harvey Annual Research Review 2008

Barts and The London
School of Medicine and Dentistry

Introduction

- Myocardial Infarction (Heart attack).
- Reduced or total failure of blood supply to myocardium.
- 1/3 die before reaching hospital
- 40% mortality including acute and long term outcome events (ventricular fibrillation and LV failure).

Introduction

- Severity of outcome

Complete/

long time

Occlusion.

Large area/

full thickness

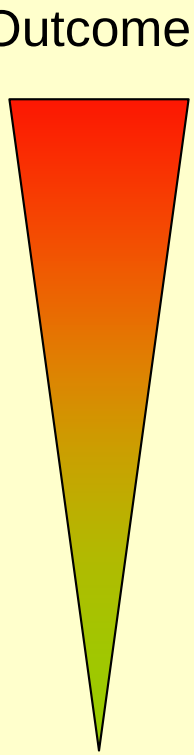
Infarct.

Incomplete/

short time

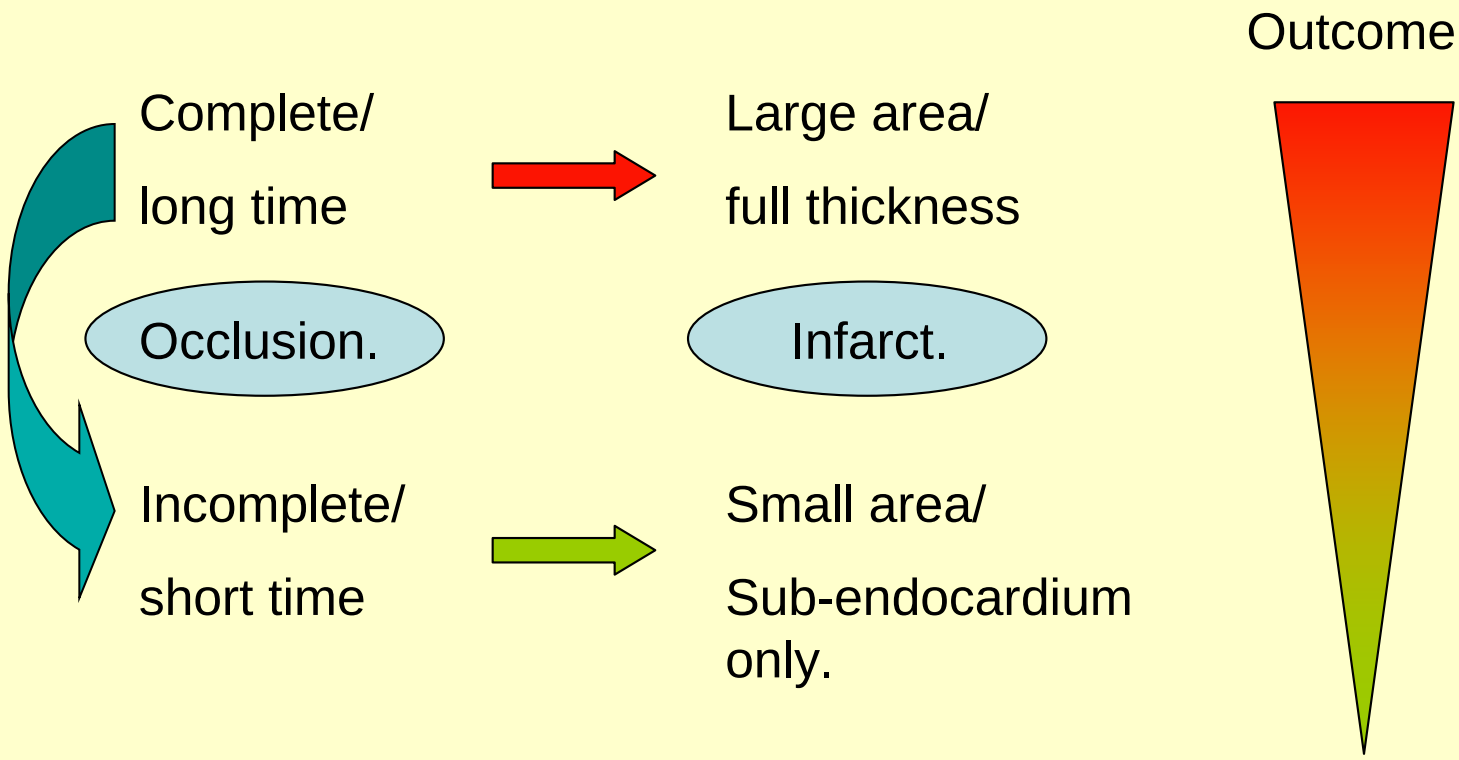
Small area/

Sub-endocardium only.



Introduction

- Current intervention options

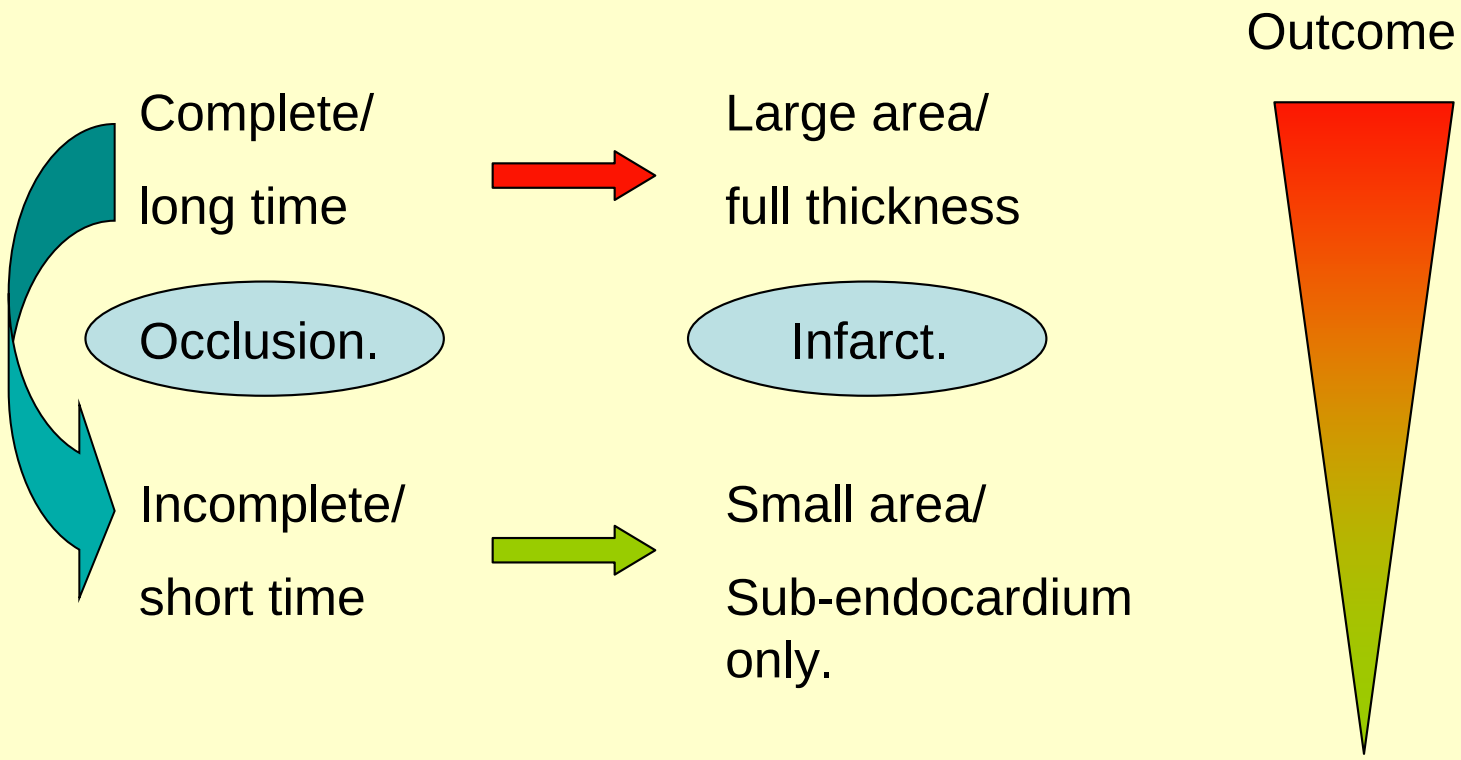


Reduce period and severity of occlusion through recanalisation:

- ❑ Angioplasty
- ❑ Thrombolysis

Introduction

- Current intervention options

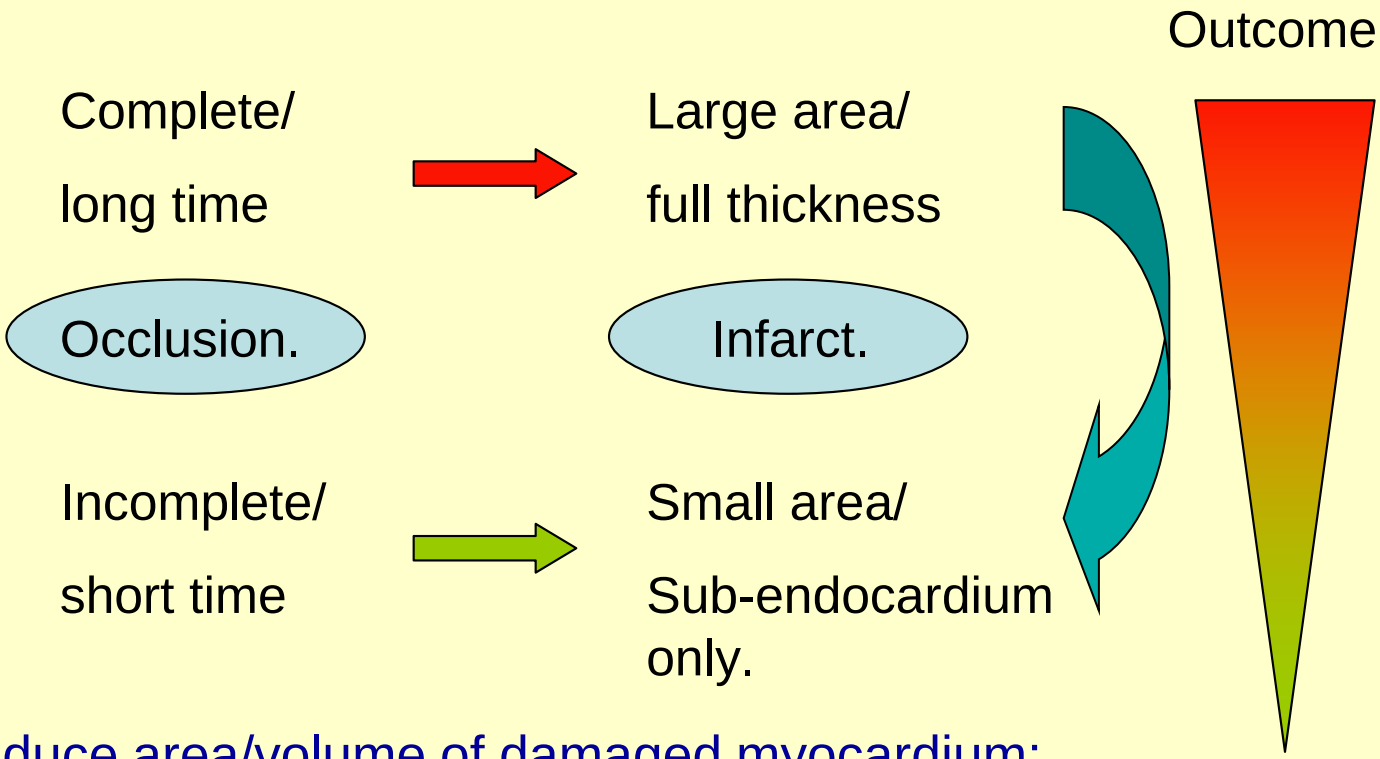


Reduce period and severity of occlusion through recanalisation:

- ❑ Angioplasty
- ❑ Thrombolysis

Introduction

- Other Intervention options



Reduce area/volume of damaged myocardium:

- ❑ Regeneration/rejuvenation of myocardium.
- ❑ Re-vascularisation

Introduction

- Stem cell therapy.
  - Embryonic and adult stem cell populations.
  - Adult stem cells - convenient and autologous.
  - Bone marrow derived stem cells.

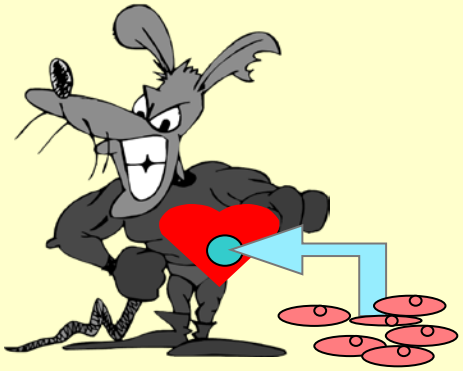
Previous Investigation

- Model:

3 groups (n=3)

SHAM

MI



MNC

- Methods:

- ❑ I/R model: left anterior descending coronary artery
- ❑ Bone marrow derived mononuclear cells (MNCs)
- ❑ Ischemia 30 minutes – cells (intravenous) – reperfusion 2 hours
- ❑ Cell migration/homing, apoptosis/necrosis and cardiac function (ECHO)

Personal communication, M.Lovell and M.Yasin.

Previous Investigation

- Findings:
  - ❑ BMCs home-in on infarct zone.
  - ❑ Reduction in apoptosis and necrosis.
  - ❑ 40% reduction in infarct size.
  - ❑ Improved myocardial functioning.

However, mechanism is as yet unknown….

Personal communication, M.Lovell and M.Yasin.

Current Study

- Our aims:

  - ❑ Use gene expression profiling to look for differentially expressed genes.

  - ❑ Use comparative 2D-Gel electrophoresis (2DGE) methods to identify differentially expressed proteins.

  Hypothesis is that differentially expressed genes and proteins will indicate a mechanism.

Transcriptomics

- Illumina (Genome Centre)
- RatRef12 beadchips (~22,000 genes)
- Linear modelling (Limma, Bioconductor)

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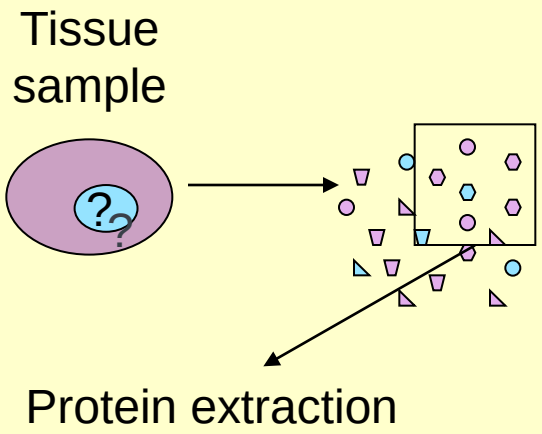
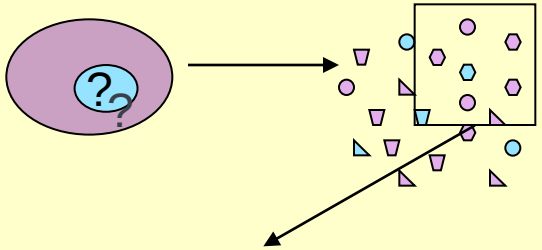
No significant differences

Methods - Proteomics



Methods - Proteomics

Tissue sample

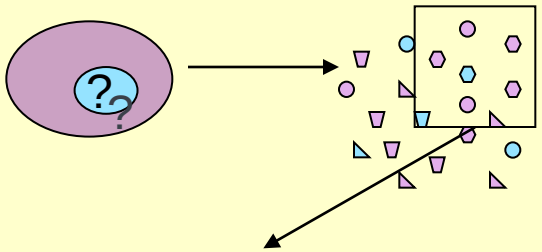


Protein extraction

1st dimension separation.

Methods - Proteomics

Tissue sample



Protein extraction

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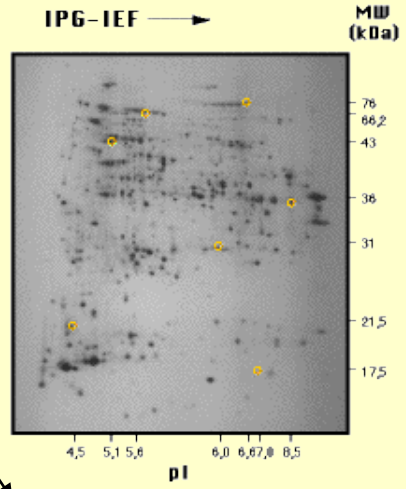

2nd dimension separation.

Methods - Proteomics

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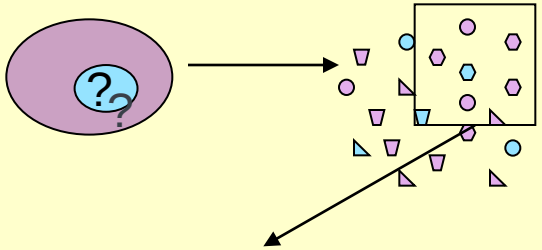

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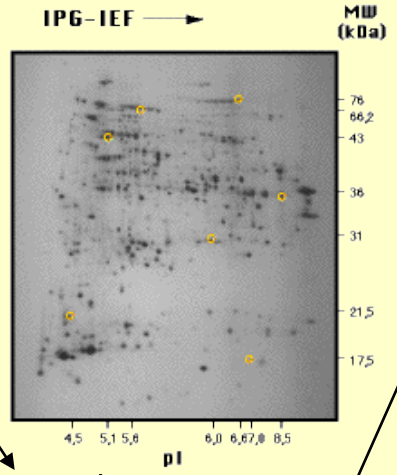
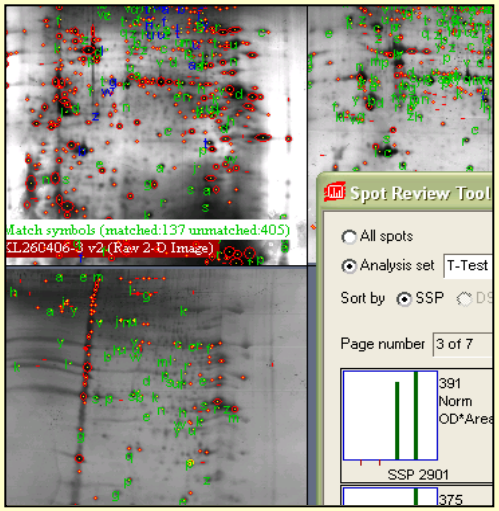


2nd dimension separation.

Image analysis



Methods - Proteomics

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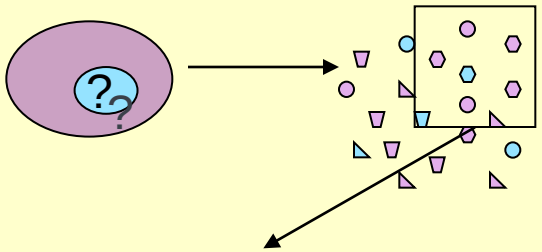

Protein extraction

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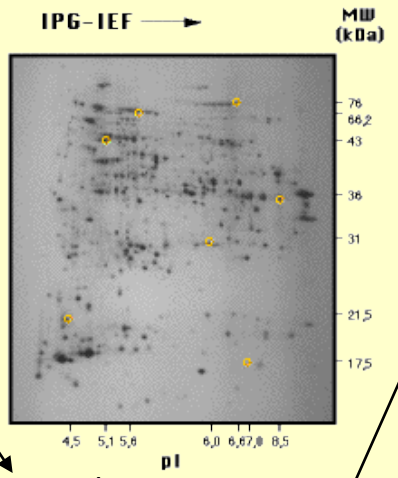
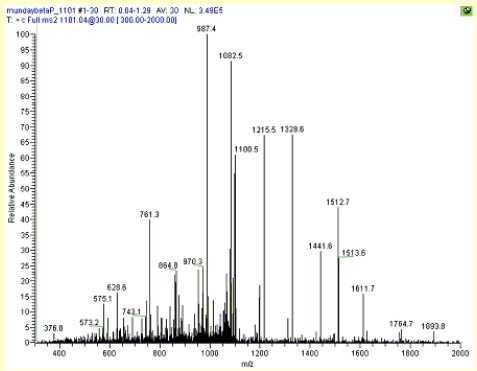
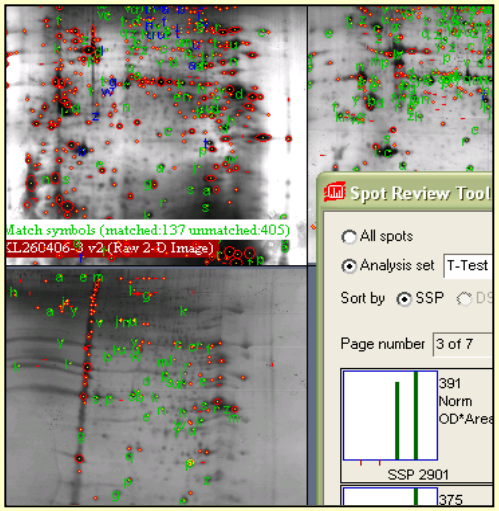


2nd dimension separation.



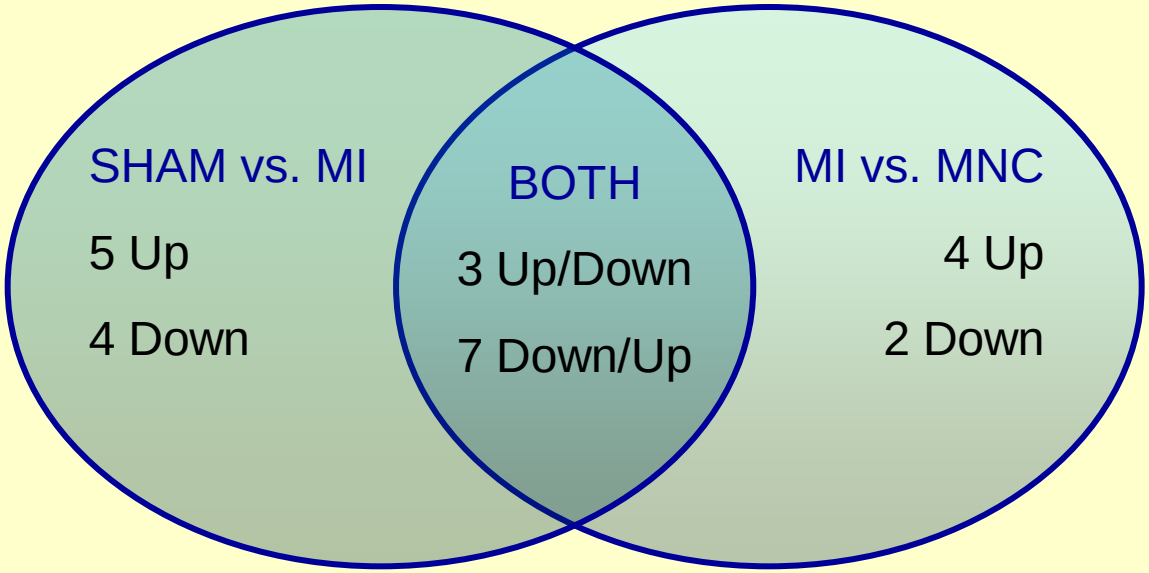
Protein Identification (LC-MS/MS)

Image analysis



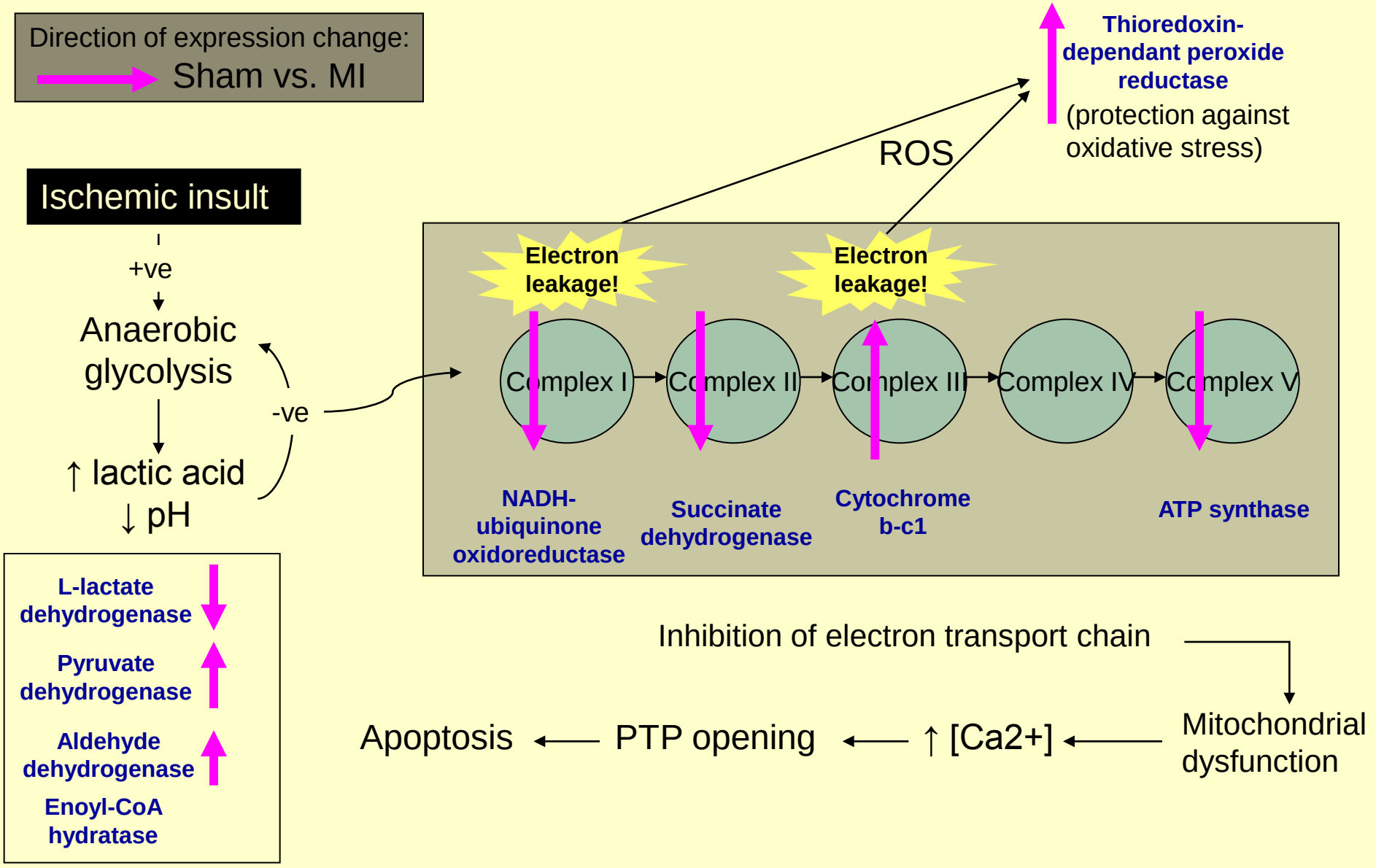
Results – Proteomics

- Differentially expressed proteins:
  - ❑ ‘SHAM vs. MI’ and ‘MI vs. MNC’.
  - ❑ >2.5 fold and p≤0.05*
  - ❑ 25 different proteins



* in either Student’s T-test or Wilcoxon Rank sum test.

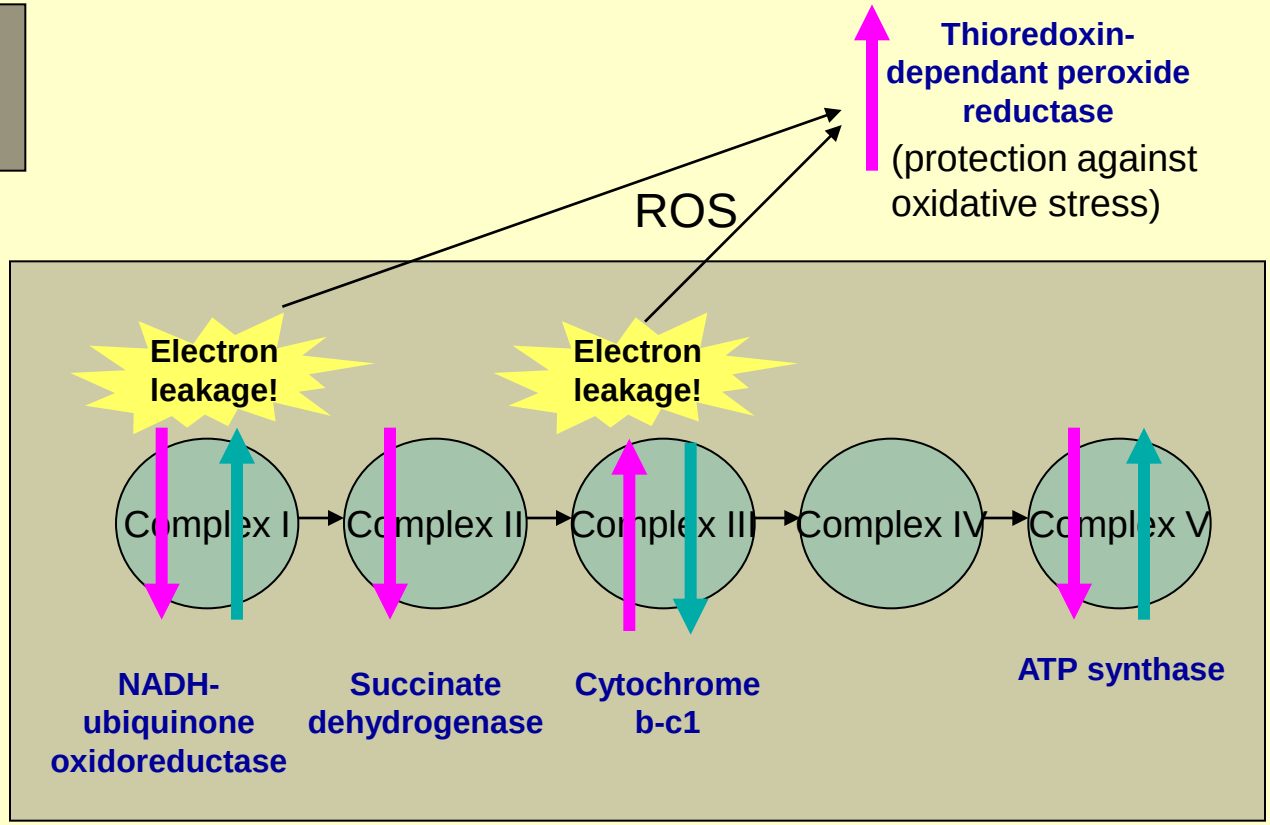
Results – Proteomics



Results – Proteomics

Direction of expression change:
Sham vs. MI
MI vs. MNC

Bone Marrow Stem Cells ?



Restoration of electron transport chain

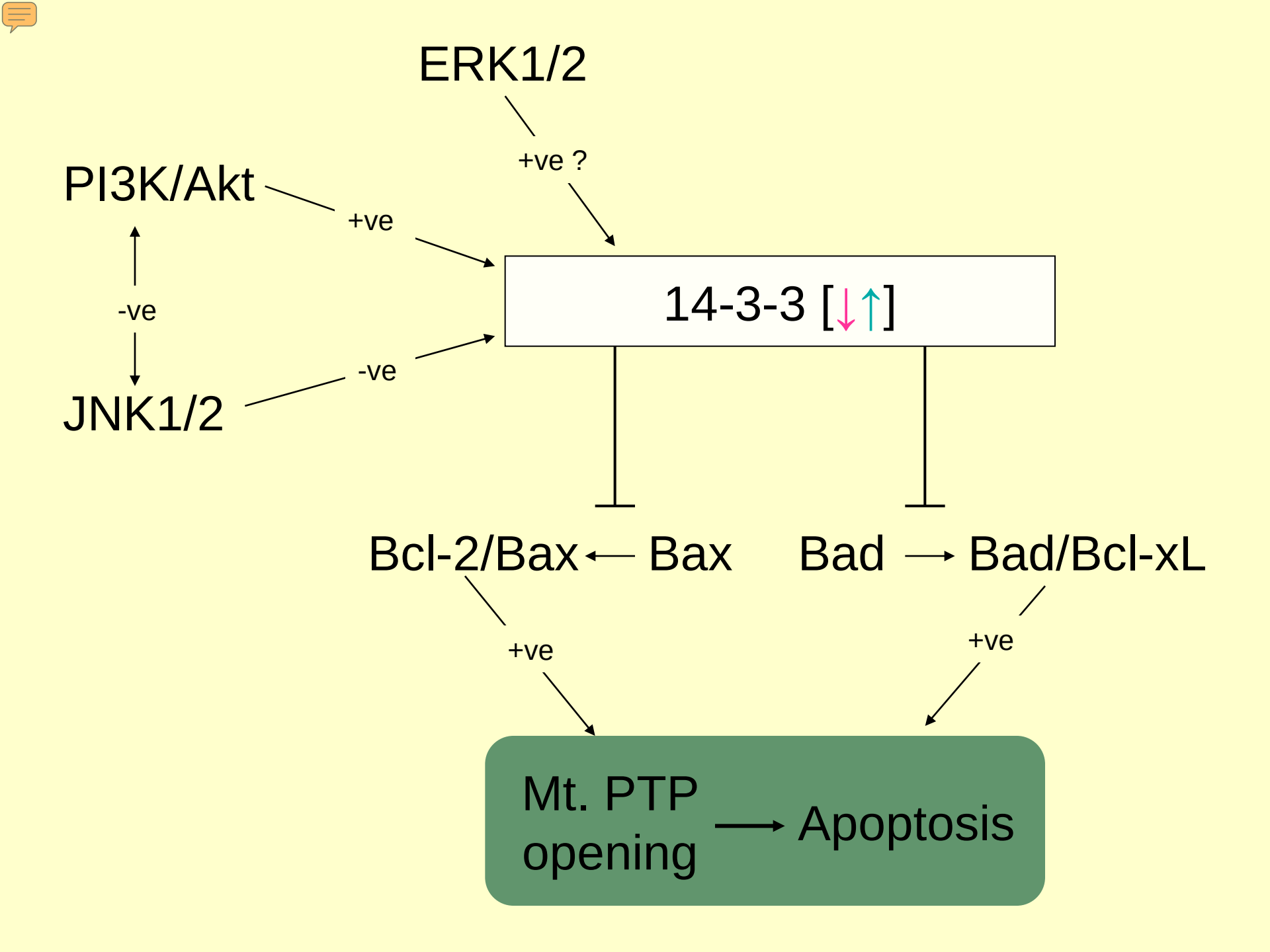
L-lactate dehydrogenase
Pyruvate dehydrogenase
Aldehyde dehydrogenase
Enoyl-CoA hydratase

Results - Proteomics

- Other proteins of interest:

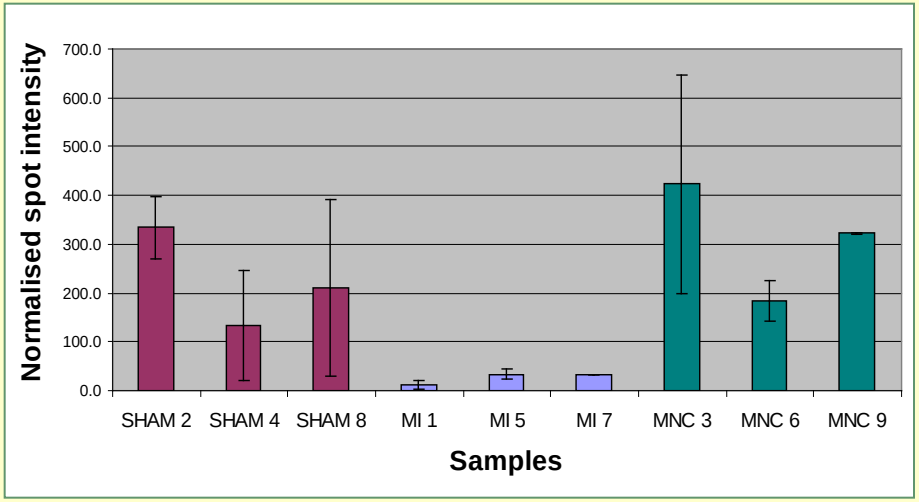
		SHAM vs. MI			MI vs. MNC		
Protein ID	**Symbol**	**FC**	**p-value ^{t}**	**p-value ^{w}**	**FC**	**p-value ^{t}**	**p-value ^{w}**
HEAT SHOCK							
Stress-70 protein	HSPA9	-3.30	0.011	0.026	2.71	0.006	0.009
Heat shock cognate 71Da	HSPA8	-	-	-	2.92	0.048	0.082
STRUCTURAL							
Desmin	DES	-	-	-	2.64	0.003	0.004
OTHER							
Alpha-crystallin B chain	CRYAB	16.25	0.013	0.010			
Adenylyl cyclase-associated protein 1	CAP1	-4.11	0.010	0.030	6.21	0.002	0.004
14-3-3 protein epsilon	YWHAQ	-9.11	0.027	0.126	12.51	0.007	0.004

FC= Fold change; T= Students t-test, W= Wilcoxon's test


ERK1/2
+ve ?
PI3K/Akt
+ve
-ve
14-3-3 [↓↑]
-ve
JNK1/2
Bcl-2/Bax
Bax
Bad
Bad/Bcl-xL
+ve
+ve
Mt. PTP opening
Apoptosis

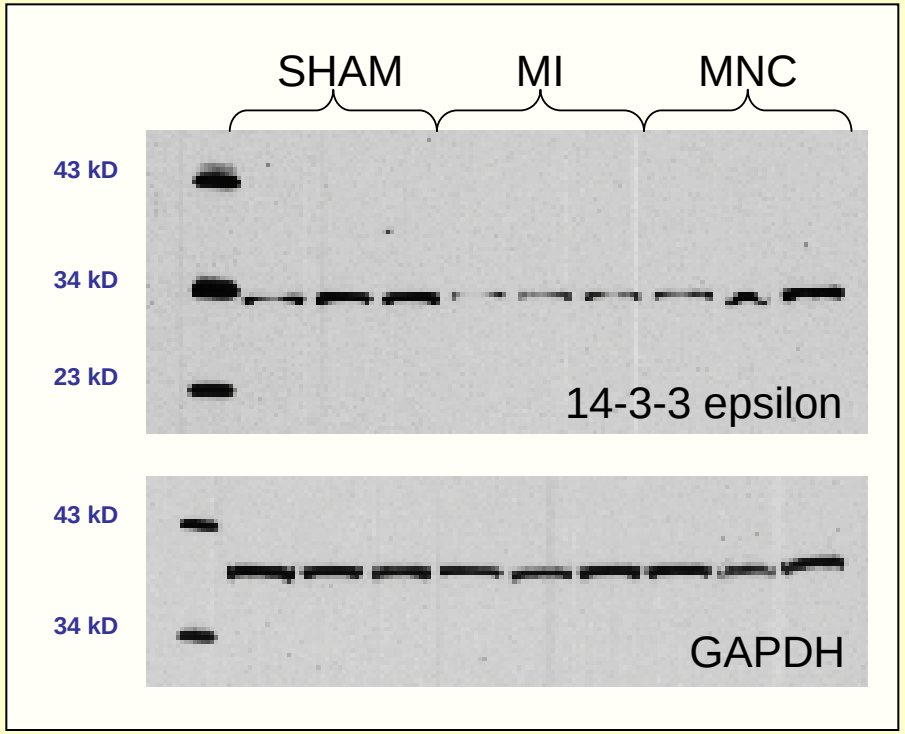
Results – Western Blot

- Western blot confirmation of 2DGE 14-3-3 epsilon results



Graph showing 2D spot intensities

Western blot image



Discussion

- Lack of significant gene expression changes.
- Proteomics approach obtained differentially expressed proteins.
- Molecular reflection on cellular and myocardial scale restoration.
- Potential clues to mechanism (14-3-3).
- Naturally occurring cardioprotective mechanisms .

What next

- Validation.
- Targeted gene expression.
- Investigate phosphorylation patterns.
- Narrow range 2D gel analysis.

Acknowledgements

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Richard Dobson

Leading science for better health