Bystander effect of Oncothermia

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Introduction

Oncothermia (OTM) is an active hyperthermia modality, a long-time since 2005 applied method in oncology with great clinical success. OTM changes the paradigm of hyperthermia by targeted microspores heat liberation in the membranes of the nanogranules. This method creates homogeneous heating, microscopic temperature differences far from thermal equilibrium. The tumor destruction efficacy and the role of temperature independent effects of the OTM was proven earlier by laboratory research, and proven by clinical trials.

Hypothetic bystander effect (or direct effect) means that a single tumor treatment can affect the behavior of the tumor metastases. It was first discovered by radio- oncologists and remained highly controversial topic until recent years. New, intensive research is conducting to reveal the molecular and cell biological basis of OTM bystander effect.

Methods

Animal model:
HT29 human sigmoid carcinoma cell line derived xenograft tumor model in nude mice.

Experimental setup and treatment:

A single 10 or 20 min Oncothermia treatment was done, reaching maximum AT 42°C metastatic temperature using the LabTHERM system (Oncothermia Ltd) under precise temperature control using targeted temperature measurement system Eurotherm 6400.

Study design:
Time course study was performed. After a single single-shot treatment, sampling was made after 0, 1, 4, 11, 24, 48, 72, 120, 180, 216 hours. 3 mice were sacrificed at each time point, keeping 5 sham treated animals.

Tumor sample processing I:

24 h later, the treated and control tumors were removed and stained in paraffin.

Tumor sample processing II:

Due to the extremely high tumor density, tumor microarray (TMA) technology was used to perform accurate immunohistochemical reactions on many samples in one block.

Immunohistochemistry (IHC):

The following reactions and IHC analysis were performed on the TMA samples: TUNEL (Envirogen), TRAIL, DR4/5 (Cell Signaling), Myosin heavy chain (Dako), CD10 (DAKO), CD73 (Medical Imaging).

Digital microscopy analysis:

All histological slices were scanned using Hamamatsu G1 Scanner (12 MPix) and special software was used for imaging and evaluation.

Results

1. Histomorphological changes:

Morphologically the first significant sign of cell destruction was seen 4h after the treatment. Drain and selective Tumor-destruction was detected 24h after OTM which became more emphasized after 48h. 72 hours after the treatment a significant edematous infiltration connected with red arrows appeared around the destroyed tumor tissue and reached its maximum 150 hours after the treatment.

2.1 Apoptotic body formation

Oncothermia treatment induced apoptotic cell death. Indeed all the cell nuclei of the treated tumor cells are TUNEL positive. In the process of this programmed cell death many apoptotic bodies were formed (crushed with red arrows).

2.2. TRAIL (DR4S) expression

TRAIL/DR4S is a highly immunogenic cell surface receptor. Expression was increased in the treated side 4h after the treatment and became more emphasized after 144h.

2.3. HSP70 expression changes and molecular dynamics

(Oncothermia) increase of the HSP70 expression was observed 4h hours after the treatment. After 24 hours, unusual molecular dynamic changes of the increased amount of HSP70 can be mobile: intracellular condensation (1) and relocation to cell membra. After 48 hours the membrane relocation of the HSP70 became more emphasized, especially in the region of the leukocyte invasion (1).

2.4. Strong local immune reaction

The leukocyte invasion ring which appears at 72h and became very characteristic at 168h around the destructed tumor area, contains high number of MIF positive cells: (neutrophils, macrophages).

Conclusions

1. Oncothermia can induce programmed cell death which create many apoptotic bodies
2. Oncothermia induced cell death is highly immunogenic, showing all the key molecular pattern dynamic changes what is characteristic of immunogenic tumor cell death
3. Oncothermia treatment can induce strong and very unusual immune reaction at the site of the treatment
4. The local anti tumor immune reaction can be systemic, if the host has an intact immune system, and this process can control the distant metastases by bystander effect, making possible the systemic control of the malignant disease with local treatment.

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