Molecular mechanisms of radiation-induced cell fate decisions

Molecular and Clinical Radiobiology Workshop
McGill University Health Centre,
June 17-19, 2015

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Molecular and cellular biology of senescence:
- DDR, senescence and microenvironment

Complex diseases/cell therapy - Translational research:
- Impact of MSC/HSPC senescence for Rheumatoid arthritis

Cancer Cell fate decisions - Translational research:
- Impact of cell fate decisions in OvCa treatment
- Advanced chemotherapy for PrCa
Exploring the role of Therapy-Induced Cell Fate Decisions in cancer

- Transient Cell cycle arrest
- DDR
- DNA repair/mutations
- Extracellular signals

- Stress or Damage From therapy
- Cellular Senescence
- Apoptosis
- Necroptosis
- Mitotic catastrophe

- DDR

BIOBANKS: Three important type of information from patients

- **Life history**
- **Observations and Imaging**
- **Biological/biochemical samples**
  - More difficult for solid cancers
How to strategize: Select Appropriate Clinical Models for Cancer Biobanking

How to strategize: Select Appropriate Clinical Models for Cancer Biobanking

Sample/Data Collection

Negative clinical outcome

Positive clinical outcome

• The molecular side of TICFD
Treatment Black Box: Exploring the role of Therapy-Induced Cell Fate Decisions in cancer (TICFD)

Hallmarks of cancer: next generation Hanahan Cell 2011
Timing is critical to study Therapy-Induced Cell Fate Decisions

Do not mix cell TICFD caused by the therapy and long-term proliferation or “colony formation”, which is the consequence of earlier survival following the summation of all TICFDs.
How to assay Therapy-Induced Cell Fate Decisions? What can you see early: TICFD – i.e. senescence

HFF > 99% senescence...

Human normal diploid fibroblasts
The DDR influences TICFD

- Transient Cell cycle arrest
- Stress or Damage From therapy
- DNA repair/mutations
- Extracellular signals
- Cellular Senescence
- Apoptosis
- Necroptosis
- Mitotic catastrophy

Cell fate: survival and perfect repair or mutation fixation, elimination (programmed cell death), senescence and autophagy, checkpoint bypass (cancer cells or low damage).
The DDR influences TICFD

DNA repair
- NER, BER
- NHEJ, HR
Chromatin remodeling

DNA Strand breaks
N nucleotide damages
DNA replication fork collapses
Oxidative damages
B Bulky lesions
Chromatin stress

Detection

Apical kinases

Signal amplification / checkpoint kinases

Cell cycle
- checkpoints
DNA replication
- Entry
- progression
Extracellular signals
- Inflammatory
- kill me
Metabolism
- mitochondria
- autophagy
Cell fate decisions
- apoptosis
- necroptosis
- senescence

Negative feedback

SOURCE

SENSORS

TRANSUDCERS

MEDIATORS

EFFECTORS

TERMINATORS
The DDR influences TICFD

More effectors: Genes regulated by p53

The DDR influences TICFD

Deciding between survival and apoptosis

Nature Reviews Microbiology 7, 144-155 (February 2009)
Establishing senescence in cells that resist apoptosis

ACTIVATED ONCOGENES

GENOTOXIC DAMAGE

DNA-SCARS

DDR (H2AX/NBS1-ATM-CHK2)

SECRETION/INFLAMMATION

STRESS?

ROS

Chronic damages

Mitogenic stress

ROSA

CDK

(�(380,558),(533,619)

CDK

(CDK2-Cyclin E/A)

pRb

CDK

(CDK4,6-Cyclin D)

SAHF

Cell proliferation

p19

p53

p21

p16

pRb

E2F

Cell proliferation

SENESCENCE

Establishing senescence in cells that resist apoptosis

ACTIVATED ONCOGENES

GENOTOXIC DAMAGE

DNA-SCARS

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Cell proliferation

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p53

p21

p16

pRb

E2F

Cell proliferation

SENESCENCE
DOES Therapy-Induced Cell Fate Decisions Influences LONG-TERM OUTCOME?

Human normal diploid fibroblasts

Early cell death (apoptosis, senescence autophagy, necrosis)

Mitotic Catastrophe

Clonogenic survival

Late cell death (apoptosis, senescence autophagy, necrosis)
DOES Therapy-Induced Cell Fate Decisions Influences LONG-TERM OUTCOME?

WHAT HAPPENS IF WE SWITCH CELL FATE?

Outcome?

Human normal diploid fibroblasts
DOES Therapy-Induced Cell Fate Decisions Influences LONG-TERM OUTCOME?

WHAT HAPPENS IF WE SWITCH CELL FATE?

HDF > 99% senescence...

P53-Dn > 95% mitotic cat.
DOES Therapy-Induced Cell Fate Decisions Influences LONG-TERM OUTCOME?

WHAT HAPPENS IF WE SWITCH CELL FATE?

Conclusion, in some context senescence is a better TICFD to reduce long-term proliferative capacity in damaged cells.
The DDR influences TICFD
Hallmarks of senescence

- no DNA synthesis (G0/G1)
- Essentially irreversible growth arrest (p53/pRb)
- Altered morphology (Qualitative)
- Senescence-associated-Beta-Gal (SA-B-Gal)
- Persistent DNA damage signaling (DNA-SCARS)
- Heterochromatin formation (SAHF)
- Altered biological activity (SASP)
Senescence inducers

*Carcinogens*

“Culture stress”

*OIS: Oncogene-induced senescence*
Senescence-associated secretory phenotype (SASP)

**Antibodies array**

**SEN**

**PRE**

**OVER secretion of pro-inflammatory factors**

- **Cytokines**: IL-6, IL-8, Gro-1, MCP-1...
- **Growth factors**: HGF/SF, PDGF, KGF...
- **Proteases**: MMPs, uPA, PAI-1...

**LOSS of structural matrix components**

- **Fibrillar proteins**: collagen, elastin...
- **Glycoproteins**: Fibronectin, SPARC, Decorin...

*Coppé et al, PLoS Biol, 2008; Bavik et al, Cancer Res, 2006; Malaquin et al, Plos One 2013; unpublished data*
The senescence program is dynamic

Rodier & Campisi “four faces of senescence” JCB 2011
Tissue repair: Senescent cells stimulate their own clearance

Krizhanovsky, Cell 2008
Senescence-associated secretory phenotype (SASP)
Detrimental Paracrine activities of the SASP

The SASP alters the microenvironment contributing to aged-related diseases
Summary: Potential benefits and problems of the SASP

Disruption of normal tissue structure and function

Immune cell (NK cell, etc)

Clearance

Reinforced growth arrest

Bystander effects
Malaquin 2015
Frontiers in Genetics

Angiogenesis

Senescent cell

SASP factors

Involution

Proliferation

Freund 2010
Trends in Molecular Medicine 2010
Clearance of p16^{Ink4a}-positive senescent cells delays ageing-associated disorders

Darren J. Baker1,2,3, Tobias Wijshake1,4, Tamar Tchkonia5, Nathan K. LeBrasseur1,5, Bennett G. Childs1, Bart van de Sluis6, James L. Kirkland7 & Jan M. van Deursen1,2,3

Cancer cells often retain their capacity to undergo senescence in response to radio-chemo therapy.
Cell Senescence: The Dr. Jekyll and Mr. Hyde of TICFD?

**Diagram:**
- Normal Tissue vs. Cancer Tissue
- Growth Arrest:
  - Normal Tissue: p53/pRb dependent
  - Cancer Tissue: p53/pRb independent (Therapy-induced)
- SASP:
  - Normal Tissue: Wound response activation, immune microenvironment modulation, clearance of senescent cells, wound resolution
  - Cancer Tissue: Wound response activation, immune microenvironment modulation, clearance of senescent cancer cells, stimulation of surviving cancer stem cells

**Questions:**
- Tumor promotion or elimination?
• What does it look like (TICFD) in real human cancer?
The example of radiation-treated sarcomas

GTVs grow before responding to RT

Change in RPS volume during RT

Weeks of RT (approximate cumulative Gy)
The example of radiation-treated sarcomas

Spatial and volumetric changes of retroperitoneal sarcomas during pre-operative radiotherapy.
Wong P1, Dickie C1, Lee D1, Chung P1, O'Sullivan B1, Letourneau D1, Xu W2, Swallow C3, Gladdy R3, Catton C4.

Is it human tumor senescence?
Is it beneficial for patients?

Weeks of RT
Ovarian Cancer (OvCa) model

How to strategize: Clinical Model to select samples for cancer biobanking

Sample/Data Collection

Tumor Mass

Time

OvCA tissue bank at the CRCHUM
Dr. Mes-Masson and Provencher

Exploring senescence-associated molecular networks in human cancer:

UNDERSTANDING THE IMPACT OF CANCER CELL FATE DECISIONS DURING OVARIAN CANCER TREATMENT

Shuofei Cheng, Llillians Calvo Gonzalez, Michael Skulimowski, Lise Portelance, Guillaume Cardin, Diane Provencher, Anne-Marie Mes-Masson and Francis Rodier
Ovarian Cancer (OvCa) model

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Death</th>
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<tbody>
<tr>
<td><strong>Females</strong></td>
<td><strong>Lung &amp; bronchus</strong></td>
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<tr>
<td>Breast</td>
<td>232,340</td>
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<tr>
<td>Lung &amp; bronchus</td>
<td>110,190</td>
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<tr>
<td>Colorectum</td>
<td>69,140</td>
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<tr>
<td>Uterine corpus</td>
<td>49,560</td>
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<tr>
<td>Thyroid</td>
<td>45,310</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>32,140</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>31,630</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>24,720</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,480</td>
</tr>
<tr>
<td><strong>Ovary</strong></td>
<td><strong>Liver &amp; intrahepatic bile duct</strong></td>
</tr>
<tr>
<td></td>
<td>22,240</td>
</tr>
<tr>
<td>All Sites</td>
<td>805,500</td>
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</table>

CA: A Cancer Journal for Clinicians

Volume 63, Issue 1, pages 11-30, 17 JAN 2013 DOI: 10.3322/caac.21166

http://onlinelibrary.wiley.com/doi/10.3322/caac.21166/full#fig1
Ovarian Cancer (OvCa) model

- High lethality (5 years survival rate below 30%) mainly due to late diagnostic
- Traditional treatments with high failure rates and severe side effects (surgery followed by platinum- and taxol- based chemotherapy)

How to improve the survival:
- Better early diagnostic (new and predictive biomarkers)
- Improvement of treatment (understand the effects)
Question: What happens to OvCa cells/tumors that undergo chemotherapy treatment?

Hypothesis: Multiple cell fates could occur, perhaps particular cell fate decisions can be linked to treatment outcome and eventually manipulated for the benefit of the patient.

Potential results:

- Predictive biomarkers
- Follow-up biomarkers
- Drug targets
Ovarian Cancer (OvCa) model
Using primary tumors-derived established cell lines

Sample/Data Collection

OvCA tissue bank at the CRCHUM
Dr. Mes-Masson and Provencher

OvCa clinical model to study senescence in cancer

Spontaneous immortalization: 33 cell lines panel

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<th>Histopathologie</th>
<th>Grade, stade</th>
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<tr>
<td>TOV 2835EP</td>
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<td>TOV 2929D</td>
<td>3, IHC</td>
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<td>3, IV</td>
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<td>3, IHC</td>
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<td>3, IHC</td>
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<tr>
<td>OV 3133</td>
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<tr>
<td>OV 3133(2)</td>
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<tr>
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<td>3, III</td>
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<tr>
<td>3121D</td>
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<table>
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<th>Histopathologie</th>
<th>Grade, stade</th>
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<tbody>
<tr>
<td>TOV 21G</td>
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<tr>
<td>Ov 90</td>
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<td>TOV 112D</td>
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<td>Ov 866(2)</td>
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<td>Mov 1078D</td>
<td>Néo endomètre</td>
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<td>Ov 1946</td>
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<td>Ov 1946</td>
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<td>Ov 2085</td>
<td>Adénocarcinome, 3, IHC</td>
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<td>Ov 2085(3)</td>
<td>Adénocarcinome, 3, IHC</td>
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<td>Ov 2295</td>
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<td>Ov 2295(2)</td>
<td>Adénocarcinome, 3-4, IHC</td>
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<tr>
<td>Tov 2295</td>
<td>Adénocarcinome, 3-4, IHC</td>
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<tr>
<td>Ov 2414</td>
<td>Adénocarcinome, 1, IHC</td>
</tr>
</tbody>
</table>
OvCa clinical model to study senescence in cancer

**cell lines – damage – cell fate measurements**

% cell survival

<table>
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<tr>
<th>Cell Line</th>
<th>Control</th>
<th>Senescent</th>
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<tbody>
<tr>
<td>TOV 21G</td>
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<td>TOV 81D</td>
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<td>TOV 1946</td>
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<tr>
<td>OV 4485</td>
<td></td>
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<td>OV 4453</td>
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<td></td>
<td></td>
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<tr>
<td>TOV 112D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = near 100% death

**Brightfield (200x)**

Control

Senescent
Clinical models to study senescence in cancer

Some Ovarian cancer cell lines undergo senescence in response to DNA damage

- **Control**
  - DAPI
  - 53BP1
  - PML

- **Senescent**
  - DAPI
  - 53BP1
  - PML

**Fluorescence (400x)**

**Zoom**

- **Control**
  - DAPI
  - 53BP1
  - PML

- **Senescent**
  - DAPI
  - 53BP1
  - PML

**IL-6 secretion**

- **Control**
  - 6000 PG/Microliters / day
- **Senescent**
  - 8000 PG/Microliters / day

**IL-8 secretion**

- **Control**
  - 750 PG/Microliters / day
- **Senescent**
  - 1000 PG/Microliters / day

**NOT so surprising, p53 is mutated > 90% in HGS-OvCa**
Ovarian Cancer (OvCa) model
Using primary tumor cell cultures

Sample/Data Collection

Ovarian Cancer (OvCa) model

- Tumor Mass
- Time
- Tumor Recurrence
- Minimal Residual Disease
- Sustained Tumor Regression

Negative clinical outcome
Positive clinical outcome


OvCA tissue bank at the CRCHUM
Dr. Mes-Masson and Provencher
Stress-induced (culture shock) premature senescence in OvCa cells

Senescence hallmarks evaluation:

Conclusions: 90%+ of primary OvCa cells can undergo robust stable senescence!
From primary cells to tissues

Exploiting TMAs
Ovarian Cancer (OvCa) model

How to strategize: Clinical Model to select samples for cancer biobanking

Sample/Data Collection

OvCA tissue bank at the CRCHUM
Dr. Mes-Masson and Provencher

TMA (Tissue MicroArray) - Concept

Examples of Medical applications:
- Samples are slow to collect, thus TMA are long to build
- So far mostly for research
- Biomarkers discovery in personalized medicine

Prostate cancer TMA – mosaic scan
PCa Tissue TMA: Image segmentation

A

MERGE OF FOUR COLOURS

B

DAPI
CK18-19
53BP1
p-H2AX

DAPI
CK18-19
53BP1
p-H2AX
Des opérations arithmétiques ("AND, NAND, OR, NOR" etc.) peuvent être réalisées sur les binary layers ce qui permet encore plus de segmentation et donc de mesures.

Il est possible d'utiliser plusieurs binary layers permettant la détection de plusieurs régions morphologiques.

Etc... (colors...)
Software-based tissue segmentation

\[
\text{DNA (nuclei)} \cap \text{Intersec} = \text{Epithelial nuclei} = \text{Stromal nuclei}
\]

Mesures possibles:

• % de surface d'ADN contenu dans l'épithélium et stroma (proportionnelle au # de cellules)
• L'expression de CD73 détectée et normalisée par rapport au contenu d'ADN
PCa Tissue TMA: Image segmentation

FOUR COLOURS RAW IF DATA

SEGMENTED IMAGE: Epithelial – Stromal – Nuclear masks
DDR Test-TMA: Software-based quantification

(Mean Fluorescence Intensity-MFI)/MFI of 0h x 100%

Time (h)

pH2AX
53BP1
p21
pCHK2

Control 0.5 h 2 h 8 h 24 h

pH2AX

53BP1

N=950  N=1866  N=1755  N=2334  N=1316

N=950  N=1866  N=1755  N=2334  N=1316
OvCa conclusions: At least 90% of the patients could probably benefit from senescence-manipulation strategies, either by

- Reinforcing the senescence growth arrest
- Re-directing senescent cells to death
- Manipulating the SASP

- Senescence in IR-treated cancers?
- Promising evidence for Prostate, HPV-induced, Sarcoma...
How can we acquire and exploit knowledge about TICFD?

Knowledge acquisition:

Discovery and Validation using tumor biobanks and TMAs

Uses:

**Predictive biomarkers**

- Predict what the cancer cell will do and inform whether this is good or bad

- Inform the use of Targeted/Personalized therapies

based on the prediction, select appropriate treatment combinations to optimize favorable TICFD

**Treatment follow-up using non-invasive markers**

Monitor TICFD biomarkers in real-time to rapidly assess treatment success
Acknowledgements

CURRENT LAB MEMBERS and FUNDING:

**Graduate Students**
- Stéphanie Nadeau PhD
- Sabrina Ghadaouia MsC
- Lillians Calvo Gonzalez MsC
- Aurélie Martinez PhD
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- Ophélie Lescat M2

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- Fred Saad (Prostate)
- Diane Provencher (Ovary)
- Ines Colmegna (Rheumatoid Arthritis)
- Vanessa Samouelian (Cervix)
- Apostolos Christopoulos (Head and neck)
- Jean-François Cailher (Macrophage)
- Philip Wong (Radio-oncology)

**Investigators:**
- Anne-Marie Mes-Masson (TMAs)
- Réjean Lapointe (Immuno-oncology)