The mTOR-LARP1 axis and the anabolic reservoir of tumor cells: A new therapeutic target in colorectal cancer and beyond

Antonio Gentilella
Laboratory of Cancer Metabolism
Bellvitge Biomedical Research Institute
BARCELONA
Gene Expression

- Motor neuron
- Red blood cell
- White blood cell
- Bone cell
- Cells in the inner lining of the intestine
- Ovum
- Sperm cell
The Mission of Biomedicine

To a Sailor With No Direction

No Wind is Favorable

(Seneca)
Proliferation, But Not Growth, Blocked by Conditional Deletion of 40S Ribosomal Protein S6


Insulin Activation of Rheb, a Mediator of mTOR/S6K/4E-BP Signaling, Is Inhibited by TSC1 and 2

Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity

Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpl11-translation-dependent mechanism of p53 induction

Suprainduction of p53 by disruption of 40S and 60S ribosome biogenesis leads to the activation of a novel G2/M checkpoint
The Ribosome
Protein Synthesis

- Macrolides
- Tetracyclins
- Aminoglicosides
- Chloramphenicol
Ribosome Biogenesis

Actinomycin D
5-FU
Rapalogs
Dual PI3K-mTOR inhibitors
Ribosomal Proteins and mTOR

mTOR

LARP1

TOP motif

Ribosomal Protein mRNA

RPs pool

80S
40S-LARP1-5’TOPs complex

Stable pool of 5’TOP mRNAs

Gentilella et al., Mol Cell 2017
Polysome Profiling
not translated
translated
RP mRNA (5' TOP)

Northern blot of mRNA of interest

Polysome Profiling
40S-LARP1-5’TOPs complex

OXPHOS metabolism mRNAs

<table>
<thead>
<tr>
<th>Complex V</th>
<th>Complex IV</th>
<th>Complex I</th>
<th>Complex III (bc1)</th>
<th>Other Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP5I</td>
<td>COX6B1</td>
<td>NDUFB11</td>
<td>UQCRH</td>
<td>CYC1</td>
</tr>
<tr>
<td>ATP5B</td>
<td>COX8A</td>
<td>NDUFS4</td>
<td>UQCRQ</td>
<td>SDHB</td>
</tr>
<tr>
<td>ATP5D</td>
<td>COX7C</td>
<td>NDUFA4</td>
<td>UQCRB</td>
<td>TOMM7</td>
</tr>
<tr>
<td>ATP5G2</td>
<td>COX4I1</td>
<td>NDUFA3</td>
<td></td>
<td>TOMM22</td>
</tr>
<tr>
<td>ATP5L</td>
<td>COX5A</td>
<td>NDUFB9</td>
<td></td>
<td>TOMM20</td>
</tr>
<tr>
<td>ATP5E</td>
<td>COX5B</td>
<td>NDUFS5</td>
<td></td>
<td>TIMM8B</td>
</tr>
<tr>
<td>ATP5A1</td>
<td>COX6A1</td>
<td>NDUFS3</td>
<td></td>
<td>TIMM10</td>
</tr>
<tr>
<td>ATP5O</td>
<td>COX7A2</td>
<td>NDUFB4</td>
<td></td>
<td>TIMM13</td>
</tr>
<tr>
<td>ATP5J2</td>
<td>COX6C</td>
<td>NDUFS6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP5F1</td>
<td>COX7A2L</td>
<td>NDUFA1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
40S-LARP1-5’TOPs complex upon mTOR inhibition

- low AA
- low nutrients
- mTOR inhib.
- low O₂
- low ATP/AMP

![Graph showing L5 mRNA and β-Actin mRNA per cell](image)
Translatome Protected by LARP1

Ribosome Biogenesis and Protein synthesis

Fuentes et al., Science Adv. 2021
Working Model

stable pool of 5'TOP mRNAs
Working Model

stable pool of 5’TOP mRNAs
Working Model

mTOR inactive

stable pool of 5'TOP mRNAs
stable pool of 5’TOP mRNAs
Growing cells $\rightarrow$ 48h TORi $\rightarrow$ TORi Washout (70 min)

Utilizing the Protected Translatome

Fuentes et al., Science Adv. 2021
The 40S-LARP1 complex reprograms the cellular translatome upon mTOR inhibition to preserve the protein synthetic capacity.
40S-LARP1 complex in cancer

**Tumor microenvironment**

- low AA
- low nutrients
- mTOR inhib.
- low O₂
- low ATP/AMP

**Chemotherapeutic Regimens**

**CRC patients**

**Metabolic Resistance to therapy?**

- LARP1
- 40S
- 5'TOP

Anabolic reservoir ready-to-use
LARP1 and energetic production

OXPHOS metabolism mRNAs

<table>
<thead>
<tr>
<th>Complex V</th>
<th>Complex IV</th>
<th>Complex I</th>
<th>Complex III (bc1)</th>
<th>Other Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP5I</td>
<td>COX6B1</td>
<td>NDUFB11</td>
<td>UQCRH</td>
<td>CYC1</td>
</tr>
<tr>
<td>ATP5B</td>
<td>COX8A</td>
<td>NDUFS4</td>
<td>UQCRQ</td>
<td>SDHB</td>
</tr>
<tr>
<td>ATP5D</td>
<td>COX7C</td>
<td>NDUFA4</td>
<td>UQCRB</td>
<td>TOMM7</td>
</tr>
<tr>
<td>ATP5G2</td>
<td>COX4I1</td>
<td>NDUFA3</td>
<td></td>
<td>TOMM22</td>
</tr>
<tr>
<td>ATP5L</td>
<td>COX5A</td>
<td>NDUFB9</td>
<td></td>
<td>TOMM20</td>
</tr>
<tr>
<td>ATP5E</td>
<td>COX5B</td>
<td>NDUFS5</td>
<td></td>
<td>TIMM8B</td>
</tr>
<tr>
<td>ATP5A1</td>
<td>COX6A1</td>
<td>NDUFS3</td>
<td></td>
<td>TIMM10</td>
</tr>
<tr>
<td>ATP5O</td>
<td>COX7A2</td>
<td>NDUFB4</td>
<td></td>
<td>TIMM13</td>
</tr>
<tr>
<td>ATP5J2</td>
<td>COX6C</td>
<td>NDUFS6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP5F1</td>
<td>COX7A2L</td>
<td>NDUFA1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What defines the 40S-LARP1 ribosomes?

1. Growing
2. TORi
3. SS
4. Growing

LC / MS-MS
Are 40S-LARP1 ribosomes different in make up than 40S?
c-MYC and Ribosome Biogenesis

MYC

Pol I  Pol II  Pol III

rRNA  RPs  tRNAs

Ribosome Biogenesis

Protein Synthesis

Anabolic Power
Hyperactivation of Ribosome Biogenesis in CRC (CMS2-3)

Wnt \[\rightarrow\] MYC

\[\begin{align*}
\text{Pol I} & \quad \text{Pol II} & \quad \text{Pol III} \\
\text{rRNA} & \quad \text{RPs} & \quad \text{tRNAs}
\end{align*}\]

Ribosome Biogenesis

Protein Synthesis

Anabolic Power

p53 stabilization

IRBC Complex

Morcelle et al., Cancer Res. 2019
To a Sailor With No Direction

No Wind is Favorable

(Seneca)