Micro RNAs regulation of atherosclerosis

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Discovery of small RNAs

- The first small RNA:
- In 1993 Rosalind Lee (Victor Ambros lab) was studying a non-coding gene in C. elegans, lin-4, that was involved in silencing of another gene, lin-14, at the appropriate time in the development of the worm C. elegans.

Rosalind Lee
# RNA types & functions

<table>
<thead>
<tr>
<th>Types of RNAs</th>
<th>Primary Function(s)</th>
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</thead>
<tbody>
<tr>
<td>mRNA - messenger</td>
<td>translation (protein synthesis) regulatory</td>
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<tr>
<td>rRNA - ribosomal</td>
<td>translation (protein synthesis) &lt;catalytic&gt;</td>
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<tr>
<td>t-RNA - transfer</td>
<td>translation (protein synthesis)</td>
</tr>
<tr>
<td>hnRNA - heterogeneous nuclear</td>
<td>precursors &amp; intermediates of mature mRNAs &amp; other RNAs</td>
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<tr>
<td>scRNA - small cytoplasmic</td>
<td>signal recognition particle (SRP) tRNA processing</td>
</tr>
<tr>
<td>snRNA - small nuclear</td>
<td>mRNA processing, poly A addition &lt;catalytic&gt;</td>
</tr>
<tr>
<td>snoRNA - small nucleolar</td>
<td>rRNA processing/maturation/methylation</td>
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<td><strong>regulatory RNAs</strong> (siRNA, miRNA, etc.)</td>
<td>regulation of transcription and translation, other??</td>
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- **Two small non-coding RNA:**
  1. Micro RNA (miRNA)
  2. Short interfering RNA (siRNA)
Micro RNA transcription and maturation

PATHOGENESIS OF ATHEROSCLEROSIS
LDL synthesize by De Novo
(A) In normal cells, ABC1 helps cholesterol exit the cell where it combines with lipid-poor Apo-A1 lipoprotein to form high density lipoprotein (HDL). HDL picks up more lipids from low-density (LD) and very low density (VLDL) lipoproteins and transfers the cholesterol to the liver, where it is processed.

(B) In Tangier disease, mutations in ABC1 cause cholesterol to accumulate within the cell.

[Adapted from Young and Fielding (1999) Nat Genet., Aug;22(4):316-8, with permission]

Response to Injury

1. Chronic endothelial "injury":
   - Hyperlipidemia
   - Hypertension
   - Smoking
   - Homocysteine
   - Hemodynamic factors
   - Toxins
   - Viruses
   - Immune reactions.
2. Endothelial dysfunction (e.g., increased permeability, leukocyte adhesion)
   Monocyte adhesion and emigration.

Fatty Streak

4. Macrophages and smooth muscle cells engulf lipid

Fibro-fatty Atheroma

5. Smooth muscle proliferation, collagen and other ECM deposition, extracellular lipid

Fibrofatty atheroma

Lymphocyte

Collagen

Lipid debris
Role of Micro RNAs

1. Regulation of lipoprotein Homeostasis.

2. Regulation of Endothelial Cell inflammation & plaque progression.
   - A - Cytokine-responsive MiRNAs.
   - B - Mechano-sensitive EC MiRNAs.
   - C - Leukocyte Migration & activation.

3. Implication in Vascular Smooth Muscle Function

I-Lipoprotein regulation

- **MiR-223** represses gens in chol. Biosynthesis HMGS1 & HDL uptake SR-B1 \(\uparrow\) HDL-C
- **MiR-30c** potent target LPGAT1 \(\downarrow\) apo-B lipoprotein & LDL-C
- **MiR148a,128,130b** target in LDL receptor helping LDL clearance
- **MiR148a,128,33,758,26,106,144** target ABCA1(HDL transporter) helping HDL biogenesis & removal of cholesterol.
Micro RNA 33

Several studies of MiR 33 inhibition or deletion for 4 weeks have been conducted in mouse models of atherosclerosis. Moreover, **antisense inhibition of miR-33 resulted in a regression of the atherosclerotic plaque volume in LDL-receptor-deficient mice.** Antisense inhibition of miRNA function is an important tool for elucidating miRNA biology and evaluating its therapeutic potential.

![miR-33 deficiency increased HDL-C.](image)

Takahiro Horie et al. J Am Heart Assoc 2012;1:e003376
miR-33 deficiency reduced lipid accumulation and macrophage content in atherosclerotic plaque.

Takahiro Horie et al. J Am Heart Assoc 2012;1:e003376

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miR-33 deficiency reduced atherosclerosis.

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MicroRNA (miRNA) orchestration of cholesterol homeostasis and macrophage activation in atherosclerosis.


II-Regulation of Endothelial Cell inflammation & plaque progression.
A-Cytokine-responsive Endothelial Cell MiRNAs

**MiR-181b** inhibits: NF signaling in EC only & decrease its activation leading to marked suppression of leucocyte recruitment & atherosclerosis. So it can be used as anti-inflammatory replacement therapy

**MiR-146a** inhibits:
- NF signaling in both EC & macrophages.
- TNF & IL-1b in EC.
- MAPK signaling pathway (inhibitor effect in EC- NO synthase).

B- Mechano-sensitive EC Micro RNAs

- Atherosclerotic lesions develop at arterial branch points, bifurcations, and the lesser curvature of the aorta due to:
  - Presence of disturbed flow (d-flow) in these region, which increases endothelial permeability.
  - Pro-inflammatory activation as (NF & adhesion molecule) thereby enhance leucocytes accumulation and lesion formation.

- In contrast laminar flow (l-flow) has anti-inflammatory, anti-adhesive, and anti-thrombotic properties.

- Large number of MiRNAs have been identified as shear stress responsive by either d-flow (MiR 92,712,126) or l-flow (MiR 143/145,MiR 155).
**MiR-92a** expression is: targets on the transcription factors Kruppel-like factor 2 (KLF2) and 4 (KLF4) & suppressor of cytokine signalling (SOCS5) expression, an effect that decreases anti-inflammatory pathways and increases monocyte chemo attractant protein (MCP)-1 and interleukin (IL)-6 that further activate EC.

Deletion of it protect from neointimal formation

**MiR-712:** suppresses tissue inhibitor of metalloproteinase-3 (TIMP3), thereby increasing the expression of matrix metalloproteinases (MMPs).

Its neutralization may decrease atherosclerosis progression

**MiR 126-5p:** d flow reduce its expression, thereby de-repressing its target gene delta-like1 (Dlk-1), a negative regulator of endothelial proliferation.

**MiR143/145:** extracellular release are atheroprotective properties in VSMCS & IV delivery block atherosclerotic lesion progression

**MiR155:** reduce atherosclerotic lesion formation by targeting MAP3K10 & improving endothelial barrier dysfunction and monocyte migration.
Endothelial microRNAs (miRNAs) regulate vascular inflammation.

C- MicroRNAs regulation of Leucocyte recruitment

1- Macrophage chol. Homeostasis is maintained by the balance between Chol. uptake, endogenous synthesis, esterification/hydrolysis, and efflux

- **MiR 27a**: targeting on genes involved in chol. Esterification (LACT), uptake (LDL), and efflux (ABCA1)

- **MiR 125a**: decrease lipid uptake & cytokines release in oxidized LDL-stimulated macrophages.

- **MiR 26-33-106-128**: targeting of ABCA1, so it reduce Chol. Efflux and help foam cell formation.
2- Macrophages activation programs

M1 phenotype
Increase atherosclerotic progression

M2 phenotype
Associated with anti-inflammatory profile

MiR-33,155 sustains the inflammatory M-1 like macrophage phenotype, so its inhibition reprograms macrophages to the M-2 phenotype which promotes accumulation of athero-protective T-cell

MiR-223: suppress M1 (pro-inflammatory pathway & enhance alternative activation
MiR-27a: promote markers of M2 macrophages
MicroRNA (miRNA) orchestration of cholesterol homeostasis and macrophage activation in atherosclerosis.


### III Implication in Vascular Smooth Muscle Function

- **Contractile phenotype**
  - Maintains the vascular wall function

- **Synthetic phenotype**
  - Promote migration, proliferation, and inflammation

Switch to

Vascular injury
**MiR143/145**: maintains contractile functions
- Its deficiency reduces vessel wall medial thickness and gene ACE
- Delivery of it reduces atherosclerotic plaque size and necrotic core area and increases fibrous cap

**MiR-221/222**: increased in response to injury.
- Its deficiency reduces VSMC proliferation and neointimal lesion formation after mechanical injury

**MiR 21**: enhances VSMC proliferation in response to mechanical injury.
- Its neutralization reduces neointimal proliferation after balloon injury.

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**MicroRNA (MicroRNA) regulation of vascular smooth muscle cell phenotype.**

CONCLUSIONS

* Atherosclerosis is a multifactorial disease driven in part by chronic inflammation in response to cholesterol accumulation.

* Micro RNAs is a class of short (22 nucleotides) non-coding RNA that serve as important regulators of pathophysiological cellular and molecular pathways involved in atherosclerosis.

* We provide an update on the potential use of MiRNAs diagnostically for detecting increasing severity of CAD.

* Finally, we provide a perspective on therapeutic opportunities and challenges for MiRNAs delivery in the field.