Title: The NAD kinase HSP20 complex

Background

Heat shock protein 20 (HSP20) is a multifaceted protein which is involved in various physiological and pathophysiological processes such as cardioprotection, anti-platelet aggregation, chaperone activity, smooth muscle relaxation, prevention of β-Amyloid plaque toxicity and apoptosis. These versatile functions of HSP20 are now being investigated in cancer. Accumulating evidences have revealed the implication of HSP20 in tumour progression in numerous types of cancer, and that HSP20 expression could influence tumour growth (Noda et al., 2007; Matsushima-Nishiwaki et al., 2011, 2013; Nagasawa et al., 2014; Ju et al., 2015). Collectively, these studies suggest the potential for HSP20 as valuable cancer biomarkers and that targeting HSP20 may serve as a potential therapeutic strategy for cancer therapy.

Prior work within the Baillie group using proteomics screening has identified several novel HSP20 binding partners including NAD kinase (NADK), the only known enzyme which regulates the intracellular NAD levels in mammalian cells. NADK can modulate responses to oxidative stress-related pathologies by regulating the intracellular balance of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) through phosphorylation. NADP which is subsequently reduced to NADPH by dehydrogenases is important for neutralising high levels of reactive oxygen species (ROS) generated by increased metabolic activity especially in hyperproliferating cancer cells (Tedeschi et al., 2015). Several hallmarks of cancer, including uncontrolled proliferation, angiogenesis, and genomic instability, are promoted by the increased ROS levels commonly found in tumour cells. Considering the implications of these two proteins in cancer, it will be interesting to study the molecular mechanism linking NADK-HSP20 interaction.

We have now validated the association between NADK and HSP20 through co-immunoprecipitation and immunocytochemistry. We have also mapped the interaction site of NADK on HSP20 utilising peptide array technology. In this study, the docking site of NADK on HSP20 encompassing residues $A^{17}PGRLFDQR^{25}$ is used to manufacture a cell-permeable analogue of a 9-mer disruptor peptide for displacement studies. A 9-mer peptide consists of a scrambled version of the disruptor peptide is used as a control.

Aims & Objectives

The aim of this study is to further characterise the interaction between NADK and HSP20 and elucidate their possible mechanisms of action.

Two main objectives:

- to verify the peptide array data by evaluating the effectiveness of the peptide to disrupt the NADK-HSP20 complex
- to examine the effect of disruptor peptide on subcellular distribution of NADK-HSP20 complex

Experimental design and methods

1. Co-immunoprecipitation experiments using lysates from HeLa cells and co-transfected HEK293 cells to assess the ability of the disruptor peptide to displace NADK-HSP20 interaction.
2. Use of proximity ligation assay and/or immunocytochemical studies to further examine NADK association with HSP20 in cancer cell lines.