Functional genomics of cardioprotection: role microRNAs and exosomes

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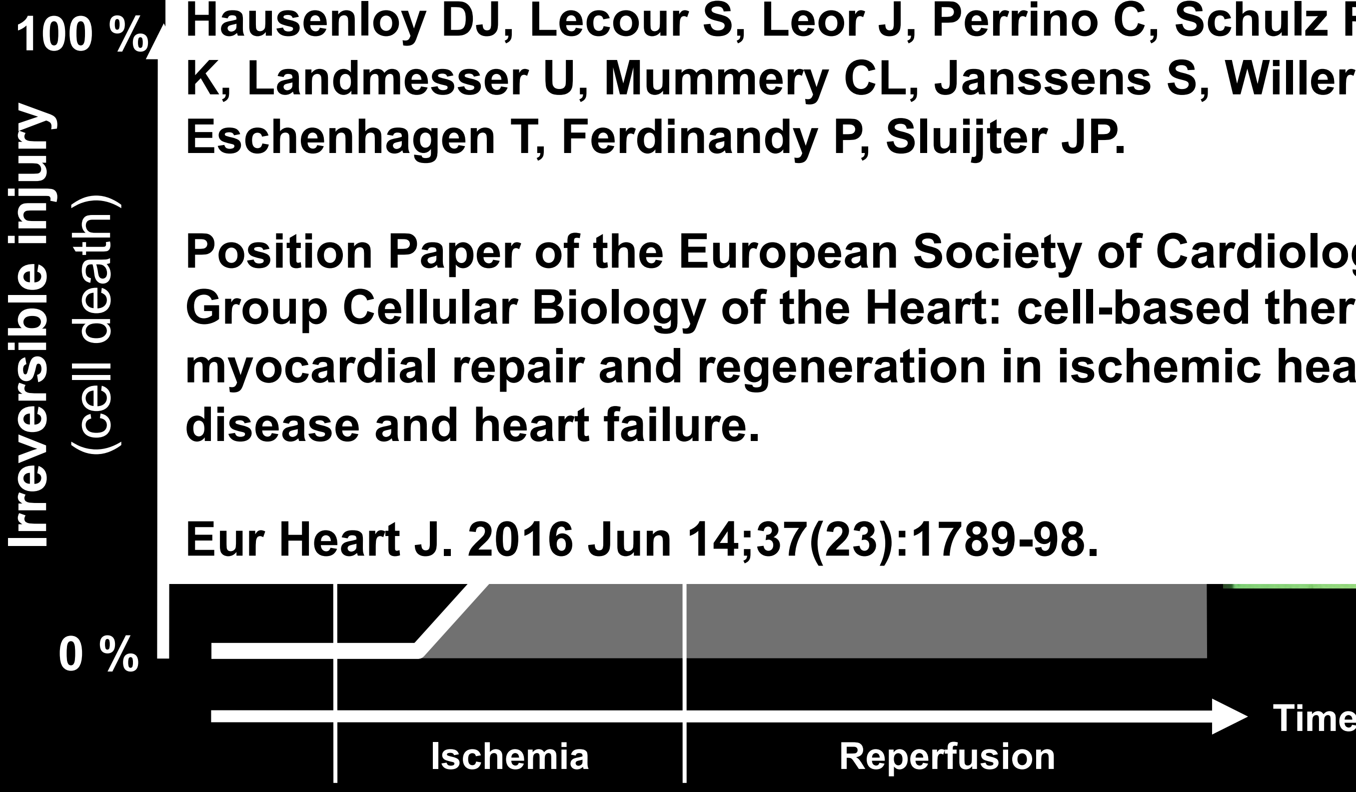
Cardioprotection: reduction of irreversible ischemia/ reperfusion injury – prevention of HF

Cardiac regeneration by cell therapy: importance of protecting the therapeutic cells:

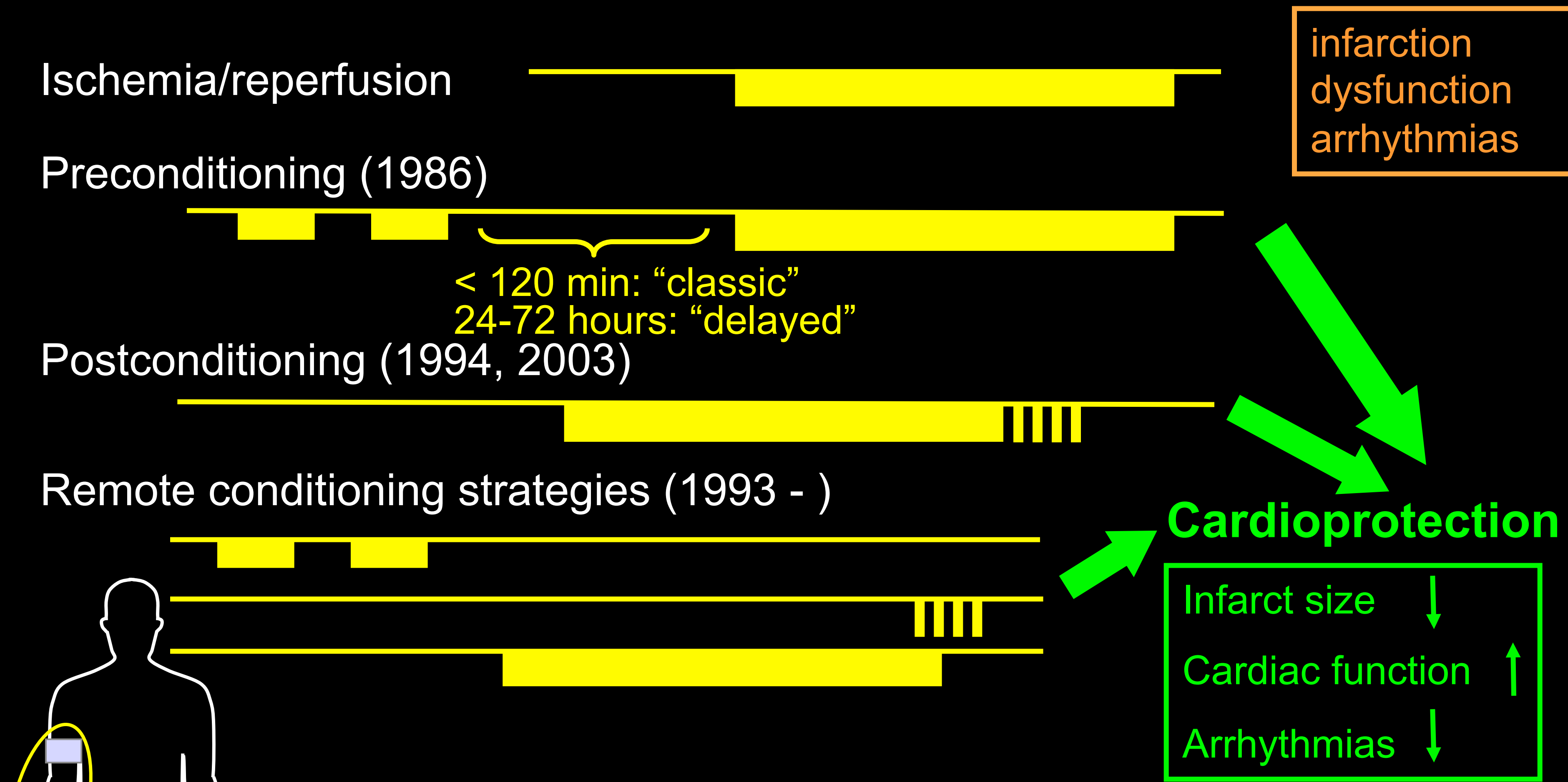
Madonna R, Van Laake LW, Davidson SM, Engel FB, Hausenloy DJ, Lecour S, Leor J, Perrino C, Schulz R, Ytrehus K, Landmesser U, Mummery CL, Janssens S, Willerson J, Eschenhagen T, Ferdinandy P, Sluijter JP.

Position Paper of the European Society of Cardiology Working Group Cellular Biology of the Heart: cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure.

Eur Heart J. 2016 Jun 14;37(23):1789-98.

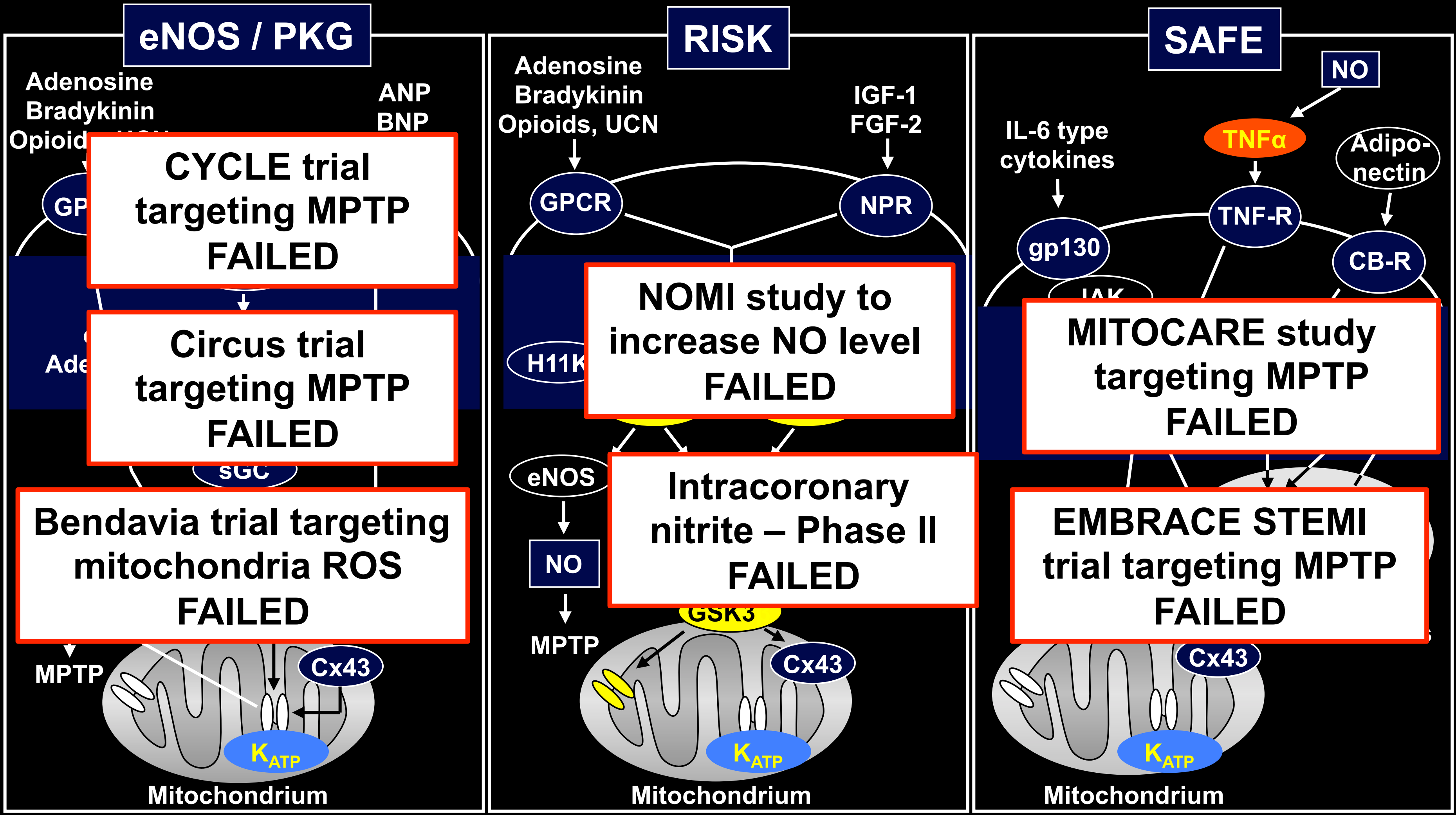


Ischemic conditioning: 30 years of hope for cardiopotection



reviews: Hausenloy et al, CVR 2013; Ovize et al, CVR, 2010
Ferdinandy et al, Pharmacol Rev, 2007; 2014

Why do we still not have cardioprotective drugs? „death valley for biotech companies”



Heusch et al., Circulation, 2008; Andreadou et al, Br J Pharmacol 2014; Ferdinandy et al, Pharmacol Rev, 2014

Why do we still not have cardioprotective drugs on the market?

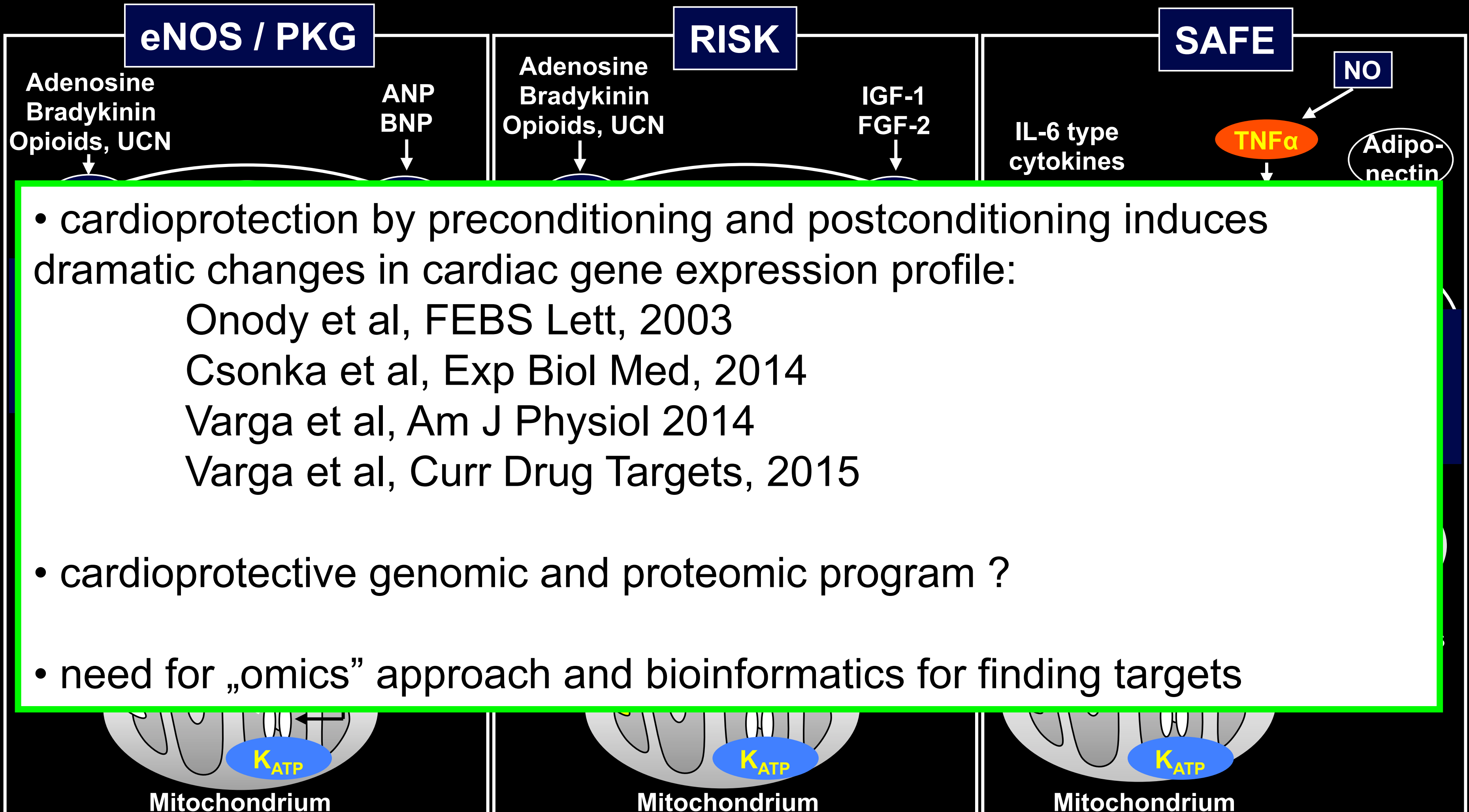
Hypothesis-driven simplified approach in target identification and validation so far?

Still neglecting comorbidities and comedications in preclinical development

1. Functional genomics of cardioprotection: unbiased novel targets?

2. Effect of co-morbidities on cardioprotection and the functional genomics of the heart

One dominant pathway or multiple ones are responsibe for cardioprotection? Importance of „omics” approach



- cardioprotection by preconditioning and postconditioning induces dramatic changes in cardiac gene expression profile:
  - Onody et al, FEBS Lett, 2003
  - Csonka et al, Exp Biol Med, 2014
  - Varga et al, Am J Physiol 2014
  - Varga et al, Curr Drug Targets, 2015
- cardioprotective genomic and proteomic program ?
- need for „omics” approach and bioinformatics for finding targets

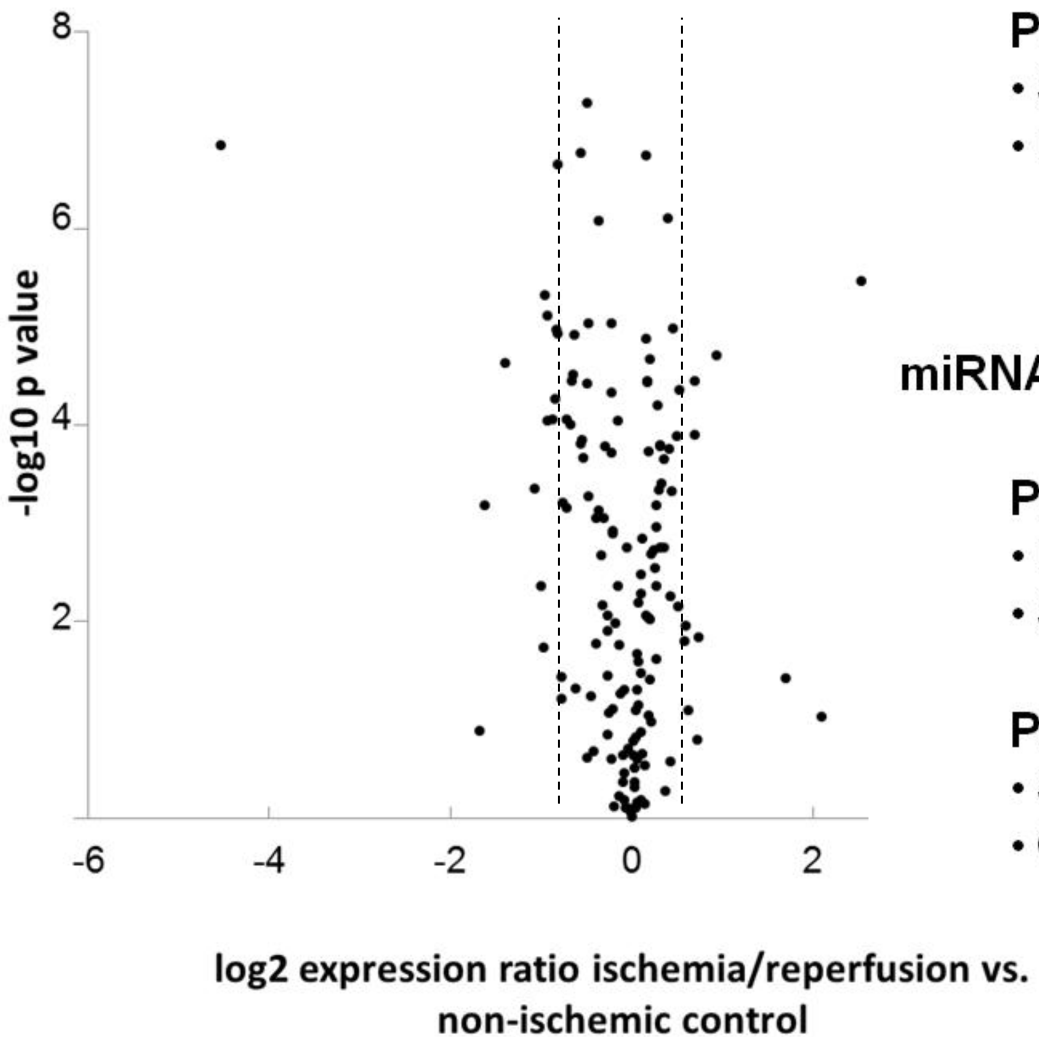
Heusch et al., Circulation, 2008; Andreadou et al, Br J Pharmacol 2014; Ferdinandy et al, Pharmacol Rev, 2014

Gene expression changes by pre- and postconditioning

DNA chip studies	Genes over-expressed	Genes repressed	Reference
3200 genes, rat			Onody et al, FEBS Lett, 2003
Normal vs. IR	28	21	
IR vs. precond	14	17	
31000 genes, rat			Csonka et al, Exp Biol Med, 2014
IR vs. postcond	50	58	
350 miRNAs, rat: altered	miRNAs		
IR vs precond	4		Varga et al, Am J Physiol, 2014
IR vs postcond	9		
IR vs pre & postcon	**5**		

„ProtectomiRs”:
miRNA-139-5p, 125b*, let-7b, antagomiR-487b

microRNA alteration patterns after I/R and cardioprotection



Pattern 1.

- Significantly affected by I/R
- Not affected by Pre/Post

miRNAs with cardioprotective potential:

Pattern 2.

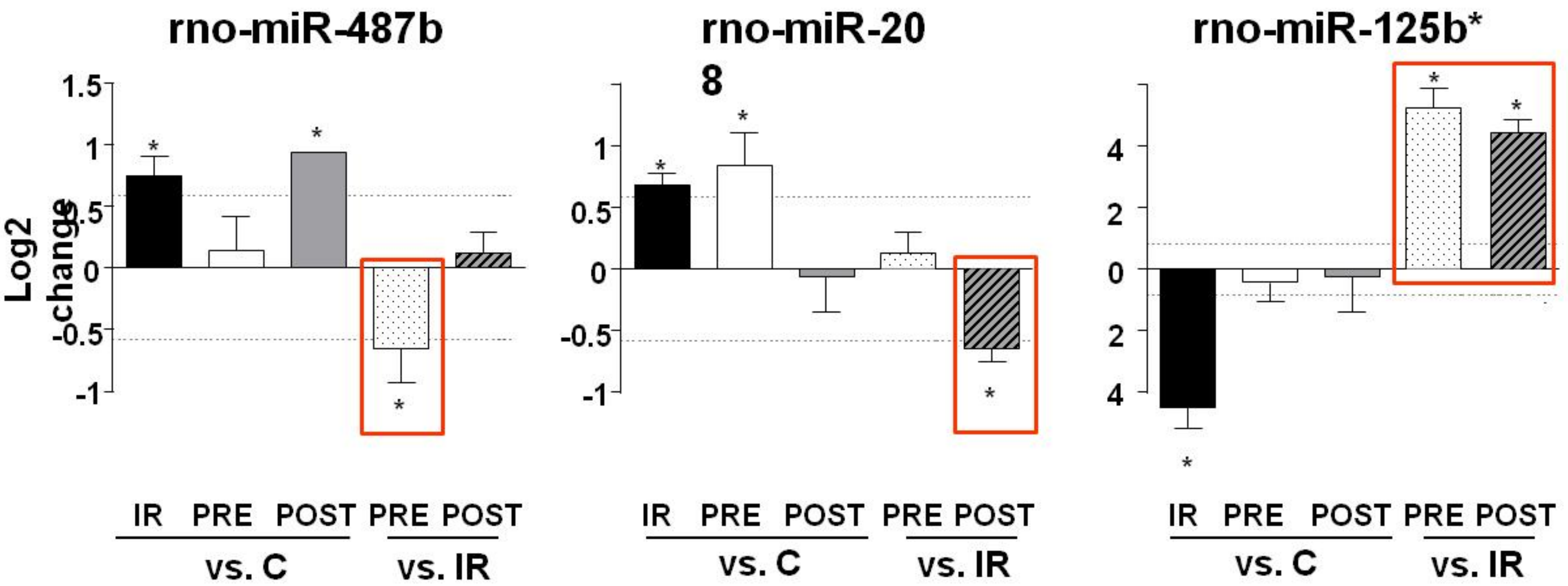
- Not affected by I/R
- Significantly affected by Pre/Post

Pattern 3.

- Significantly affected by I/R
- Counter-regulated by Pre/Post

Varga et al, Am J Physiol, 2014

Cardioprotective microRNA expression pattern:
Significantly affected by I/R and counter-regulated by Pre/Post



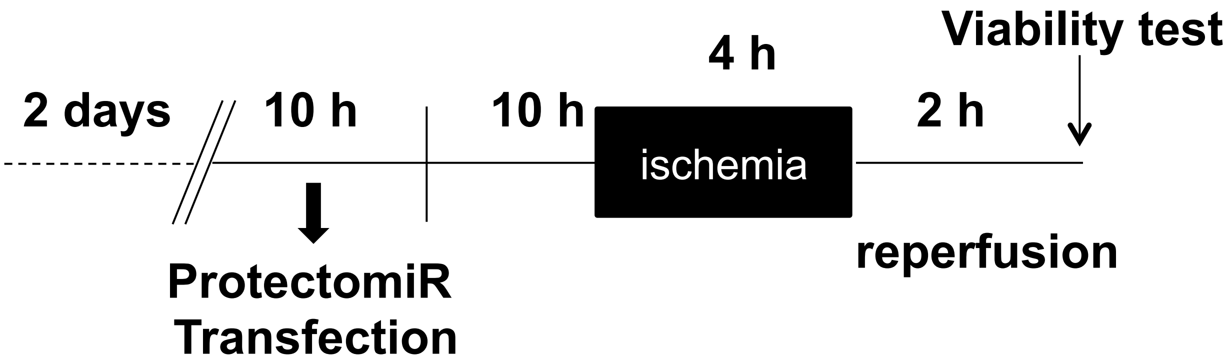
- preconditioning-induced down-regulation
- protectomiR: miR-487b antagomiR

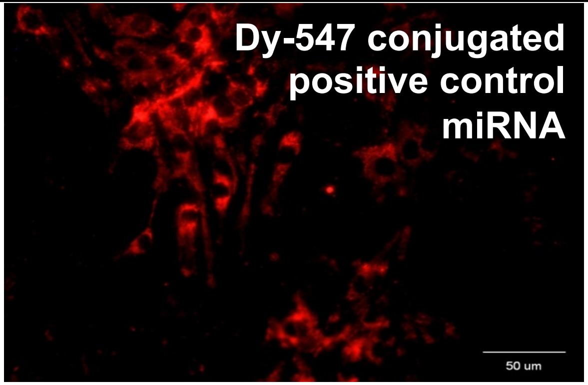
- postconditioning-induced down-regulation
- protectomiR: miR-208 antagomiR

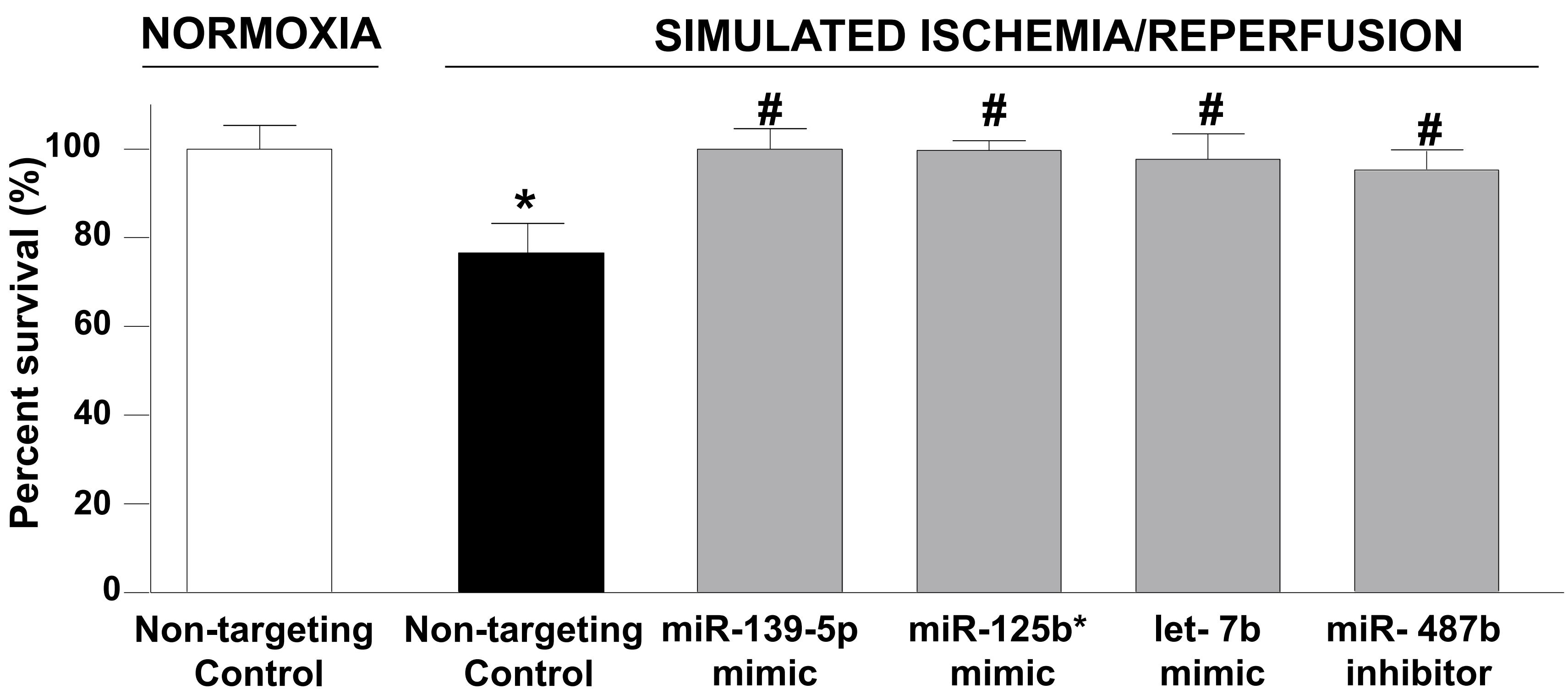
- pre- and postconditioning-induced up-regulation
- protectomiR: miR-125b* mimic

Varga et al, Am J Physiol, 2014

Validation of protectomiRs: simulated ischemia/reperfusion injury in protectomiR transfected cardiomyocytes

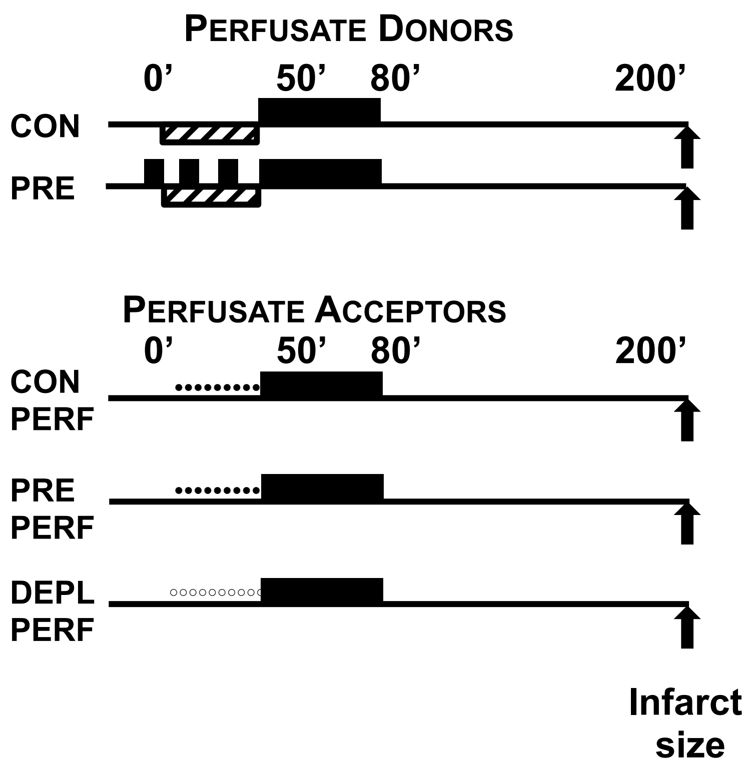


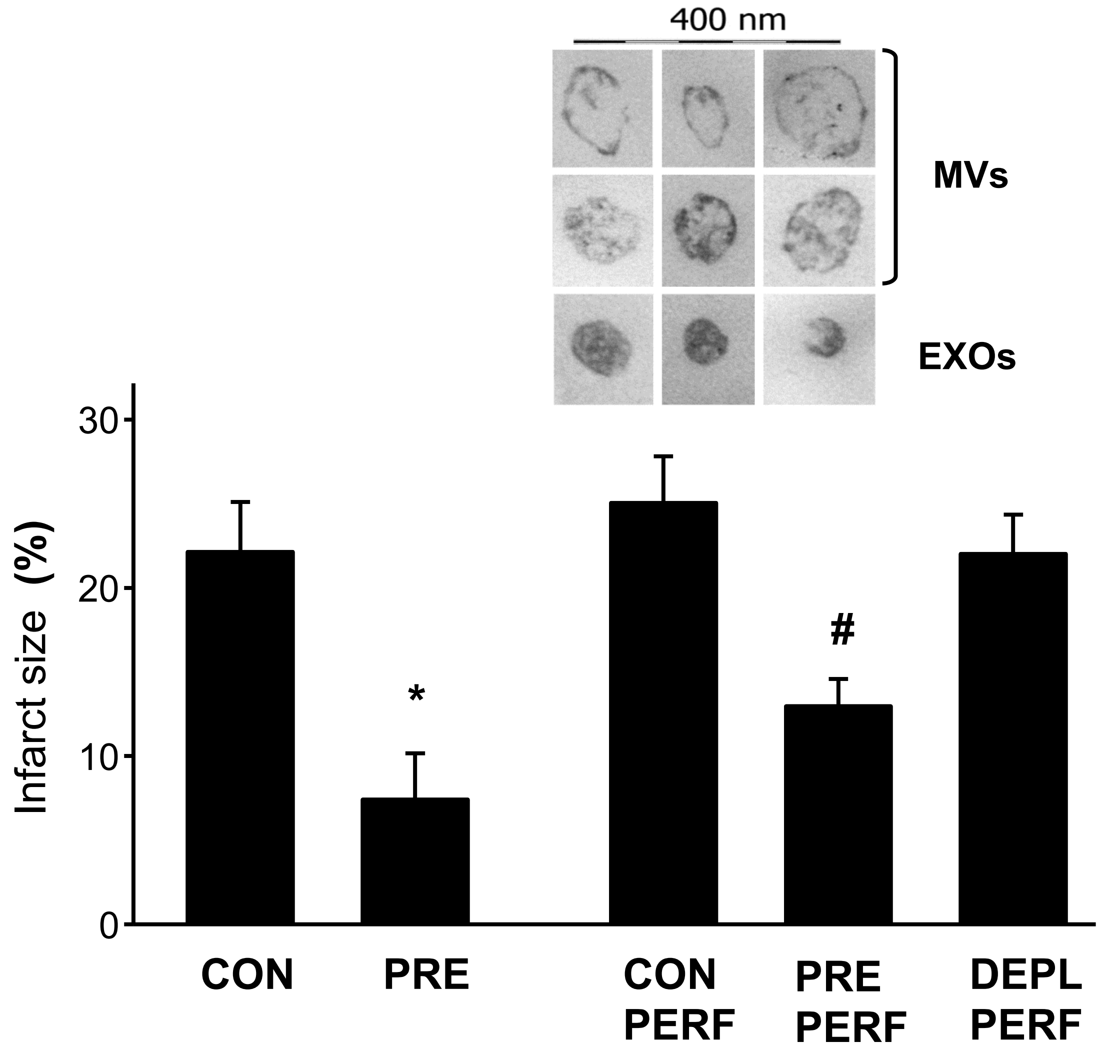




Varga et al, Am J Physiol, 2014

Extracellular vesicles are required to transfer cardioprotection in remote conditioning: carriers of ProtectomiRs?





Active substance carried by the vesicles are to be identified

Giricz et al, J Mol Cell Cardiol. 2014

Detection of extracellular vesicles from the blood: only technical difficulties?

PLOS | ONE

RESEARCH ARTICLE

Isolation of Exosomes from Blood Plasma: Qualitative and Quantitative Comparison of Ultracentrifugation and Size Exclusion

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Citation: Baranyai T, Herczeg K, Onó
Módos K, Marton N, et al. (2015) Isola

SCIENTIFIC REPORTS

OPEN

Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection

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Barbara W Sódar[1], Ágnes Kittel[2], Krisztina Pálóczi[1], Krisztina V Vukman[1], Xabier Osteikoetxea[1], Katalin Szabó-Taylor[1], Andrea Németh[1], Beáta Sperlágh[2], Tamás Baranyai[3], Zoltán Giricz[3], Zoltán Wiener[1], Lilla Turiák[4], László Drahos[4], Éva Pállinger[1], Károly Vékey[4], Péter Ferdinandy[3], András Falus[1] & Edit Irén Buzás[1]

1. Functional genomics of cardioprotection: unbiased novel targets?

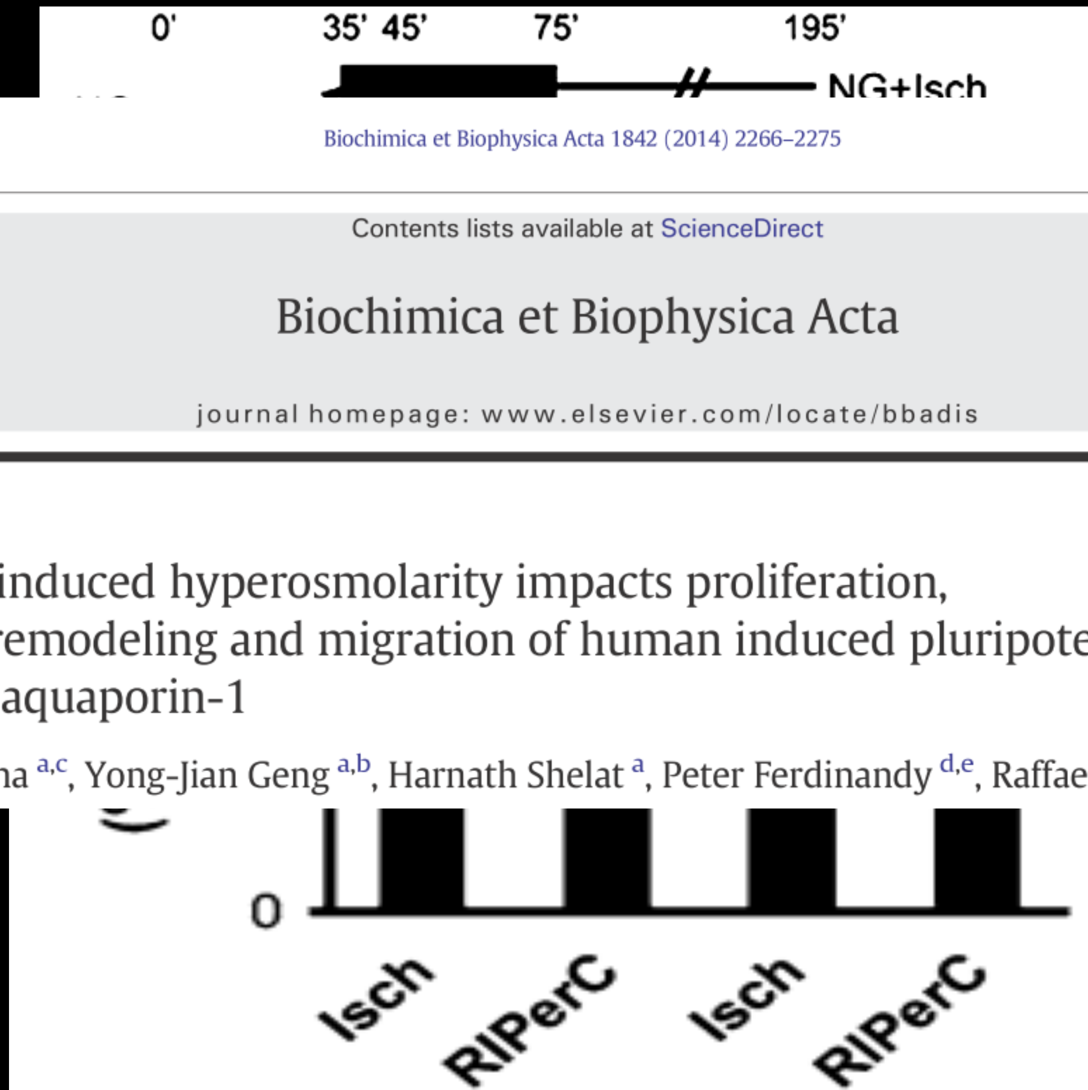
2. Effect of co-morbidities on cardioprotection and the functional genomics of the heart

Influence of co-morbidites/risk factors on I/R injury and cardioprotection by conditioning

Co-morbidities	I/R Injury ↑ increased	Protection by preconditioning ↓ attenuated	Protection by postconditioning ↓ attenuated	Protection by remote cond. ↓ attenuated
Aging	↑	↓	↓	no data
Hypertension, hypetrophy, remodelling	~	~	↓	no data
Hyperlipidemia	↑	↓	↓	no data
Diabetes	↑	↓	↓	↓
Kidney failure	↑	~	~	no data

Modified from Ferdinandy et al, *Pharmacological Reviews*, 2014

Influence of acute hyperglycemia on I/R injury and cardioprotection by remote conditioning in rats



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High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1

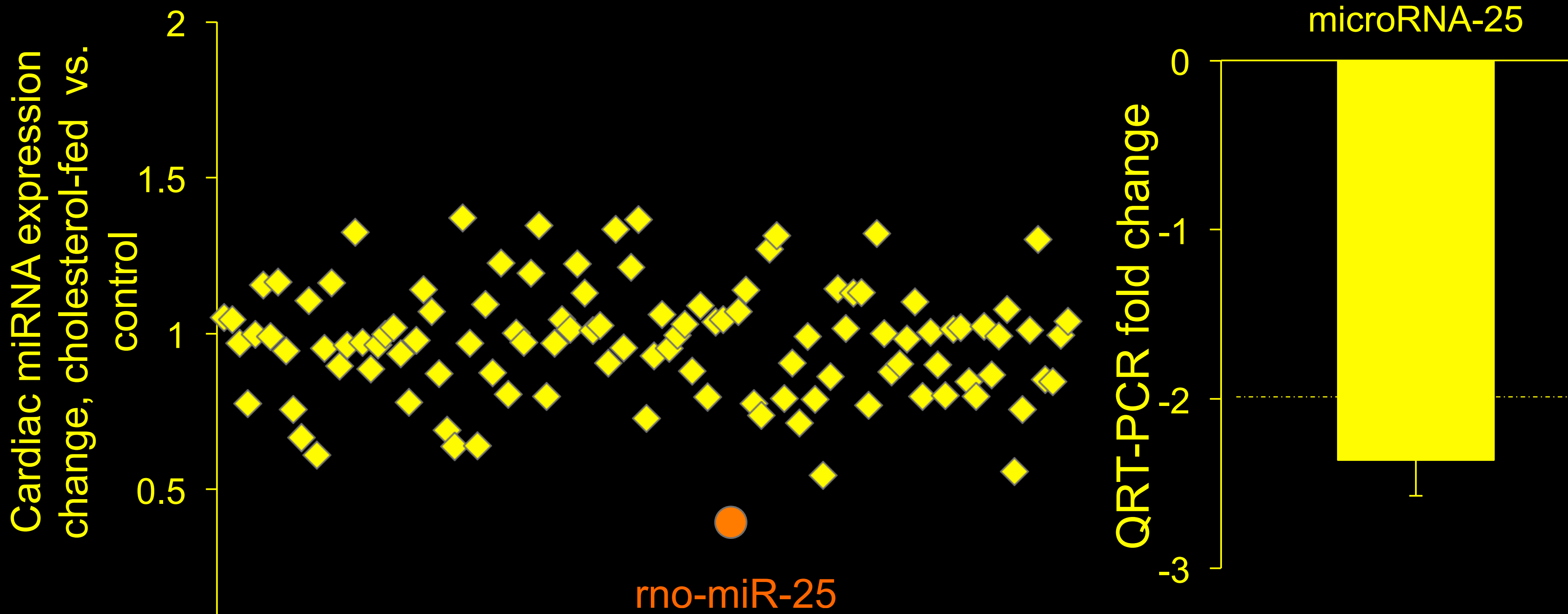
Rosalinda Madonna [a,c], Yong-Jian Geng [a,b], Harnath Shelat [a], Peter Ferdinandy [d,e], Raffaele De Caterina [c,*]

Baranyai et al, Cardiovasc Diabetol, 2015

Effects of hyperlipidemia on cardiac gene expression profile (mRNA, miRNA, and protein microarray studies in rodent hearts)

Microarray and RT-PCR	Overexp.	repressed	Reference
3200 genes, rat - hyperchol vs. normal	26	25	Puskas et al, FEBS Lett, 2004
330 antibodies, rat - hyperchol vs. normal	10	3	unpublished
16 oxidative stress genes - hyperchol vs. normal	eNOS	Phox4, MMP9	Kocsis et al, Med Sci Monit, 2010
360 miRNA, rat - hyperchol vs. normal	6	2	Varga et al, JMCC, 2013
420 miRNA, rat - Statin vs normal	7	4	Szücs et al, in preparation
15000 genes, ZDF rat - Metabolic dis vs. normal	36	42	Sárközy et al, Cardiov Diab 2013

Cardiac microRNA expression pattern is altered hyperlipidemia: biased selection of microRNA-25

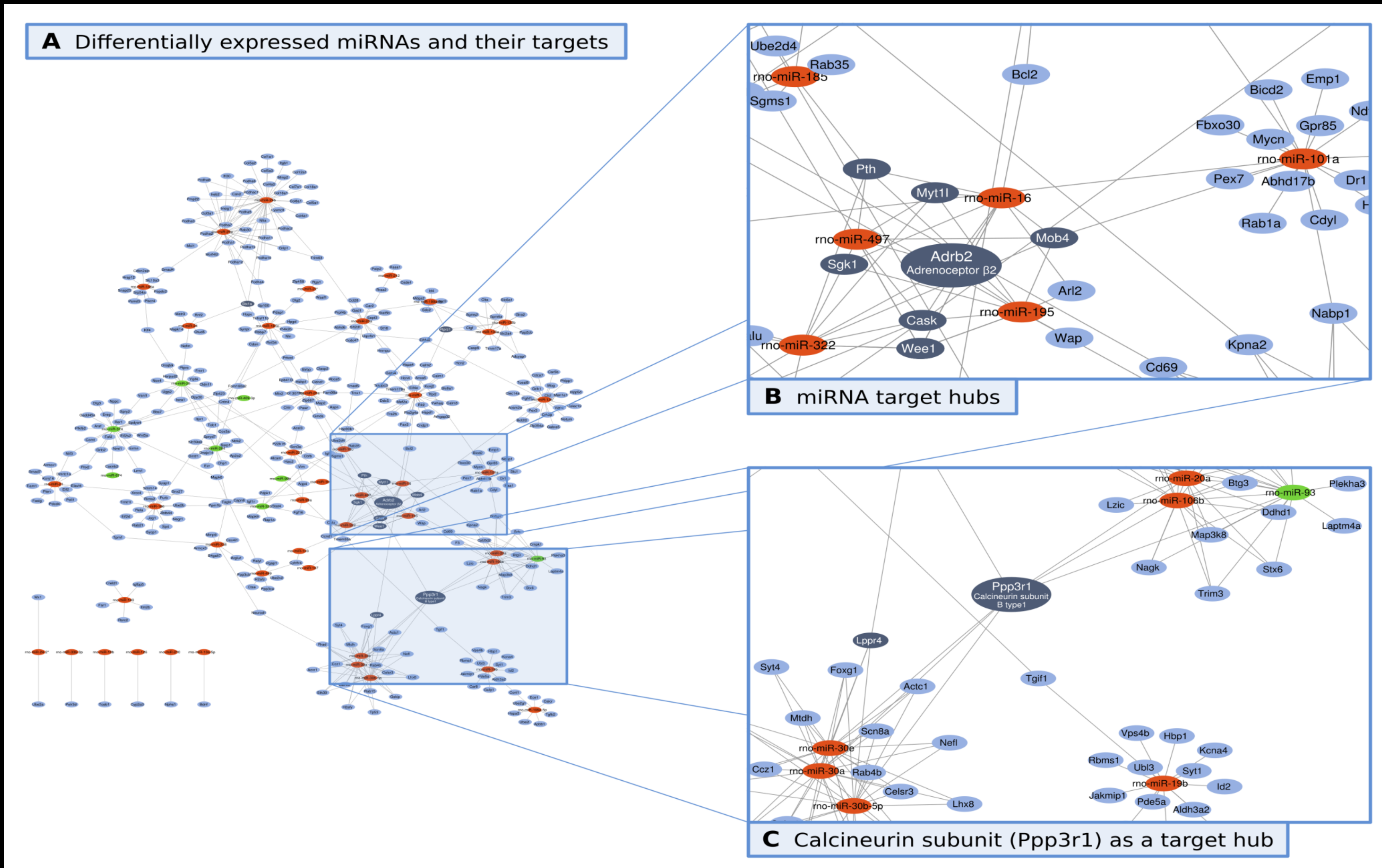


Target analysis

NADPH oxidase 4 upregulation oxidative/nitrosative stress

Varga et al, J Mol Cell Cardiol, 2013

Unbiased network biology approach: novel targets affected by hyperlipidemia?



Agg et al, in preparation

Influence of co-medications on I/R injury and cardioprotection by conditioning

Drug class	I/R Injury (↓ decreased, ↑ increased)	Protection by preconditioning (↓ attenuated)	Protection by postconditioning (↓ attenuated)	Protection by remote cond. (↓ attenuated)
Nitrate	↓	no data	no data	~
Nitrate tolerance	↑	↓	↓	no data
Statins acute	↓ ~	↓	~	no data
Statins chronic	↓ ~	~ ↑ (hyperlipidemia hyperglycemia)	↓	no data
ß-Blocker	↓ **Drug-dependent**	↓ ~ ↑ (chronic stenosis)	↓ ~ ↑ (chronic stenosis)	↓
ACE-inhibitor	↓	**Reduced threshold** (diabetes)	no data	no data

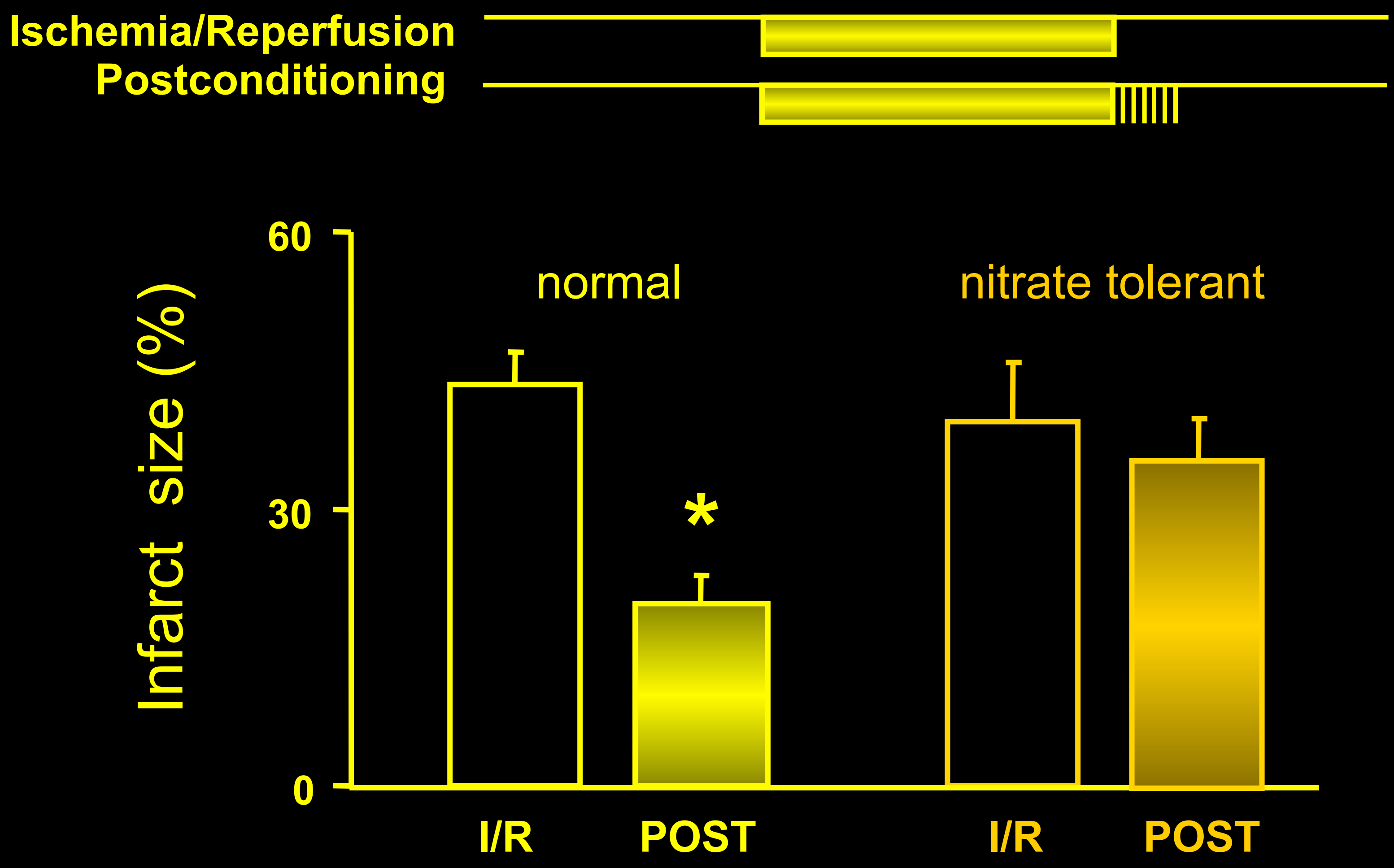
Modified from Ferdinandy et al, *Pharmacological Reviews*, 2014

Influence of co-medications on I/R injury and cardioprotection by conditioning

Drug class	I/R Injury (↓ decreased, ↑ increased)	Protection by preconditioning (↓ attenuated, ↑ increased)	Protection by postconditioning (↓ attenuated)	Protection by remote cond. (↓ attenuated)
AT_1-antagonist	↓	Reduced threshold ↑ (LVH)	no data	no data
Metformin	↓	no data	no data	no data
K_{ATP}-blocker	~	↓ Drug-dependent	↓ Drug-dependent	↓ sulfonylurea
Glitazone	↓	no data	no data	no data
DPP4-inhibitor	↓	no data	no data	no data
GLP-1 analogs	↓	no data	no data	no data
Insulin-K^+	↓	no data	no data	no data
Cox-inhibitor	↓ ~	↓	↓	no data

Modified from Ferdinandy et al, *Pharmacological Reviews*, 2014

The infarct size limiting effect of postconditioning is lost in hearts of nitrate tolerant rats



Fekete et al, J Cardiovasc Pharmacol, 2013

Altered gene expression pattern of the heart and the aorta in nitrate tolerant rats

Out of the 7742 genes analyzed by DNA microarray:

25 genes changed significantly in the heart:

- **increased:** Tas2r119, Map6, Cd59, Kcnh2, Kcnh3, Senp6, Mcpt1, Tshb, Haus1, Vipr1, Lrn3, Lifr
- **decreased:** Ihh, Fgfr1, Cryge, Krt9, Agrn, C4bpb, Fcer1a, Csf3, Hsd17b11, Hsd11b2, Ctnnbl1, Prpg1, Hsf1

14 genes changed significantly in the abdominal aorta:

- **increased:** Tas2r119, Ihh, Rrad, Npm1, Snai1
- **decreased:** Tubb2b, Usp15, Sema6c, Wfdc2, Rps21, Ramp2, Galr1, Atxn1, Lhx1

Csont et al, J Cardiovasc Pharmacol, 2015

Take home messages

- Cardioprotection involves a complex functional genomic and proteomic program:

  - mRNA, miRNA expression changes,

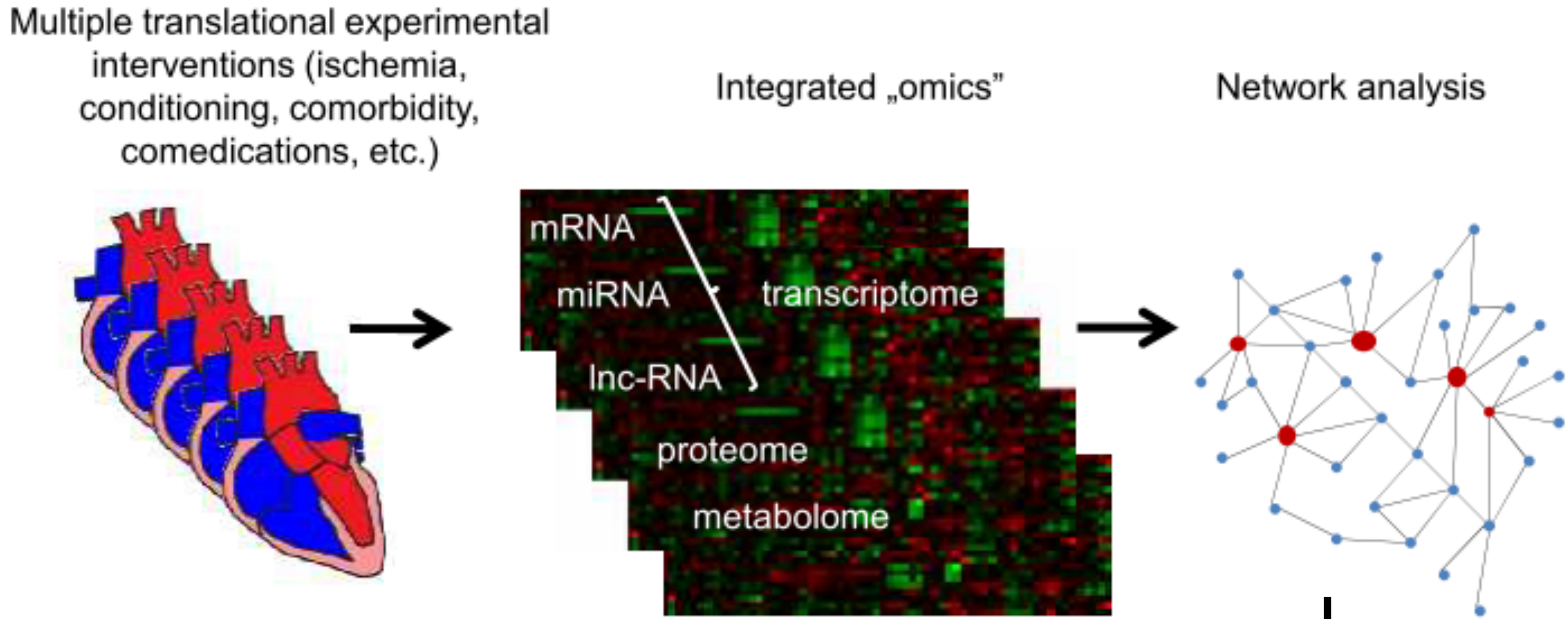
  - exosomal transport of …?

- Cardiovascular comorbidities and comedications modify cardiac functional genomics

- Relevance to protecting therapeutic cells (see Madonna et al, EHJ, 2016)

See for reviews: Ferdinandy et al, *Pharmacol Rev, 2014*
Hausenloy et al, Cardiovasc Res, 2013
Varga et al, *Curr Drug Targets*, 2015

Target finding and validation for cardioprotection: comorbidities, comedications, and multi-omics



Modified from: Varga et al, Curr Drug Targets, 2015

Target validation in comorbid, comedicated models

Financing all these studies ?

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