Pluronic Activity in Hyperthermia-induced Tumor Cell Death

David Dremann[1], Tianyi M. Krupka[1,2], Ann-Marie Broome[1,2], Agata A. Exner[1,2].
Departments of Biomedical Engineering[1] and Radiology[2], Case Western Reserve University, Cleveland, OH 44106

Statement of Purpose: Radio frequency ablation (RFA), an image guided cancer therapy, delivers thermal energy to a tumor providing localized tissue destruction. Tumor recurrence due to sublethal heat levels deposited at the tumor periphery presents a considerable challenge in this approach. The long term objective of this research is to improve RFA outcomes by developing a thermosensitizing agent to increase cancer cell susceptibility to heat injury. Pluronics are triblock copolymers of polyethylene oxide, (EO), and polypropylene oxide, (PO). Previous studies in our group have shown a promising linkage between Pluronic and increased thermosensitization of cancer cells [1-2]. We hypothesize that the observed thermosensitizing effect is, in part, due to the downregulation of heat shock protein 70 (HSP70), a chaperone protein upregulated under stress. The objective of this study was to examine the effect of Pluronic in conjunction with hyperthermia on HSP70 expression in cancer cells.

Keywords: Hyperthermia, sensitizer, HSP70, Pluronic

Methods: DHD/K12/TRb rat colorectal carcinoma cells were maintained in RPMI 1640 and passaged weekly. Pluronic L61 (EO_2-PO_{32}-EO_2) and P85 (EO_{26}-PO_{40}-EO_{26}) were used to treat the cells at previously determined optimal concentrations and times of 120 min at 0.3 mg/ml for L61 and 240 min at 10 mg/ml for P8 followed by exposure of cells to low grade hyperthermia at 43°C for 30 min [2]. Specific treatment regimens were examined: *pretreatment*: Pluronic applied prior to and during heat; *acute*: applied only during heat; *pre-acute*: applied only before heat. Protein expression was analyzed using Western blot analysis (12% SDS-PAGE). Each lane was loaded with an equal concentration of whole cell lysate. Nitrocellulose immunoblots were incubated first with mouse anti-rat HSP70, then with HRP-conjugated sheep anti-mouse and finally incubated with chemiluminescence substrate before exposure to x-ray film. β-actin staining was used to verify equal protein loading. Quantification was completed on a BioRad VersaDoc utilizing Image J software.

Results: Following hyperthermia alone HSP70 expression was initiated one hour after treatment and reached a steady state level from 9-24 hrs (Fig. 1-2). Untreated cells were found to have negligible endogenous HSP70, and neither P85 nor L61 alone induced HSP70 expression. Upon the application of Pluronic in conjunction with hyperthermia, results showed that HSP70 production was substantially downregulated. The duration of HSP70 downregulation was different for the two Pluronics (6 hrs for P85 and 3 hrs for L61) after pretreatment. This may suggest a linkage between sensitizing efficiency and structure of Pluronic. (Fig. 3-4). Pre-acute treatments also lead to HSP70 downregulation (duration: 6 hrs for both P85 and L61). However, acute treatment proved to be the most effective, with HSP70 downregulation continuing for up to 9 hrs for L61.

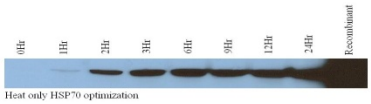

Figure 1: Heat only HSP70 optimization

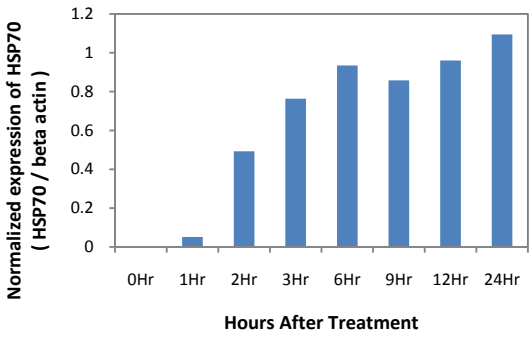

Figure 2: Quantification of HSP70 control

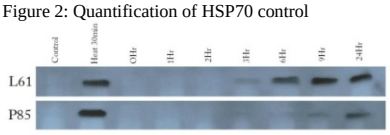

Figure 3: Representative blots for L61 and P85

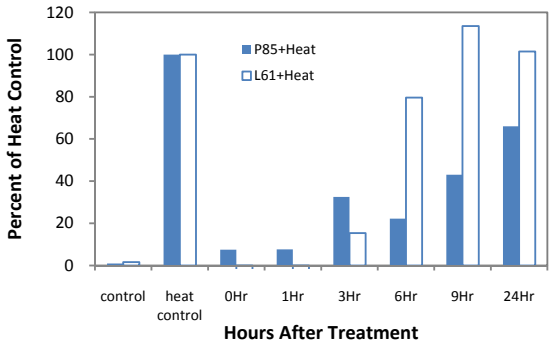

Figure 4: Quantification of Pluronic and heat treatment

Conclusions: Both Pluronic L61 and P85 result in downregulation of hyperthermia induced HSP70 expression in DHD/K12/TRb colorectal carcinoma cells in vitro. This observation may explain why cells are more vulnerable to heat related injury [2], thereby leading to improved toxicity of the combined Pluronic/ hyperthermia treatment. Ongoing studies are examining this effect in normal noncancerous cells as well as cancer cells expressing the MDR phenotype. Additional studies will examine the dependence of this activity on Pluronic structure.

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References:

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