EFFECT OF NANOPARTICLE-DRIVEN HYPERTHERMIA IN BIOMIMETIC NEUROBLASTOMA MODELS

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Introduction

Neuroblastoma (NB) is a rare developmental cancer. It is usually located in the abdominal region around adrenal glands, since it is a result of a defective evolution of the neural crest cells. Unfortunately, high-risk neuroblastoma causes relapse in approximately the 50% of affected children, eventually leading to child death [1-2].

Current translational gap created by conventional models hinders the development of efficient treatments. Therefore, there is a crucial necessity for innovative neuroblastoma models able to recapitulate complexity of human NB tumors. One of the main research fields on NB involves the production of biomimetic models resembling human tumor's core aspects. These cutting-edge models introduce complex features such as tumor composition in terms of cells and matrix, or three-dimensional structure. Additionally, they offer a more realistic representation of tumor aggressiveness, which is not achieved with traditional 2D models [3-4].

In parallel, bioengineering is also focused on the development of more efficient therapies, most of them based on nanomedicine principles. Emerging nanotherapies take advantage of the enhanced effect and selectivity provided by the addition of nanoparticles (NPs). Particularly, superparamagnetic iron-oxide nanoparticles (SPIONS) stand out as suitable candidates due to their double function of (1) absorbing near-infrared light, and (2) magnetism. SPIONS are being recently tested for hyperthermia techniques, consisting in exposing the tumor to temperatures above the physiological limits, causing cell death or cell sensitization. Therefore, we should highlight two hyperthermia techniques which will be highly favored from SPIONS's double functionality: photothermal therapy (PTT), and magnetic hyperthermia (MH). SPIONS in PTT are activated by local irradiation of a specific region of the tumor with a near-infrared light laser, and transform optical energy into heat. In MH, the whole tumor is homogeneously exposed to external alternating magnetic fields, so SPIONS convert the magnetic stimuli into heat [5-6].

Within this research, two novel nanoparticle-driven hyperthermia treatments (PTT and MH) were tested in biomimetic neuroblastoma models.

Methodology

In first place, we functionalized magnetite-based SPIONS with glucose, boosting tumor internalization of the nanoparticles. Then, three-dimensional models of human NB tumors were fabricated using tissue-engineering (TE) techniques. The TE-NB models included both the main components of human NB, collagen I and hyaluronic acid, and cells from SK-N-BE(2) NB cell line. Cells were seeded and cultured in the models for seven days. Another scientific publication by the same authors detailed these two fabrication steps [7].

Finally, glucose-modified nanoparticles were added to the TE-NB models. Samples were treated with the hyperthermia techniques (PTT or MH) 24h after the addition. Impact of the treatment was studied overtime (days 1, 2 and 5).

We evaluated the impact on cell apoptosis by immunofluorescence. Activation of caspase 3-7 apoptotic cascade was checked by staining histological slides with CellEvent™ Caspase-3/7 Green ReadyProbes™ Reagent (#R37111, ThermoFisher).

We quantified cell proliferation using the Quant-iT Picogreen dsDNA Assay Kit (#P7589, Invitrogen).

Results and discussion

Activation of caspase 3-7 apoptotic pathway

Regarding TE-NB exposed to PTT, we noticed activation of the apoptotic cascade in both scenarios with and without the nanoparticles (Figure 1). These results indicate that PTT in these experimental settings is influencing the studied apoptotic route of neuroblastoma cells independent of the addition of nanoparticles. Besides, activation in the edges of the models suggests unexpected propagation of the effect, since the models were irradiated locally only in the inner core. In contrast, TE-NB models treated with MH showed activation of the apoptotic cascade only in the condition with nanoparticles (Figure 2). These data indicate that an additional level of selectivity is being achieved with these experimental conditions for MH.

Picogreen quantification of cell proliferation

We studied cell proliferation at 1, 2, and 5 days. Regarding PTT, no significant differences were found between conditions (Figure 3A). Nevertheless, MH-treated samples showed a significant decrease of tumor cell proliferative activity with respect to the control condition at day 2 (Figure 3B).

Conclusion

Considering the experimental conditions for this project, PTT is not recommended as optimal for suppressing tumor growth. However, results from MH propose this technique as a promising nanoparticle-based hyperthermia approach.

References

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Figures

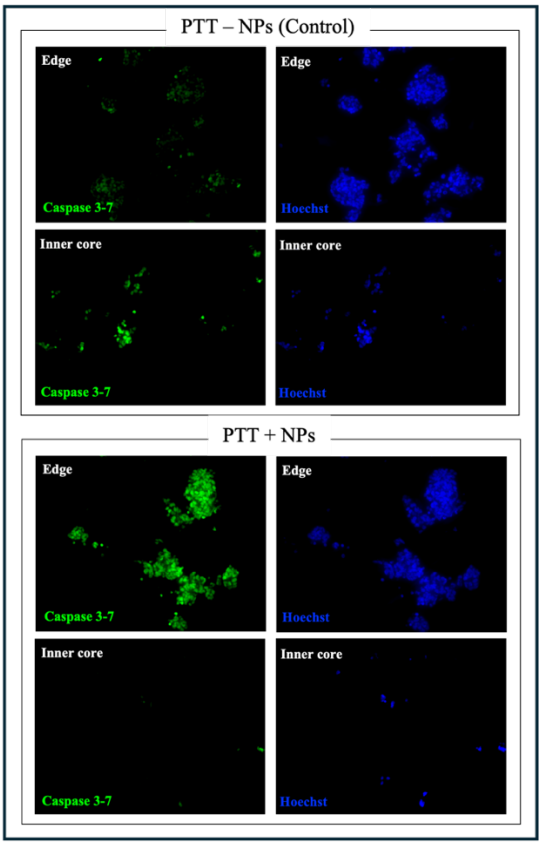

Figure 1. Activation of apoptotic caspase 3-7 cascade in TE-NB models subjected to PTT.

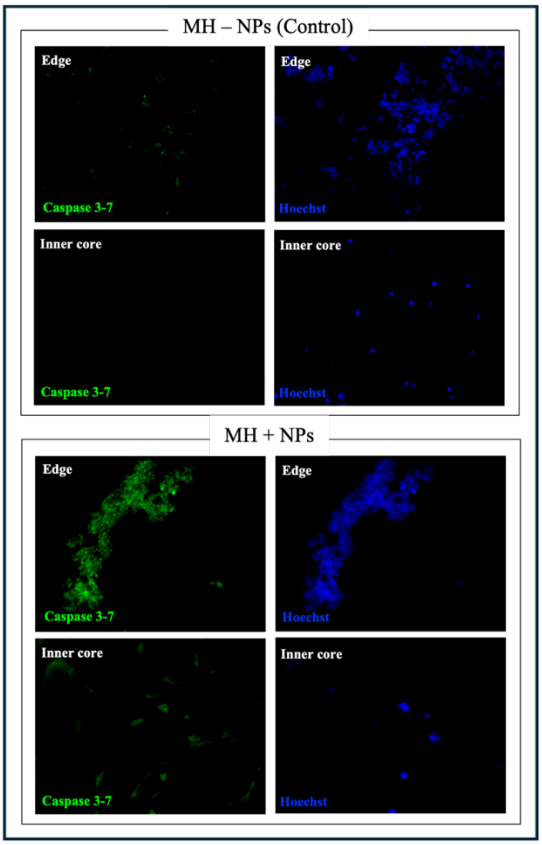

Figure 2. Activation of apoptotic caspase 3-7 cascade in TE-NB models subjected to MH.

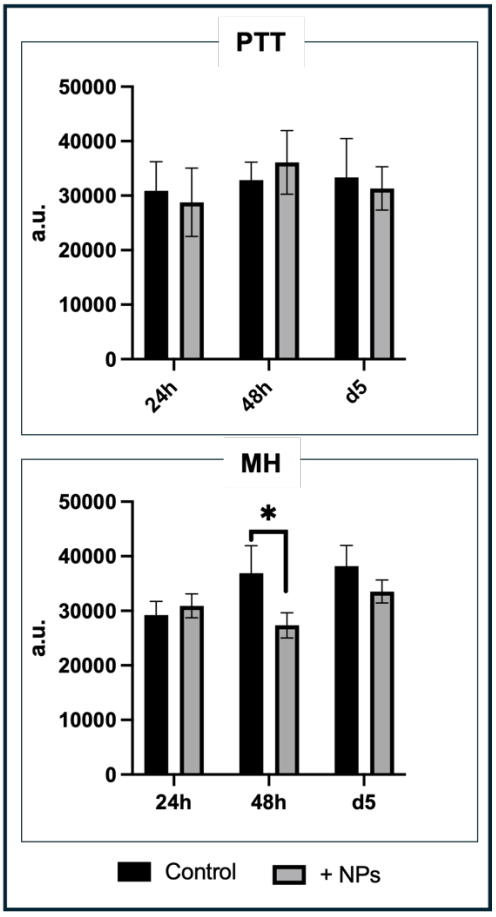

Figure 3. Picogreen quantification of cell proliferation in TE-NB models subjected to (A) PTT, and (B) MH. P-values < 0.05 are marked with "*"