COOPERATIVE EFFECT OF RADIOTHERAPY AND GOLD NANOPARTICLE-INDUCED HYPERTHERMIA IN CANCER TREATMENT

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INTRODUCTION: So called cell-based therapies, treatments in which stem or progenitor cells are induced to home within damaged or cancer tissues, and nanomedicine, which relies on the use of nanoparticles (NPs), are becoming outstanding research areas in personalized tumor therapy. Despite continuous technical advances, the radiation-induced toxic effects in adjacent healthy tissues still represent the dose-limiting factor. The aim of our study is to develop an efficient therapeutic strategy to control tumor growth and progression based on the combination of nanomedicine and cell therapy with radiation therapy.

EXPERIMENTAL: Endothelial colony forming cells (ECFCs), a subtype of Endothelial Progenitor Cells, with inherent tumor tropism capability [1,2], were chosen to carry AuNPs to tumor cells and were used in co-culture experiments with unloaded melanoma (M6-A375) or breast cells (MCF7). The long-term cytostatic/cytoxic effects of combined radiotherapy and nano-mediated hyperthermia were evaluated using clonogenic assays while the short-term effects were determined evaluating DNA damage by comet assay and cell cycle arrest and autophagy western blot analysis.

RESULTS AND DISCUSSION: We have shown how the cooperative effect between irradiation and hyphertermia is much more effective in MCF7-ECFCs co-colture than M6-ECFC cells. We observed increased levels of γH2AX, also confirmed by comet assay after the combo treatment. Moreover, the combined treatment induces a significative decrease of lc3, a well known marker of the autophagy process which desensitizes cancer cells to radio therapy

Thermographic Analysis

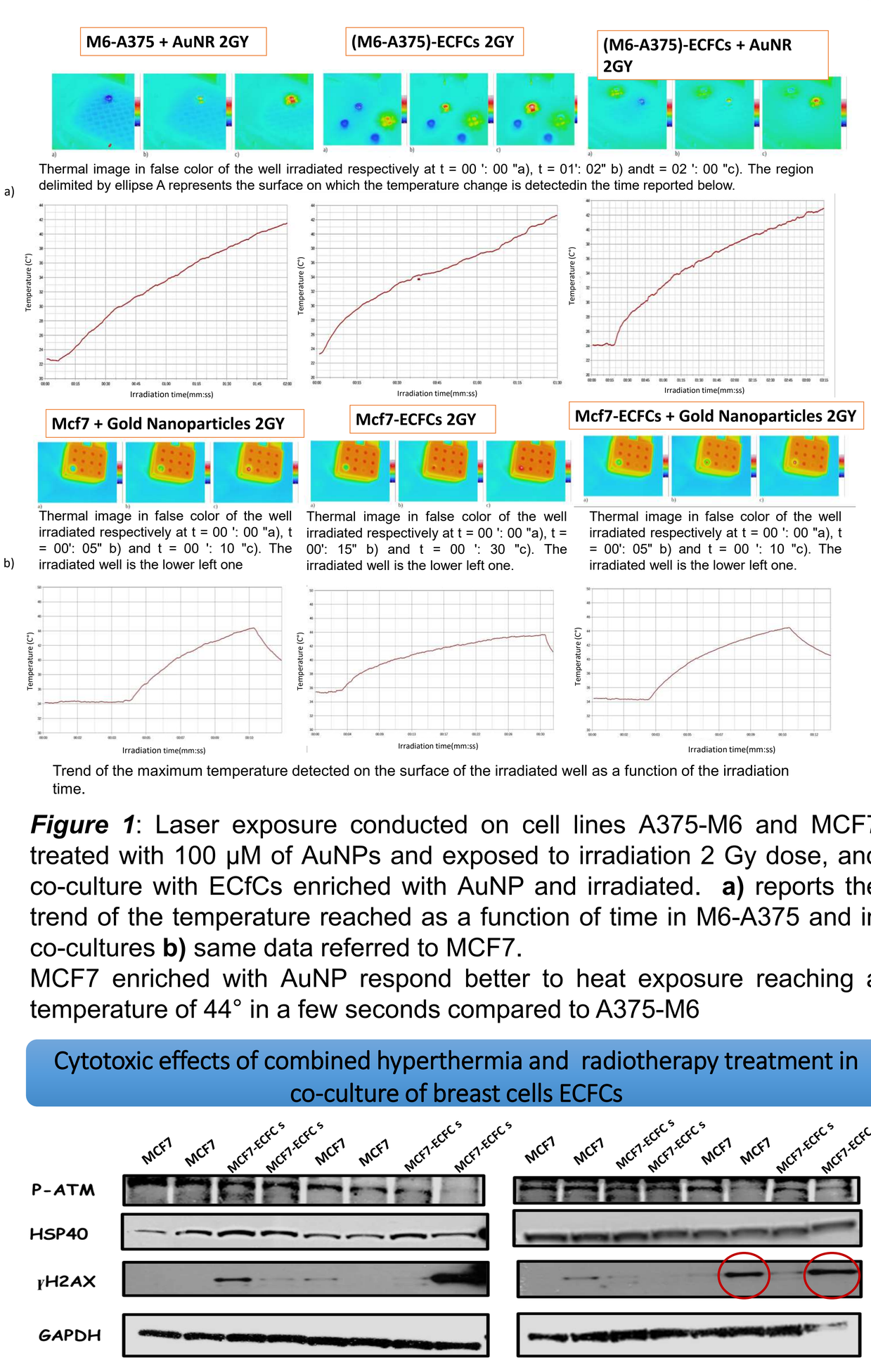

Figure 1: Laser exposure conducted on cell lines A375-M6 and MCF7 treated with 100 μM of AuNPs and exposed to irradiation 2 Gy dose, and co-culture with ECfCs enriched with AuNP and irradiated. **a)** reports the trend of the temperature reached as a function of time in M6-A375 and in co-cultures **b)** same data referred to MCF7.
MCF7 enriched with AuNP respond better to heat exposure reaching a temperature of 44° in a few seconds compared to A375-M6

Cytotoxic effects of combined hyperthermia and radiotherapy treatment in co-culture of breast cells ECFCs

Figure 4: Evaluation of cellular damage by western blot assay. Specifically, the levels of P-ATM involved in the mobilization and regulation of the cellular response to DNA double strand breaks and γH2AX were examined. Although the P-ATM levels are almost similar in the various treatments, there is instead an increase in γH2AX levels, specially in the combo treatment enriched with AuNPs. Furthermore, we evaluate an increase in Hsp40 after laser exposure

Short-term effects of combined hyperthermia and radiotherapy treatment in co-culture of breast cancer cells and ECFCs

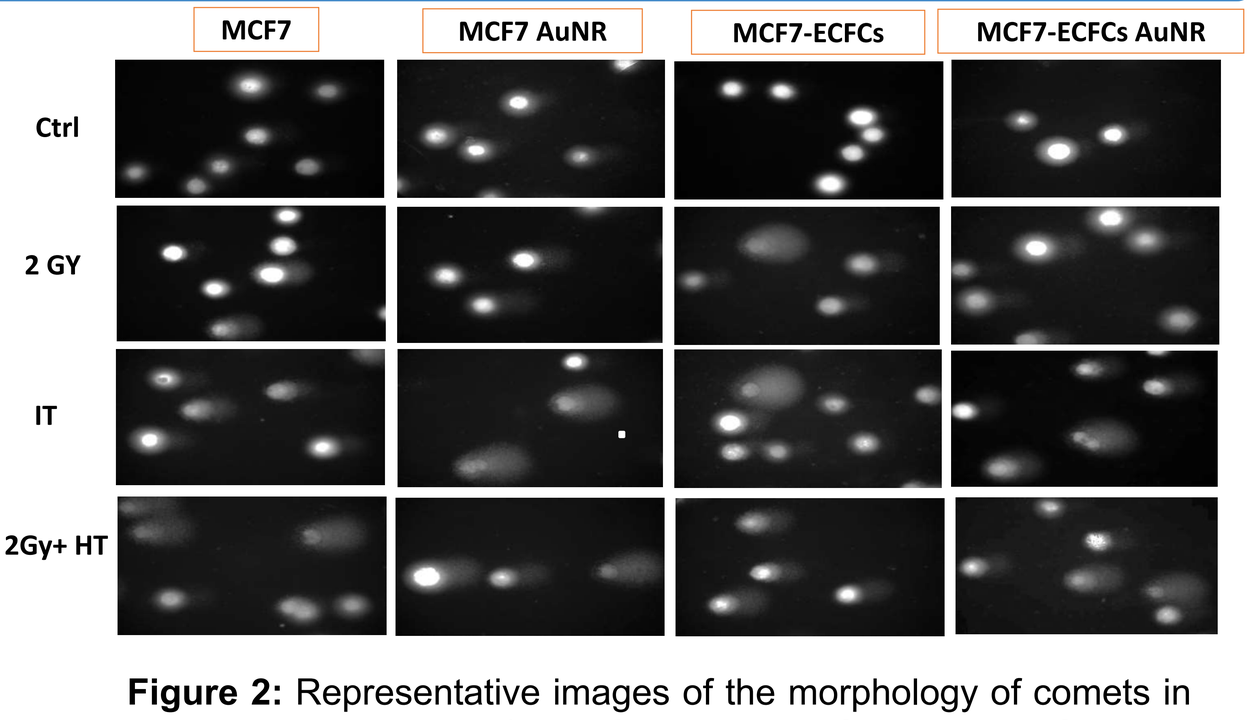

Figure 2: Representative images of the morphology of comets in the various treatments (ctrl, 2Gy, HT and 2 Gy + HT in MCF7 and co-culture MCF7-ECFCs.

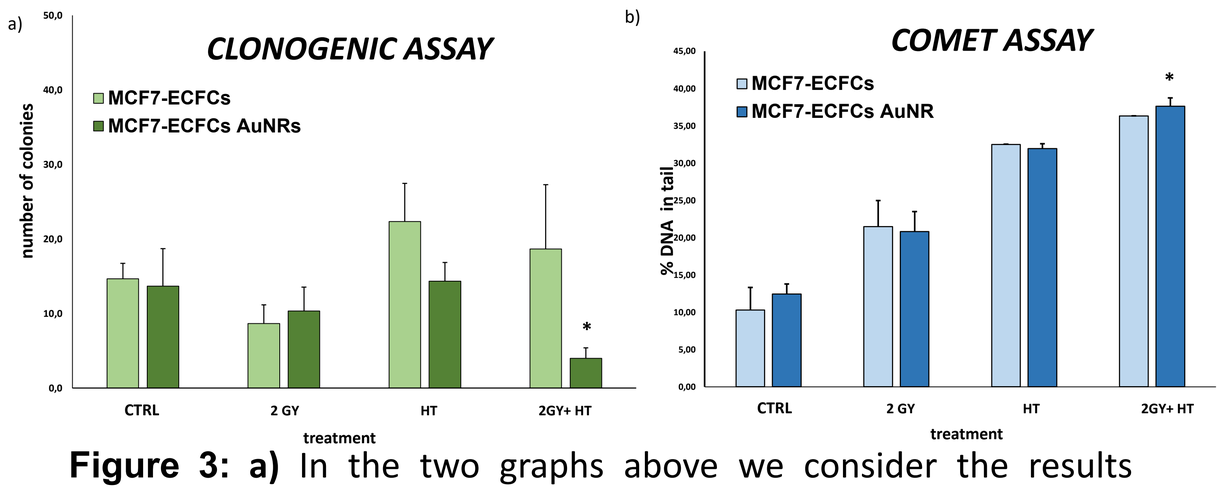

Figure 3: a) In the two graphs above we consider the results obtained in the co-cultures MCF7-ECFCs.
a)The long-term cytostatic / cytotoxic effect was evaluated by clonogenic assay: the number of colonies formed significantly decreases in the combined treatment. **b)** Considering the levels of basal breakages in the DNA obtained from the comet assay: after laser treatment and in combined therapy they significantly increase compared to untreated controls

RESULTS AND DISCUSSION: We have shown how the cooperative effect between irradiation and hyphertermia is much more effective in MCF7-ECFCs co-colture than M6-ECFC cells. We observed increased levels of γH2AX, also confirmed by comet assay after the combo treatment. Moreover, the combined treatment induces a significative decrease of lc3, a well known marker of the autophagy process which desensitizes cancer cells to radio therapy.

CONCLUSIONS: AuNPs are confirmed to be as excellent radiosentizers and thus allows to shorten the duration of the tratment and to reduce the radiation doses. The combo treatment of MCF7-AuNP enriched ECFCs inibits autophagy.

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