Exosome-mediated transfer of tumor suppressor microRNA15a supports multiple myeloma pathogenesis

Aldo M Roccaro, MD, PhD

Dana-Farber Cancer Institute
Medical Oncology
Harvard Medical School, Boston, MA, USA

2012 ISSNAF ANNUAL MEETING

October 25th, 2012
Italian Embassy
Washington, DC
DISCOLOSURE FORM

ALDO M. ROCCARO

NOTHING TO DISCLOSE
Myeloma (MM) is a plasma cell dyscrasia, the second most prevalent hematological malignancy with a median survival of 3-5 years.

MM is characterized by widespread disease at diagnosis with the presence of multiple lytic lesions and disseminated involvement of the bone marrow (BM).

Implying that the progression of MM involves a continuous re-circulation of the MM cells in the peripheral blood and re-entrance into the BM.
Cell trafficking is a hallmark of Multiple Myeloma

Multiple lytic lesions in patients with Multiple Myeloma
Cell trafficking is a hallmark of Multiple Myeloma

✓ *In vivo flow cytometry*

✓ *In vivo confocal microscopy*
In vivo flow cytometry
A laser beam is focused to a ~5µm x 50µm slit (green rectangle).

Labeled cells in the circulation (black circles) are excited and pass through the slit, giving off a burst of fluorescence (yellow circle) for each cell.
Cell trafficking is a hallmark of Multiple Myeloma

In vivo flow cytometry
Evaluation of MM tumor dissemination \textit{in vivo}

Evans Blue i.v.

\textit{In vivo} confocal imaging
Visualization of MM cell homing to the BM niches

Bone marrow microenvironment plays a key role in the pathogenesis and progression of Multiple Myeloma

Bone marrow microenvironment

- MM cells
- Bone marrow Stromal Cells
  - IL-6
  - TNFα
  - IL-1β

Interactions:
- IL-6
- TNFα
- IL-1β
- VEGF
- bFGF

Endothelial cells

Bone marrow angiogenesis

References:
- Hideshima et al. 2000; Blood
- Vacca et al. 2003; Blood
- Roccaro et al. 2006; Cancer Res
- Mitsiades et al. 2002; Blood
- Lentzsch et al. 2002; Cancer Res
- LeBlanc R et al. 2004; Blood
- Hayashi T et al. 2005; Brit J Hematol
Does BM-MSCs transfer genetic material to the tumor clone?

We used MM as a model disease to examine whether the bone marrow microenvironment may induce genetic changes in the tumor clone leading to tumor progression and dissemination.
EXOSOMES

- nanometer-sized vesicles of endocytic origin
- released in the extracellular milieu by several cell types
- physiological and pathological conditions
- Bind to cells (receptor/ligand interaction)
- Attach/fuse with the recipient cells
- Deliver their content to the recipient cells
- antigen presentation
- transmission of infectious agents (i.e.: viruses, prions...)

↓
EXOSOMES

II

✓ TUMORS

modulate and mold the host microenvironment

promote tumor cell growth

disease progression
HYPOTHESIS

Bone marrow mesenchymal stem cell-derived exosomes

Mediate interaction between the bone marrow milieu and the clonal plasma cells

Oncogenesis in multiple myeloma
Bone marrow mesenchymal stem cell-derived exosomes: characterization

Exosomes are transferred to MM cells

<table>
<thead>
<tr>
<th>DAPI</th>
<th>FITC-tubulin</th>
<th>PKH67-Exosomes</th>
<th>Merge</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Fluorescence 490/520 (fold of control)

- BMSCs-CONDITIONED MEDIUM-DERIVED EXOSOMES

- **P 0.01**
- **P 0.0002**
Exosomes modulate MM cells behaviour in vitro

**Cell proliferation**

**Cell adhesion**

- DNA synthesis (% of control)
  - MM1s
  - Active MM BMSCs
  - MGUS BMSCs
  - S-MM BMSCs
  - Normal BMSCs

- Adherent cells (% of control)
  - BSA
  - Multiple Myeloma BMSCs
  - Normal BMSCs
  - HS5
  - Poly-D-Lys

- Fibronectin
  - -
  - +

- Conditioned medium-derived exosomes
- #1, #2, #3, #4
- #1, #2, #3, #4
- #1, #2, #3, #4
Exosomal miRNA content differs between normal and MM BM-MSCs
microRNAs

- class of small, non-coding RNAs
- negative regulators of gene-expression
- first transcribed by RNA Polymerase II into large precursor RNAs (pri-miRNAs)
- subsequently processed by the RNase III-type enzyme Drosha to generate precursor miRNAs (pre-miRNAs) in the nucleus
- exported to the cytoplasm by exportin-5, where they are subjected to secondary processing by the RNase III-type enzyme Dicer
- short double-stranded RNA duplex which in turn is incorporated into a ribonucleoprotein complex (miRISC) where the mature miRNA strand is retained, which is now capable of regulating its target genes
microRNAs

By silencing targeted mRNAs

development, cell differentiation, apoptosis, cell proliferation
play roles in tumor development
both in solid and hematologic malignancies

MM cells

miRNA-15a

In vitro

In vivo studies

miRNA-15a: tumor suppressor

Roccaro et al. Blood, 2009;113:6669-6680
Pichiorri et al. PNAS;2008;105:12885-12890
Lionetti et al. Blood 114:e20-6
microRNA-15a acts as tumor-suppressors in multiple myeloma

<table>
<thead>
<tr>
<th>SFM</th>
<th>VEGF</th>
<th>CM harvested from transfected MM.1S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Scramble probe</td>
</tr>
<tr>
<td>*8±2</td>
<td>*25±3</td>
<td>*26±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-miRNA-15a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*12±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-miRNA-16-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*11±3</td>
</tr>
</tbody>
</table>

Roccaro et al. Blood, 2009;113:6669-6680
miRNA-15a: tumor suppressor

In vitro studies

In vivo

lack of trasfer of miRNA-15a from the MM BM-MSCs to the tumor clone, through exosomes

Roccaro et al. Blood, 2009;113:6669-6680
Pichiorri et al. PNAS;2008;105:12885-12890
Lionetti et al. Blood 114:e20-6
Exosomes modulate MM tumor growth and dissemination \textit{in vivo}

\textbf{Tissue- Engineered Bones}

- MM.1S GFP/+Luc$^+$ + MM BM-MSC-derived exosomes
- MM.1S GFP/+Luc$^+$ + Normal BM-MSC-derived exosomes
- MM.1S GFP/+Luc$^+$

\textbf{Tumor Growth B.L.I. (d0, +7d, +10d, +14d)}
Exosomes modulate MM tumor growth in vivo
Evaluation of MM tumor dissemination *in vivo*

Evans Blue i.v.

Parasagittal sinusoids

Central vein (cv)

Coronal vein

Lateral regions

*In vivo* confocal imaging
Exosomes modulate MM tumor dissemination *in vivo* (+ w7 post-implant)

MM cells + MM BM-MSC-derived exosomes

MM cells

MM cells + normal BM-MSC-derived exosomes
Detection of MM cells \textit{ex vivo}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Comparison of MM.1S GFP+ cells (cell count within femur) under different conditions.}
\end{figure}
SUMMARY

✓ BM-MSCs release exosomes

✓ Exosomes are transferred to MM cells

✓ Normal and MM BM-MSC-derived exosomes present with a differential biological impact on MM cells biology in vitro and in vivo

✓ Partially due to the differential miRNA content characterizes normal vs MM BM-MSC-derived exosomes (i.e.: miRNA-15a acting as tumor suppressor)

✓ The bone marrow microenvironment does not only play a supportive role in the growth of tumor cells, but also acts as a conduit of epigenetic information leading to changes in the behavior of the tumor clone
Acknowledgement

Dana-Farber Cancer Institute
Boston, MA, USA
I.M. Ghobrial

Irene Ghobrial’s Lab Members
A. Sacco
F. Azab
A.K. Azab
P. Maiso
Y. Liu
Y. Zhang
L. Flores
M. Reagan
M. Moschetta
Yuji Mishima
S. Glavey

FUNDING
NIH/NCI
Accademia Nazionale dei Lincei
Claudia Adams Barr Award
The Doctor’s Cancer Foundation Award