Bone marrow adipocytes induce metabolic reprogramming of multiple myeloma cells

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Cristina Panaroni: No competing financial interests
Obesity, adiposity are positively correlated with myeloma progression

• Myeloma is highly dependent on bone marrow (BM) tumor microenvironment (TME) which changes with age and eventually comprises >70% of volume with BM adipocytes by the median MM patient’s age of 65 years

• Obesity and lipid disorders, like Gaucher’s, are associated with an increased risk of MM development (Birmann et al., 2007; Landgren et al., 2010; Mistry et al., 2013; Chang et al., 2017)

• Obesity-induced deregulation of lipids and diet-induced obesity were found to promote a myeloma-like syndrome in mice (Lwin et al., 2015)

• Metabolomic and lipidomic profiling showed that complex fatty acids are decreased in the BM plasma samples from MM compared to MGUS patients (Gonsalve et al., 2020)
Cancer associated adipocytes support tumor cells through multiple mechanisms

Bone marrow adipocytes support MM expansion by providing FFA for their metabolic reprogramming
Adipocytes promote MM cell proliferation

A

5TGM1 Cells
Relative Proliferation

<table>
<thead>
<tr>
<th></th>
<th>Alone</th>
<th>MC3T3</th>
<th>OP9</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 5TGM1 MM Cells</td>
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</table>

B

MM.1S Proliferation

<table>
<thead>
<tr>
<th></th>
<th>Alone</th>
<th>HD</th>
<th>MGUS/SMM</th>
<th>NDMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMSC Mature Adipocytes + MM.1S MM Cells</td>
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</tbody>
</table>
MM cells induce lipolysis in adipocytes

**A**

LipidTox Staining

OP9 Alone

OP9 + OPM2

OP9 + 5TGM1

**B**

Without Isoproterenol

With Isoproterenol

Glycerol (ng/µl)

Lipid Droplet Area

OP9 Mature Adipocyte Co-culture

5TGM1 Alone

OP9 Alone

OP9 + 5TGM1

OP9 Alone

OP9 + 5TGM1

*
FA metabolism genes are altered in mature BM adipocytes from MM patients

**A**

**Genes involved in Lipolysis**

<table>
<thead>
<tr>
<th>Genes</th>
<th>HD</th>
<th>MGUS/SMM</th>
<th>NDMM</th>
<th>RRMM</th>
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<tbody>
<tr>
<td>LPL</td>
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<tr>
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<tr>
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<tr>
<td>MGLL</td>
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</table>

**B**

**Genes involved in fatty acid synthesis and desaturation**

<table>
<thead>
<tr>
<th>Genes</th>
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<th>MGUS/SMM</th>
<th>NDMM</th>
<th>RRMM</th>
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</thead>
<tbody>
<tr>
<td>ACSL1</td>
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<td>ACSL4</td>
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<td>FASN</td>
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<tr>
<td>SCD1</td>
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<tr>
<td>FASD2</td>
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</tbody>
</table>
MM cells have cellular machinery to uptake FA
FA from adipocytes are directly transferred to MM cells

A

5TGM1

OPM2

BODIPY-C12 MFI

Unstained

Bodipy-C12 1 min

Bodipy-C12 30 min

Bodipy-C12 10 min

Bodipy-C12 60 min

B

LipidTox staining in MM cells

5TGM1

OPM2

Unstained

MM Cells Stained with LipidTox

MM Cells Co-cultured with OP9 Adipocytes Stained with LipidTox
MM cells uptake FAs through Fatty Acid Transporter Proteins (FATPs)

### (A) Log2 median-centered intensity in 21 multiple myeloma cell lines

- **FATP1**
- **FATP2**
- **FATP3**
- **FATP4**
- **FATP5**
- **FATP6**

### (B) CD138+ MM cells

- **MM pt1**
  - 48.7% 34.9%
  - 16.1% 0.39%
- **MM pt2**
  - 7.90% 57.3%
  - 16.3% 18.4%

### CD138neg B-cells

- **MM pt1**
  - 23.4% 40.3%
  - 34.9% 1.38%
- **MM pt2**
  - 7.26% 42.4%
  - 34.9% 15.5%

### T-cells

- **MM pt1**
  - 13.1% 7.29%
  - 78.5% 1.06%
- **MM pt2**
  - 2.77% 1.28%
  - 94.5% 1.49%
Blocking lipolysis in adipocytes or FA-uptake through FATP in MM cells could be promising therapeutics strategies.
Low-dose intake of Arachidonic Acid (AA) increases proliferation of MM cells whereas high-dose uptake decreases viability through ferroptosis-mediated lipotoxicity.

A) 5TGM1 Cells

B) SCID plasmacytoma tumor model

C) Viable Cells (Absorbance) (Relative to DMSO)
Summary

- BMAAds promote proliferation of MM cells
- MM cells induce lipolysis in co-cultured Ad
- FFA released through lipolysis are uptaken by MM cells through FATPs
- Low-dose intake of AA increase proliferation of MM cells whereas high-dose uptake reduces viability in-vitro and in-vivo through ferroptosis mediated lipotoxicity
- Inhibiting lipolysis in adipocytes or inhibiting uptake of FFA by MM cells through blocking of FATPs could be promising therapeutic strategy
Thank you for your attention!
MM cells rapidly uptake FA

A

5TGM1

OPM2

B

% Relative Fatty Acid Uptake

BODIPY-C12 MFI

- Unstained
- Bodipy-C12 1 min
- Bodipy-C12 30 min
- Bodipy-C12 10 min
- Bodipy-C12 60 min

1 min 10 min 30 min 60 min 120 min
MM cells uptake Fatty acids through FATPs

A

Bone Marrow
Arachidonic Acid (ng/ml)

HD MGUS SMM NDMM RRMM

0 500 1000 1500

5TGM1 Cells

Proliferation (fluorescence) (Relative to DMSO)

5TGM1 Cells

Arachidonic Acid (AA)

C

5TGM1 Cells

Viability (% Relative to DMSO)

D

Linoleic Acid (LA)

Viability (% Relative to DMSO)
**MM CELLS UPTAKE FATTY ACIDS THROUGH FATPS**

A. U266, MM.1S, and H929 cells were treated with different fatty acid concentrations (µM) and the percentage of proliferating cells was measured using BODIPY-C11.

B. DMSO and Arachidonic Acid (100uM) were tested for their effect on % proliferating cells.

C. SCID plasmacytoma tumor model was used to study the tumor volume over time.

D. Ki67 IHC was performed on MM + Vehicle, MM + 500 µg/g AA, and MM + PBS.

E. Viability of cells (Absorbance) was compared between DMSO, Ibuprofen, AminBenzo, BW B70C (0.1 µM), BW B70C (10 µM), Baicalein, and Ferrostatin.

F. Lipid ROX Green and GPX4-PE were used to assess the levels of lipid peroxidation and glutathione peroxidase 4 respectively.