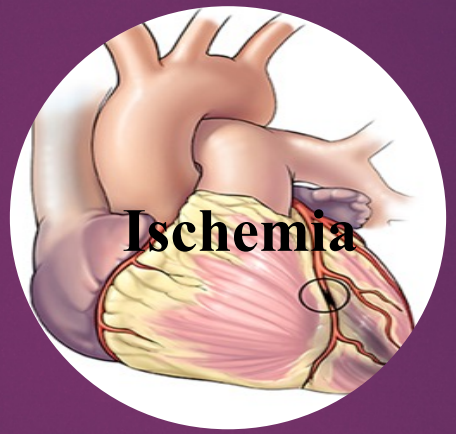
Nourin-dependent miR-106b: A Novel Early Inflammatory Diagnostic Biomarker for Cardiac Injury



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Disclosures of Authors

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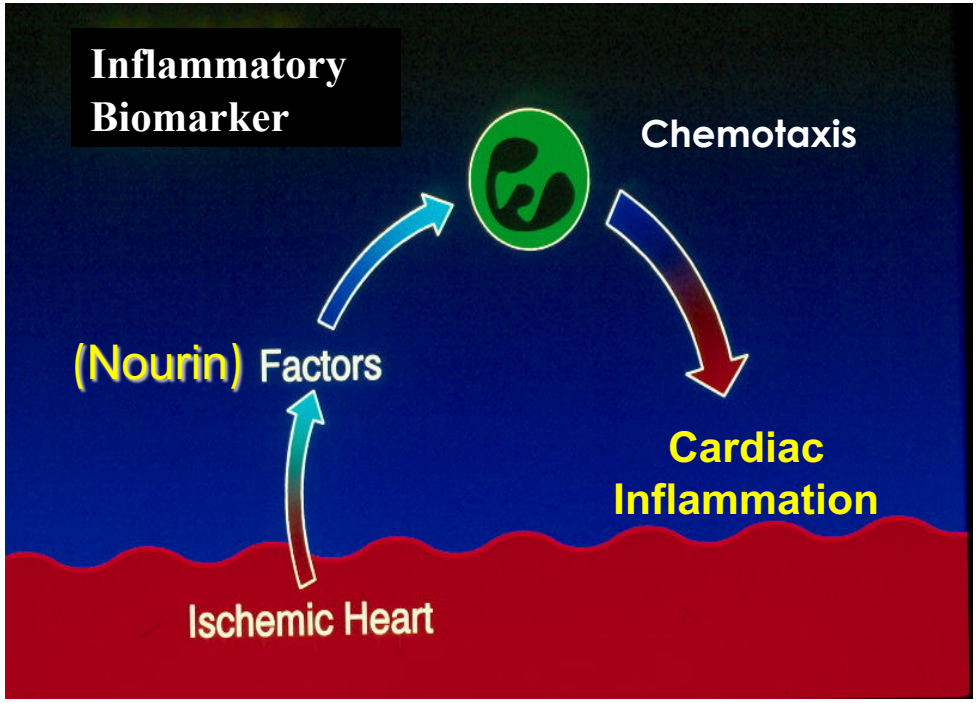
What is Nourin?

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

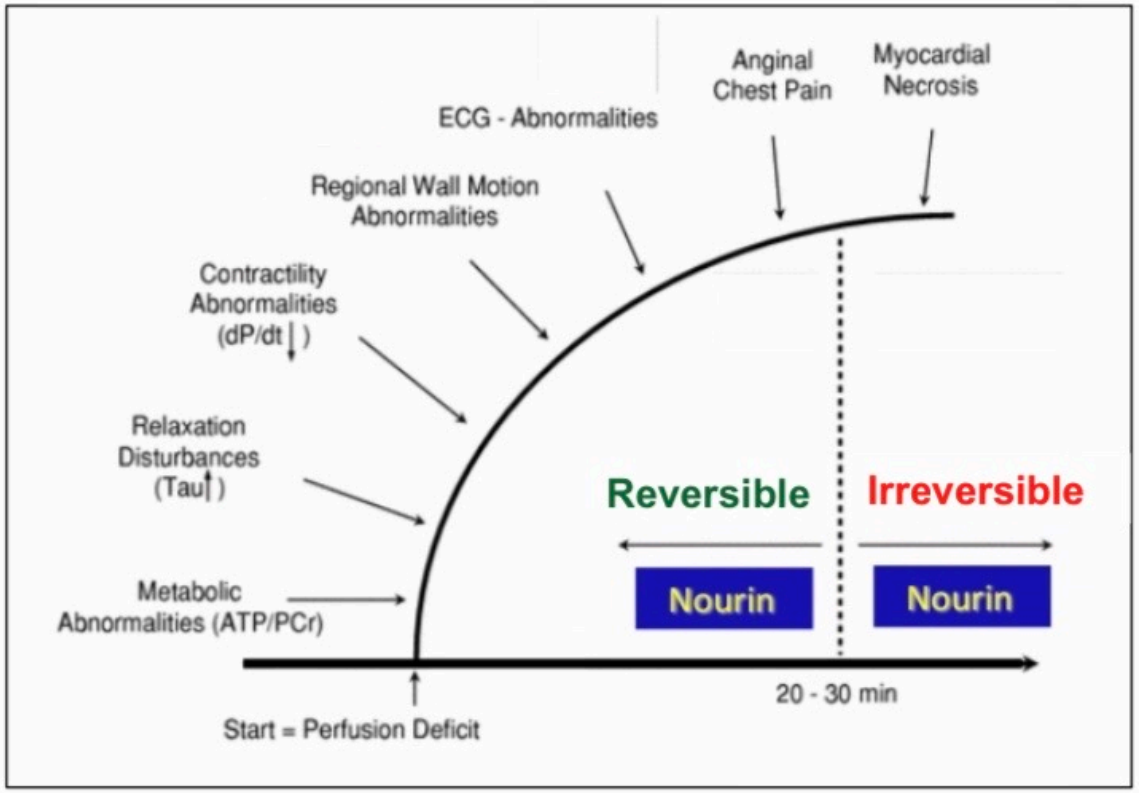


Elgebaly SA, et al. Expert Review of Cardiovascular Therapy – 2019 – REVIEW
Elgebaly SA, et al. Society for Cardiovascular Angiography and Interventions (SCAI) - 2013

What is Unique About Nourin?

Released by "Reversible" Ischemia!

- Released by "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 reversible myocardial ischemia in ACS patients. Using Nourin amino acid sequence, an integrated bioinformatics analysis was conducted and the interaction network was constructed:

lncRNA	miRNA	mRNA
CTB89H12.4	miR-106b	ANAPC11

miRNA-106b is an inflammatory-signaling pathway linked to myocardial ischemia

Hypothesis

The Nourin-dependent miR-106b (inflammatory marker linked to ischemia)

can diagnose

UA patients
STEMI patients

Regulatory mechanism of miR-106b in ACS patients involves lncRCTB89H12.4 and mRNA-ANAPC11

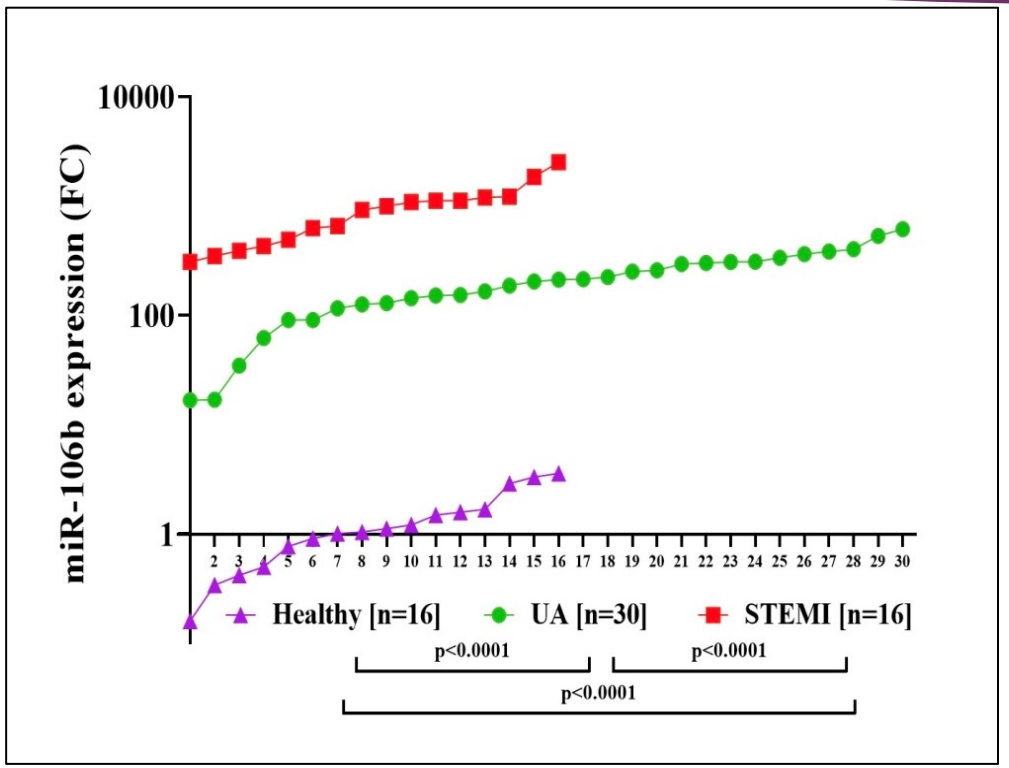
Methods

qPCR was used to measure serum expression profile of lncR-CTB89H12.4, miR-106b and mRNA-ANAPC11 in blood samples collected *once* at presentation to ED from patients with acute chest pain (first 1 to 10 hours of symptoms)

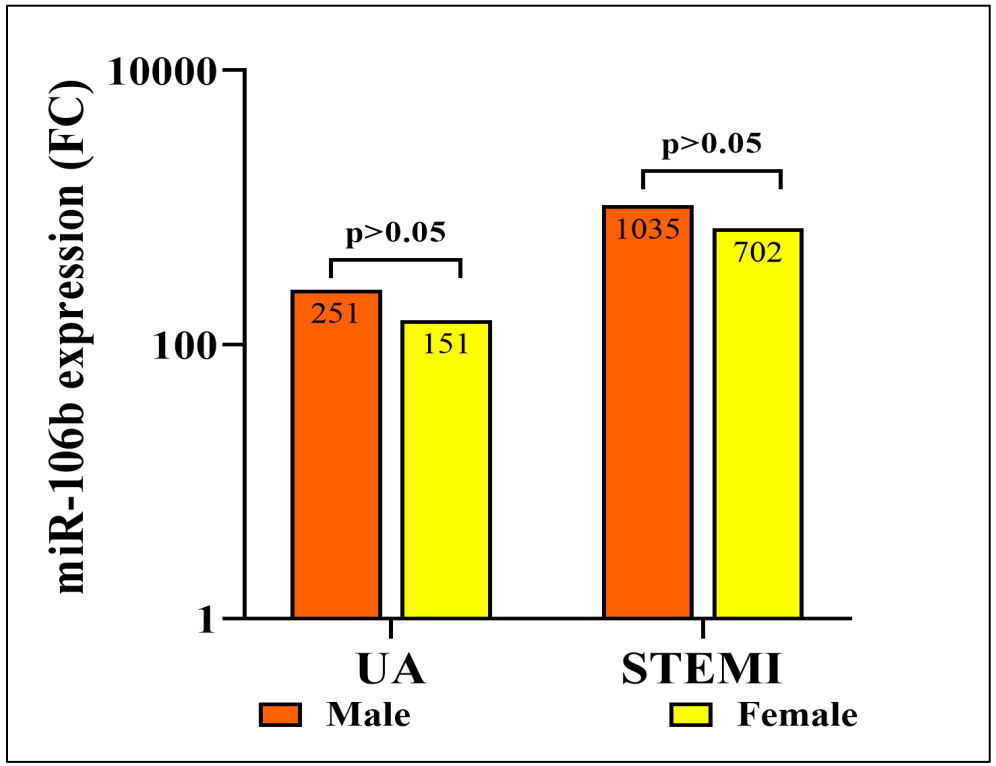
a) *UA patients (n=30)* confirmed by invasive coronary angiography and Troponin levels were below the decision limit (below 99th of URL)
b) *STEMI patients (n=16)* confirmed by positive ECG changes and elevated Troponin levels
c) *Healthy subjects (n=16)* with negative Troponin

Median expression level was used

Expression Pattern of miR-106b in UA, STEMI & Healthy

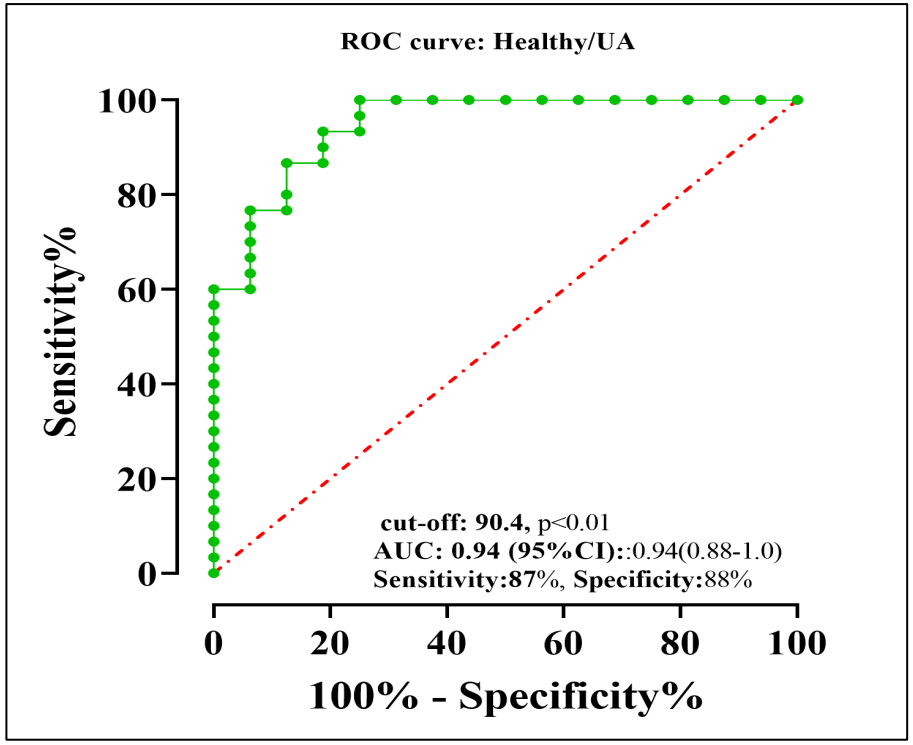


Higher expression level of miR-106b was detected in STEMI, followed by UA. Healthy subjects showed very low level of miR-106b expression

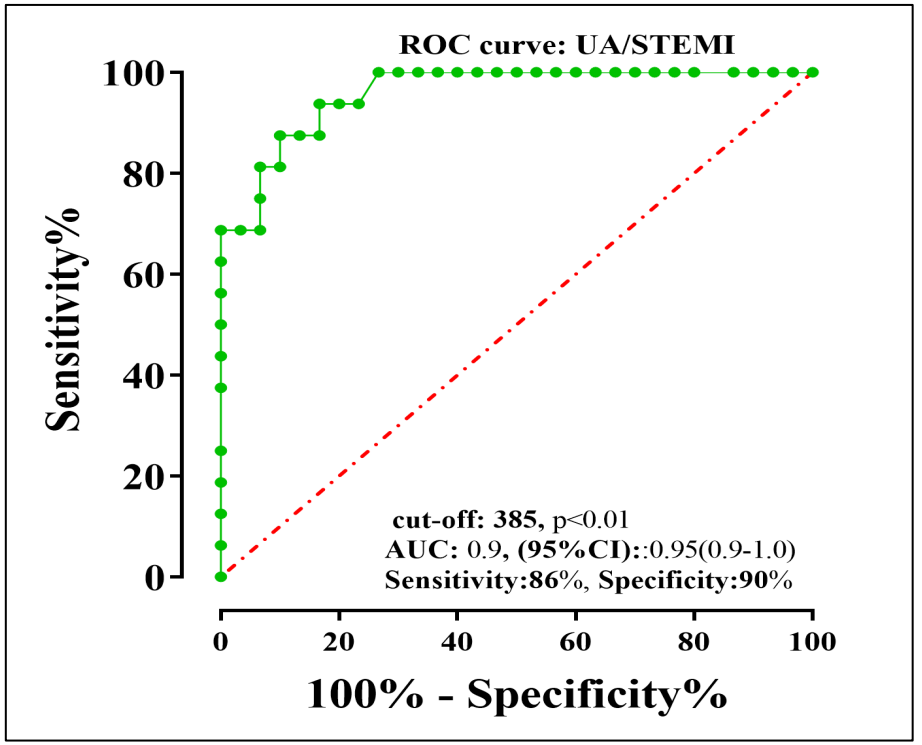


No significant statistical difference in miR-106b expression level in male and female UA patients and STEMI patients

Diagnostic Potential of miR-106b in ACS Patients (ROC Curve)

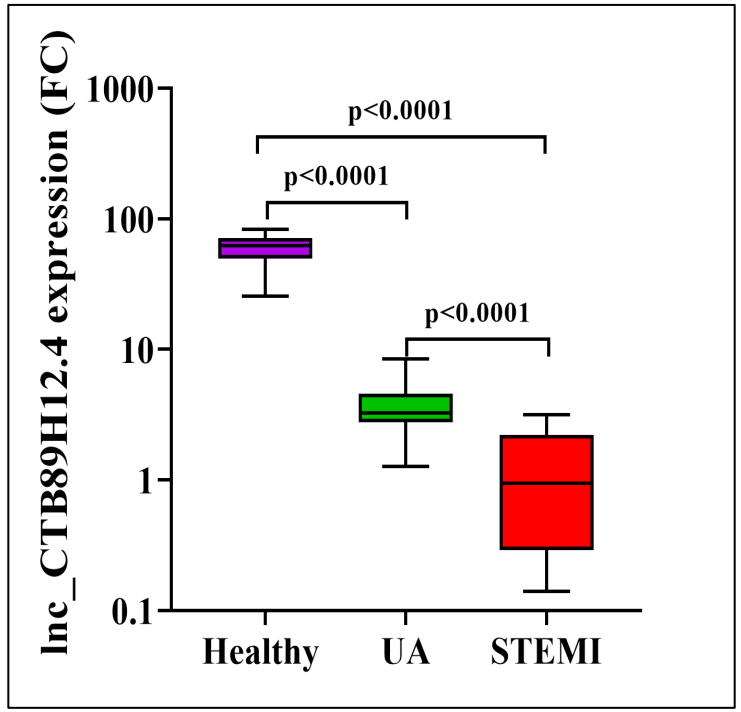


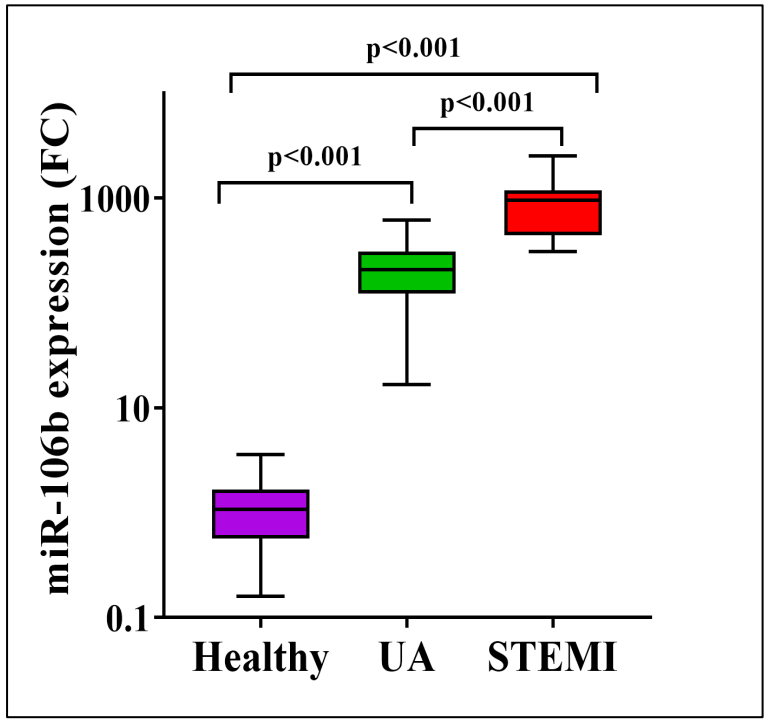
At a cut-off value of 90.4, miR-106b could discriminate UA patients from healthy with Sensitivity of 87% & Specificity of 88%

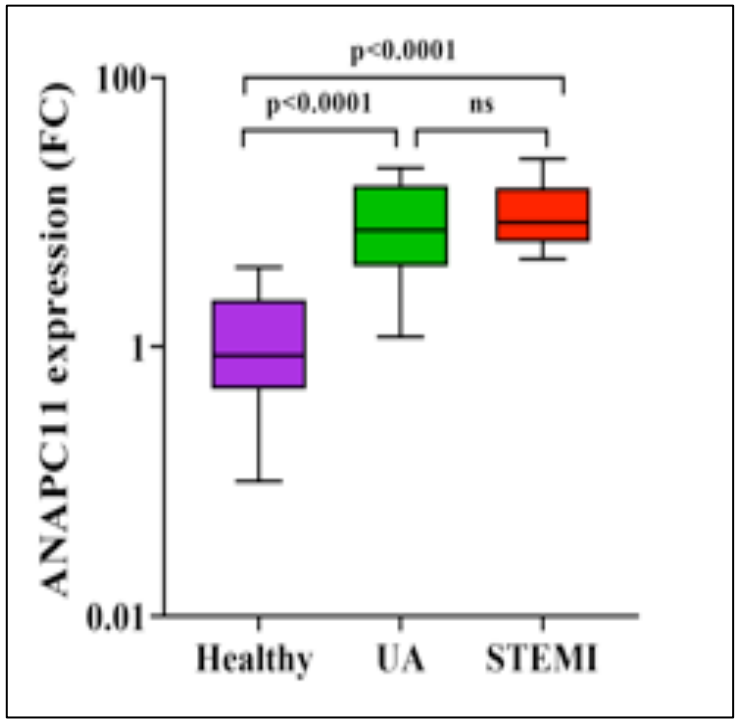


At a cut-off value of 385, miR-106b could discriminate UA patients from STEMI with Sensitivity of 86% & Specificity of 90%

Expression Level of lncR-CTB89H12.4, miR-106b and mRNA-ANAPC11 in UA, STEMI & Healthy







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

miR-106b
150-fold in UA vs. Healthy
4.6-fold in STEMI vs. UA

mRNA-ANAPC11
8.5-fold in UA vs. Healthy
9.5-fold in STEMI vs. Healthy

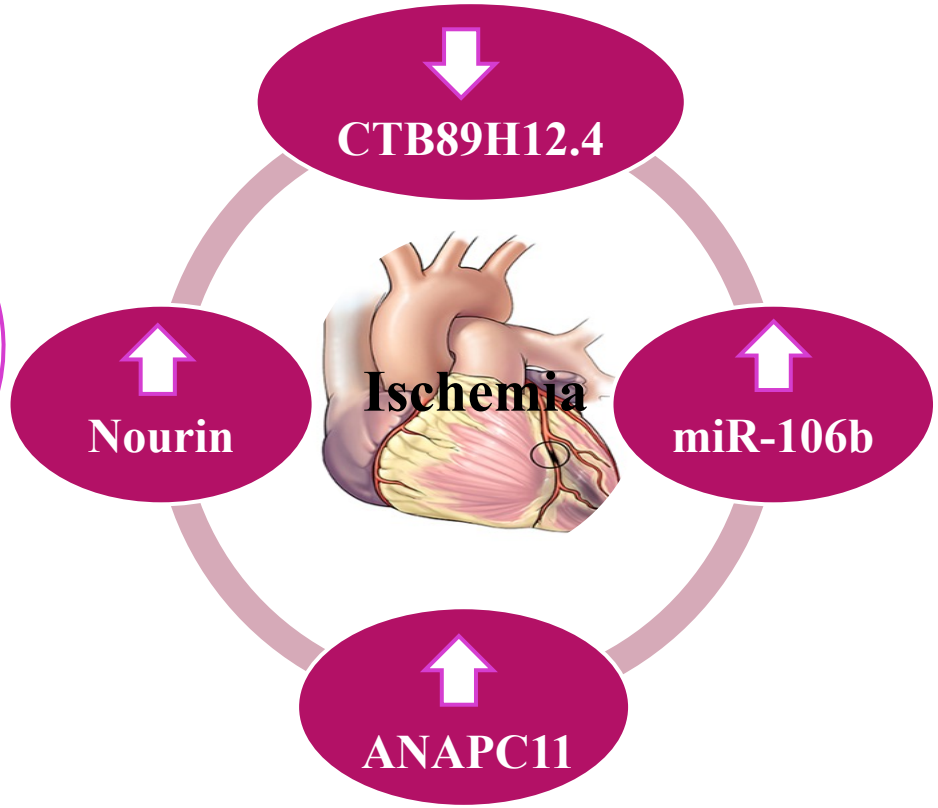
Association of IncR-CTB89H12.4/miR-106b/mRNA-ANAPC11/Nourin in ACS Patients

Spearman's Correlation Analysis in ACS Patients Between miR-106b/ mRNA-ANAPCII/ IncR-CTB8912.4

VARIABLES	ACS (n=46)
miR-106b vs mRNA-ANAPCII	r: 0.35 p=0.02
miR-106b vs IncR-CTB8912.4	r: -0.6 p=0.0001

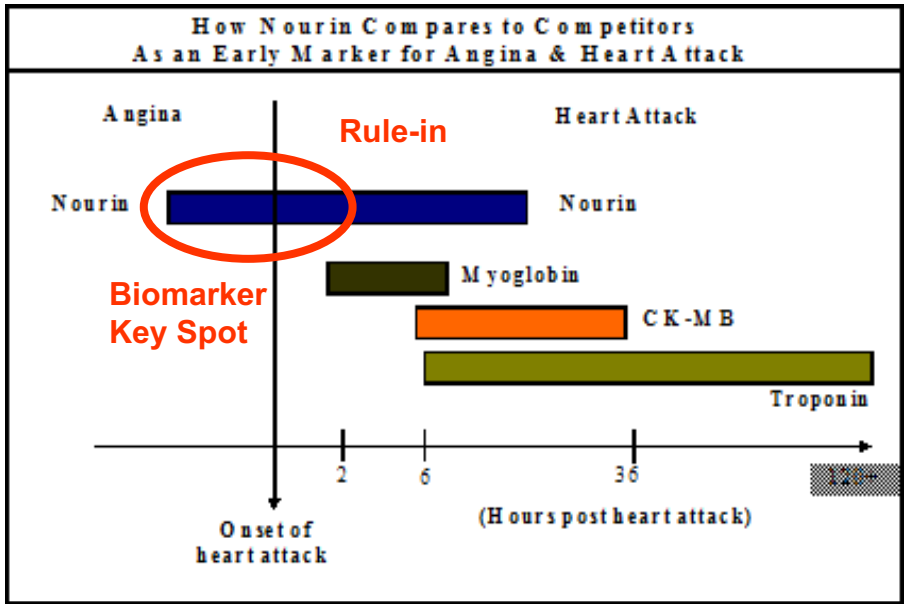
Spearman's correlation revealed a significant association between CTB89H12.4/miR-106b and ANAPC11 in ACS patients

Down-regulation of CTB89H12.4 due to ischemia, resulted in up-regulation of miR-106b and activation of ANAPC11 with an increased translation and production of Nourin protein



Conclusions

- **Results support the Ontology bioinformatics evidence that lncR-CTB89H12.4/miR-106b/mRNA-ANAPC11 network synergistically regulates the Nourin protein expression in myocardial ischemia, and thus, provides a novel molecular mechanism in ischemic heart disease**
- **Nourin-dependent miR-106b is an inflammatory marker that:**
  - **Diagnosed ischemia-induced cardiac injury in UA and STEMI**
  - **Discriminated between UA, STEMI and Healthy**
- **The Nourin-dependent miR-106b 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-106b expression level can be measured using serum or plasma samples (fresh or frozen)**



Thank You.

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