Extracellular Ribonucleic Acids (RNA) in Stroke and Inflammation: Novel Targets for Therapy

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2015 JCR Joint meeting of Coronary Revascularization

JCR - Busan 2015

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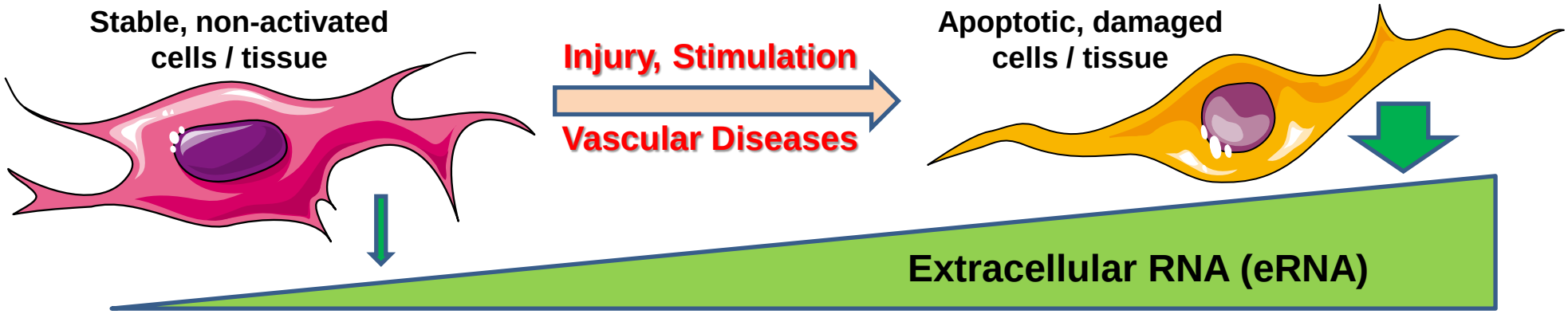
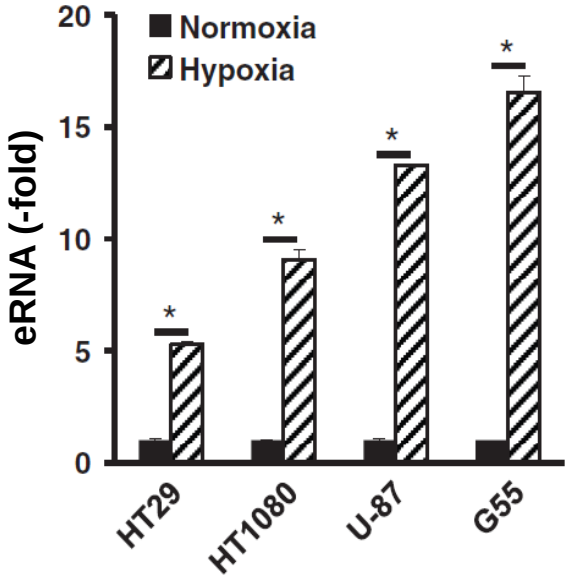
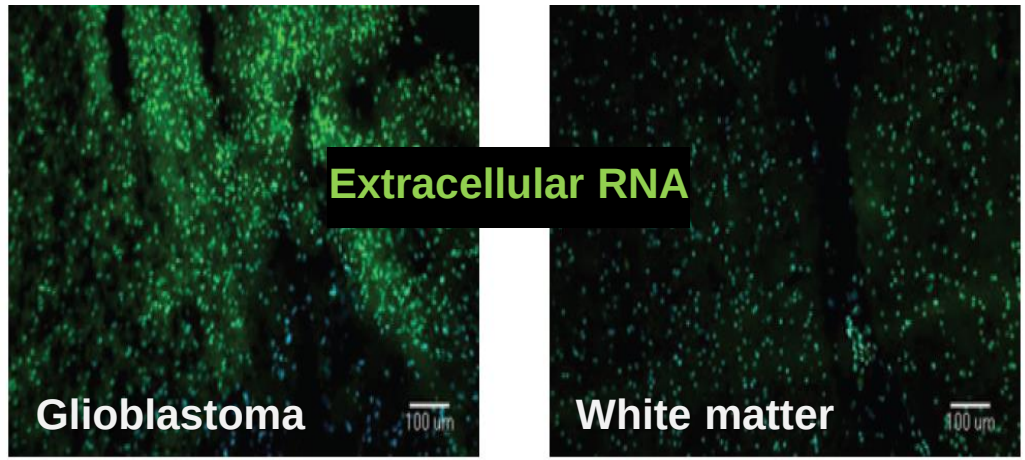
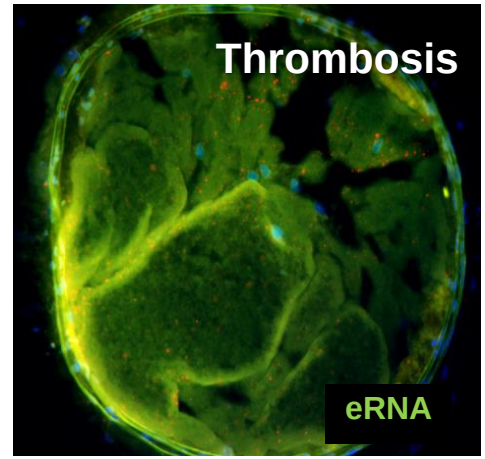
Distribution and Cellular Release of Extracellular RNA



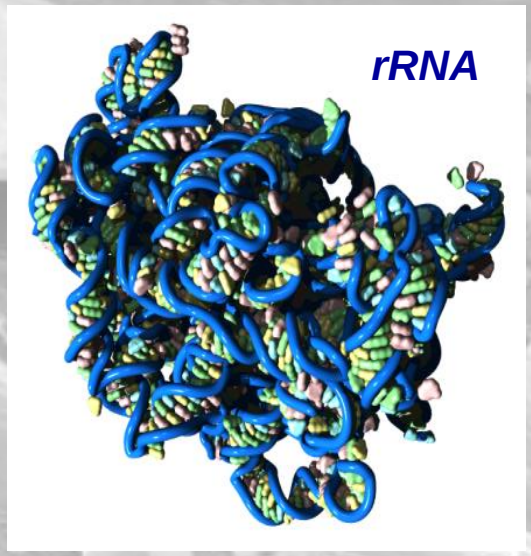
eRNA liberation from tumor cells (hypoxia)

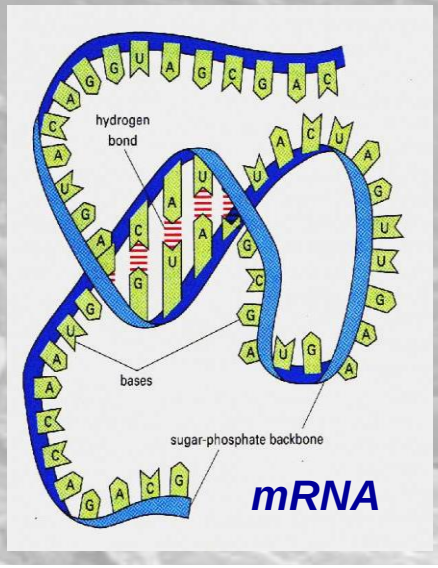


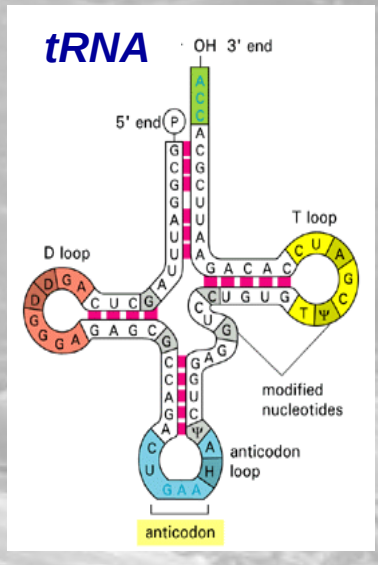


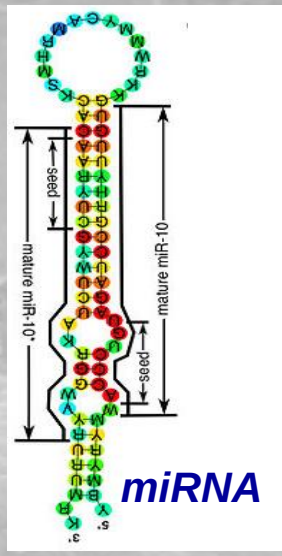


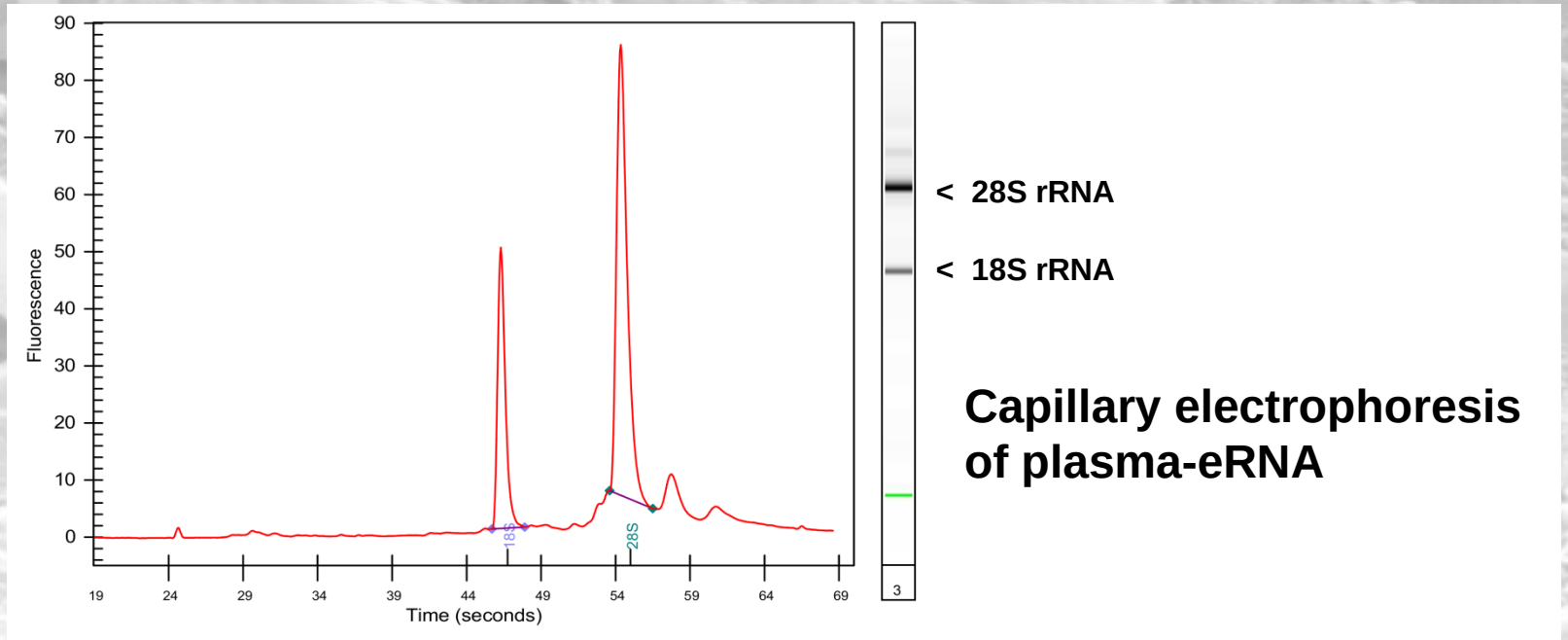
Natural Appearance of Self Extracellular RNA (eRNA)



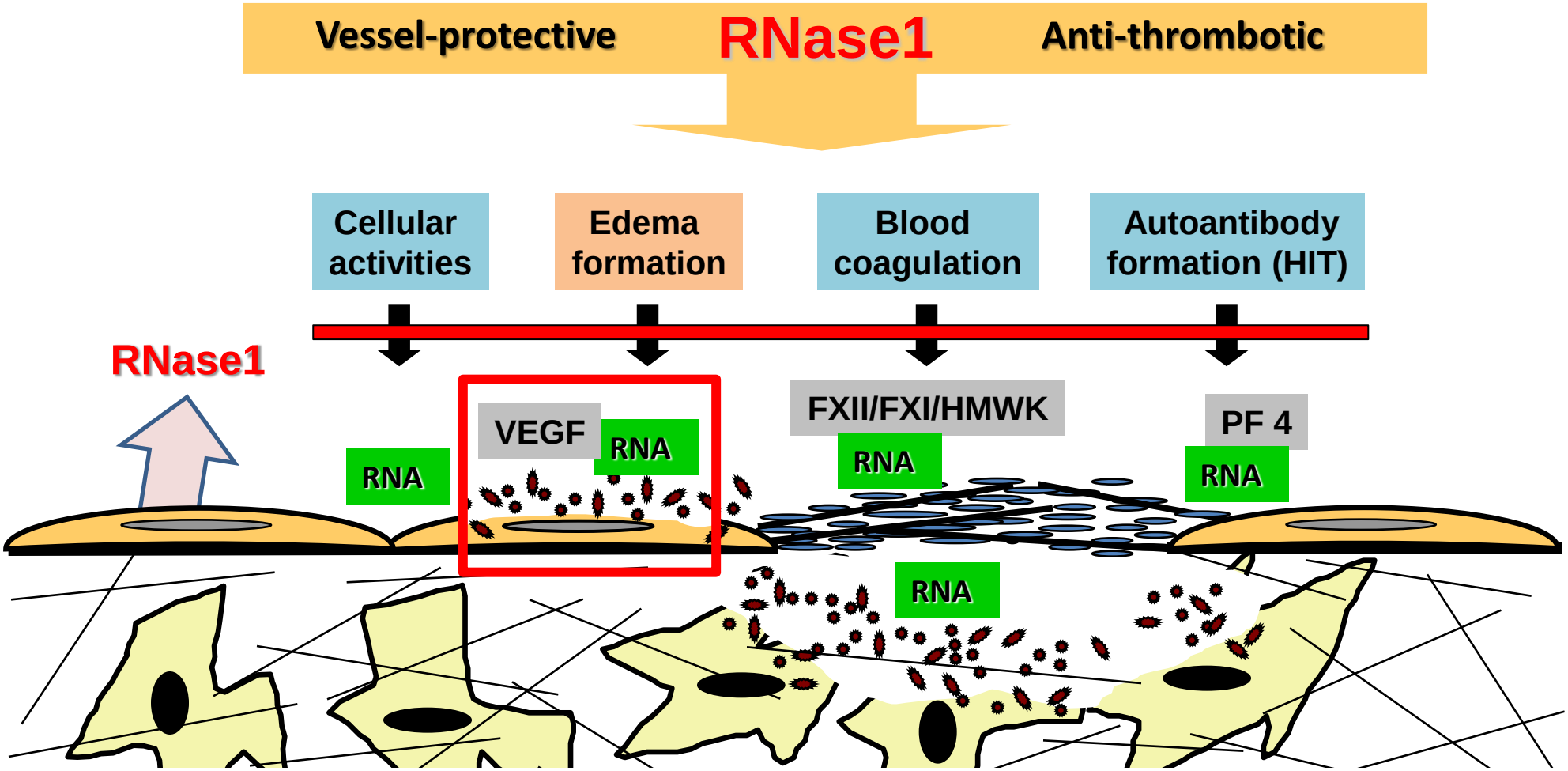






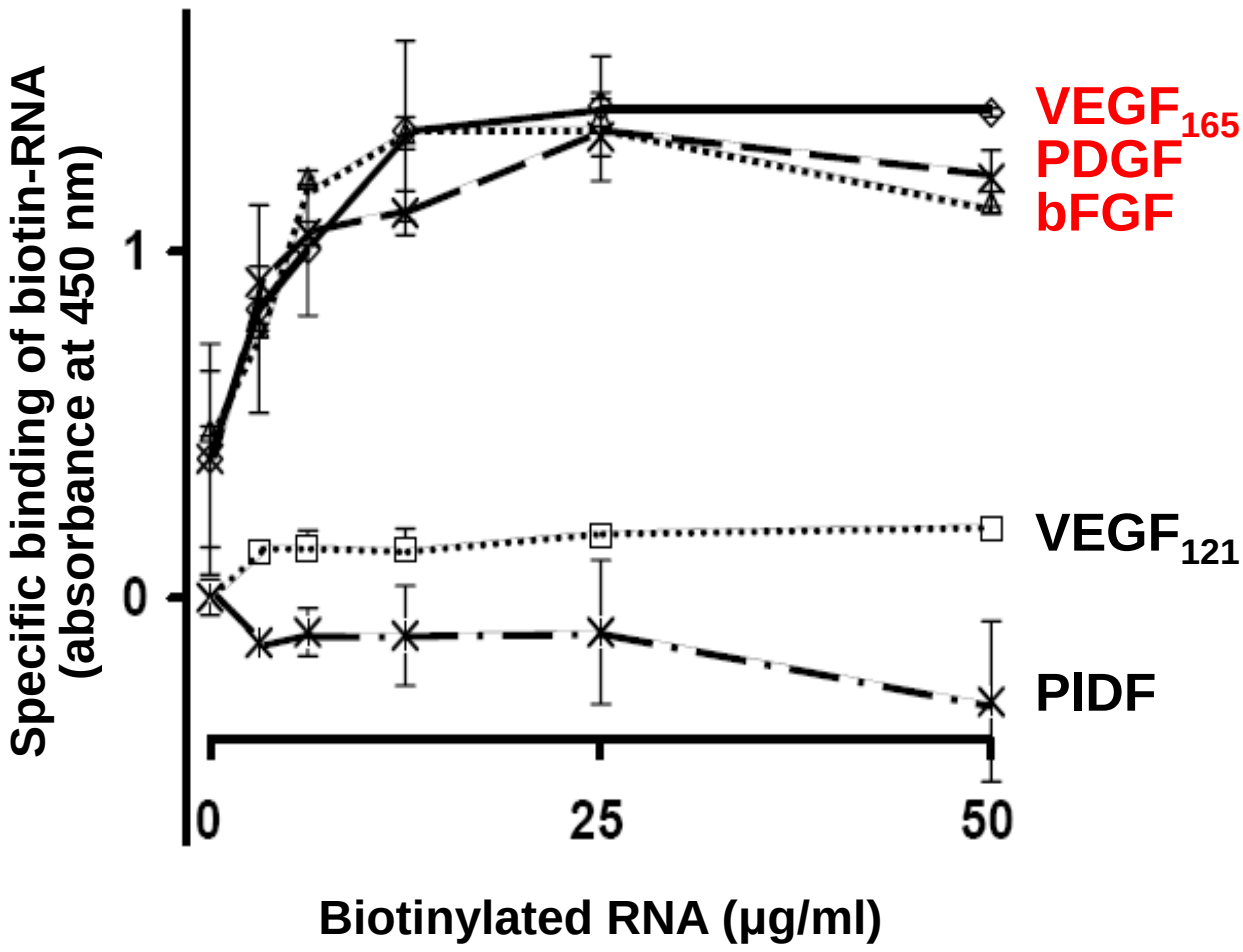


The Extracellular RNA / RNase System and Vascular Homeostasis



Fischer et al., Blood 2007; Kannemeier et al., PNAS 2007; Shibamiya et al., Blood 2009; Fischer et al., T&H 2011; 2012; Can Res 2013; Jaax et al., Blood 2013; Cabrera-Fuentes et al. T&H 2014

Specific Binding of Proteins to Extracellular RNA (eRNA)



Fischer et al., Blood 2007

eRNA-Binding Proteins

Cytokines
VEGF, PDGF, bFGF, ...

Chemokines
Platelet factor 4, SDF-1, ...

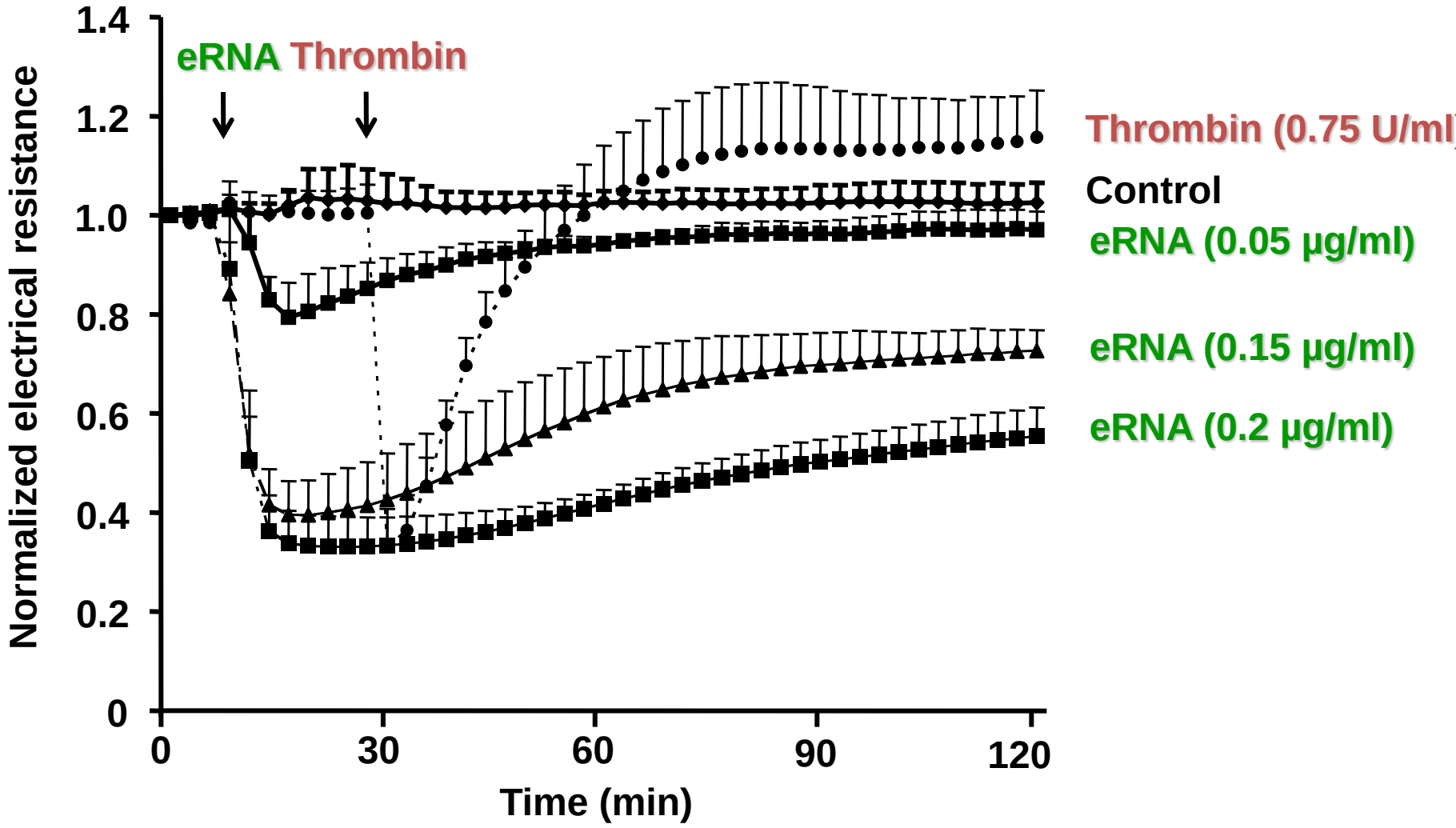
Serine proteases
FSAP, F-XII, F-XI, prekallikrein

Serine protease inhibitors
Plasminogen activator inhibitor-1

ECM molecules
Fibrillar collagens, fibrin, vWF

Induction of Endothelial Cell Permeability by Extracellular RNA

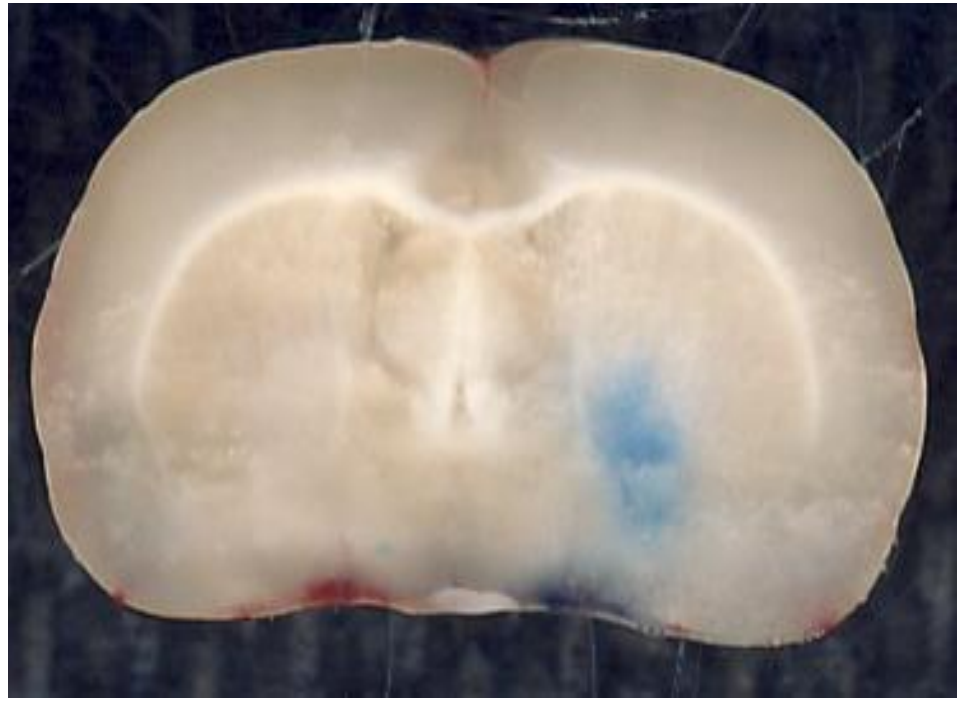
(Porcine brain microvascular endothelial cells)



Fischer et al., Cell Tissue Res 2014

Influence of RNase1 Administration on Brain Edema in a Stroke Model (Ligation) in Rats (I)

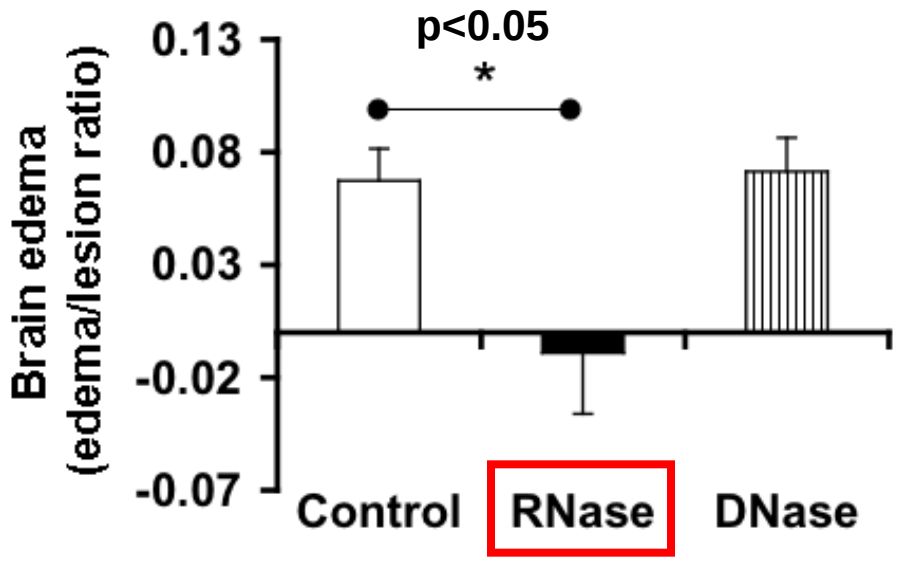
Ischemia (Control)



Ischemia, RNase1 Treatment

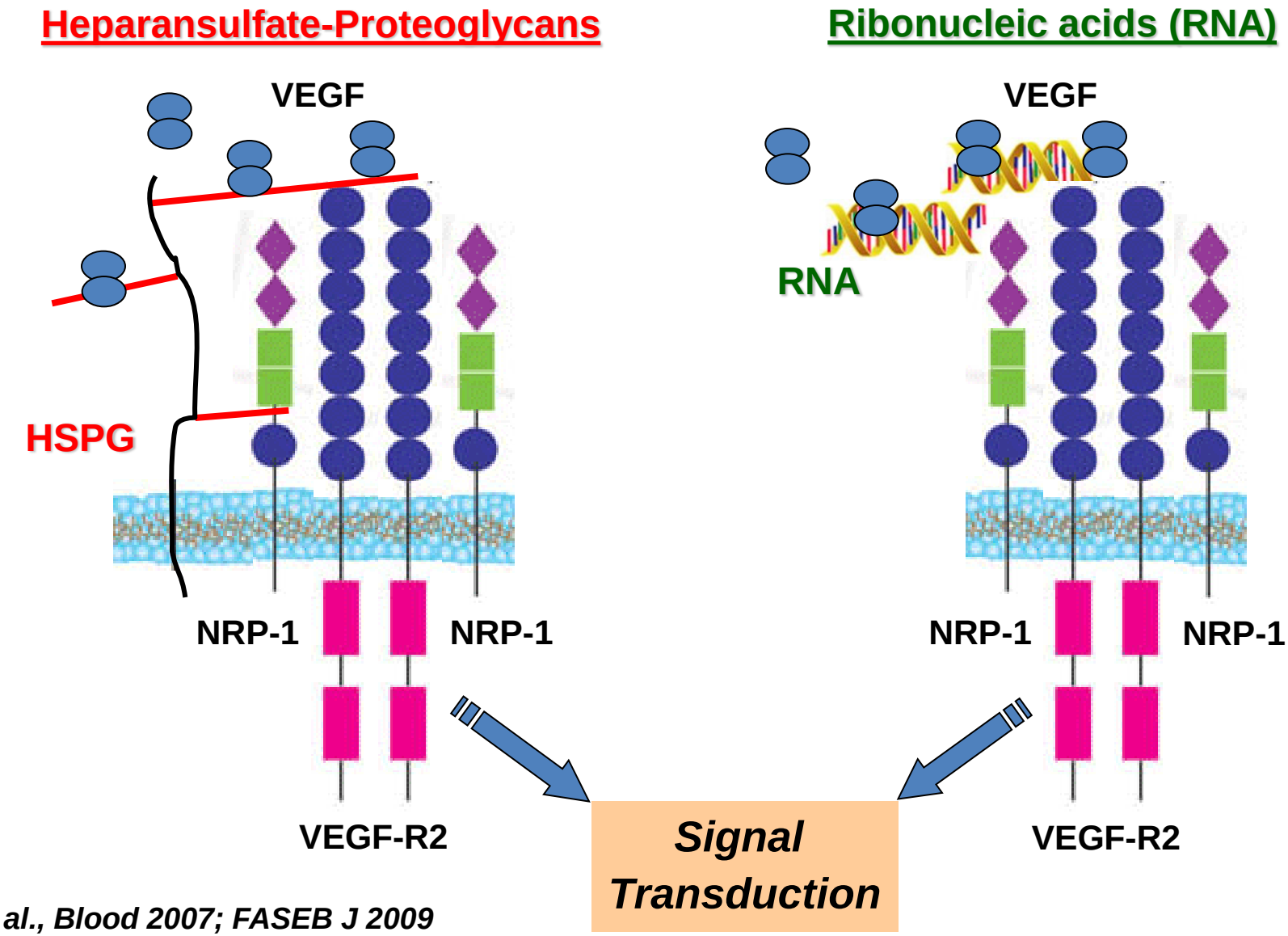
Influence of RNase1 Administration on Brain Edema in a Stroke Model (Ligation) in Rats (II)

A. Brain edema



Walberer et al., Curr Neurovasc Res. 2009

Extracellular RNA-dependent Cell Signaling in Endothelial Cells:
A Vascular Endothelial Growth Factor (VEGF)-dependent Process

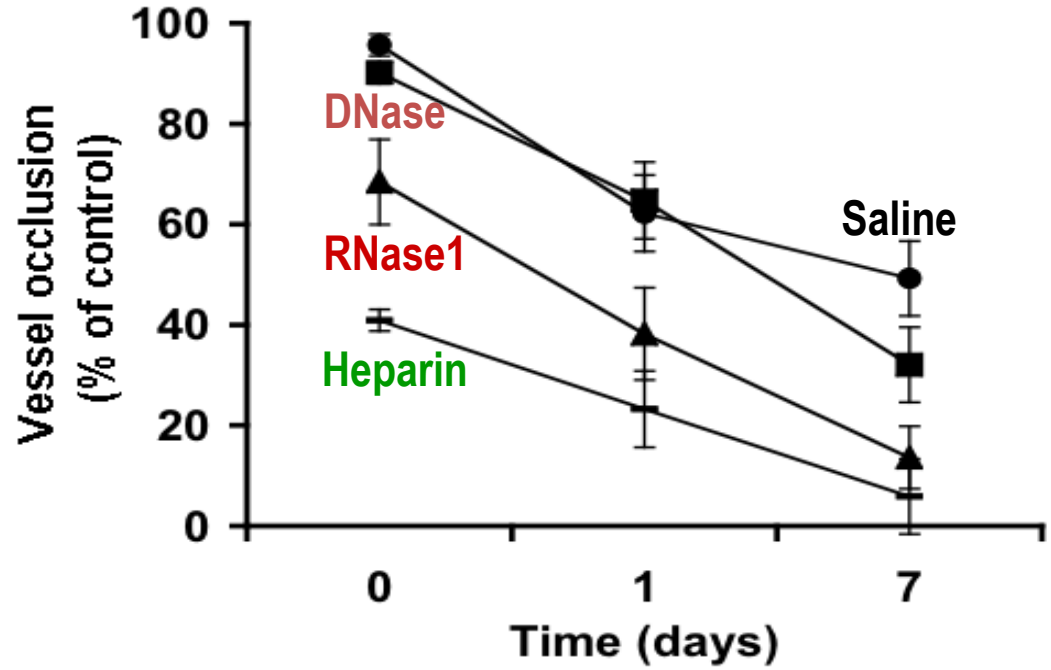


Fischer et al., Blood 2007; FASEB J 2009

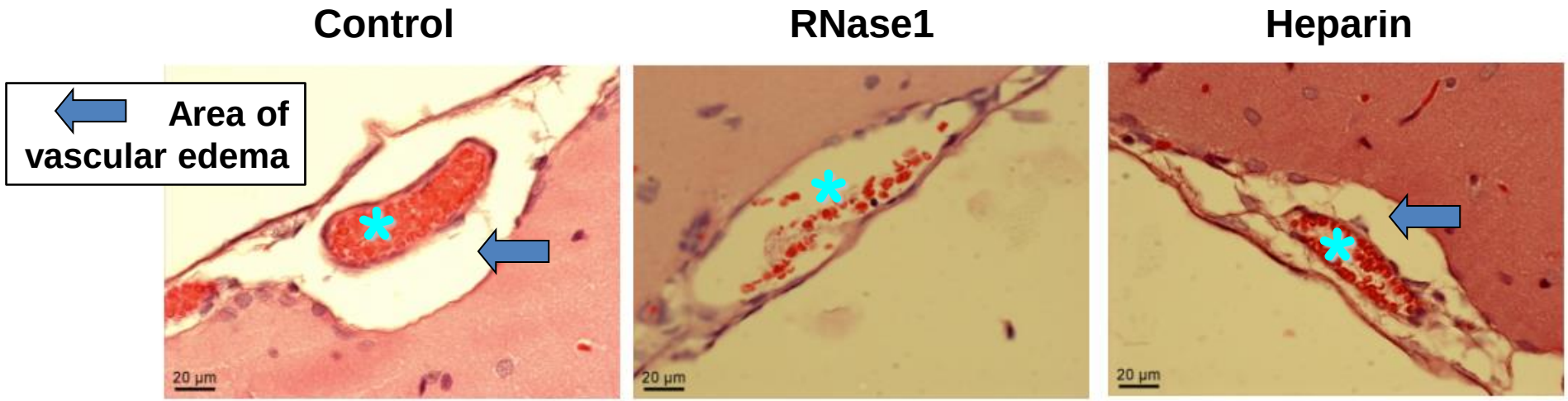
Extracellular RNA-mediated Thrombus / Edema Formation *in vivo*:

(Rat model of $FeCl_3$-induced *Sinus sagittalis* thrombosis)

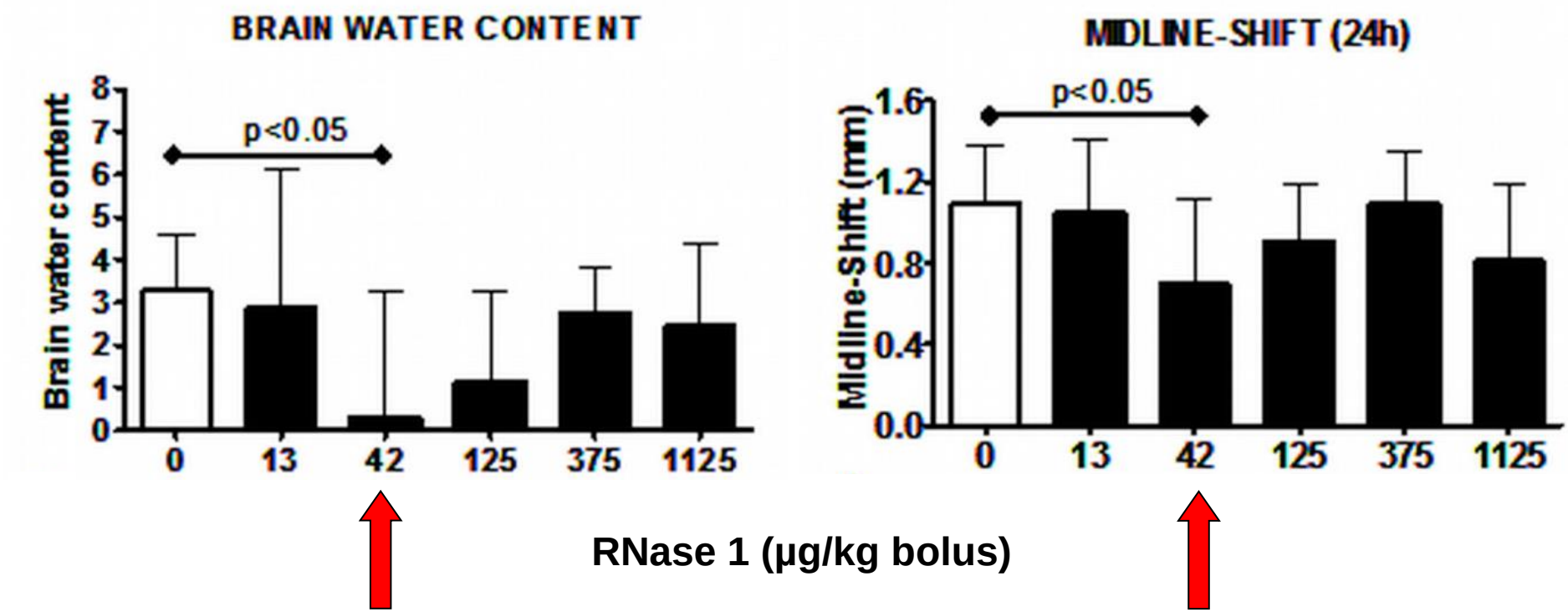
A. Venous occlusion rate



B. Histology



RNase 1 Administration in a Stroke Model in Rats: Dose-Response Relationship

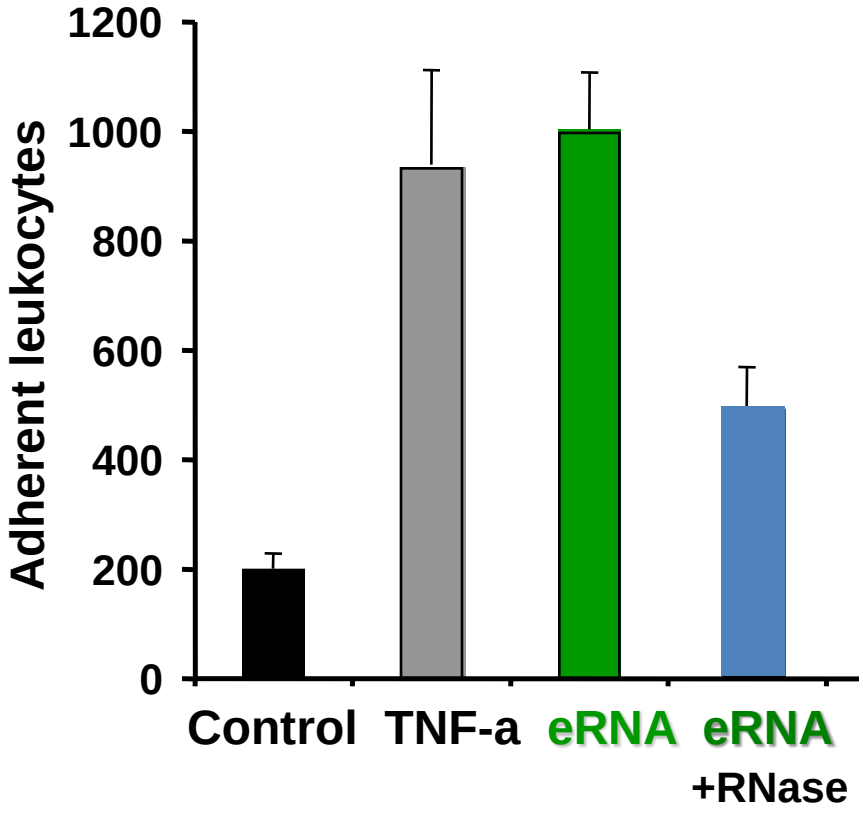


RNase1 serves as natural neuro- and vessel-protective factor in a dose-dependent manner: Point of application ?

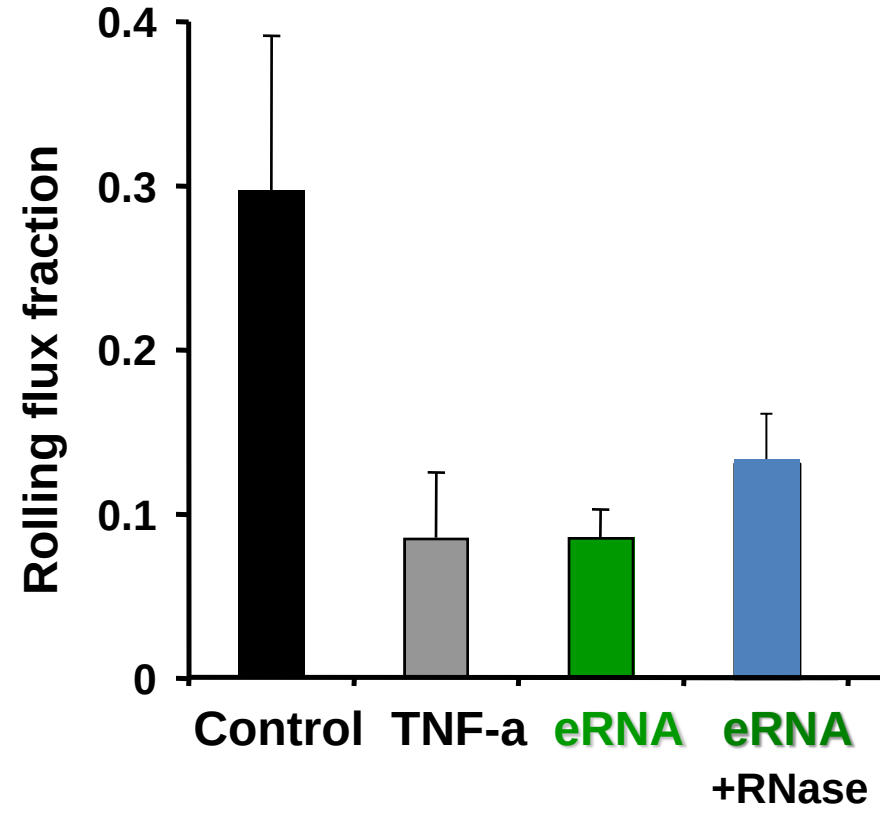
Influence of Extracellular RNA on Leukocyte – Vessel Wall Interactions *in vivo*

(Cremaster muscle vascular model)

Leukocyte Adhesion

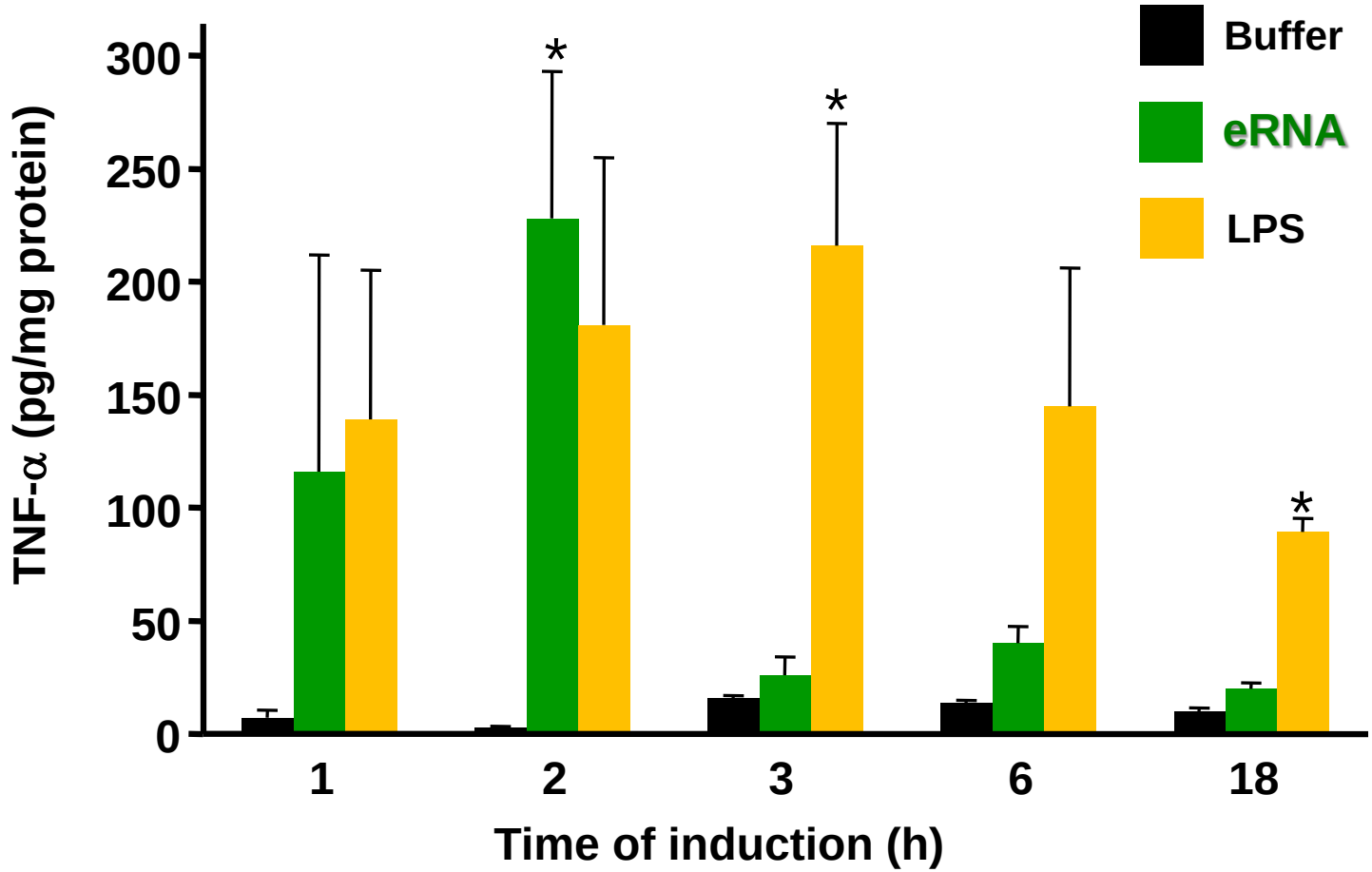


Leukocyte Rolling



Extracellular RNA acts as cell adhesion-promoting factor, in part by a VEGF-receptor-dependent mechanism

Extracellular RNA-induced Release of TNF-α from Monocytes



Extracellular RNA induces TNF-α to promote leukocyte adhesion

Diverse Functions of Self-Extracellular RNA

- Blood coagulation, thrombosis
- Vascular permeability, oedema formation
- Inflammation, leukocyte trafficking
- Cardiac ischemia-reperfusion injury
- Tumor progression and metastasis

Patho-physiological Role in Atrial Fibrillation ?

Kannemeier et al., PNAS 2007; Fischer et al., Blood 2007; FASEB J 2009;
Thromb Haemost 2012; Cancer Res 2013; Jaax et al., Blood 2013

Acknowledgements .....

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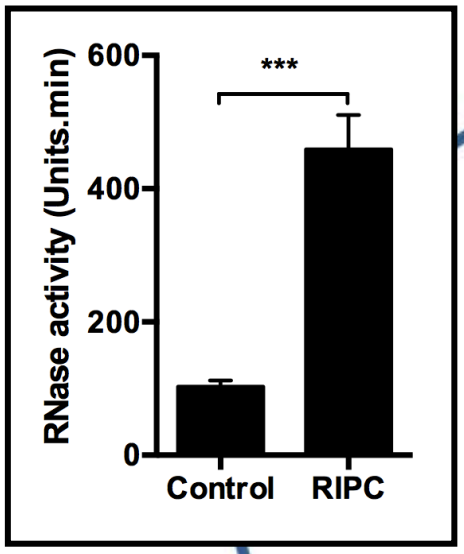
Damaging Interplay Between eRNA and TNF-α in Cardiac Ischemia/Reperfusion Injury



TNF-α
proTNF
TNF-R1
TAPI
TACE
RNase1
eRNA
eRNA
eRNA
RNA
RNA
Hypoxia
Cardiomyocyte
ROS
Prdx3
SOD 1-3
IκBα degradation,
NF-κB translocation
mPTP
iNOS, MCP1
Inhibition of ATP synthesis
Mitochondrial rupture
CELL DEATH

RIPC: Remote Ischemia Preconditioning

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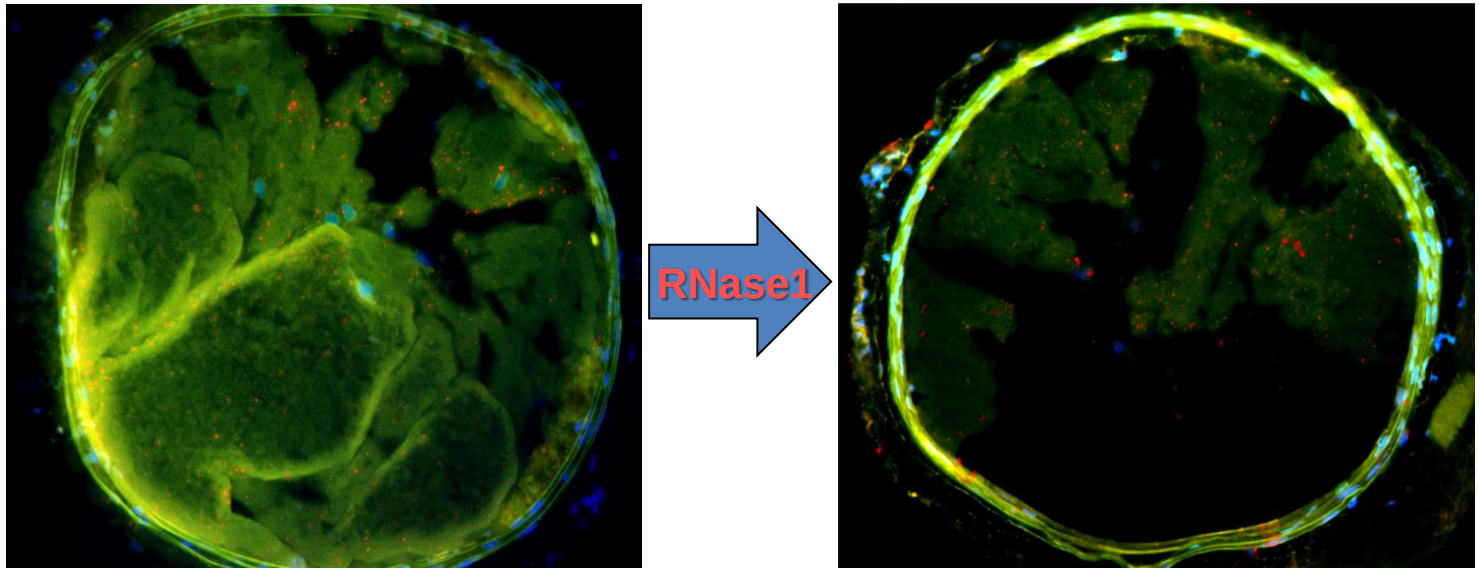
International Society for Thrombosis and Hemostasis

(Damaging) Functions of Self-Extracellular RNA

- **Extracellular RNA:**
  - Blood coagulation, thrombosis
  - Vascular permeability, edema formation
  - Inflammation, atherosclerosis, ischemia/reperfusion injury
  - Tumor progression and metastasis

(*Kannemeier et al., PNAS 2007; Fischer et al., Blood 2007; FASEB J 2009; Thromb Haemost 2012; Cancer Res 2013; Jaax et al., Blood 2013; Simsekyilmaz et al., Circulation 2014*)



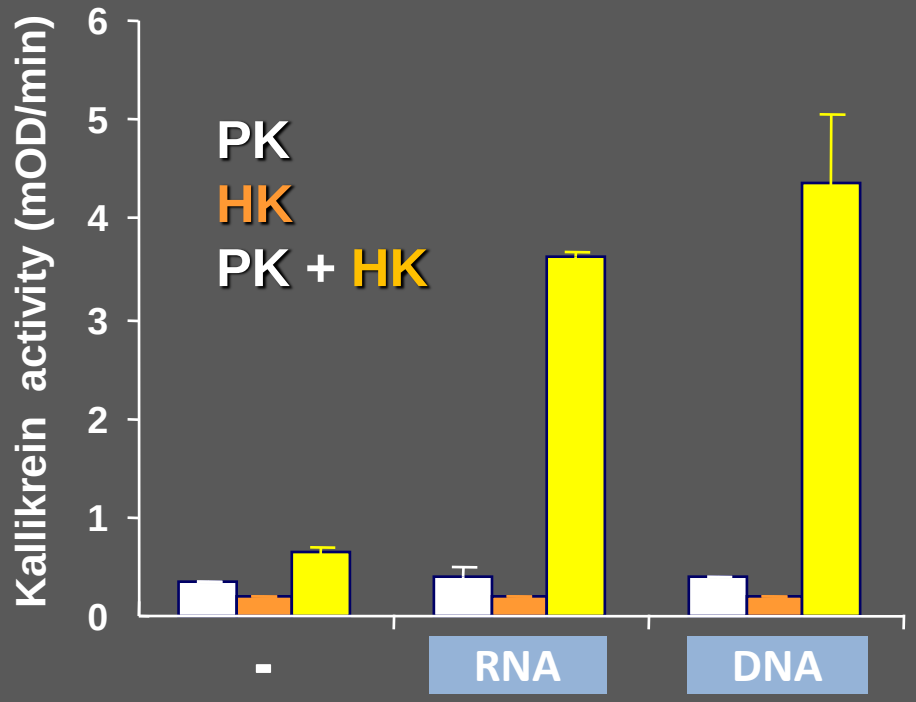
Kannemeier et al., PNAS 2007

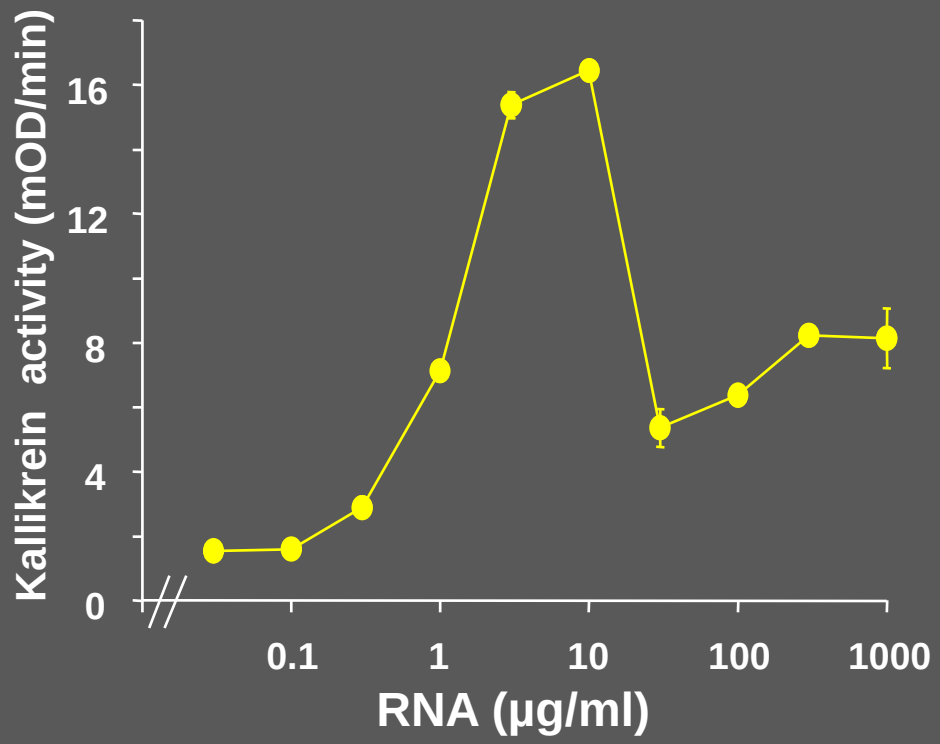
Proposed mechanism

exRNA is a natural auto-activation factor for proteases of the CONTACTPHASE of blood coagulation.

Nucleic Acids and Contactphase Activation

Prekallikrein (PK) —Kininogen (HK) / Nucleic acids→ Kallikrein



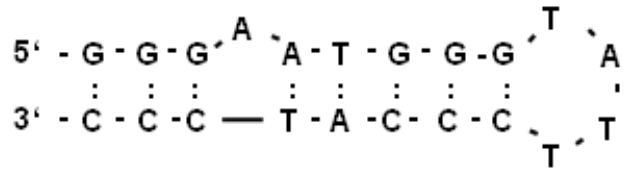


Procoagulant RNA / DNA Oligonucleotides

hairpin structures

linear structures

DNA

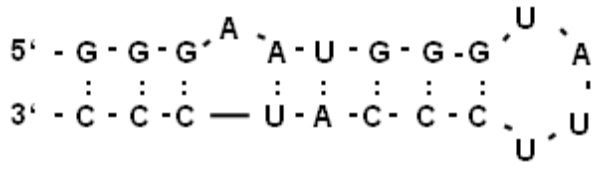


21mer-H1 (ΔG<0)

5‘-A-A-G-A-A-T-A-C-G-A-C-C-A-C-C-T-A-G-C-T-C-3‘

21mer-L1 (ΔG>0)

RNA

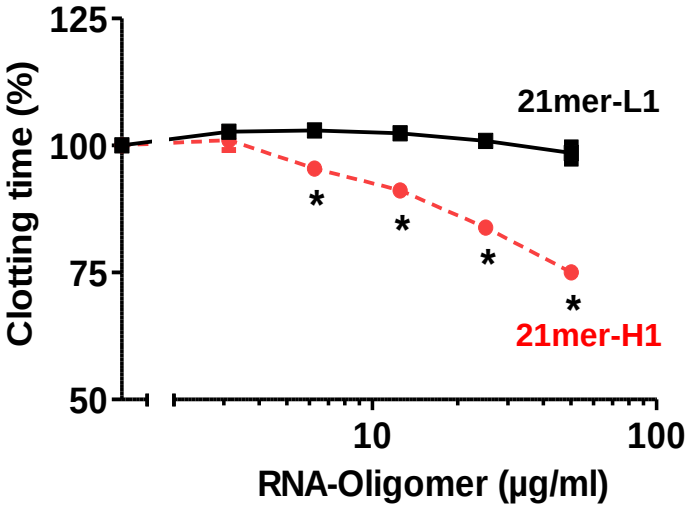


21mer-H1 (ΔG<0)

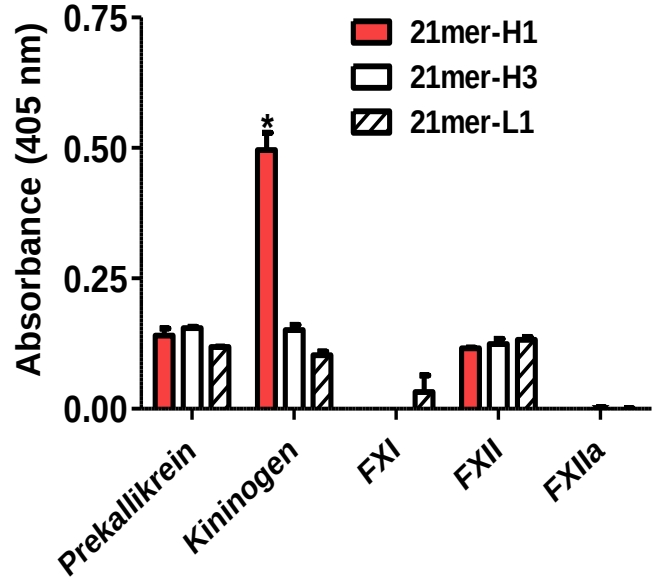
5‘-A-A-G-A-A-U-A-C-G-A-C-C-A-C-C-U-A-G-C-U-C-3‘

21mer-L1 (ΔG>0)

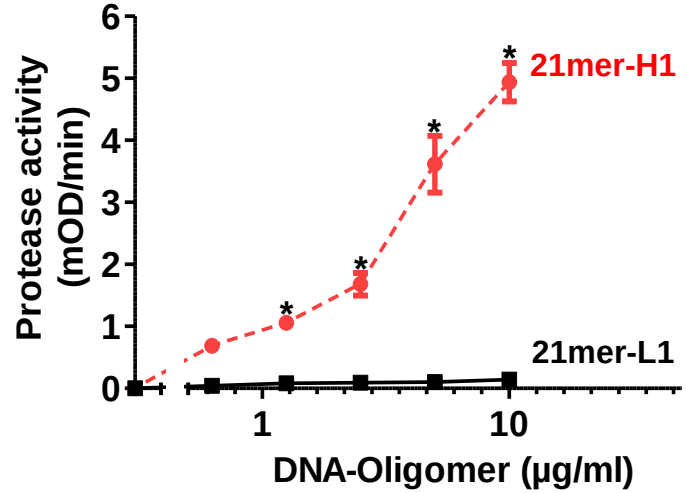
Clotting time



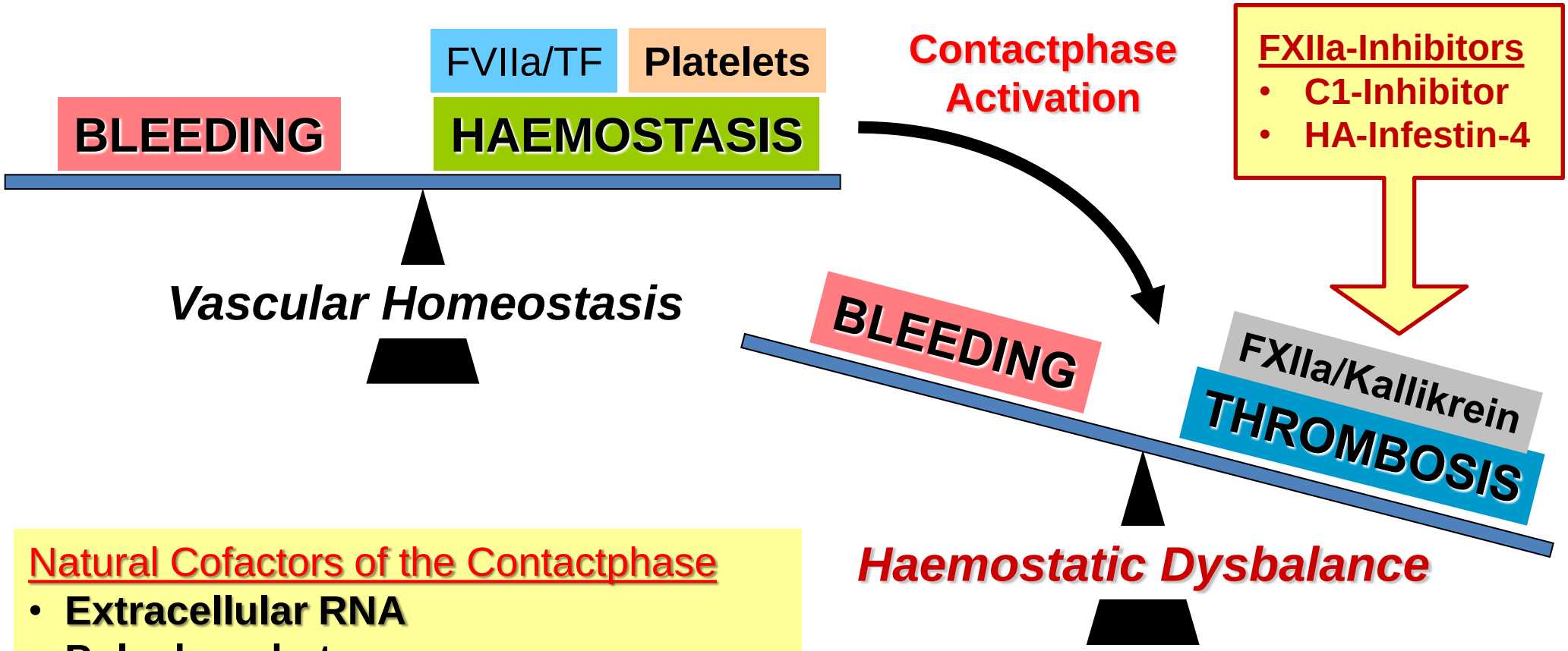
Kininogen binding



Protease activity



The Transition from Haemostasis to Thrombosis: Factor XIIa Makes the Difference



Natural Cofactors of the Contactphase

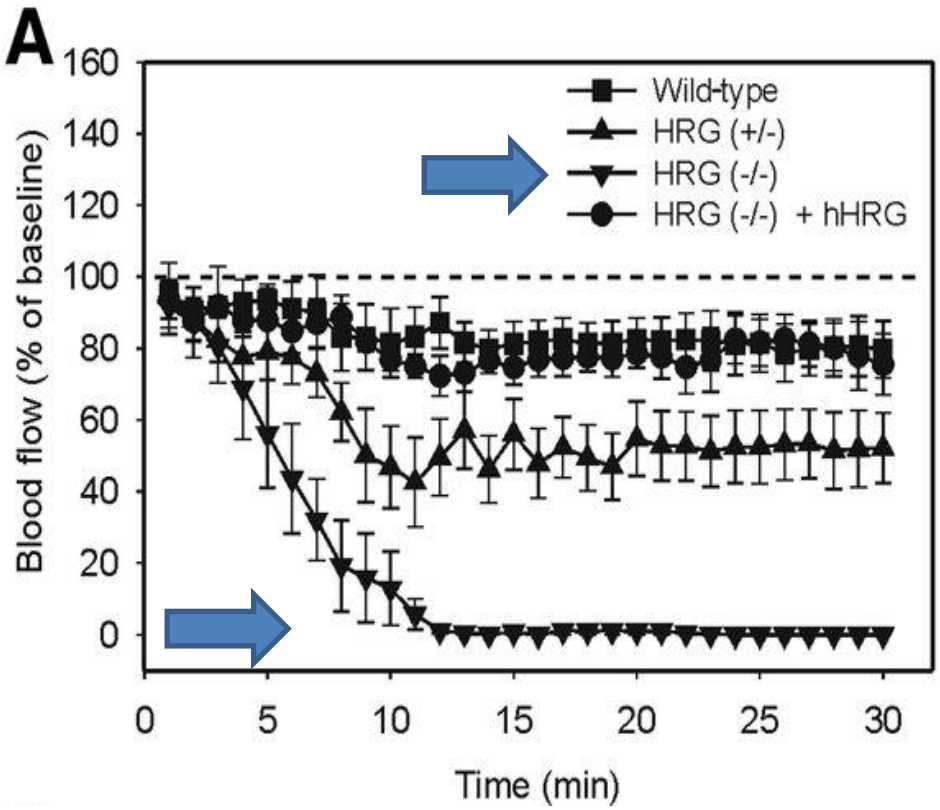
- **Extracellular RNA**
- **Polyphosphates**
- **Neutrophil Extracellular Traps (NETs)**
- **Denatured Proteins**

HA-Infestin-4: Albumin-peptide fusion product from *Triatoma infestans* bug

Histidine-rich Glycoprotein (HRG) Deficiency: Accelerated Thrombosis After $FeCl_3$-induced Arterial Injury

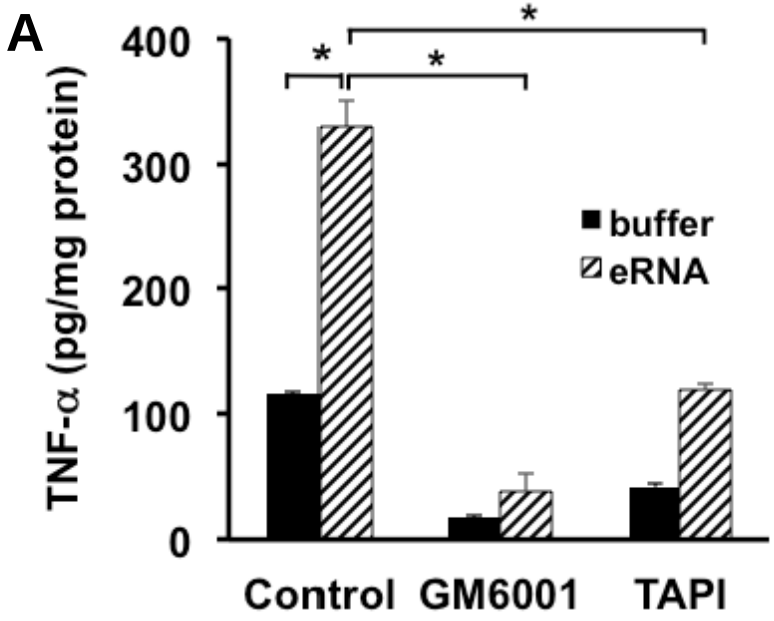
Histidine-rich Glycoprotein (HRG):

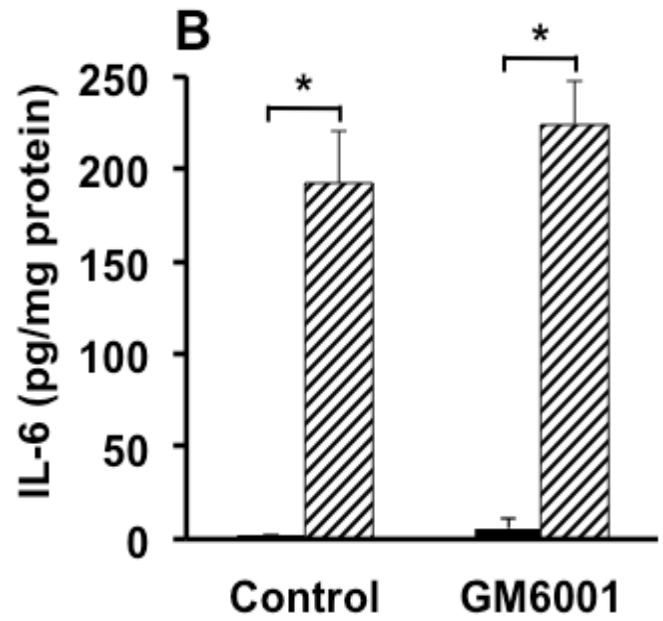
- **Plasma and platelet protein, deficiency is associated with thrombophilia**
- **Binds to heme, heparinoids, thrombospondin, plasminogen, divalent metal ions**
- **Regulates cell adhesion, angiogenesis, coagulation and fibrinolysis**

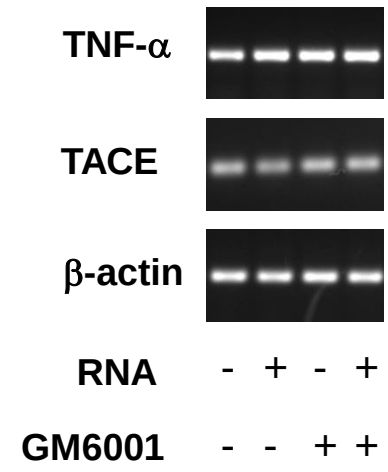


Vu et al., Blood 2015

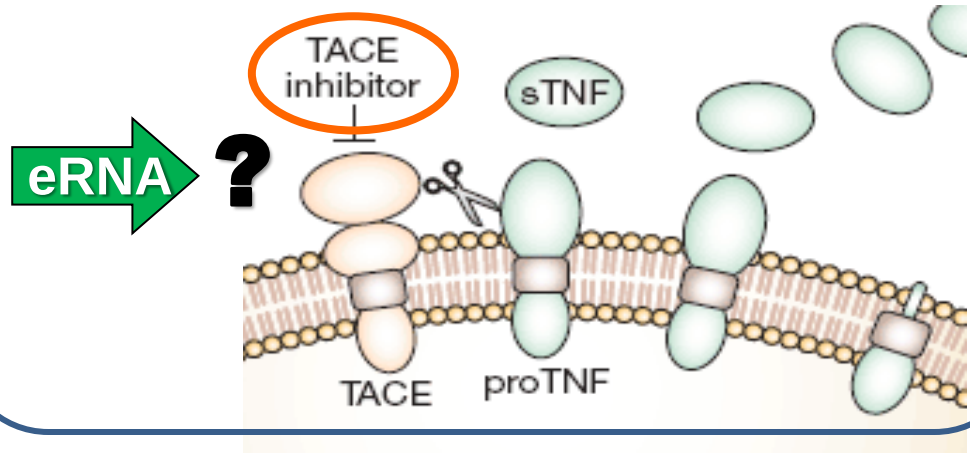
Extracellular RNA-induced TNF-α Release: Contribution of the Sheddase TACE / ADAM-17







Proposed mechanism

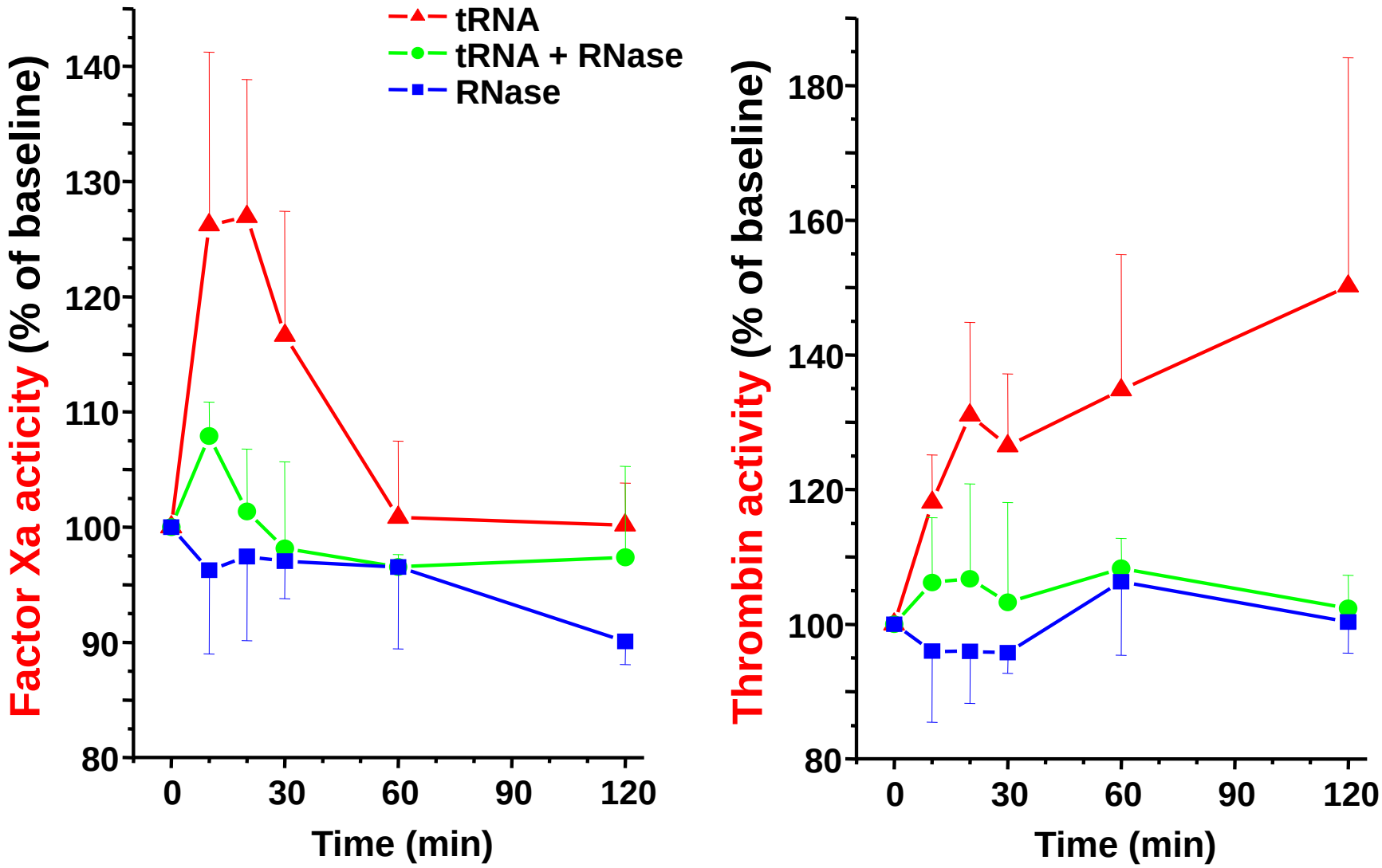


Extracellular RNA promotes TACE-induced cleavage of membrane-bound pro-TNF.

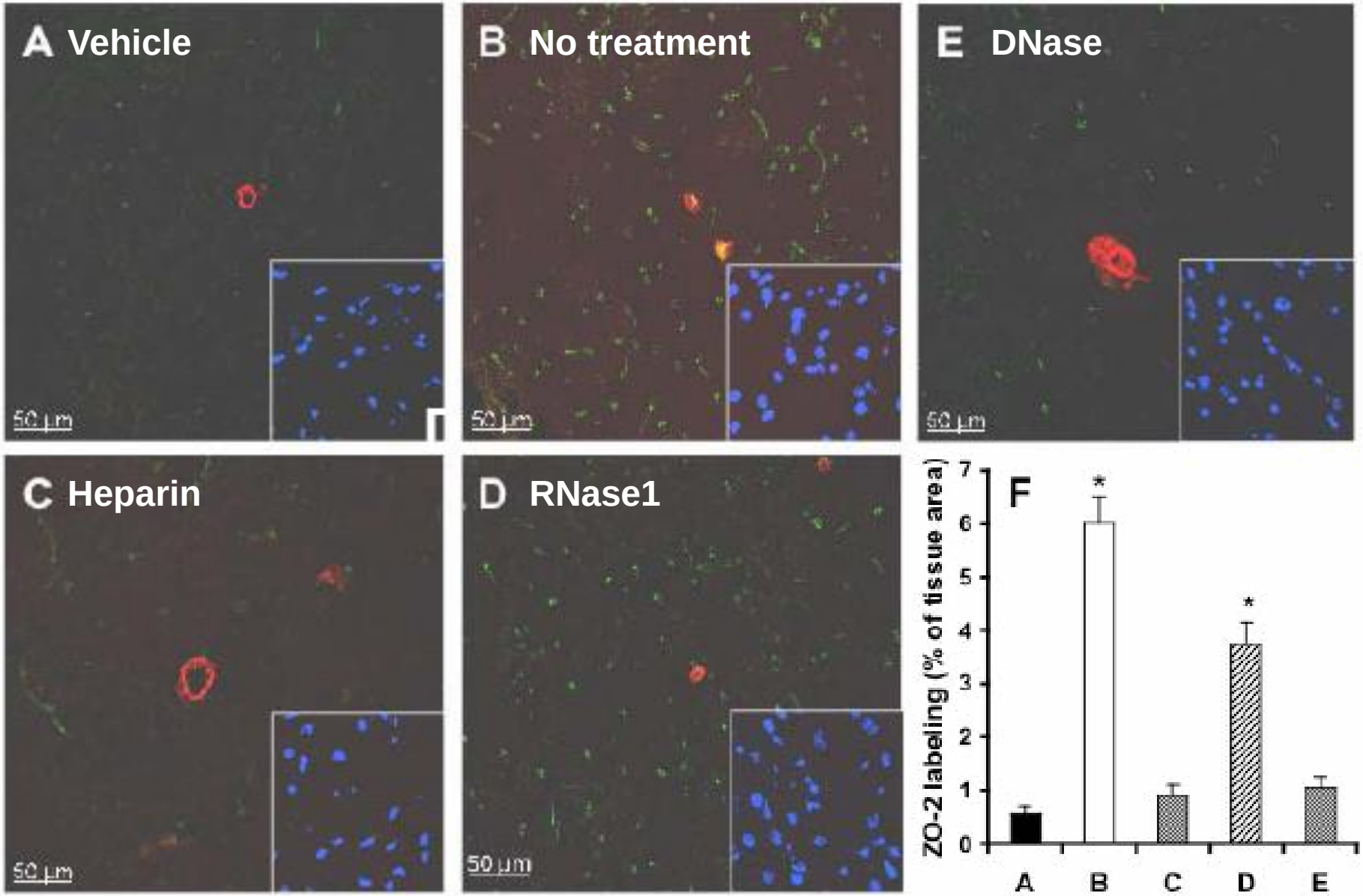
TACE / ADAM-17: Tumor Necrosis Factor-α Converting Enzyme has >70 substrates.

Fischer et al., Thromb Haemost 2012

Procoagulant Activity of Extracellular RNA *in vivo*



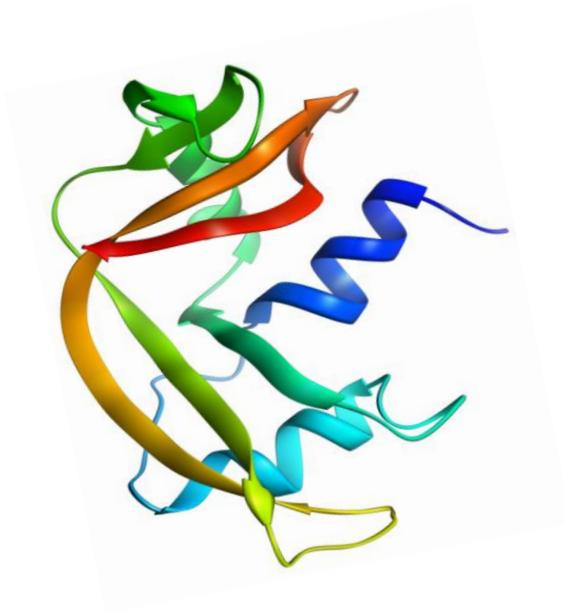
Influence of RNase on Vessel Integrity:
Analysis of ZO-2 in Ischemic Brain Regions

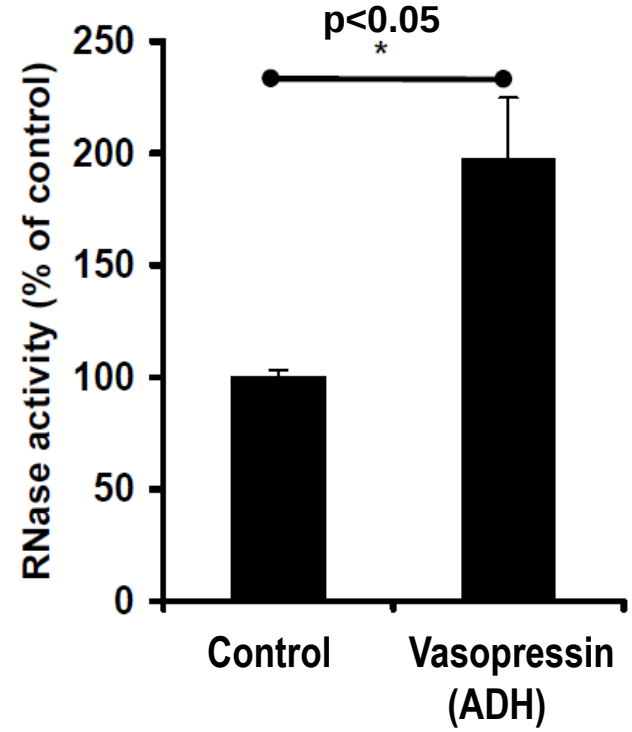


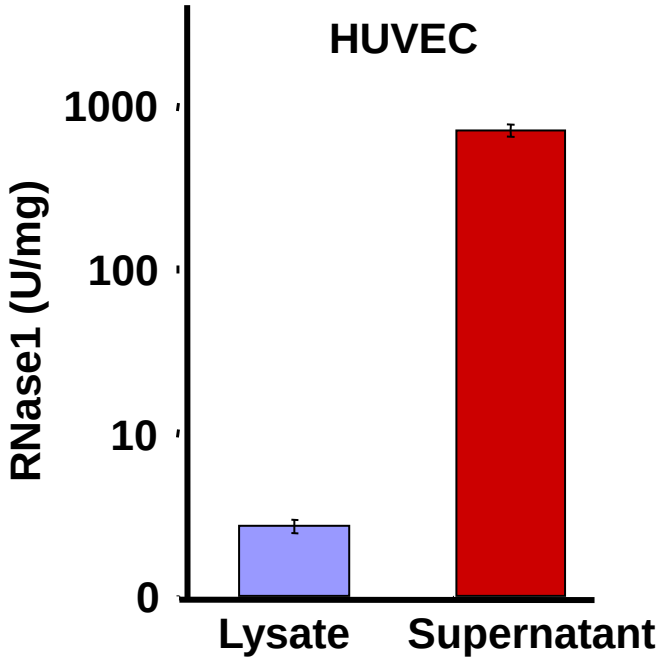
Expression and Distribution of Vascular RNase1

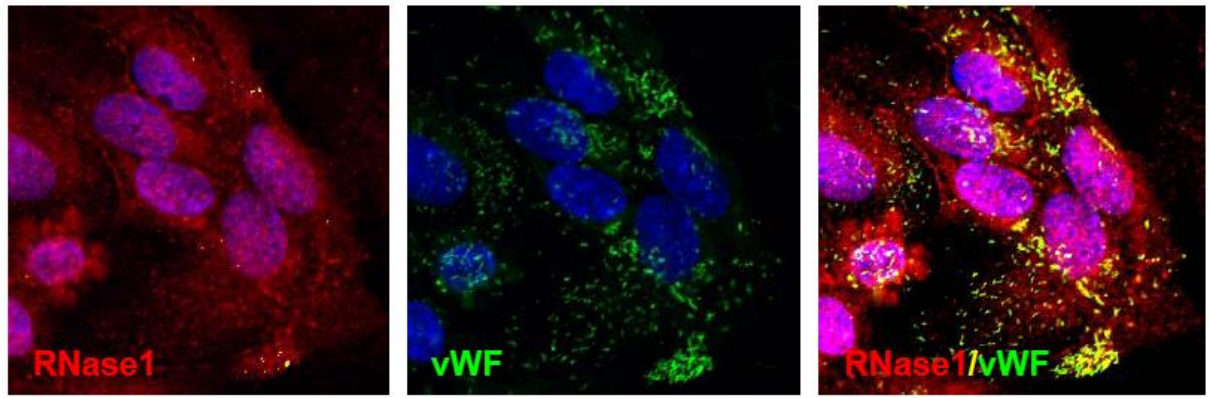
Extracellular RNase1

- Member of the RNaseA-family
- Thermostable enzyme (17 kDa)
- Major pancreatic ribonuclease
- Non-toxic factor for host cells









Fischer et al., T&H 2011