Genetic risk and anti-inflammatory effects of low doses of different types of ionizing irradiation (GREWIS-project)

Studies using cell and animal models and samples of radon patients

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Emission of $\alpha$-particles during Radon decay: densely ionizing radiation

Noble gas $\rightarrow$ chemically inert; evaporates from rocks

Radioactive decay chain

**Photons**
- $\gamma$, X-rays
  - Sparsely ionising
  - Dose contribution/ photon very low
  - Homogeneous distribution in the tissue
  - All cells are irradiated

**Charged particles**
- $\alpha$-particles, protons, HI
  - Densely ionising
  - Major dose contribution
  - Dose deposition/ $\alpha$-particle/nucleus 0.2-0.5 Gy
  - 0.5 Gy = 20% of the cells in the tissue are irradiated

$\rightarrow$ Enhanced biological efficiency
$\rightarrow$ Increased risk for lung cancer at high doses
Entries of radon in the organism

Organs with epithelial tissue
1. Lung
2. Skin
3. Gastrointestinal tract

Diffusion/transport (radon)

→ Fast passage of Rn through the body
  Only 0.2 % of the Rn atoms decay during the passage
  Relevant half-life time of the daughter nuclei ~ 50 Min.

+ Deposition of short lived progeny

α-particles: 30 - 70 µm
c.a. 100 µm
Radon exposure: genetic risk?

No enhanced risk?
Epidemiological evidence for an increased risk for lung cancer
Anti-inflammatory effects?
Pain relieve?

WP3: N. Paz, E. Nasonova, S. Ritter
Reference dose-response curves for $\alpha$-exposure (in vitro)
→ high number of aberrant cells
→ high number of break points/ cell
→ complex aberrations
(A,B: 4 breaks in 3 chromosomes)

Effective dose/ year
Sv

Mice bone marrow

Healthy donor

Patients Bad Steben Bad Gastein

$\alpha$-source (Americium)
**Dosimetry and risk assessment**

- Physical dosimetry
  - penetration depth and diffusion of radon
    - AP 1 Kraft

- Biological dosimetry
  - formation of γH2AX foci
    - AP 2 Löbrich

- Estimation of dose and radiation risk
  - cytogenetic analyses
    - AP 3 Ritter

**Inflammation and associated processes (cell death, pain)**

- functional changes and signal transduction (humoral/ cholinergic)

  - Humoral mechanisms
    - cellular and molecular interactions in blood vessels and bones
      - AP 4 Fournier
  - Cholinergic mechanisms
    - anti-inflammatory reactions
      - AP 6 Layer

- Intracellular signal transduction
  - regulation of adhesion molecules
    - AP 5 Cardoso
  - role of NFκB
  - role of NFκB
  - role of NFκB

- Discontinuous dose-dependency
  - AP 8 Rödel

- Immunological danger signals and inflammation
  - AP 9 Gaipl

**Model systems**

- **mouse**
  - wild type
  - hTNF transgenic
  - rheumatoid arthritis

- **human**
  - healthy donors
  - patients

  - primary cells
    - (e.g. blood, bone marrow)
    - mono- and co-cultures

- primary cells
  - (blood)
  - mono- and co-cultures

**Radiation**

- low and high dose ionizing irradiation
  - (in vitro exposure)
    - photons
    - radon (AP 1 Kraft)
    - α-particles (AP 2 Löbrich)
    - carbon ions (AP 1 Kraft)
Modulation of the chronic inflammation after radon exposure: immune status of the patients

100 patients with musco-skeletal diseases (Bad Steben)
Blood samples
Medical examination (CVD + pain)
Medication

30 subtypes of immun and stem cells (surface marker)
Markers of inflammation (cytokines, chemokines), cell death

Shift of the balance between immune cells with antagonistic functions?

Human primary cells CD4+
Photons (X-ray) + TGF-β
7 days

→ $T_{H17}$ cells unchanged (inflammatory stimulation)
→ $T_{reg}$ cells: increase after irradiation

WP 9: P. Rühe, B. Frey, U. Gaipl
WP 4: A. Groo, C. Fournier
Radon exposure under defined conditions

Radon chamber is ready to be used (stability tests, cell growth curves) →
Irradiation of cells started+ small animals (rodents, start November 2013)
Activity: 40 kBq/m\(^3\) (galleries) → 600 kBq/m\(^3\)
Dose rate ~1.3 mGy/h (will be increased → source with higher activity)

First measurements of activity:
• Absorption of Radon on 4.8g carbon/ water (under work)
• γ-spektrum → activity
Noble gas radon: distribution in the organism?

Passiv transport
Diffusion
Respiratory tract (inhalation)
Skin (contact)
Gastrointestinal tract (ingestion)

Active transport
Vascular system

Physical dosimetry
1. Measurements of activity after exposure of different tissues (under work)
   → Solubility coefficients (ratio of activity in different tissues)
   → Retention time (activity in a tissue over time)
2. Dose estimations: Lung >> Alveolar capillaries > Red bone marrow > Fatty tissue

Biological dosimetry
1. In vitro exposure of HeLa cells to α-particules (241Americium)
2. In vivo exposure of C57BL/6 mice to carbon ions (Radon scheduled for Nov. 2013)
Rheumatoid arthritis: disease of the bones and joints

Bones and cartilage  →  target of Radon exposure?

[Morbus Bechterew Journal 2006 No. 107]

→ Changes in differentiation and activity of bone resorbing cells?
Noble gas radon: accumulation in bone tissue?

Immunofluorescence staining of DNA damage markers (CLSM)

WP2: M. Steinlage, M. Löbrich
Low dose exposure influences bone erosion and density

Mouse model for human polyarthritis

Improvement of joint function?

X-ray (α-particles radon).

Inflammation? H&E staining

Bone erosion? TRAP assay

→ Reduction of inflamed areas and bone erosion
→ Increase in bone density (not shown)
Radiation exposure modifies differentiation of osteoblasts and activation of osteoclasts

**Human primary cells: MSC/ monocytes**

**WP4: A. Groo, D. Kraft, C. Fournier**

**Inactivation of osteoclasts by irradiation?**
- TRAP pos. > 3 nuclei / F-Actin ring / integrin
- → Cell number and differentiation unchanged

**Release of factors modifying osteoclast activity by osteoblasts?**
- → Accelerated differentiation
- → Release of OPG ↑
..and for your attention!

DoReMi Workshop, Budapest, 5th of November 2013