Breast cancer: an environment story and/or a micro-environment story?

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Introduction

• Breast cancer (BC) is a major public health problem
• More than 2M new cases diagnosed and more than 600,000 deaths in 2018 (World)
• Different stages: T1-4
• 5-year survival rate
  • Without metastases: 99%
  • With metastases: 26%
Introduction

- Breast cancer (SC) is a major public health problem
- More than 2M new cases diagnosed and more than 600,000 deaths in 2018 (World)
- Different stages: T1-4
- 5-year survival rate
- Without metastases: 99%
- Gangli.: 85%
- With metastases: 26%

https://www.ncbi.nlm.nih.gov/books/NBK65969.2/figure/CDR0000062955_396/?report=objectonly
The breast

• Mammary gland
• Secondary sexual organ
• Endocrine gland
• Lactation
Multiple cells

- Epithelial cells
  - luminal cells (ER+, PR+)
  - contractile myoepithelial cells.
  - progenitor cells
- Micro-environment
  - Fibroblasts
  - Adipocytes and pre-adipocytes
An intercellular communication

• Paracrine communication
  • Cytokines, interleukins
  • Micro-vesicles
  • Growth factors....

• What role for the environment in this communication?
An intercellular communication

- Adipocytes
  - Endocrine cells
  - Rich in lipids (droplet)
  - Contribution -> milk
- POPs storage site
What is a POP?

Take a step back -> detoxication processes in animals
A general overview of the detoxication process
One example: the AhR (Aryl hydrocarbon R)

Many pollutants are well metabolized
- Ex: polycyclic aromatic hydrocarbons
- Benzo(a)pyrene
One example: the AhR (Aryl hydrocarbon R)

Many pollutants are well metabolized
- Ex: polycyclic aromatic hydrocarbons
- Sulfo-Benzo(a)pyrene
One example: the AhR (Aryl hydrocarbon R)

Many pollutants are well metabolized
- Ex: polycyclic aromatic hydrocarbons
- Benzo(a)pyrene

But some pollutants…. resist
POPs or Persistent Organic Pollutants

- Organic molecules
  - High Octanol/Water (or Kow) partition coefficient
  - 'Affinity' -> fatty substances (lipid chutes)
  - Unmetabolized
  - Storage in adipose tissue, liver, brain and
- Which molecules? Dioxins, PCBs, polybrominated, organochlorines...

TCDD: an AhR ligand
Main question

- What joint influence?
- POP
- Microenvironment

Tumor cell phenotype
Cancer-associated adipocytes

Wang et al. *Cancer letters*, 2012
Part 1: association between [POPs] in adipose tissue and mammary tumor phenotype?

- In Paris (T3S)
  - Preliminary results:
    - MCF7 (human breast cancer line): minimally invasive at the base, low migration
    - TCDD
  - EMT (Epithelial-mesenchymal transition)

- Collaborations
  - Cliniciens (HEGP , T3S) - prélèvements / caractéristiques : cancer du sein + TA
  - Analyticiens (LABERCA) - détermination [POP] – tissus adipeux
  - Biologistes (T3S) - analyses / synthèse
Part 1: association between [POPs] in adipose tissue and mammary tumor phenotype?

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Diry et al, Oncogene 2007
Epithelial-mesenchymal transition

- Gastrulation
- Fibrosis
- Metastases
Part 1: association between [POPs] in adipose tissue and mammary tumor phenotype?

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    - TCDD
    - EMT (Epithelial-mesenchymal transition)

- Collaborations
  - Clinicians (HEGP, T3S) -> samples / characteristics: breast cancer + BP
  - Analyticians (LABERCA) -> determination [POP] – adipose tissue
  - Biologists (T3S) -> analyses / synthesis
Methodology

• Exploratory case-controls study
  • concentrations of 49 POPs were measured in samples of
    • Adipose tissue
    • Serum
  • Patients with breast cancer, with or without lymph node metastasis,
  • BMI : considered as a factor
  •

Koual M et al, 2019, Environmental International
Obesity and breast cancer

- Risks factors for Breast Caner
  - +9% increase in risk for 5 units of BMI (BMI>25kg/m2)
  - +30% in morbidly obese patients
- More aggressive cancers, more advanced stages
  - Delayed diagnosis
  - Particular intrinsic properties of the tumors
- Response to treatments: chemotherapy/hormone therapy
  - Doses unsuitable for volume of distribution and body surface area
- Decreased overall survival and increased risk of recurrence
  - A dose-response effect in premenopausal patients
  - Increased risk of metastasis by 46%
Methodology

• Exploratory case-control study
  • Concentrations of 49 POPs were measured in samples of
    • Adipose tissue
    • Serum
  • Patients with breast cancer, with or without metastasis of lymph nodes,
  • BMI : considered as a factor
  • Adjusted and unconditional logistics models
• Main Q: Associations between [POPs] and the risk of metastasis ?

Koual M et al, 2019, Environmental International
Population

- 91 patients operated on in the HEGP department
  - Women
  - Age >18 years
  - Palpable tumour
  - Informed consent
- 2 groups: With or without metastatic lymph node involvement

Koual M et al, 2019, Environmental International
Sample analysis

Dosage of 49 POPs
- 19 Dioxins
- 18 Polychlorinated biphenyls (PCBs)
- 8 Polybrominobiphenyl ethers (PBDEs)
- Hexabromocyclododecane (HBCD)

Koual M et al, 2019, Environmental International
Results

Homogeneous population between both groups
Except
* The size of the tumor
* Ki67 marker

<table>
<thead>
<tr>
<th>Histopathological examinations</th>
<th>Metastatic N = 38</th>
<th>Non-metastatic N = 53</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple negative</td>
<td>4 (11%)</td>
<td>6 (11%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Estrogen receptor positive</td>
<td>33 (87%)</td>
<td>44 (83%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Progesterone receptor positive</td>
<td>27 (71%)</td>
<td>36 (68%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>0.1–2 cm</td>
<td>12 (32%)</td>
<td>31 (58%)</td>
<td></td>
</tr>
<tr>
<td>3.1–5 cm</td>
<td>20 (53%)</td>
<td>22 (42%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>6 (16%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Affected lymph nodes ≤ 3 nodes</td>
<td>26 (66%)</td>
<td>0 (0%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>4–9 nodes</td>
<td>9 (24%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>≥ 10 nodes</td>
<td>3 (8%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>No applicable</td>
<td>0 (0%)</td>
<td>53 (100%)</td>
<td></td>
</tr>
<tr>
<td>Grade histology</td>
<td></td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>1</td>
<td>5 (13%)</td>
<td>10 (19%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21 (55%)</td>
<td>25 (47%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12 (32%)</td>
<td>18 (34%)</td>
<td></td>
</tr>
<tr>
<td>Histological classification</td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>Infiltrating ductal carcinosa</td>
<td>31 (82%)</td>
<td>40 (75%)</td>
<td></td>
</tr>
<tr>
<td>Infiltrating lobular carcinosa</td>
<td>6 (16%)</td>
<td>9 (17%)</td>
<td></td>
</tr>
<tr>
<td>Papillary carcinosa</td>
<td>0 (0%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Mucinous (Collloid) carcinosa</td>
<td>1 (3%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Apocrine carcinosa</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>HER2 Positive</td>
<td>2 (5%)</td>
<td>6 (11%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>&lt; 20%</td>
<td>12 (32%)</td>
<td>29 (55%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20%</td>
<td>26 (68%)</td>
<td>24 (45%)</td>
<td></td>
</tr>
</tbody>
</table>

Koual M et al, 2019, Environmental International
Results

Concentrations in adipose tissues

- 2.3.7.8-TCDD (dioxine de Seveso) (OR=1.76)
- PCB 77
- PCB 169

All ligands of the AHR

Positive associations (p<0.05)

- Risk of lymph node metastasis
- Tumor size

Koual M et al, 2019, Environmental International
Results

- Subgroup of overweight patients BMI > 25kg/m²

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<th>Adipose tissue concentrations</th>
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<td>2.3.7.8-TCDD</td>
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Odds ratio = 4.48 (1.32-20.71)

Risk of lymph node metastasis

N=43

Koual M et al, 2019, Environmental International
Results

- 10 patients with triple-negative cancer:
- Higher POPs concentrations in the metastatic group

  - HpCDF $p < 0.1$
  - PCB 101
  - PCB 138
  - PCB 167 $p < 0.05$
  - Sum of PCB non dioxin like
  - PCB 153 (non dioxin like)
  - Sum de 8 PBDE

Koual M et al, 2019, Environmental International
Conclusion

- Exploratory study
- Limited sample (91 patients)
- Limitations of statistical models

2.3.7.8-TCDD and some PCBs are associated with more aggressive breast cancers

Koual M et al, 2019, Environmental International

- Need for broader longitudinal studies
- Need to understand the underlying mechanisms

Overweight patients
What potential mechanisms?

- Positive associations with a high BMI
- Role of the adipose tissue (AT)
  - AT: storage of POPs such as TCDD
- TCDD is an AhR ligand

Koual M et al, 2020, Environmental Health
Larigot L, Benoit L et al, 2021, ARPT
Part 2: Modeling the metastasis process

- Breast cancer cells

- Pre-adipocyte cells (hMADS=Human multipotent adipose-derived stem cells)
Part 2: Modeling the metastasis process

- Breast cancer cells
- Pre-adipocyte cells (hMADS)

Results: XCelligence

Drug Discovery Today Volume 22, Issue 12, December 2017, Pages 1808-1815
Results: XCelligence

Results: growth of mamospheres

Treatment with conditional medium

MCF-7 cells in agarose-coated wells (low adhesion)

Results: growth of mamospheres

Contribution of pre-adipocytes to a change in the phenotype of tumor cells

Effect of TCDD?
Unsupervised study - proteomics

Collaboration with C. Guerrera
Univ. Paris Cité
Results: proteomics

Increased expression of ALDH1A3
Contribution of pre-adipocytes to a change in the phenotype of tumor cells

TCDD -> stem cell biomarkers

Co-exposure leads to the acquisition of a "cancer stem cell" phenotype by cancer cells
Co-exposure and metastasis?

Necessity to validate the results with an \textit{in vivo} model
In vivo zebrafish experiments

• Advantages of the zebrafish model
  • 70% of genes in common with humans
  • Absence of immune system in the embryo (<10j)
  • Excellent reproductive abilities, ectopic gestation
  • Large number of transparent embryos
  • Lower cost than rodent
  • Short experimental protocol (<7 days)
Results: metastasis in zebrafish

Co-exposure increases the formation of metastases in a xenograft model in zebrafish embryo

Any features of aggressiveness observed with the cellular model?
Results: microscopy of the cancer cells

Co-exposure increases the formation of giant polynuclear cells (GCPs)

Conclusions


Co-exposure

Phenotypical changes of breast cancer cells

- Stem cell
- Metastasis
- PGCC
Overall conclusion

• Convergence of results
  • Clinics: association [TCDD] peritumor adipocyte & aggressiveness of breast K.
  • Experimental: TCDD linked to the development of an 'aggressive' cellular phenotype

• Remaining questions
  • What are the effects of environmental mixtures? Work on cigarette smoke (submitted)
  • What role does AhR play? **Building an AOP**
  • What measures at the clinical level? RNAseq Biomarker Characterization - [2019]
Adverse Outcome Pathway

Regulatory purposes -> OECD  
Benoit L et al, 2021, Environ. International
Overall conclusion

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Thanks to you and...

- T3S: C. Tomkiewicz, M. Koual, L. Benoît, A-S Bats, F. Lecuru, R. Barouki
- Université Paris Cité: C. Guerrera, S. Dupuy, Ima
- Université de Boston: D. Sherr
Protocol