Expression of Hsp27, Hsp60, and Hsc70 in paediatric c-ALL-, normal precursor B- and B-cell populations

Peder Skov Wehner

The PhD dissertation was accepted by the Faculty of Health Sciences of the University of Aarhus, and defended on November 25, 2005.

Official opponents: Hans Erik Johnsen, Prof., PhD, DSc Suresh Rattan og Svend Birkelund Andersen.

Tutors: Marianne Hokland og Bendt Nielsen.

Correspondence: Peder Skov Wehner, Skodshøjen 43, 8240 Risskov, Denmark.

E-mail: pedersko@dadlnet.dk

Dan Med Bull 2006;53:80

ABSTRACT

This PhD dissertation is based on three manuscripts, two of which were published in international peer-reviewed journals. The work presented in this dissertation has been carried out mainly during my time as research assistant at the Department of Medical Microbiology and Immunology, University of Aarhus, Aarhus, Denmark.

A better knowledge of the signalling pathway involved in cell differentiation and cell survival in the most common (c) form of acute lymphoblastic leukaemia (ALL) in children (c-ALL) may lead to more rationally tailored therapy. Inhibition of apoptosis is a hallmark of c-ALL cell transformation, and apoptosis by receptor selection takes place at the normal bone marrow (BM) counterpart B-cell precursor (BCP) stage of development. Heat shock proteins (Hsp's) such as Hsp27, Hsp60 and Hsc70 are regulators of protein folding (chaperones) at different intracellular locations. Thereby they are suspected of functioning at key regulatory points in the control of apoptosis, e.g. during differentiation (Hsp27). In addition, the many reports of induced expressions of such Hsp's in solid tumours suggest their involvement in tumour genesis.

The aim of the Ph.D. disertation was to increase our knowledge of the regulation of selected Hsp's at clinically relevant incidents in immunomagnetic bead-enriched subpopulations of c-ALL BCP's, normal BCP's, and B-cell populations from peripheral blood and BM. The dissertation focuses on the possible role of such Hsp's in the normal B-cell developmental programme, in normal B-cell physiology, and in B-cell leukemo genesis.

By using quantitative 2-D PAGE, subcellular fractionation, and double immunoflurescent analysis the expressions of the Hsp27, Hsp60, and Hsc70 genes at the protein level were compared in the B cell subpopulations, and in the case of Hsp27 at its level of phosphorylation and at the subcellular level.

The collective data presented in this dissertation constitute the first reports on comparative investigations at the protein level of Hsp27, Hsp60, and Hsc70 expression within such well-defined subpopulations from normal and diseased children. In paediatric c-ALL, no overall aberrant expression of Hsp27, Hsp60, and Hsc70 was detectable in c-ALL BCP's at initial presentation or at relapse. In contrast, our results indicate that levels of such Hsp's are developmentally regulated during normal B-cell ontogenesis. With regard to Hsp27, this study points to additional levels of regulation in c-ALL BCP's and non-c-ALL BCP's. The very high constitutive expression of Hsp27 demonstrated, and its differential phosphorylation distributed at cytoplasmic and membranous levels combined with its reported cytoprotective activities at such locations make it a potential part of c-ALL hallmarks. The predicted differential enzymatic activity behind the increased Hsp27 phosphorylation in paediatric c-ALL BCP's at relapse may be favourable to such cells during cytotoxic stresses.

Future studies may determine the role of Hsp27 in the mechanism of apoptosis inhibition in paediatric c-ALL cells and may indicate whether Hsp27 or its pathways of enzymatic phosphorylation regulators are future c-ALL treatment targets.