Adipose derived stem cells in endometrial cancers

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Advances in Endometrial Cancer Epidemiology and Biology

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Tumor Stroma

Where does it come from?
Bone marrow origin of tumor stroma

$\beta$-gal Tg RAG-1$^{-/-}$, H-2b

XRT
SCID
H-2d

BMT

Pancreas Ca S.C.

40% of the myofibroblasts in the cancer-induced stroma were of BM origin at 4 weeks.

Ishii et al, BBRC, 309:232, 2003
MSC/ASC

Marrow stromal cells/
Adipose stromal cells
Mesenchymal stem cells

Therapeutic
- Immune modulation for hematopoietic transplantation
- Tissue engineering

Definition
- Plastic adherent
- Mesenchymal markers
- Multipotent

Endogenous functions
- Support hematopoiesis
- Wound repair

Characteristics
Cells can be readily, expanded and retransplanted. Migrate to sites of injury and tumors

Resident and circulating
- Bone marrow
- Fat
Bone-marrow derived MSC engraft in MDA231 Breast Metastasis

Murine Lungs with MDA231 metastasis

Normal Murine Lungs

Studeny, Marini, et al., Vol. 96, No. 21, November 3, 2004
Adipose is also a source of MSC/ASC which form tumor stroma

Zhang, Kolonin Cancer Research 2009
Local fat tissue
Provide vascular and fibrovascular support

Tissue remodeling and structure and promote cell motility and metastasis

Bone marrow derived cells recruited via circulation

Kidd Plos 2012
Does obesity increase ASC engraftment?

Tumors in obese mice contain more ASC marker positive cells

Zhang, Kolonin Cancer Research 2012
Hypothesis: visceral adipose tissue provides ASC to intraperitoneal tumors
Visceral adiposity vs. BMI

A

B

VFV vs. BMI

R-Square = 0.314

Observed
Linear
Visceral adiposity and endometrial cancer risk in post-menopausal women

<table>
<thead>
<tr>
<th>Waist to hip ratio</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.74</td>
<td>1.0</td>
</tr>
<tr>
<td>0.74-0.78</td>
<td>1.09</td>
</tr>
<tr>
<td>&gt;0.78-0.83</td>
<td>1.22</td>
</tr>
<tr>
<td>&gt;0.83</td>
<td>1.55</td>
</tr>
<tr>
<td>P-trend</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Friedenreich et al Cancer Causes Control
<table>
<thead>
<tr>
<th>Visceral adipose</th>
<th>Peripheral adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common site of metastasis of intra-abdominal cancers</td>
<td>Rare metastasis</td>
</tr>
<tr>
<td>Increases hyperglycemia, hyperinsulinemia, hyperTG, impaired glucose tolerance</td>
<td>Less metabolic effects on insulin resistance</td>
</tr>
<tr>
<td>Drains through the liver</td>
<td>Venous drainage through systemic veins</td>
</tr>
<tr>
<td>Larger adipocytes (more insulin resistance)</td>
<td>Smaller adipocytes (higher uptake of FFA and TG)</td>
</tr>
<tr>
<td>Well vascularized and innervated</td>
<td>Less well vascularized and innervated</td>
</tr>
<tr>
<td>More inflammatory cell infiltrating</td>
<td>Less inflammatory cell infiltrate</td>
</tr>
<tr>
<td>Higher expression of pro-inflammatory cytokines (TNF-alpha, CRP, IL-6)</td>
<td>Less inflammatory cytokines</td>
</tr>
<tr>
<td>Accumulation supported by lower estrogen</td>
<td>Accumulation promoted by estrogen</td>
</tr>
<tr>
<td>Specimen</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>OSC1</td>
<td>AdenoCA of endometrium and ovary</td>
</tr>
<tr>
<td>OSC2</td>
<td>Granulosa cell tumor of the ovary</td>
</tr>
<tr>
<td>sc-ASC</td>
<td>lipoaspirate</td>
</tr>
<tr>
<td>BM-MSC</td>
<td>Normal bone marrow donors</td>
</tr>
<tr>
<td>Wi38</td>
<td>Fetal lung</td>
</tr>
</tbody>
</table>
Unanswered questions

• Do visceral adipose derived stromal cells engraft and support endometrial cancers?

• Does the tissue source of ASC (visceral vs. subcutaneous) change the ASC phenotype?

• Does obesity support cancer progression through ASC?
  – by altering the phenotype of ASC?
  – or just increasing the availability of ASC?

• Is ASC population abnormal in patients with metastatic cancer?
Mince omentum

Enzymatic digestion (collagenase and dispase) for 1 hour on shaker at 37 degrees

Centrifuge and resuspend in DMEM/10%FBS

Filter through 100μm and 40μm Nylon cell strainer

Centrifuge and resuspend pellet in red cell lysis buffer
Incubate in 37 degree water bath

Count and plate cells
In-vivo effects of OSC on endometrial xenograft formation

HeLa cells
1.6 x 6

Stromal cells
$10^4$ cells (sc)/mouse 3x/week
In-vivo effects of O-ASC on endometrial xenograft formation

![Graph showing tumor volume over weeks post-injection for different treatments: O-ASC2, O-ASC1, BM-MSC, SC-ASC, WI38, PBS.](Klopp Clinical Cancer Research 2012)
O-ASC engraft in endometrial xenografts

Periphery

Center
O-ASC increase tumor vascularity

A
CD31  Ki67  Nuclei

O-ASC1  O-ASC2  BM-MSC
SC-ASC  WI38  PBS

Klopp Clinical Cancer Research 2012
O-ASC increase tumor vascularity
O-ASC increase tumor vessel maturity

Desmin Nuclei

Tumor edge

A

O-ASC1  O-ASC2  BM-MSC

SC-ASC  WI38  PBS
O-ASC increase tumor vessel maturity
**VEGF-A**

- Concentration pg/ml vs Day
- Lines represent different samples: hBM-MSC, hO-ASC 1, hSQ-ASC, WI38

**SDF-1 alpha**

- Concentration pg/ml vs Day
- Lines represent different samples: hBM-MSC, hO-ASC 1, WI38, hSQ-ASC

**FGF-2**

- Concentration pg/ml vs Day
- Lines represent different samples: hBM-MSC, hO-ASC 1, hSQ-ASC, WI38

**HGF**

- Concentration pg/ml vs Day
- Line represents different samples: WI38, hBM-MSC

*Klopp Clinical Cancer Research 2012*
Conclusions

• Omental ASC promote the growth of endometrial xenografts by supporting tumor vasculature.

Questions

• Could differences in O-ASC and sc-ASC be attributed to isolation techniques?
Omental vs. subcutaneous derived ASC: Paired clinical samples

Days

Days after 300uM carboplatin

Fluorescence

Absorbance @ 590nm

hO-ASC S10
hSQ-ASC S10
hO-ASC S15
hSQ-ASC S15
hO-ASC S21
hSQ-ASC S21
hO-ASC S22
hSQ-ASC S22
Conclusion

• O-ASC have similar effects as SC-ASC on endometrial cancer cell proliferation, migration and chemosensitivity in-vitro.

Questions

• Are differences in O-ASC and sc-ASC seen in-vivo?
• Does obesity alter the phenotype of ASC or just increase the availability of ASC?
C57 mice fed high-fat diet develop obesity and metabolic syndrome
ID8 ovarian cancers grow more rapidly in mice with diet-induced obesity
ASC isolation

Lean

Obese

SubQ

Visceral
ASC proliferation

![Graph showing ASC proliferation over days]

- Le-SC-ASC
- Le-V-ASC
- Ob-SC-ASC
- Ob-V-ASC
In-vitro adipocyte differentiation

**Optical Density**

- Lean-SC-ASC
- Ob-SC-ASC
- Lean-V-ASC
- Ob-V-ASC

*adipogenesis* (total OD)
In-vitro osteocyte differentiation

SubQ

Lean

Obese

Visceral

![Images of cellular differentiation](image1)

** Osteogenesis (total OD)

- Lean-SC-ASC
- Ob-SC-ASC
- Lean-V-ASC
- Ob-V-ASC

Optical Density

- Lean-SC-ASC: **
- Ob-SC-ASC: **
- Lean-V-ASC: 
- Ob-V-ASC: 

*Significant difference.*
SubQ

Obese

Lean

P=0.05

Visceral

Lean

Obese

P=0.321

Leans

Obese
Ki67 Vessel Nuclear

**SubQ**

Lean

Obese

**Visceral**

<table>
<thead>
<tr>
<th>groups</th>
<th>percent Ki67+ pixels</th>
<th>percent GSL I isolectin B4+ pixels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le-SC-ASC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ob-SC-ASC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Le-V-ASC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ob-V-ASC</td>
<td></td>
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</table>

**p**

* (*p < 0.05)

** (**p < 0.01)
VisceralSubQ

Lean

Obese

Perilipin Vessel Nuclear

Perilipin (pixel)

**

percent perlipin+ pixels

Le-SC-ASC  Ob-SC-ASC  Le-V-ASC  Ob-V-ASC

groups
Analysis of malignant ascites

**Ascites**

- Le-SC-ASC
- Ob-SC-ASC
- Le-V-ASC
- Ob-V-ASC

**Macrophage in ascites**

- Le-SC-ASC
- Ob-SC-ASC
- Le-V-ASC
- Ob-V-ASC
Lean

Obese

SubQ  F4/80 Vessel Nuclear  Visceral

Lean

Obese

Percent F4/80+ pixels

Le-SC  Ob-SC  Le-V  Ob-V

*  *  ***  *
MSC increase MCF-7 mammosphere formation

Day 5

MCF-7 alone

20% MSC

HMEC

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<thead>
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<th>Protein</th>
<th>MSC</th>
<th>MSC CM+</th>
<th>MSC CM</th>
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<tr>
<td>E-Cadherin</td>
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<td>N-Cadherin</td>
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</tr>
<tr>
<td>Fibronectin</td>
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<td></td>
<td></td>
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<tr>
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<tr>
<td>Actin</td>
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MCF-7

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<th>MSC CM+</th>
<th>MSC CM</th>
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</tr>
<tr>
<td>Actin</td>
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</table>

ASC spheroid formation in ID8-CM

**Visceral**

<table>
<thead>
<tr>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>SubQ</td>
<td></td>
</tr>
<tr>
<td>Visceral</td>
<td></td>
</tr>
</tbody>
</table>

**Numbers**

- Lean-SC-CM
- Obese-SC-CM
- Lean-V-CM
- Obese-V-CM

**Size (um)**

- Lean-SC-ctrl
- Obese-SC-ctrl
- Lean-V-ctrl
- Obese-V-ctrl
ID8 spheroid formation in ASC-CM

SubQ

Visceral

Lean

Obese

![Image of spheroid formation](image)

![Graph showing numbers and size](graph)

*P-value: 0.05
## Characterizing ASC samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Diagnosis</th>
<th>Omentum involved</th>
<th>BMI</th>
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<tbody>
<tr>
<td>OSC1</td>
<td>AdenoCA of endometrium and ovary</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>OSC4</td>
<td>Serous ovarian cancer</td>
<td>+</td>
<td>32.5</td>
</tr>
<tr>
<td>OSC5</td>
<td>Serous ovarian cancer</td>
<td>+</td>
<td>34</td>
</tr>
<tr>
<td>sc-ASC</td>
<td>lipoaspirate</td>
<td>n/a</td>
<td>unknown</td>
</tr>
<tr>
<td>BM-MSC</td>
<td>Normal bone marrow donors</td>
<td>n/a</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Nowicka PLOS 2013
O-ASC and cancer cell migration

Nowicka PLOS 2013
Hec1A spheroid formation

![Graph showing Hec1A spheroid formation](image)
## ASC secretome: Mass spectrometry

<table>
<thead>
<tr>
<th></th>
<th>Present in O-ASC</th>
<th>Present in O-ASC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory cytokines</td>
<td>TGF-B, IL-6</td>
<td></td>
</tr>
<tr>
<td>Extracellular matrix</td>
<td>Fibronectin, TIMP1, thrombospondin1,</td>
<td>Periostin, vitronectin, thrombospondin2, lysyl oxidase, and collagens (1,2,3,5,6)</td>
</tr>
<tr>
<td>modeling</td>
<td>Plasminogen activator inhibitor</td>
<td></td>
</tr>
<tr>
<td>Mesenchymal markers</td>
<td>Vimentin, fibronectin</td>
<td></td>
</tr>
<tr>
<td>cytoskeletal</td>
<td>transgelin</td>
<td></td>
</tr>
<tr>
<td>Migration/metastasis</td>
<td>Profilin-1</td>
<td>Petraxin</td>
</tr>
</tbody>
</table>
Conclusions

- Omental ASC promote the growth and vascularization of endometrial xenografts.
- Individual heterogeneity in ASC isolates
  - Candidate secreted factors include periostin, MMP2, LOXL2
- Obesity and tissue source of murine ASC does not alter ASC morphology, MSC cell surface marker expression or alter in-vitro effects of ASC on ID8 proliferation or migration
- Obesity impairs differentiation potential of ASC from both subcutaneous and visceral adipose
- Obesity had most consistent effects of ASC from subcutaneous tissues
  - Increasing
    - In-vivo ID8 tumor growth
    - ASC sphere formation and tumorisphere formation
    - Macrophage infiltration
  - Decreasing
    - Intra-tumoral adipocytes
Future directions

• Identify critical ASC <-> endometrial cancer signals
  – Characterizing ASC from patients with and without cancer
• Determine if effects of tissue source and obesity are seen in clinical ASC samples.
• Investigate impact of weight loss and exercise on ASC.
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