

## **HYPERTHERMIC REGULATION OF HIF-1 $\alpha$ IN MACROPHAGES**

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Macrophages play an important role in tumor angiogenesis by generating reactive oxygen species (ROS), VEGF and other tumor promoting factors. Newly formed vessels are generally immature and non-functional resulting in tumor hypoxia, which is associated with treatment resistance and more aggressive tumor phenotype. Under hypoxia, transcriptional regulation of VEGF is controlled primarily by HIF-1 $\alpha$ . Recent studies have demonstrated HIF-1 $\alpha$  stabilization and transcriptional activity can be modulated by ROS signaling. Hyperthermia (HT) is known to improve tumor oxygenation. However, little is known on the effect of heat on macrophage ROS production and HIF-1 $\alpha$  regulation. The objective of this study is to evaluate the mechanism of hyperthermic regulation of HIF-1 $\alpha$  accumulation and transcriptional activity in macrophages.

In this study macrophages were incubated under normoxic or hypoxic (0.5% O<sub>2</sub>) conditions for one hour at target temperature (37-43°C). Superoxide and nitric oxide levels were determined by reduction of cytochrome *c* and Griess reaction, respectively. pVHL and/or HSP90 binding to HIF-1 $\alpha$  were evaluated by co-immunoprecipitation and Western blot. HIF-1 $\alpha$  accumulation was analyzed by immunofluorescence and transcriptional activity detected by VEGF ELISA.

HT increases superoxide and nitric oxide production in macrophages under hypoxic conditions. In the range of 39-41°C, HT has the ability to significantly attenuate HIF-1 $\alpha$  accumulation and transcriptional activity in hypoxic cells, which is reversed at 43°C. By repressing HIF-1 $\alpha$  activity, mild HT has the potential to increase tumor oxygenation and downregulate pro-angiogenic responses in macrophages. Although the mechanism of this phenomenon is not clear, ongoing studies are testing the following hypotheses: HT-induced production of nitric oxide restores prolyl hydroxylase activity and increases pVHL binding to HIF-1 $\alpha$  and its degradation. Furthermore, HSP90 has been shown to enhance stabilization of HIF-1 $\alpha$  at elevated temperatures; therefore, we hypothesized HSP90 could reverse the effect of nitric oxide on HIF-1 $\alpha$  at temperatures > 43 °C.

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