TELOMERE SHORTENING IN
HAEMANGIOMA COHORT

TELOMERES IN RADIOBIOLOGY AND CANCER DEVELOPMENT

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LAS VEGAS 2014
Combining epidemiology and radiobiology to assess cancer risks in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with cumulated equivalent doses of the order of 100 mSv or below

- Effect of low dose irradiation?
  - Based on model calculation or *in vitro* experiments

- High interest in analyzing data of *in vivo* studies

- Searching for **biomarkers** for cancer risk assessment

Assessing cancer risks of low-dose radiation
Combining epidemiology and radiobiology to assess cancer risks in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with cumulated equivalent doses of the order of 100 mSv or below

Cohort

Criteria's for a good cohort

- Exposure to low doses
- Comparable age at exposure
- Healthy individuals (no other genotoxic treatments)
- Homogeneous population (one country)
- Long follow-up
- Access to medical records
Combining epidemiology and radiobiology to assess cancer risks in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with cumulated equivalent doses of the order of 100 mSv or below.

Cohort

Criteria's for a good cohort

- Exposure to low doses
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Effect on chromosomal stability: number and type of chromosomal aberrations

Gold standard: Giemsa staining
TELOMERES - A BIOMARKER FOR STRESS, AGEING, CANCER AND CARDIO-VASCULAR DISEASES

Modifications of telomere length have been associated with cancer.
TELOMERES IN RADIOBIOLOGY

telomere shortening after low-dose irradiation and preservation of these changes even 20 years after exposure – Chernobyl workers

Analysis on peripheral blood lymphocytes

Exp Oncol 2011, 33,4,235–238

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**CHOICE OF COHORT**

French haemangioma cohort

**Haemangioma:**
- benign, and usually self-involuting mass of endothelial cells that line blood vessels (estrogen induced?!)  
- 3–5% of new born are having a haemangioma  
- 2-3x more often for girls than for boys  
- usually appears the first weeks of life and generally resolves by age 10  
- approximately 80% are located on the face and neck (organ: liver)

- Treatment between 1940-1973 ➔ (age in 2014: 40-73 years old)
- 8335 patients in total (4767 patients chosen for EpiRadBio)
- Treatment for EpiRadBio-candidates before the age of 3
- In the cases of radiation therapy: $^{226}$Ra, X-rays, $^{32}$P, $^{90}$Y or $^{90}$Sr
- Thereby, only low doses in regions developing tumors (i.e. breast, skin and thyroid)
- Some without treatment or cryotherapy

**Increased tumor-incidence:** 3 times higher than expected

→ Effect of radiation for breast, thyroid and skin

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### Incident tumors

<table>
<thead>
<tr>
<th>Sites</th>
<th>Malignancy</th>
<th>Beginn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucal cavity</td>
<td>Total</td>
<td>W/o RT</td>
</tr>
<tr>
<td>Digestive system</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Trachea, bronchus, lung</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Malignant skin melanoma</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Breast: women</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Uterus</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Ovaries and annexes</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Testis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brain and CNS (except meningiomas)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Other endocrine gland</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hodgkin disease</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Other neoplasm of lymphoid tissue</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Leukemia</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Secondary and ill defined</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Skin: epitheliomas and carcinoma</td>
<td>44</td>
<td>8</td>
</tr>
</tbody>
</table>

**Total**

<table>
<thead>
<tr>
<th>Incident tumors</th>
<th>Malignancy</th>
<th>Beginn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>134</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>356</td>
<td>271</td>
</tr>
</tbody>
</table>

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CHOICE OF COHORT

French haemangioma cohort

Advantage of the French haemangioma cohort:

1. Homogeneous cohort: in fact normal population, just characterized by having a haemangioma
2. Medical documents of haemangioma radiotherapy available
3. Long follow-up
4. Volunteers that filled already a questionnaire for epidemiological studies
5. Dose estimation for every major organ was performed

Dosimetry:
- Mathematical computer models are used to simulate a person of any age, based on body-size measurements
- Taking into account the surface of the applicators (cm²)
CALCULATION OF THE DOSE

Dosimetry - ITCA software for brachytherapy

For each child treated by curie-therapy or radiotherapy, whole body dose estimation -> (at least one site in each of the main organs or structures in the body).

Dose Estimation was performed between 1985 and 1995, using:

- ICTA software for radium and beta treatments
- Dos_EG software for X-ray treatments

These estimation were carried out in the INSERM in collaboration with department of Radiophysics at IGR.

The ICTA software was developed especially for epidemiological studies of cohorts of patients who received $^{226}$Ra and beta treatments for skin haemangioma.
Mathematical computer model can be used to simulate a person of any age, based on body-size measurements.

The phantom is divided into sections: head, neck, trunk, legs.

Parameters can be changed to fit individual patient dimensions using atlases of anatomy, locations of organs are specified within the phantom.
Mathematical computer model can be used to simulate a person of any age, based on body-size measurements.

<table>
<thead>
<tr>
<th>Site</th>
<th>Dose (mGy)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IGR study</td>
<td>Lundell</td>
<td>Karlsson</td>
</tr>
<tr>
<td>Active marrow</td>
<td>53</td>
<td>160</td>
<td>-</td>
</tr>
<tr>
<td>Brain</td>
<td>89</td>
<td>70</td>
<td>72</td>
</tr>
<tr>
<td>Breast</td>
<td>110</td>
<td>480</td>
<td>160</td>
</tr>
<tr>
<td>Colon</td>
<td>15</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>150</td>
<td>120</td>
</tr>
<tr>
<td>Thyroid</td>
<td>39</td>
<td>290</td>
<td>120</td>
</tr>
</tbody>
</table>

The phantom is divided into sections: head, neck, trunk, legs.

Parameters can be changed to fit individual patient dimensions using atlases of anatomy, locations of organs are specified within the phantom.
Long-term effect of low-doses of radiation during infancy on the telomere driven genomic instability.

- All type of irradiation (\(^{226}\)Ra, X-rays, \(^{32}\)P, \(^{90}\)Y or \(^{90}\)Sr) and non-exposed (with or without cryotherapy)
- equal numbers of subjects of each gender (not in all cases possible)
- Treatment for EpiRadBio-candidates before the age of 3
- In the cases of radiation therapy: only low doses at bone marrow

- Study of telomere length in nucleated blood cells with focus on lymphocytes

### Dose groups

<table>
<thead>
<tr>
<th>Category</th>
<th>Dose at bone marrow (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Treated/no dose at BM</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 0 &lt; 0.001</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 0.001 &lt; 0.01</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 0.01 &lt; 0.05</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 0.05 &lt; 0.1</td>
</tr>
<tr>
<td>6</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

Exposed (\(^{226}\)Ra, X-rays, \(^{32}\)P, \(^{90}\)Y or \(^{90}\)Sr)
Non-exposed w/wo cryotherapy

- Chromosomal aberrations
- Changes in telomere length
- Changes in heterogeneity
PROGRESS FORMATION OF FHC-BIOBANK

- First letters sent to FHC donors December 2012
- All authorisations present Sept. 2012
- Start of the project in April 2011
- First blood tube kits sent December 2013
- 32 months
- 185 samples with reply
- FIRST SAMPLE 17.12.2013
- 395 acceptances

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INDIVIDUAL AND ENVIRONMENTAL RISK FACTORS

Type of work

Smoking and consumption of alcohol

Radiological procedures during lifetime

Chronic diseases

Phototype

Skin type

Information about cardio-vascular diseases

Medical data from health insurance

Etude sur les hémangiomes traités à l’Institut Gustave-Roussy

For women: Number of pregnancies

Cancer/benign tumour

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FHC-BIOBANK – BLOOD LYMPHOCYTES

1. Biobank of frozen cells
   - 3x10^6 cells/mL
   - For future culture

2. Further analysis like DNA isolation
   - 3x10^6 cells/mL

3. Cells frozen for FACS analysis
   - 3x10^6 cells/mL

4. Preparation of metaphase spreads; 5 slides per B- and T-lymphocytes, Freezing of remaining cells in Fixativ
   - 5 slides per B- and per T-lymphocytes

- 200 μL blood for the analysis of blood lead concentration

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TECHNIQUE TO ANALYZE TELOMERES

PNA-Telomere-Centromere -FISH

Dicentrics – telomere instability scoring: loss, doublets

Peptide nucleic acid (PNA)

Telomeres in red
Centromeres in green
DNA in blue
ANALYSIS OF CYTOGENETIC SLIDES

Analysis with the microscope

- nuclei

- metaphase

Southern blot

Using of control cell lines allow comparison of different experiments and translation of fluorescent signals in kilobases

Q-FISH

Solely relative signals for telomere length obtained

Quantification of telomere signals: MEAN TELOMERE LENGTH

Required for determination of effective telomere length
TELOMERE SHORTENING IN FUNCTION OF AGE

Vaziri et al., 1993

FH donors treated between 1940 and 1973 ➔ 40-73 years old now

Loss of telomeric DNA as a function of age in peripheral blood lymphocytes
### PRELIMINARY RESULTS

<table>
<thead>
<tr>
<th>Category</th>
<th>Dose at bone marrow (Gy)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>&gt; 0 &lt; 0.001</td>
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<tr>
<td>3</td>
<td>&gt; 0.001 &lt; 0.01</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 0.01 &lt; 0.05</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 0.05 &lt; 0.1</td>
</tr>
<tr>
<td>6</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

**Number of donors per dose group**

- **First analysis**: 60 donors
- **Second analysis**: 80 donors

60 donors first analysis

80 donors second analysis

21
ANALYSIS OF CYTOGENETIC SLIDES

Quantification in nuclei

• Mean telomere length
• Comparison of non-exposed vs. exposed
• Comparison of T- and B-lymphocytes

10,000 nuclei/donor in average analyzed

Variations in telomere length in function of age + dose dependence

Chromosomal instability

1000 metaphases/donor analyzed

Chromosomal instability is not detected – level is lower than those observed in in vitro analysis in blood lymphocytes directly after irradiation
MAYAK COHORT – LONG TIME EXPOSURE DURING PROFESSIONAL LIFE
WOMEN WITHOUT CANCER / FEASIBILITY STUDY

Comparable dose at bone marrow as dose group 5 and 6 of FHC

Radiotherapy
Exposed during work
Age at blood donation

French haemangioma cohort
Non-exposed controls
NO exposure
Age range: 67-96

Mayak cohort
Mayak workers
External and internal exposure
Age range: 73-86
External dose (cumu.): >0 - 2.6 Gy
Internal dose (cumu.): >0 – 1.1 kBq

Differences in telomere shortening in function of age depending on exposure

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PRELIMINARY RESULTS

FHC recruitment has started
119 /400 FHC donors have been analyzed

- Exposed FHC donors without cancer have longer telomeres than non-exposed
- Exposed Mayak workers without cancer seem also to preserve longer telomeres

- Exposed FHC donors for doses (>50mGy) without cancer seem to preserve longer telomeres

? are exposed donors of the FHC with short telomeres those with cancer?
? the number of cancer increasing with age. Are exposed donors of the FHC without cancer those with longer telomeres (according to their age)?
? does the exposure to low doses in combination with short telomeres lead to a higher risk of getting cancer?
? does the exposure to low doses eliminate lymphocytes with short telomeres?
FUTURE

- Increase the size of the cohort (119 → 400)
- Intra- and inter-individual distribution of telomere length
- Intracellular heterogeneity of telomere length
- Chromosomal instability
- Telomere instability

Early effect or Telomere shortening or Telomere elongation
homogenous or heterogenous
Thank you

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