

## EARLY GLOBAL GENE EXPRESSION *IN VIVO* AND *IN VITRO* AFTER TREATMENT WITH HYPERTHERMIA.

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**Purpose.** Early global gene expression was analyzed after treatment with hyperthermia *in vivo* and *in vitro*. We have previously presented that both a metronomic dosage regimen of CTX ( $p = 0.006$ ) and hyperthermia ( $p < 0.001$ ), significantly delayed the time for the tumor to reach four times the initial volume in an experimental brain tumor model. A combination of the two regimens exhibited significantly better tumor control than the two modalities separately (both  $p < 0.001$ ).

**Materials and methods.** BT<sub>4</sub> An aggressive rat glioblastoma-like tumors were serially transplanted orthotopically on the right hind foot in BD IX rats. One group was treated with a metronomic dosage regimen of the alkylating agent cyclophosphamide (CTX) at doses of 35 mg/kg i.p. administered three times a week for two weeks. Another group received local water-bath hyperthermia at 44.1 +/- 0.1 °C administered for one hour. A third group had both modalities combined, and a fourth group served as placebo treated controls. In a separate experiment tumor cells were also treated *in vitro* with hyperthermia at 43°C for one hour. We collected samples *in vivo* and *in vitro* up to three hours after completed treatment day zero, isolated mRNA according to standard protocols, and analyzed early global gene expression using Applied Biosystems Rat Genome Survey Microarray analyzing 26.857 genes.

**Results.** SAM analyses revealed 1213 genes that were differently expressed after treatment with hyperthermia *in vivo*. 127 of these we differently expressed also *in vitro* (t-test). Sorting the genes after biological processes according to the database of the Gene Ontology Consortium, we found significantly increased number of genes involved in processes like apoptosis, transcription, immune response, angiogenesis, cell signaling and protein metabolism. Interestingly the immune system (both lymphocyte and myeloid leucocytes) seems to be depressed by hyperthermia in our model system. There is upregulation of genes involved in protein folding (among others heat shock proteins) and overrepresentation of a cluster of genes involved in protein amino dephosphorylation (among them dual specificity phosphatases) indicating that protein modifications relevant for signal transduction, as well as transcriptional regulation may be important mediators of the effect of hyperthermia. An interesting observation was a significant upregulation of DNAJB4 mRNA after treatment with hyperthermia. DNAJB4 is recently shown to be a novel tumor suppressor.

**Conclusion.** Global gene expression reflects direct cell damage and simultaneous cell survival responses. To our best knowledge, this is the first full genome microarrays analysis focused hyperthermia versus placebo in the treatment in a malignant tumor *in vivo* and *in vitro*. The data may provide a basis for new hypotheses for the mechanisms involved.