CHAPTER 2

HSP70 PEPTIDE ACTING AS A DANGER SIGNAL FOR NATURAL KILLER (NK) CELLS

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Abstract: Similar to full length Hsp70 protein the 14-mer Hsp70-peptide T-K-D-N-N-L-L-G-R-F-E-L-S-G (TKD), representing part of the C-terminal domain, could be identified as a danger signal for human natural killer (NK) cells. Following incubation with TKD plus low dose IL-2 the cytolytic activity of resting NK cells against Hsp70 membrane-positive tumors was initiated. Concomitantly the cell surface density of activatory receptors including the C-type lectin receptor CD94 was found to be up-regulated. In contrast to normal tissues, tumors frequently present Hsp70 on their cell surface as a tumor-specific recognition structure for IL-2/TKD-activated NK cells. The adoptive transfer of ex vivo IL-2/TKD-activated NK cells into tumor-bearing mice resulted in the control of primary tumors, in prevention of distant metastases, and an improved survival. Based on these results tolerability, feasibility, and safety of an adoptive transfer of IL-2/TKD-activated NK cells was tested in a clinical phase I trial in patients with progressive tumor diseases which were refractory to standard therapy. Apart from an excellent safety profile, the cytolytic activity of patient-derived NK cells could be stimulated in 10 of 12 patients by IL-2/TKD. Furthermore, two of five patients receiving more than four treatment cycles showed clinical responses.

Keywords: Heat shock protein 70 peptide, NK cells, tumor, immunostimulation, cell-based immunotherapy

Abbreviations: APC, antigen presenting cell; HLA, human leukocyte antigen; HSP heat shock proteins; IL-2, interleukin-2; ILT, immunoglobulin-like transcripts; KIR, killer cell immunoglobulin receptor; MHC, major histocompatibility complex; NK cell, natural killer cell; NCR, natural cytotoxicity receptor; TKD, Hsp70 peptide T-K-D-N-N-L-L-G-R-F-E-L-S-G

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INTRODUCTION

Heat shock proteins (HSP) are highly conserved proteins inhabiting nearly all subcellular compartments. Already under physiological conditions they are overexpressed in a broad range of human tumors and play crucial roles in tumor invasion, metastasis, cell proliferation, differentiation and cell death (Ciocca and Calderwood, 2005). Depending on their localization HSP either exert immune activation or protection against environmental stress. Our laboratory identified membrane-bound Hsp70, the major stress-inducible heat shock protein, as a tumor-specific recognition structure for natural killer (NK) cells (Radons and Multhoff, 2005a, 2005b). In the cytosol, Hsp70 functions as a molecular chaperone supporting folding and transport of a great variety of polypeptides and proteins under both, physiological conditions and following chemical or physical stress stimuli (DeNagel and Pierce, 1992; Hartl, 1996; Lindquist and Craig, 1988). Hsp70 was also found to protect cells from apoptosis by an inhibition of the permeabilization of lysosomal membranes (Jaattela et al., 1998; Nylandsted et al., 2004; Nylandsted et al., 2000). In contrast, extracellular HSP elicit a potent anti-cancer immune response mediated either by the adaptive or innate immune system (Gehrmann et al., 2005). This review will focus on the immunological role of membrane-bound Hsp70 serving as a tumor-specific recognition structure for NK cells. We further present data on the immunostimulatory capacity of an Hsp70-derived peptide, termed TKD, which initiates an immune response against Hsp70 membrane-positive tumor cells in human NK cells. In a phase I clinical trial the adoptive transfer of \textit{ex vivo} IL-2/TKD-activated, autologous NK cells into patients with progressive colorectal and non-small cell lung carcinoma, refractory to standard therapy, was found to be safe, feasible and well tolerated. The encouraging immune and clinical responses of patient-derived NK cells warrant future clinical trials.

NK CELLS AND TUMOR CELL KILLING

Apart from their intracellular chaperoning functions, HSP have been found to play key roles in tumor immunity. Most immunotherapeutic approaches exploit the carrier function of HSP for tumor-derived peptides. Following cross-presentation of HSP-chaperoned peptides on MHC class I molecules (Arnold-Schild et al., 1999; Basu et al., 2001; Binder et al., 2000; Binder et al., 2004; Sondermann et al., 2000) an antigen-specific CD8+ T cell response is thus initiated (Binder et al., 2001; Doody et al., 2004; Schild et al., 1999; Srivastava et al., 1998). However, even in the absence of immunogenic peptides, HSP serve as danger signals for the host’s immune system (Asea et al., 2000). Exosomes have recently been described as export vehicles for Hsp70 from the endosomal compartment into the extracellular milieu (Bausero et al., 2005; Gastpar et al., 2005; Lancaster and Febbraio, 2005).

NK cells comprising 5–20% of peripheral blood lymphocytes (PBL) are important players of the innate immune system that control bacterial, virus infections and mediate protection against cancer (Trinchieri, 1989). The low affinity Fcγ receptor