7 Summary

Poly (ADP-ribosyl)ation is a posttranslational modification, known to be involved in several cellular processes as regulation of cell cycle, DNA repair and cell death. While synthesis of poly (ADP-ribose) (PAR) and the function of Poly (ADP-ribose) polymerases (PARPs) were studied extensivly, only little is known about the polymere degrading enzyme Poly (ADP-ribose) glycohydrolase (PARG). First aim of this study was the identification of PARG interacting proteins. Further characterizations were intended to provide more insightes into the cellular functions of the PARG.

Initially, the human PARG cDNA from the cancer cell line HeLa S3 was cloned. The analysis of the primary protein structure revealed the presence of all amino acids, essential for cellular distribution and the mechanism of catalysis. For the first time human PARG, catalytic domain ($PARG_{65}$) as well as full length protein ($PARG_{110}$), were expressed as soluble polyhistidin tagged fusion proteins in *E. coli*. Purification of the catalytic domain of PARG using Ni-NTA affinity chromatography enabled the generation of polyclonal antibodies directed against human PARG. Since purification of the full length PARG using Ni-NTA affinity chromatography was impossible, an affinity matrix composed of sepharose beads covalently linked to gallotannins, which are described as PARG inhibitors, was established. Subsequently, interactions between gallotannin sepharose immobilized PARG and other nuclear proteins were studied. A direct protein protein interaction between the enzymes PARP-1 and PARG was demonstrated. Using deletion mutants of both proteins the automodification domain of PARP-1 and the catalytic domain of PARG were identified as interacting regions. Functional experiments indicated that PARG downregulates the enzymatic activity of nuclear PARP-1.

In addition, PARG interacts with *X-ray repair cross-complementing 1* (XRCC1), a DNA repair factor which is recruited by PARP-1. To study the impact of XRCC1 on PAR metabolism and regulation of cell death hamster ovary cell lines either deficient in XRCC1 (EM9-V) or overexpressing XRCC1 (EM9-XH) were used. Treatment with supralethal doses of the alkylating agent MNNG caused a considerable accumulation of PAR only in EM9-XH cells. Consequently, MNNG triggered in XRCC1-proficient cells the translocation of apoptosis inducing factor (AIF) from mitochondria to the nucleus. This was followed by nuclear shrinkage and caspase-independent cell death. In XRCC1-deficient cells, a same MNNG treatment caused ATP and NAD depletion, resulting in necrotic cell death without AIF translocation and nuclear shrinkage.

Efficient progression of both DNA repair and apoptosis are essential for genomic integrity. The results of present study demonstrate, that the interactions between PARG, PARP-1 and XRCC1 are important for the regulation of both processes.