Metastasis Initiating Cells: A Link Between Metastasis And What We Eat

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How is homeostasis maintained and why is it broken?

- Daily business
- Retention of identity during damage
- Change of identity under stress
- Loss of function during ageing
- Neoplastic transformation
Behavior of several types of stem cells is predominantly stochastic.

**LETTERS**

A single type of progenitor cell maintains normal epidermis

Elizabeth Clayton¹, David P. Doupé¹, Allon M. Klein², Douglas J. Winton³, Benjamin D. Simons² & Philip H. Jones¹

**ARTICLE**

Distinct contribution of stem and progenitor cells to epidermal maintenance

Guilhem Mascre³, Sophie Dekoninck¹, Benjamin Droga³, Khalil Kass Youssef¹, Sylvain Brohée¹,², Panaglota A. Sotiropoulou¹, Benjamin D. Simons³,⁴ & Cédric Blanpain¹,³
But their timing isn’t...
Premature ageing of adult stem cells lacking BMAL1

Bmal1KO arrhythmic mice

Control 5 months
KO 5 months

Janich et al., Cell Stem Cell 2013
Toufighi et al., Plos Comp Biol 2015
Janich et al., Nature 2011
CIRCADIAN REPROGRAMMING IN AGED STEM CELLS

Solanas/Peixoto et al., Cell (accepted)
Sato/Solanas, et al., Cell (accepted)
Several models show that cancer stem cells arise from adult stem cells.

**Identifying the cellular origin of squamous skin tumors**
Gaëlle Lapouge, Khalil Kass Youssef, Benoit Vokaer, Younes Achouri, Cindy Michaux, Panagiota A. Sotiropoulou, and Cédric Blanpain

**LETTERS**

**Crypt stem cells as the cells-of-origin of intestinal cancer**
Nick Barker, Rachel A. Ridgway, Johan H. van Es, Marc van de Wetering, Harry Begthel, Maaike van den Born, Esther Danenberg, Alan R. Clarke, Owen J. Sansom & Hans Clevers
What is the origin of metastasis?

Primary tumors are heterogeneous

Which cells initiate metastasis?

What distinguishes them molecularly?
SUMMARY OF RESULTS

Identified cells uniquely capable of initiating metastasis (MICs)

MICs are defined by high expression of metastatic and lipid metabolism genes

MICs are CD36+ (fatty acid transporter)

MICs are highly sensitive to dietary fatty acids (i.e. palmitic acid)

MICs depend on CD36 for their metastatic potential (therapeutic target)

Pascual et al., Nature (2017)
Orthotopic model of oral cancer patient-derived tumors

Oral lesion  LN metastasis  Lung metastasis

Collaboration with Vall D´Hebron Hospital (Barcelona)
Dormant CSCs are defined by a **METASTATIC** and **LIPID METABOLISM SIGNATURE**

**Biological Categories in Dormant cells**

- Psoriasis
- Neoplasms, Squamous Cell
- Skin Diseases, Papulosquamous
- Stomach Neoplasms
- Carcinoma, Squamous Cell
- Prostatic Diseases
- Stomach Diseases
- Lymphatic Metastasis
- Neoplasm Metastasis
- Skin Neoplasms

**Lipid Metabolic Process**

<table>
<thead>
<tr>
<th>Fatty Acid uptake</th>
<th>CD36</th>
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<tbody>
<tr>
<td></td>
<td>ABCA1</td>
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<tr>
<td></td>
<td>SLC10A1</td>
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</tbody>
</table>

| Lipid Catabolism        | ACSL1 |
| Fatty Acid beta oxidation| ACSBG1 |
| Fatty Acid alpha oxidation| PPAR-alpha |
|                         | PNPLP3 |
|                         | LIPH |
|                         | PLA2G4E |
|                         | PNPLA2 (ATGL) |
|                         | HSD17B2 |
|                         | FA2H |

| Lipid Biosynthesis      | ACSL1 |
|                         | ACSBG1 |
|                         | FA2H |
|                         | HSD17B2 |
|                         | CYP4F3 |

| Triglyceride Synthesis/Lipid Storage | DGAT2 |
|                                      | SEC14L2 |
CD36

- Multifunctional membrane protein

- Internalizes FFAs, oxidized lipids and LDL/HDL

- CD36 internalizes fatty acids and can activate their oxidation at the mitochondria
CD36+ cells are uniquely capable of initiating metastasis
Metastasis-initiating cells depend on CD36 to induce metastasis (LNs, lung)
The pro-metastatic activity of CD36 relies on its ability to internalize FAs.
Inhibition of CD36 is a potential anti-metastatic therapy
Two different neutralizing antibodies
...and inhibit metastasis progression (in immunodeficient/competent models)
Inhibition of CD36 inhibits metastasis in different tumor types

Lung SCC  |  Bladder  |  Breast (luminal)  |  HNSCC  |  Melanoma

Melanoma

Luminal breast cancer

Melanoma
So, is there then a specific link between **DIET** and **METASTASIS**?
High fat diet boosts metastasis in a CD36-dependent manner
Is it any fatty acid?
Palmitic and Linoleic acid do not exert the same effect on MICs

Unpublished
Some worrying facts...
...and other countries are **not doing much better**

At this rate, 1/3 of the population of several EU countries (and UK...) will be obese in 2030
We consume more fatty acids than we think
Palmitic acid potently induces CD36+ metastatic-initiating cells (15X increase)

Palm oil is heavily used (and consumed) in processed (supermarket) food

Treat cells with PA for only 2 days
Wash out PA
Inoculate cells in mice fed with normal diet

Unpublished
Dnmt3a and Dnmt3b activate superenhancers in adult stem cells

Rinaldi et al., Cell Stem Cell (2016)
Dnmt3a/Dnmt3b-dKO shortens latency, increases tumor burden and promotes metastasis

Rinaldi et al., eLife (2017)
Dnmt3a/Dnmt3b-KO derepresses the expression of lipid metabolism genes.

### Downregulated in Dnmt3a cKO tumors: 114 genes

<table>
<thead>
<tr>
<th>Biological Processes</th>
<th>Fatty acid metabolic process</th>
<th>Lipid storage</th>
<th>Positive regulation of cell proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptotic process</td>
<td>1.63E-03</td>
<td>3.72E-06</td>
<td>7.35E-04</td>
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<tr>
<td>Locomotion</td>
<td>2.94E-03</td>
<td></td>
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<tr>
<td>Cell-cell adhesion</td>
<td>6.69E-03</td>
<td></td>
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<tr>
<td>Toll like Receptor</td>
<td>9.02E-04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOPTOSIS</td>
<td>1.59E-03</td>
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</tbody>
</table>

### Upregulated in Dnmt3a cKO tumors: 277 genes

- MgiL, Sod1, Phash, Adh7, Elov13, Lpl, Pa2h, N11h3, Acot1, Aocob, Fad4, Ppara, Ephp2, Cd36, Acot5, Ayp1a, Acox2, Ppara

### Signal Transduction Pathways

- **PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA and ALPHA**
- **WNT SIGNALLING PATHWAY**

**Note:** The table and diagram illustrate the downregulated and upregulated gene expressions in Dnmt3a/Dnmt3b-KO tumors, focusing on lipid metabolism and related biological processes.
Loss of Dnmt3a/Dnmt3b derepresses the expression of lipid metabolism genes

Rinaldi et al., eLife (2017)
Rinaldi et al., *eLife* (2017)
Rinaldi et al., *Cell Stem Cell* (2016)

**New Questions**

Why do tumors have CD36+ cells?
Where are they?
Where do they bind at the metastatic site?
Can changing the fatty acid content in the diet affect metastasis in patients?

Importantly: can we convince *Nutella* to go back to their original palm oil-free recipe?
Funding agencies
The Stem Cells and Cancer Lab

Marion Salzer (PhD student)
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MECHANISMS OF METASTASIS
12-14 March, 2018

Chairs
Joan Massagué (Memorial Sloan Kettering Cancer Center)
Salvador Aznar-Benitah & Roger Gomis (IRB Barcelona/ICREA)

Speakers
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- Eduard Batlle (Barcelona, Spain)
- Mohamed Bentires-Alj (Basel/Switzerland)
- Cedric Blanpain (Bruxelles, Belgium)
- Arkaitz Carracedo (Derio, Spain)
- Christian Fedeza (Cambridge, UK)
- Johanna Joyce (NY, USA)
- Yebin Kang (Princeton, New Jersey, USA)
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- Charles Swanton (London, UK)
- Andreas Trippp (Heidelberg, Germany)
- Zena Werb (San Francisco, CA, USA)
- Xiang Zhang (Berkeley, CA, USA)

Registration deadline
15 January, 2018
http://www.irbbarcelona.org/mechanisms-metastasis