

IN VIVO IMAGING OF MEMBRANE-BOUND HSP70 ON TUMOR CELLS

Gabriele Multhoff^{1,2}

¹Klinikum rechts der Isar, Dpt. Radiation Oncology, TU München
Ismaningerstr. 22, 81675 Munich, Germany

E-mail: gabriele.multhoff@lrz.tu-muenchen.de

²Clinical Cooperation group "Innate Immunity in Tumor Biology", Institute of Pathology,
Helmholtz Zentrum München, German Research Center for Environmental Health,
Neuherberg, Germany

Heat shock proteins (HSP) are highly conserved molecules that are predominantly localized in the cytosol where they fulfill a variety of chaperoning functions. The major stress-inducible Hsp70 (new nomenclature: HspA1A) is also present on the cell membrane of tumor and metastases but not normal cells, and thus provides a tumor-specific target structure for imaging and for therapeutic intervention [1]. We generated an Hsp70 specific IgG1 monoclonal antibody (mAb cmHsp70.1, patent) which detects membrane-bound Hsp70 on the cell surface of tumor cells *in vitro* and *in vivo*. Kinetic studies revealed an enrichment of the Cy5.5-conjugated mAb cmHsp70.1, but not of an isotype-matched control antibody, already 30 min after intravenous (iv) injection into the tail vein of a tumor-bearing mouse. Remarkably the mAb cmHsp70.1 did not bind to any of the normal mouse tissues. These data indicate that mAb cmHsp70.1 might provide an innovative *in vivo* imaging reagent for intraoperative detection of tumors and metastases in the future.

Environmental stress, such as ionizing irradiation, has the capacity to enhance the density of Hsp70 selectively on the cell surface of tumors [2]. A direct interaction of Hsp70 with phosphatidylserine (PS) and its translocation to the outer membrane leaflet was identified as one potential mechanism for the transport and anchorage of Hsp70 in the plasma membrane of tumor cells [3]. Apart from Hsp70, which acts as a recognition structure for Hsp70-peptide activated NK cells [4], the expression of other NK ligands such as MICA/B and ULBP-1,2,3 have been found to be up-regulated by the DNA damage response induced by irradiation [5]. By a dual approach which consists of radiotherapy and an NK cell based immunotherapy it might be possible to improve local tumor control and prevent spread of distant metastases in the future. Regarding our promising results from a phase I clinical trial using *ex vivo* Hsp70-peptide activated NK cells for the treatment of patients with metastasized colorectal and lung cancer [6] we presently plan a proof-of-concept phase II clinical study. In this trial we will exploit the irradiation-induced up-regulation of activatory NK ligands for an immunotherapeutic intervention with Hsp70-peptide activated, autologous NK cells in patients with non-small lung cell carcinomas (NSCLC).

References

- [1] Schmitt E et al. Intracellular and extracellular functions of HSPs: repercussion in cancer therapy. *J Leucocyte Biol* **81**, 15 (2007)
- [2] Gehrman M et al. The therapeutic implications of clinically applied modifiers of Hsp70 expression by tumor cells. *CSC* **13**, 1 (2008)
- [3] Schilling D et al. Binding of Hsp70 to extracellular PS promotes killing of normoxic and hypoxic tumor cells. *FASEB*, in press (2009)
- [4] Stangl S et al. Control of metastasized pancreatic carcinomas in SCID/beige mice with human TKD-activated NK cells. *J Immunol* **176**, 6270 (2006)
- [5] Tesniere A et al. Molecular characteristics of immunogenic cancer cell death. *Cell Death Differ* **15**, 3 (2008)
- [6] Krause S et al. Treatment of cancer patients with autologous *ex vivo* Hsp70-peptide activated NK cells: a clinical phase I trial. *Clin Can Res* **10**, 3699 (2004)