

## DIFFERENTIAL EFFECTS OF 42°C-HYPERTHERMIA ON RADIATION RESPONSE OF BREAST CANCER SPHEROIDS VS. NORMAL HUMAN SKIN EXPLANTS

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### Abstract

**Background:** Recurrent tumors in previously irradiated regions remain a challenging situation, in which hyperthermia (HT) of 42°C serves to sensitize cancer cells to radiotherapy. For radiosensitization of superficial tumors, loco-regional HT can be applied in a contact-free manner by using water-filtered infrared A-(wIRA-) radiation. Starting from January 2018, our institution is treating patients with locally recurrent breast cancer using hypofractionated re-irradiation (5 x 4 Gy; 1x/wk.), shortly following wIRA-HT. Despite distinct response of cancer lesions, only grade I skin toxicities were observed. The biology underlying such favorable effects of combined treatment remains poorly understood. Therefore, this study aimed to follow the clinical protocol in vitro.

**Methods:** Human breast cancer cell lines were cultured as multicellular aggregates and treated weekly with HT at 42°C for one hour in a water bath. Hyperthermia was applied with or without simultaneous wIRA radiation (75 mW/cm<sup>2</sup>) to assess possible non-thermal effects of wIRA. Cultures were subsequently  $\square$ -irradiated with a single dose of 4 Gy up to a total dose of 16-20 Gy. Additionally, T47D breast cancer cells were treated with different treatment schedules. Response was monitored by cell aggregate volume measurements for a total of 8 weeks and evaluated by paraffin histology. As a normal tissue model, skin biopsies were treated with a single fraction of water bath HT +/- wIRA radiation, subsequent  $\square$ -irradiation and subjected to an ex vivo wound healing assay.

**Results:** Growth rates of normal cells and tumor cell aggregates were not impaired by HT or wIRA alone. When HT and  $\square$ -irradiation were combined, a significantly higher 'cure rate' of breast cancer spheroids was found, compared to irradiation alone. Maximum treatment effects were obtained with simultaneous hyperthermia and irradiation, while increasing intervals between the two treatments continually reduced 'cure rate'. In skin explants, cell outgrowth declined with increasing irradiation doses, but was not significantly reduced by addition of HT +/- wIRA.

**Conclusion:** Hyperthermia (42°C, 1 hour, induced by water bath) radiosensitizes 3D breast cancer cell cultures. Simultaneous wIRA radiation did not cause any additional effect, so non-thermal effects of wIRA could not be observed in the in vitro models used. The synergistic effect of HT and  $\square$ -irradiation was inversely correlated with time intervals between the two modalities, which emphasizes the role of adequate timing. Interestingly, the function of normal epidermal keratinocytes was not significantly impaired by addition of HT. These data thus support the clinical findings and may help to reveal the differential effect of thermo-radiation on malignant and normal tissue.