Assessment of molecular effects caused by magnetic hyperthermia in cultured cells and in an invertebrate animal model

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In recent years, nanoparticle-mediated hyperthermia has been proposed as a valid alternative to conventional thermoablation, which is currently associated to more invasive treatments for cancer therapy. So far, many nanostructured materials with adequate physical properties (optical, electrical, magnetic, thermal) have been synthesized to improve hyperthermia efficiency and targeting [1]. Noteworthy, magnetic nanoparticles (MNPs) have demonstrated superior heating capabilities, striking features for biofunctionalization together with negligible hazard effects *in vitro* [2,3]. Current research is focused on tuning NP heating properties, enabling controlled hyperthermia in a space and time selective fashion. While most of the studies relies only on *in vitro* cell culture assays, massive use of animal models with few bioethics restrictions is striving required to bridge from cell research to vertebrates and pre-clinical studies. Herein, we propose a gradual and comparative study performed on the highly metastatic murine melanoma cell line B16-F10 and the freshwater polyp Hydra vulgaris as a novel invertebrate model for reliable screening and validation of nano-heaters properties.

In this study, while B16 cells are proposed as an easy model of carcinogenesis *in vitro*, a further step is the use of animal models. Hydras, whose body organization and the lack of organs allows easy uptake and tracking of any test NPs, may enable immediate evaluation of hyperthermia effect induced upon remote magnetic activation of the NP [5].

B16 cells as well as Hydra animals were incubated with 20nm MNPs synthetized by thermal decomposition [6]. Following NPs internalization (assessed by confocal microscopy), alternant magnetic field was applied to MNP-treated cells/animals. After the thermal treatment potential cell/tissue damages induced by local heating were monitored by a multitude of approaches: i) in vivo through optical microscopy (in case of animals) ii) in vitro on cells using apoptotic/necrosis cell markers (ex vivo on Hydra isolated cells), and iii) at molecular level, by assessment of heat shock gene expression via qRT-PCR.

We provided evidences that applying alternant magnetic field does not induce any morphological changes or apoptosis/necrosis damages in case of cells and animals. Instead of that, the elevated expression of heat shock protein hsp70 was observed, showing different expression profiles in animals than in cells. What is more, it was demonstrated that increased hsp70 expression induced by magnetic hyperthermia treatment can be also obtained by incremented incubation temperature.

Overall, our results indicate that at the molecular level exists a strong correlation between cells and Hydra animals. In addition, Hydras as a small, easy to handle invertebrates are excellent tools to test nanomaterials before reaching other steps as vertebrate animals.

References

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Figures

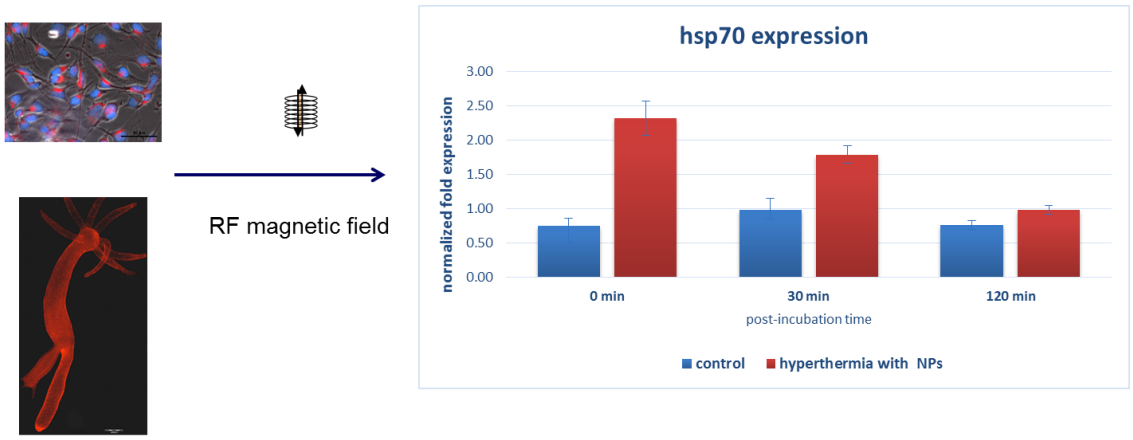

Fig.1 Molecular analysis of heat shock protein hsp70 expression after hyperthermia treatment in B16 cells and in Hydras.