Oncothermia in laboratory

Dr. Nora Meggyeshazi¹, Dr. Tibor Krenacs¹, Dr. Gabor Andocs², Dr. Oliver Szasz³

(1) 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
(2) Department of Veterinary Clinical Medicine, Faculty of Veterinary Science, Tottori University, Tottori, Japan
(3) Department of Biotechnics, Faculty of Engineering, St. István University, Budapest, Hungary
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Theoretical background: The hallmarks of cell death

When is the cell dying? (=point-of-no-return)
- Massive caspase activation
- Mitochondrial transmembrane potential decreases (mitochondrial membrane permeabilization)
- PS appears in the outer membrane of the cytoplasm.

When is the cell dead?
- The integrity of plasma membrane is lost
- The cells is fragmented
- The surrounding cells phagocytizing the dead ones.

How many ways to die?
A LOT…
... Extrinsic apoptosis, Caspase dependent intrinsic apoptosis, Caspase independent intrinsic apoptosis, Necroptosis (regulated necrosis), Autophagic cell death, Mitotic cathastrophe, Netosis, Parthantos, Pyroptosis Entosis

BUT
What can happen after the cell death? (possible interacions with the immune system)
- Inflammation can occure after accidental necrosis (professional phagocytes are involved)
- No immune reaction (usually in physiological apoptosis)
- Immunogenic cell death can occur after specific apoptosis inducers (photodynamic therapy, chemotherapy)
- Calreticulin exposure
- Membrane appearance of hsp70
- ATP release
- HMGB1 release

} Temporospatial pattern on the tumor cells in ICD
Material and method I: the model and treatment

BALB/c (nu/nu) mice inoculated with HT29 (human colorectal adenocarcinoma) in both femoral region (3*10^6 cells/0.1 ml) of 6-8 week old females

18 days later single shot treatment for (30 min), temperature between 41-42 °C. Sampling was carried out: 0, 1, 4, 8, 14, 24, 48, 72, 120, 168, 216h post-treatment

18 days later 30 min single shot treatment (treated tumor core temperature: 41-42 °C)

Material and method II: molecular biology

<table>
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<tr>
<th>Protein name</th>
<th>Clon</th>
<th>Producer</th>
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<tbody>
<tr>
<td>TRAIL-R2</td>
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<tr>
<td>CRT</td>
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<td>Cell Signaling</td>
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</tbody>
</table>
Results: Apoptotic bodies and DNA fragmentation

Are there signs of immunogenic cell death?
Hallmarks of ICD: CRT
Hallmarks of ICD: membrane hsp70
Heat shock protein 70 (HSP70)

![Graph showing HSP70 expression and molecular changes](image)

**Hallmarks of ICD: HMGB1 release**

**Summary**

Oncothermia causes programmed cell death (as an obligatory event in ICD) with concomitant TRAIL-R2, calreticulin, heat shock protein upregulation and HMGB1 release from the nuclei.