New Mechanisms of Collateral Formation: The Role of Perivascular Cells and Extracellular RNA in Arteriogenesis

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Reactions and Players in Arteriogenesis

Arteriogenesis: Formation of collateral vessels upon ischemia (Elisabeth Deindl, Markus Sperandio - Munich)

Changes in blood flow, shear stress; role of platelets?

Mast cells (eRNA), cytokines

Proteases

Cytokines, chemokines

Leukocyte extravasation

Ligation of A. femoralis (R); sham-operation (L); quantification by Laser-Doppler analysis
Platelets and Reactive Oxygen Species (ROS) Contribute to Mast Cell Stimulation and Arteriogenesis

![Graph showing the effect of various treatments on degranulated mast cells/collateral.]

- **Platelet GPIbα** significantly increases degranulated mast cells/collateral.
- **ROS** also significantly increases degranulated mast cells/collateral.

Key treatments and their effects:
- **Control**: Moderate increase in degranulated mast cells/collateral.
- **Cromolyn**: Minimal effect on degranulated mast cells/collateral.
- **IL4-R/lba**: Increase in degranulated mast cells/collateral.
- **uPA−/−**: Small increase in degranulated mast cells/collateral.
- **UK122**: Significant increase in degranulated mast cells/collateral.
- **1A8**: Moderate increase in degranulated mast cells/collateral.
- **NAC**: Minimal effect on degranulated mast cells/collateral.
- **Apocynin**: Significant increase in degranulated mast cells/collateral.
- **Nox2−/−**: Increase in degranulated mast cells/collateral.
- **Isotype Fab**: No significant change.
- **GPIbα Fab**: Significant increase in degranulated mast cells/collateral.

Note: n.s. indicates no statistically significant difference.
Reactive Oxygen Species (ROS)-dependent Mast Cell Stimulation: The Role of Nox2-deficiency

Day 1 following *femoral artery ligation*:
Number of perivascular, degranulated mast cells

*Chandraratne et al., ATVB 2015*
Mast Cell Degranulation Promotes Arteriogenesis (I)

Collateral vessel formation

Collateral vessel formation

Pericellular mast cell degranulation

Degranulated mast cells per collateral (% control)

Chillo et al., 2014 (unpublished data)
Mast Cell Degranulation Promotes Arteriogenesis (II)

Chillo et al., 2014 (unpublished data)
Mast Cell Products Recruit Leukocytes in Arteriogenesis

Chillo et al., 2014 (unpublished data)
Mast Cell Degranulation Promotes Collateral Vessel Growth

Control

Ki67-pos. cell

C48/80+dipA

100 µm

cromolyn+dipA

Ki76+ cells / collateral

Control  C 48/80 + Diprotin A  Cromolyn + Diprotin A

* *
Mast Cell Degranulation Promotes Collateral Vessel Enlargement

Day 3

Control

C 48/80 + Diprotin A

Day 7

Luminal Diameter of Collaterals

Vessel diameter (μm)

<table>
<thead>
<tr>
<th></th>
<th>d3</th>
<th>d7</th>
<th>d3</th>
<th>d7</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>C 48/80 + Diprotin A</td>
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Chillo et al., 2014 (unpublished data)
Mast cells: Major Source of Extracellular RNA

- Agonists:
  - Anaphylatoxins
  - Bacterial factors
  - Cytokines
  - IgE

INFLAMMATION (TNF-α, etc.)

Fischer et al., 2014 (unpublished data)
Induction of Vasculogenesis/Angiogenesis by Extracellular RNA

Sharifpanah et al., Free Rad Biol Med 2015
The Extracellular RNA – RNase1 System and Arteriogenesis

Novel molecular relations between extracellular RNA, mast cells and arteriogenesis
Mast Cells Orchestrate Arteriogenesis

1. Mast cell degranulation >>> Vascular remodeling & cell proliferation

- Shear stress, platelet-neutrophil conjugates
- Activation of inflammatory cells > Cytokines
- eRNA
- ROS
- MMP
- FGF-2
- PDGF-BB
- RNase1

Activated mast cell
Innate Immunity Reactions as Template for Tissue Regeneration

1. Shear-stress-mediated platelet-neutrophil conjugates
2. ROS-mediated mast cell activation, degranulation
3. Liberation of eRNA, cytokines, histamine, ...
4. Recruitment of neutrophils and monocytes
5. Growth of vessel wall cells
6. Vessel remodeling
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Players and Reactions in Innate Immunity and Defense

1. Haemostasis, wound repair
2. Pathogen recognition
3. Histamine, leukotrienes, chemokines
4. Complement
5. Leukocyte recruitment
6. Phagocytosis, clearance

Endogenous Alarmsignals:
- HMGB-1
- S100-proteins
- Heat shock proteins
- Self - Nucleic Acids
RNase1-mediated Hydrolysis of RNA: Generation of Vaso- and Tissue-protective Products?

RNase1

RNA → ss-Oligonucleotides + 3`Nucleotides

5`Nucleotides

Nucleotidases (CD73) → Nucleosides (e.g. Adenosine)

Medical RNA-omics
<table>
<thead>
<tr>
<th>Disease/Condition</th>
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<tr>
<td>Atherosclerosis</td>
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<td>Acute inflammation</td>
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<tr>
<td>Ischemia/reperfusion injury</td>
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<tr>
<td>Stroke, edema formation</td>
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<tr>
<td>Tumor development</td>
</tr>
<tr>
<td>Transplantation (?)</td>
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</tbody>
</table>
Damaging Nature of Extracellular RNA and Induction of Endogenous Inflammatory Pathways

- Atherosclerosis
- Acute inflammation
- Ischemia/reperfusion injury

- Stroke, edema formation
- Tumor development
- Transplantation (?)

Development of atherosclerotic lesions (LDLR-/- mice) in an eRNA-dependent manner

Simsekylmaz et al., Circulation 2014
RNase1 Treatment of Atherosclerosis-prone Apo-E⁻/⁻ Mice

Atherosclerosis-prone apo-E⁻/⁻ mice: Wire-induced vessel injury
(Alma Zernecke, Aachen/Würzburg)

Simsekylmaz et al., Circulation 2014
Mast Cell Degranulation, eRNA Release and Cytokine Storm

- Mast cell degranulation
- Cytokine release

- Mechanisms
- Signal Transduction

Monocyte / macrophage

- Cytokine expression
- Cytokine release

Ribosomal RNA

Dvorak et al., Histol. Histopathol. 2003
Extracellular RNA Drives Macrophages Towards M1-Polarization

Bone marrow-derived macrophages:
Pretreatment with M-CSF > M2

Cabrera-Fuentes et al., T&H 2015
Intracellular Processing of Immature TACE / ADAM17 by Furin-mediated Removal of Auto-inhibitory Prodomain

Wong et al., JBC 2015 (modified)
Extracellular RNA-induced TNF-α Release: Contribution of the Sheddase TACE / ADAM-17

A

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

Fischer et al., T&H 2012
In vivo Release of eRNA Following Ischemia-Reperfusion in the Mouse Heart

Cabrera-Fuentes et al., T&H 2014

> eRNA is mainly derived from cardiomyocytes <
Ischemia/Reperfusion Injury in Isolated Rat Hearts: RNase1 Prevents Tissue Damage and Reduces Infarct Size

LDH release (UI/g tissue) vs. Time (min)

- I/R
- 0.1 RNase1 ( fileSize/ml)
- 1.0 RNase1 ( fileSize/ml)
- 10 RNase Inhibitor

Cumulative LDH release (UI/g tissue/120 min)

- Control RNase1 RI - I / R

Infarct size (% of total area)

- Control
- RNase1
- RI

*** ns

 RNase1 (킽/ml) I/R 0.1 1.0 10

Ischemia
Reperfusion

Reperfusion
LDH and TNF-α Release in Isolated Rat Hearts Submitted to I/R: Influence of TACE/ADAM17-Inhibition by TAPI

A. LDH release

![Graph showing LDH release over time with and without TAPI administration during ischemia and reperfusion.](image)

**Administration of TAPI**
Distribution and Cellular Release of Extracellular RNA

Stable, non-activated cells / tissue

Injury, Stimulation
Vascular Diseases

Apoptotic, damaged cells / tissue

Extracellular RNA (eRNA)

eRNA liberation from tumor cells (hypoxia)

![Graph showing eRNA liberation from tumor cells under normoxia and hypoxia](image)

- **Normoxia**
- **Hypoxia**

<table>
<thead>
<tr>
<th>eRNA (fold)</th>
<th>HT29</th>
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<th>U-87</th>
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<tbody>
<tr>
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<tr>
<td>Hypoxia</td>
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![Images of extracellular RNA in different tissues](image)

- **Glioblastoma**
- **White matter**
- **Thrombosis**

Extracellular RNA
The Endogenous Inflammatory Cascade

Cellular source

Alarmins, ligands

Receptors

Inflammatory mediators

INFLAMMATION

Chan et al., JCI 2012 (modified)
Extracellular RNA-mediated Inflammatory Cascade Involving TACE/ADAM17

TACE / ADAM-17 cleaves more than 70 substrates. Examples are:

- Pro-TNF-α
- Pro-EGF
- NOTCH
- ICAM-1
- VEGF-R2
- GPIbα
- IL6-R
- L-selectin
- IGF2-R
- L-selectin
- ErB-ligand
- CD44
Extracellular RNA-mediated Cytokine Production in Bone Marrow-derived Macrophages (BMDM)

Simsekylmaz et al., Circulation 2014
Mechanism of TNF-α Shedding: The Role of TACE / ADAM-17

- TAPI, GM 6001
- TACE or ADAM-17
- Active TNF-α
- TNF-α receptor (1, 2)
- Membrane-bound Pro-TNF-α
- Intracellular signal transduction
- TACE / ADAM-17: TNF-α Converting Enzyme
- TAPI, GM 6001: TACE-inhibitors

Monocyte, Macrophage

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

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