“Genome-Wide CRISPR interference Screen Identifies RNA Regulator of Lipogenesis (RROL) as a Leading LncRNA Dependency in Multiple Myeloma”

18th International Myeloma Workshop
Eugenio Morelli, M.D.
Munshi Lab

LncRNAs: the Dark that Matters
Disclosures

- I do not have conflicts of interest.
LncRNAs & Myeloma: a new source of genetic vulnerabilities?

**Rationale of the study**

LncRNA are dysregulated in MM

- Up (n=474)
- Dn (n=395)

LncRNA signatures can predict the clinical outcome of MM patients

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To explore LncRNA Dependency in Multiple Myeloma

**Systematic mapping of LncRNA dependencies**

**Molecular mechanisms & Regulatory functions**

**Therapeutic targeting**
CRISPR interference (CRISPRi) viability screen identifies MIR17HG as a leading lncRNA dependency in MM.

**Screening Approach**

1. RNA-seq analysis
2. CRISPRi Primary screening
3. CRISPRi Validation screening
4. Multiple Myeloma IncRNA dependencies

**Ranked Analysis**

- IRF4
- MIR17HG
- MYC

To Characterize MIR17HG Dependency in MM
RNA Regulator of Lipogenesis (RROL) mediates MM dependency to MIR17HG

1. RROL depletion + Overexpression of pri-mir-17-92 = NO RESCUE

2. RROL depletion + Knockout of DROSHA = NO RESCUE

Lack of correlation
RROL promotes MYC transcriptional activity via direct RNA-protein interaction

MIR17HG cooperates with MYC in tumorigenesis... via RROL?
RROL provides chromatin scaffold for MYC occupancy at the ACC1 promoter

FOCUS on ACC1

Top Target of RROL

Top Target of MYC

Vulnerability in MM

RROL Top Targets
(30)

RROL-MYC Coregulated Targets
(22)

AMO1
H929

IL23M
ITGBB
MIR1206
IFNG
PJA2
IFNO
SCF2D2
CEP170P1
CEP170P2
ANO6
KIAA1109
EXT1
EPT1
ZYG11A
CDC91
SNORA23r
ZBTB20
SNORA24r
FER
CPN5
ACC1

LogFC (ASO1/NC)

H929

RN65C-1
SNORD38c
MIR1206
MIR528t
MIR546A
MIR548A2
MIR548H4
SLC38A2
SNORD30d
CTB-43P14.1
SNORD116-f
MIR548Q2
MIR546F3
MIR546S
MIR548-A1
MIR546A3

ChIP-seq

Signal

ACC1

Rank

Tumor volume (mm$^3$)

shACC1-3:
Uninduced
Induced

Days after induction

*
RROL provides chromatin scaffold for MYC occupancy at the ACC1 promoter

FOCUS on ACC1

Chromatin Isolation by RNA Purification (ChIRP)

RNA FISH / IF

MYC ChIP-qPCR w/o RROL depletion

RROL Top Targets
(30)

RROL-MYC Coregulated Targets
(22)
RROL assembles a MYC-WDR82 complex at the ACC1 promoter.

Proximity-dependent biotin identification (BioID) w/o RROL depletion

MYC Co-IP/MS w/o RROL depletion

WDR82 promotes MYC occupancy at the ACC1 promoter

MYC ChIP-qPCR w/o WDR82 depletion

WDR82 ChIP-qPCR w/o RROL depletion

WDR82 occupy the ACC1 promoter in RROL-dependent manner

RNA Yeast-3-Hybrid

H3K4me3

ACC1

Max

Myc

H3K4me3

Hybrid Protein 2: WDR82

Hybrid RNA

Hybrid Protein 2: WDR82

Hybrid RNA
The RROL-ACC1 transcriptional axis promotes de novo lipogenesis in MM cells.

(RNA Regulator of Lipogenesis (RROL))

Proposed model of RROL Dependency in MM
RROL is Therapeutically Actionable using Lipid-conjugated ASOs

Development of Therapeutic ASOs

- Multistep ASO screen (>70 molecules)
- Derived 2 leading candidates
  - Steric-Blocker (SB)
  - Degrader or gapmeR (G)
- Lipid-conjugation for improved PK

Subcutaneous MM xenograft

- Tumor growth
- qRT-PCR

BLI-based MM xenograft

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<th>NC1</th>
<th>NC2</th>
<th>G</th>
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<td>RROL</td>
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<td>ACC1</td>
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NC: NC1, G: G, SB: SB

**Statistical significance:**
- **: p < 0.05
- ***: p < 0.001
- ns: not significant
• LncRNA are a relevant source of genetic vulnerabilities to be molecularly, functionally and therapeutically explored.
• RROL is a leading LncRNA dependency in MM.
• RROL is susceptible to therapeutic intervention.
Munshi Lab

N. Munshi
M. Fulciniti
M. Kemal Samur
A. Aktas Samur
S. Talluri
M. Shammas
All lab members

Anderson Lab

K. Anderson
A. Gullà
Y. Tai
T. Hideshima
D. Chauhan
G. Bianchi
M. Johnstone
All lab members

Loda Lab

M. Loda
C. Ribeiro

Young Lab

R. Young
J. Henninger

Lin Lab

C. Lin
W.D. Park

Tassone Lab

P. Tassone
N. Amodio
C. Federico
F. Scionti

Penn Lab

L. Penn

Novina Lab

C. Novina
L. Wert Lamas

Neri Lab

A. Neri
K. Todoerti

S. Gryaznov

All our patients!!!