Nourin-dependent miR-137: A Novel Early Diagnostic Biomarker for Unstable Angina Patients

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Disclosures of Authors

- **S. A. Elgebaly (Univ. of Connecticut Faculty of Medicine):** Founder, Nour Heart, Inc.
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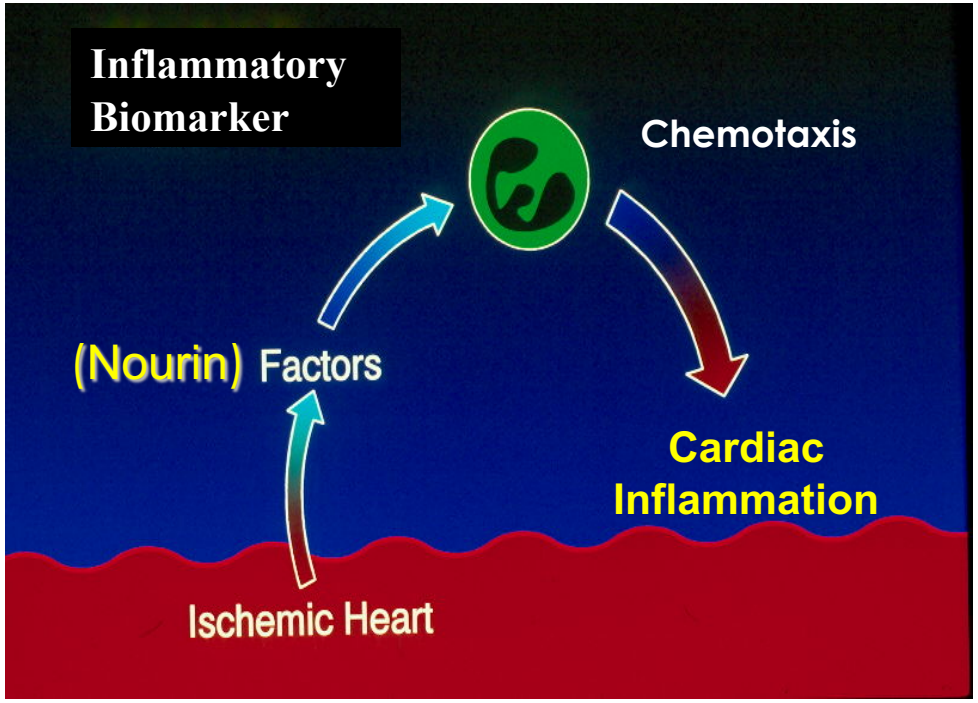
What is Nourin?

A Novel "Injury Response" Molecule!

NOURIN:

- Released within 5 minutes by ischemic hearts (human & animals)
- A 3 Kda formyl peptide potent inflammatory mediator
- Stimulates leukocyte chemotaxis and is associated with cardiac inflammation in early ischemia/reperfusion
- Activates human leukocytes & vascular endothelial cells (VECs) to express cytokine storm mediators, enzymes and free radicals
- Binds to formyl peptide receptor (FPR) on leukocytes & VECs
- Competitive antagonists (listed below) inhibited Nourin chemotactic activity and reduced tissue inflammation:
  - Cyclosporin H
  - Spinorphin
  - t-Boc-Phe-D.Leu-Phe-D.Leu-Phe
  - Soluble FPR fragment 17 aa loop peptide
- The bioenergetic compound, Cyclocreatine Phosphate (CCrP) prevented ischemic injury, thus, reduced Nourin intracellular formation/circulating levels, and post-ischemic cardiac inflammation

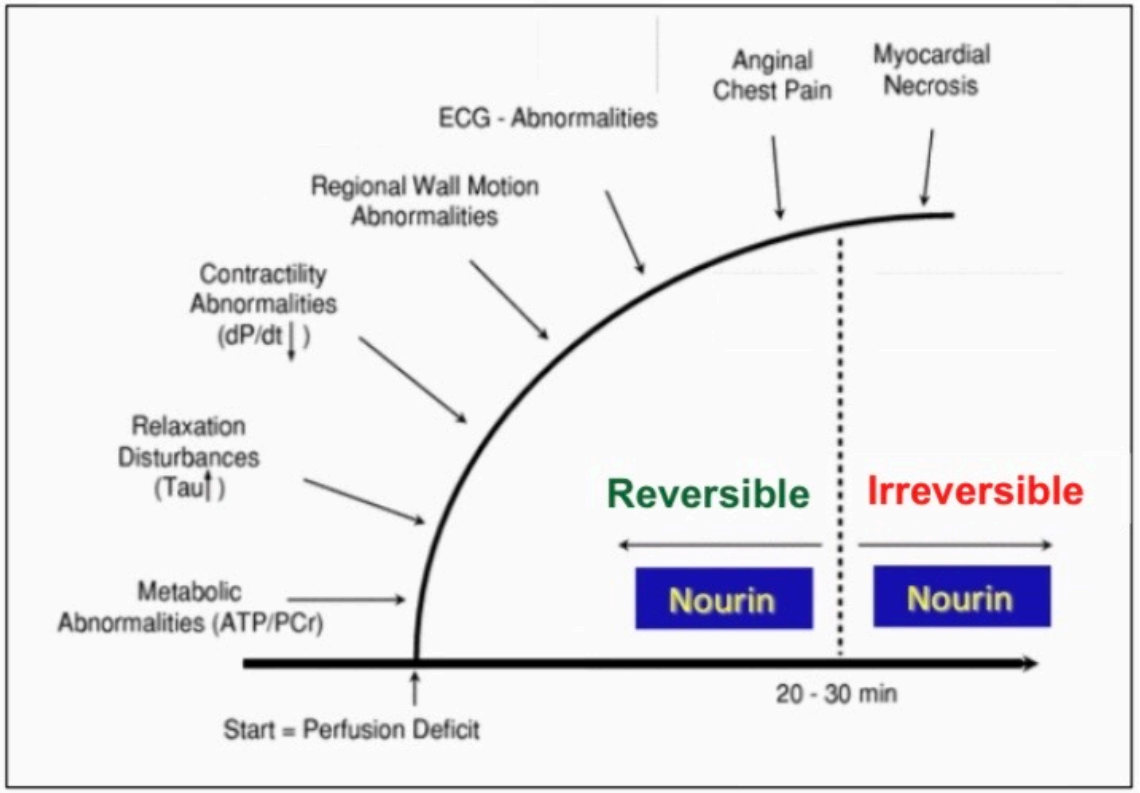


Elgebaly SA, et al. Expert Review of Cardiovascular Therapy – 2019 – REVIEW
Elgebaly SA, et al. Society for Cardiovascular Angiography and Interventions (SCAI) - 2013

What is Unique About Nourin?

Released by "Reversible" Ischemia!

- Released after "reversible" ischemic myocardium when cells are still "sick", but not dead
- Clinically, high levels at presentation to hospital ED:
  - ACS
  - STEMI
  - NSTEMI
- Very low levels in:
  - Symptomatic Non-Cardiac
  - Healthy
- Measured by ELISA & Chemotaxis assay using:
  - Serum and plasma samples
  - Fresh and frozen (-70 °C for 3 years) samples

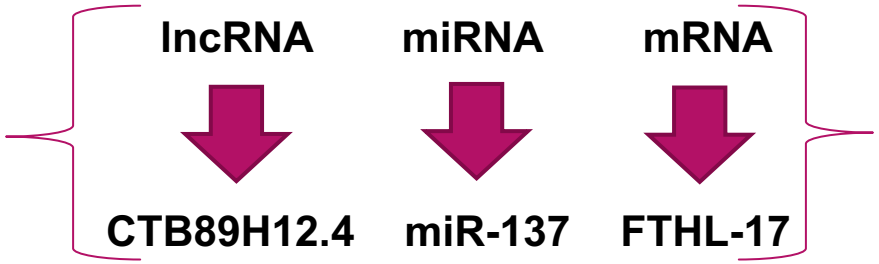


Modified from Dymarkowski S, et al. In Clinical Cardiac MRI 2005 (pp. 173-216). Springer, Berlin, Heidelberg.

Rationale, Hypothesis & Methods

Rationale

No blood biomarkers exist that can diagnose Unstable Angina (UA) patients. Using Nourin amino acid sequence, an integrated bioinformatics analysis was conducted and the interaction network was constructed:



miR-137 is a marker of cell damage and a hypoxia responsive autophagy-signaling pathway linked to myocardial ischemia and Coronary Artery Disease (CAD)

Hypothesis

The Nourin-dependent miR-137 (cell damage marker linked to ischemia)

can diagnose

UA patients
STEMI patients

Regulatory mechanism of miR-137 in ACS patients involves lncRCTB89H12.4 and mRNA-FTHL-17

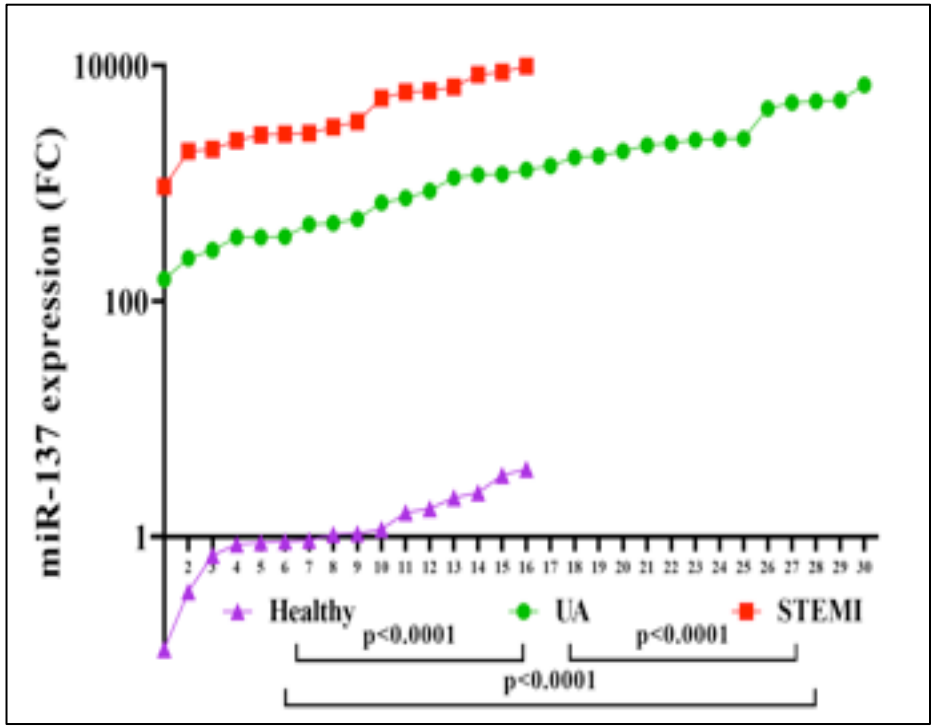
Methods

qPCR was used to measure serum expression profile of lncR-CTB89H12.4, miR-137 and mRNA-FTHL-17 in blood samples collected *once* at presentation to ED from patients with acute chest pain (first 1 to 10 hours of symptoms)

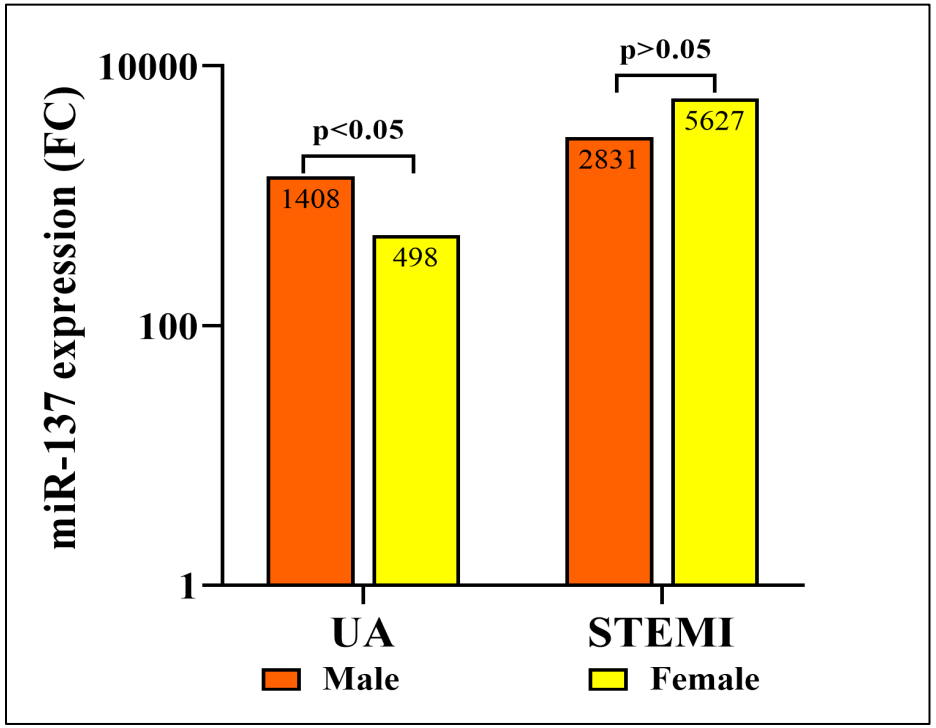
a) *UA patients (n=30)* confirmed by invasive coronary angiography and Troponin levels were below the decision limit (below 99th of URL)
b) *STEMI patients (n=16)* confirmed by positive ECG changes and elevated Troponin levels
c) *Healthy subjects (n=16)* with negative Troponin

Median expression level was used

Expression Pattern of miR-137 in UA, STEMI & Healthy

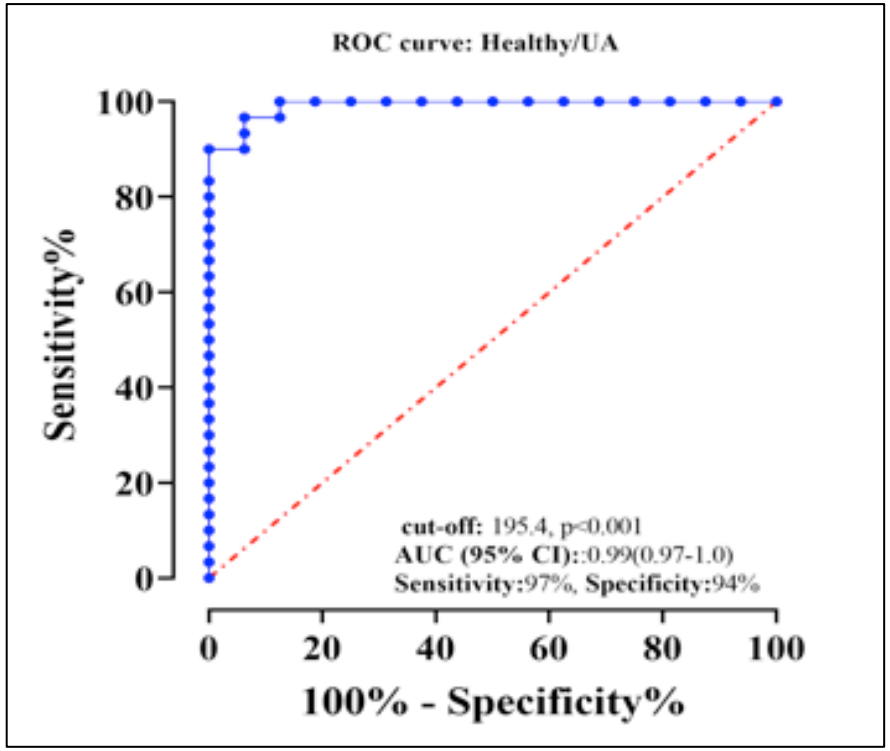


Higher expression level of miR-137 was detected in STEMI, followed by UA. Healthy subjects showed very low level of miR-137 expression

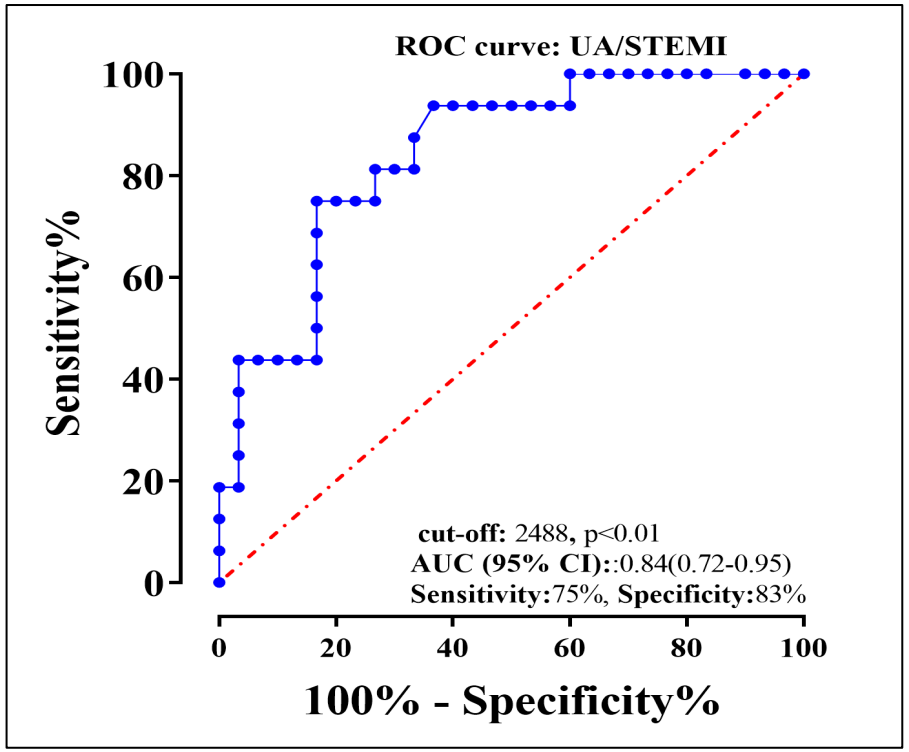


Higher miR-137 expression level in male UA patients compared to female patients ($p<0.05$). No significant statistical difference was observed between genders in STEMI patients

Diagnostic Potential of miR-137 in ACS Patients (ROC Curve)

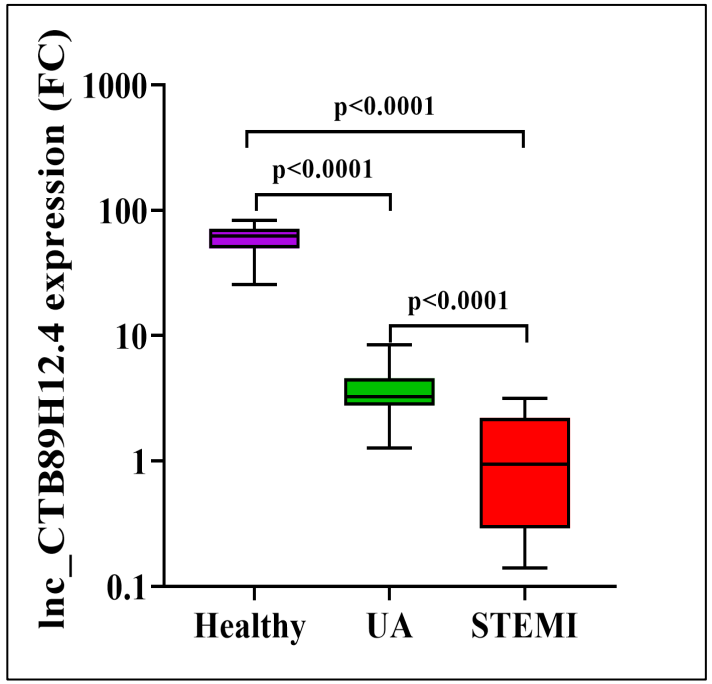


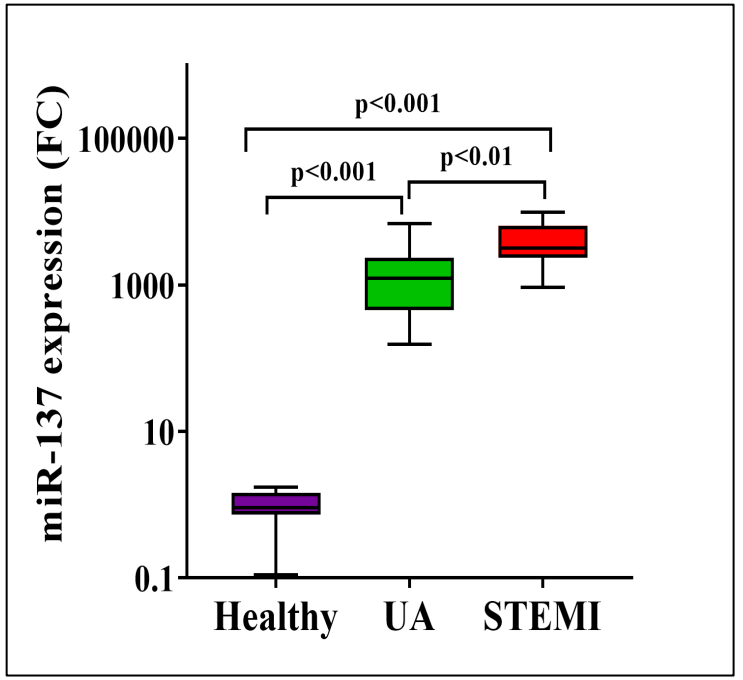
At a cut-off value of 195.4, miR-137 could discriminate UA patients from healthy with Sensitivity of 97% & Specificity of 94%

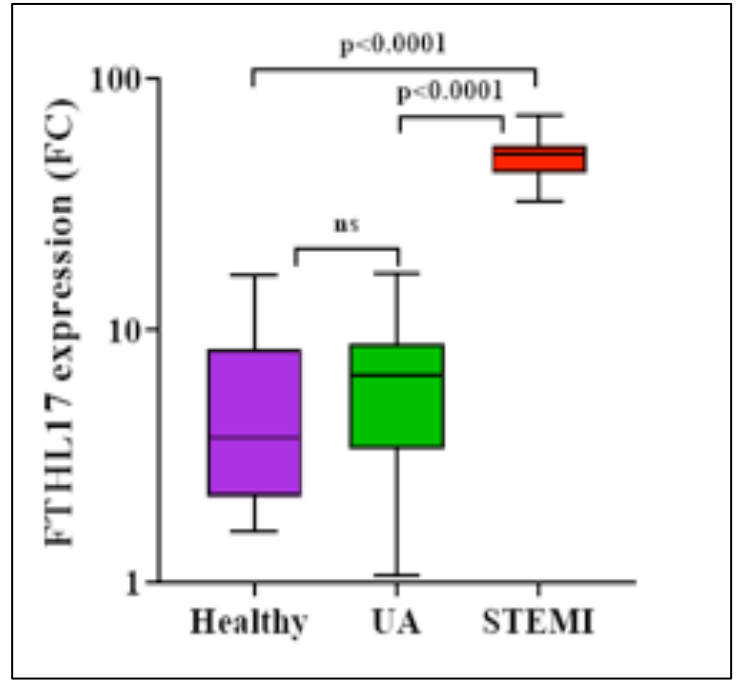


At a cut-off value of 2488, miR-137 could discriminate UA patients from STEMI with Sensitivity of 75% & Specificity of 83%

Expression Level of lncR-CTB89H12.4, miR-137 and mRNA-FTHL-17 in UA, STEMI & Healthy







lncR-CTB89H12.4
19-fold in UA vs. Healthy
3.4-fold in STEMI vs. UA

miR-137
1,185-fold in UA vs. Healthy
2.5-fold in STEMI vs. UA

mRNA-FTHL-17
1.7-fold in UA vs. Healthy
7.5-fold in STEMI vs. UA

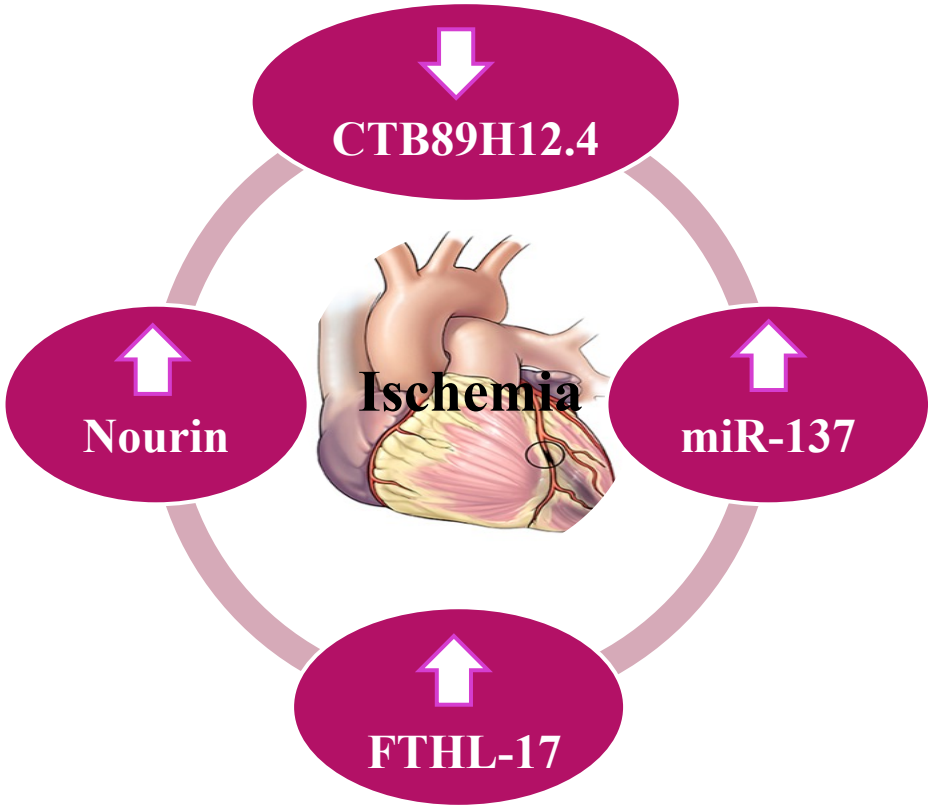
Association of IncR-CTB89H12.4/miR-137/mRNA-FTHL-17/Nourin in ACS Patients

Spearman's Correlation Analysis in ACS Patients Between miR-137/ mRNA-FTHL-17/lncR-CTB8912.4

VARIABLES	ACS (n=46)
miR-137 vs mRNA-FTHL-17	r: 0.53 p=0.0005
miR-137 vs lncR-CTB8912.4	r: -0.34 p=0.02

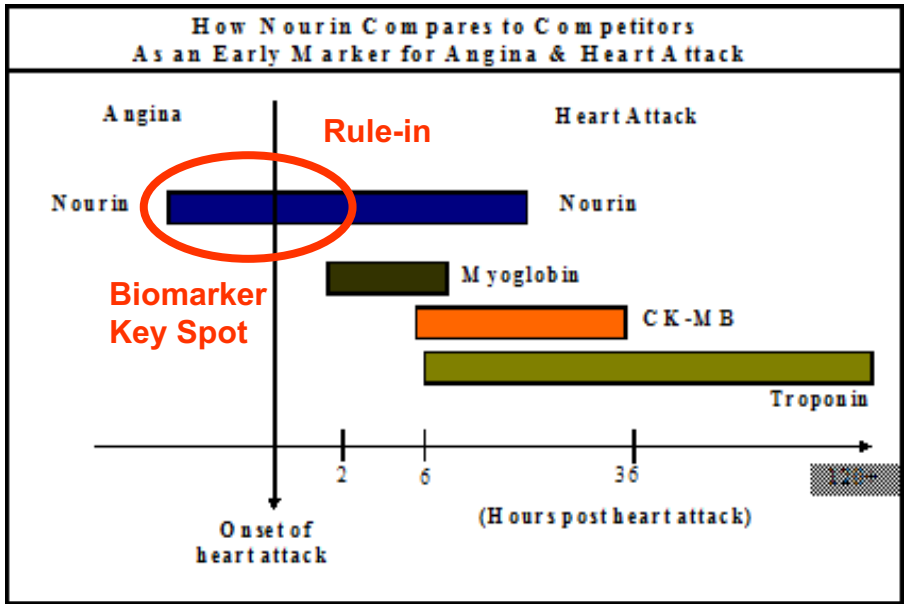
Spearman's correlation revealed a significant association between CTB89H12.4/miR-137 and FTHL-17 in ACS patients

Down-regulation of CTB89H12.4 due to ischemia, resulted in up-regulation of miR-137 and activation of FTHL-17 with an increased translation and production of Nourin protein



Conclusions

- Results support the Ontology bioinformatics evidence that lncR-CTB89H12.4/miR-137/mRNA-FTHL-17 network synergistically regulates the Nourin protein expression in myocardial ischemia, and thus, provides a novel molecular mechanism in ischemic heart disease
- Nourin-dependent miR-137 is a cell damage marker that:
  - Diagnosed ischemia-induced cardiac injury in UA and STEMI
  - Discriminated between UA, STEMI and Healthy
- The Nourin-dependent miR-137 is a promising *early* diagnostic biomarker to:
  - Diagnose symptomatic UA and AMI patients "at presentation" to hospital ED
  - Stratify severity of myocardial ischemia - higher in STEMI compared to UA
  - Rule-out ACS for symptomatic patients having non-cardiac causes
- miR-137 expression level can be measure using serum or plasma samples (fresh or frozen)



Thank You.

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