Radiation biomarkers in papillary thyroid cancer

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Post-Chernobyl papillary thyroid cancer

- **Chernobyl accident**: ~6000 radiation-associated thyroid cancer cases in young patients in Belarus, Russia and Ukraine

- **Epidemiology**: relative risk of 2-6 per Gy absorbed dose during childhood related to iodine-131 exposure (Tronko et al., JNCI 2006; Brenner et al., EHP 2011)
Post-Chernobyl papillary thyroid cancer

**Post-Chernobyl PTC as a model for mechanisms in radiocarcinogenesis**

- Young age at exposure and age at diagnosis → likelihood of radiation-induced cancer
- Tumour tissue bank storing exposed cases and age-matched controls
- Individual dose estimates
DoReMi and EpiRadBio

Integrating radiation biomarkers into the epidemiology and risk models of post-Chernobyl thyroid cancer

WP6, task 6.9: INT-Thyr
WP5, task 5.4:
Mathematical models to link experimental findings and epidemiological data
Radiation-associated DNA gain 7q11/CLIP2 overexpression

Gain of chromosome band 7q11 in papillary thyroid carcinomas of young patients is associated with exposure to low-dose irradiation

Hess et al., PNAS. 108(23):9595-600, 2011
Radiation-associated CLIP2 protein overexpression

Differential protein expression and standardized CLIP2 typing
CLIP2 typing in three independent cohorts

<table>
<thead>
<tr>
<th>Number of cases in</th>
<th>Genrisk-T</th>
<th>Genrisk-T-PLUS</th>
<th>UkrAm</th>
</tr>
</thead>
<tbody>
<tr>
<td>cohorts Exposed</td>
<td>16</td>
<td>32</td>
<td>76</td>
</tr>
<tr>
<td>Non-exposed</td>
<td>17</td>
<td>7</td>
<td>-</td>
</tr>
</tbody>
</table>

- CLIP2 marker with similar frequency in exposed cases of UkrAm and GENRISK-T

Selmansberger et al., Oncogene. 2014, in press
Dose dependency of CLIP2 marker

Dose relationship of CLIP2 in PTC (AaE < 5 years) with individual dose estimates

- **Dose-dependent** occurrence of **CLIP2 marker** in patients at young age at exposure (< 5 years)
- **Increasing frequency** of **CLIP2+ cases** in categories of **moderate and high** doses
- **Different mechanisms at low and moderate doses** compared to high doses?

**Talk by Ch. Kaiser - Session F**
INT-Thyr: Integration of epidemiology and molecular biology (partners from Spain, Germany, Belarus)

INT-Thyr aims to:
- validate 7q11 and CLIP2 biomarkers in a cohort of post-Chernobyl childhood thyroid cancer patients from Belarus
- study the dose-response relationship
- evaluate possible effect modifications of iodine deficiency, age, gender
INT-Thyr: CLIP2 typing in Belarusan PTC (AaE <19 years)

- What is the **dose-response relationship** for this biomarker in Belarusan cases?
- Which factors can lead to **variations of biomarker levels**?
INT-Thyr: CLIP2 typing in Belarussian PTC (AaE <19 years)

**Achievements to date:**

- Biomarker typing of 90 cases
- Identification and tracing of 113 Belarussian patients through roster of operated cases at the Belarus Repulican Centre of Thyroid Cancer (BelMAPO)
- Collection of data about settlements of residence and factors related to radiation dose and iodine deficiency through questionnaires

**Work in progress:**

- Individual dose reconstruction
- Analysis of dose-response relationship with CLIP2 marker
- Iodine deficiency index attribution
- Evaluation of effect modifiers
CLIP2 gene regulatory network

Reconstructed CLIP2 network: global mRNA expression data of UkrAm cases (Abend et al, 2012) – 1st neighbours

- Interaction with RGS4/BAG2/NEURL1 leads to deregulation of MAPK signalling

- KIF3C: link to “genomic/chromosomal instability“

- Validation (qRT-PCR) of all CLIP2 interactions except for GOLM1

Selmansberger et al., Oncogene. 2014, in press
CLIP2 gene regulatory network

Reconstructed CLIP2 network: global mRNA expression data of UkrAm cases (Abend et al, 2012) – 2nd neighbours

- 2nd neighbourhood consists of 218 nodes and 1304 edges
- LMO3 (associated with radiation dose in the study by Abend et al. 2012) is part of the CLIP2 interactome

Selmansberger et al., Oncogene. 2014, in press
DoReMi and EpiRadBio: Mathematical models to link epidemiology and molecular biology

Molecular biology measurements

Epi-data → Molecular biology measurements → PTC risk after exposure to IR

Talk by Ch. Kaiser - Session F
DoReMi and EpiRadBio: Mechanistic models to link epidemiology and molecular biology

Two path model of carcinogenesis for radiation-induced PTC

Molecular surrogate markers for inclusion into models
CLIP2 as surrogate for genomic instability

1st neighbours of CLIP2 suggests that CLIP2 is involved in chromosomal/genomic instability, a hallmark of cancer development

Hypothesis:

CLIP2 overexpression in PTC tissue represents a surrogate marker of genomic/chromosomal instability
CLIP2 measurements on UkrAm cases

**Tumour tissue**

- Biomarker classification
- UkrAm
- Sensitivity: 72.4%

**Normal tissue**

- Continuous

**Binary (negative/positive)**

- CLIP2 marker in PREVALENCE and INCIDENCE of UkrAm
- Prevalence (n=12)
- Incidence (n=47)
MAPK activation signature in normal UkrAm tissues

Hypothesis:

Active MAPK signalling in differentiated non-malignant thyroid tissue indicates early molecular changes during tumourigenesis
MAPK activation signature in normal UkrAm tissues

- Deregulated MAPK signalling is known to be involved in the majority (> 70%) of PTCs (RET/PTC, BRAF V600E)
- active MAPK induces cell proliferation which is a hallmark in cancer development
MAPK activation signature in normal UkrAm tissues

170 genes
MAPK pathway*

match

145 genes

20 top variant genes

hierarchical clustering

top 10 informative MAPK activation genes

* Wikipathways

http://www.wikipathways.org/index.php/Pathway:WP382
MAPK activation signature in normal UkrAm tissues

FOS, DUSP1, DUSP6, JUN, NR4A1, SRF, HSPA1A, GADD45A, MYC
MAPK activation score in normal UkrAm tissues

* BRAF mutation (deep sequencing)
Handing over molecular data to risk modelers

Talk by Ch. Kaiser - Session F
Summary and conclusion

- A novel approach of integrating genomic copy number and gene expression data resulted in the identification of a radiation-specific copy number gain of 7q11 and overexpression of CLIP2 (patients at young AaE).
- An independent validation of CLIP2 was achieved at genomic, transcriptomic and proteomic level.
- A standardized CLIP2 classification workflow was established.
- CLIP2 network reconstruction identified the CLIP2 interactome with functional links to genomic instability and MAPK signaling.
- CLIP2 typing in UkrAm and Genrisk-T showed a dose relationship for patients at young age at exposure (<5 years).
- DoReMi/INT-Thyr: similar CLIP2 results in Belarussian cases compared to UkrAm/Genrisk-T cases.
- EpiRadBio: Molecular measurements of CLIP2 and MAPK activation (normal and tumor tissues) for the development of risk models based on molecular/biological data.
Thank you ....

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MAPK activation signature in normal UkrAm tissues

http://www.wikipathways.org/index.php/Pathway:WP382
Dose dependency of CLIP2 marker

Logistic regression analysis

- CLIP2 negative
- CLIP2 positive

Probability of positive CLIP2 overexpression

- AaE < 5 years
- AaE ≥ 5 years

95% confidence intervals