THE INFLUENCE OF NVP-HSP990, IN COMBINATION WITH HYPERTHERMIA AND IRRADIATION ON THE VOLUME OF PANCREATIC CARCINOMA SPHEROIDS

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Background: Heat shock protein 90 (Hsp90) is an evolutionary conserved molecular chaperone which participates in protein folding, intracellular transport, maintenance and degradation of proteins under physiological conditions. In neoplastic tissue, Hsp-90 is expressed at 2-10 fold higher levels than in normal tissue. Several clients of Hsp-90, such as EGFR, Erb-B2, Akt, BCR-ABL, and VEGFR2 play an important role in the regulation of radiosensitivity of tumor cells. Heat is a very potent radiosensitizer in vitro and in vivo; several clinical studies have demonstrated that the combination of conventional radiation therapy (RT) with hyperthermia leads to significantly better tumor control. Interestingly, Hsp90 is heat-inducible in tumor cells. This induction could limit desirable effects of ionizing radiation (IR) through activation of client proteins involved in the regulation of the radiosensitivity. We postulated that inhibition of Hsp90 might increase the effect of hyperthermia and thereby enhance radiosensitivity. In previous work (Milanovic et al. Radiation Oncology 2013, 8:42), we demonstrated that novel Hsp90 inhibitor, NVP-HSP990 increased the thermosensitivity, radiosensitivity and radiothermosensitivity of MIA PaCa-2 cells (citation here). This study investigated whether inhibition Hsp90 with NVP-HSP990 increases the effect of hyperthermia and IR of human 3D cell models, MIA PaCa-2 human pancreatic carcinoma spheroids.

Methods: A colony-forming assay was used to determine colony formation of MIA PaCa-2 cells after treatment with increasing drug concentration. Spheroids were formed and cultivated as reported by Friedrich et al. (Nat Protoc 2009, 4:309-24). Upon addition of 0.5 µM NVP-HSP990, cells were incubated for 1 hour at 42°C and then 37°C. 24 hours later, the growth medium was replaced and cells were irradiated with a single dose of 5 Gy without NVP-HSP990. After 5 days, MTT staining was performed to assess the volume of spheroids.

Results: Treatment with 0.01 or 0.02 µM NVP-HSP990 did not influence colony formation of MIA PaCa-2 cells, while the treatment with 0.05, 0.1, 0.2 and 0.5 µM NVP-HSP990 significantly reduced colony numbers in compared to untreated controls. MTT staining showed that the triple combination (NVP-HSP990 + 42°C + 5 Gy) had the strongest effect on spheroid volume. The double combinations, NVP-HSP990 + 42°C or NVP-HSP990 + 5 Gy also decreased spheroid volume compared to the individual treatments.

Conclusion: NVP-HSP990, fully synthetic, orally available Hsp90 inhibitor exhibits strong antitumor effects on MIA PaCa-2 pancreatic carcinoma spheroids through an increase of sensitivity towards heat and ionising irradiation. Further preclinical studies are warranted to clarify the complex mechanisms of its action and to explore the therapeutic potential of this approach in vivo.