Abstract

Tumour necrosis factor-Related Apoptosis Inducing Ligand (TRAIL), a membrane-bound ligand from the TNF family, has attracted significant attention due to its rather specific and effective ability to induce apoptotic death in various types of cancer cells via binding to and activating its pro-apoptotic death receptors (DRs). However, a significant number of primary cancer cells often develop resistance to TRAIL treatment, and the signalling platform behind this phenomenon is not fully understood.

In the first paper we focused on the influence of endosomal acidification. Upon blocking endosomal acidification by the vacuolar ATPase (V-ATPase) inhibitors bafilomycin A1 (BafA1) or concanamycin A (CCA) we observed a significantly reduced initial sensitivity of several, mainly colorectal, tumour cell lines to TRAIL-induced apoptosis. In cells pre-treated with these inhibitors, the TRAIL-induced processing of caspase-8 and the aggregation and trafficking of the TRAIL-receptor complexes were temporary attenuated. The cell surface expression of TRAIL receptors and their TRAIL-induced internalization were not affected by V-ATPase inhibitors. NF-κB or MAP kinase signalling from the activated TRAIL receptors remained unchanged, and neither possible lysosomal permeabilization, mitochondrial amplification loop nor acid sphingomyelinase were involved in this process. Altogether, the obtained data provide the first evidence that endosomal acidification could represent an important regulatory node in the proximal part of TRAIL-induced pro-apoptotic signalling.

In the second presented project we studied regulation of death receptors expression on the plasma membrane and we identified and characterized the novel TRAIL-R1/DR4 interacting adaptor protein ARAP1, which is involved in receptor trafficking to the cell surface. The last study was aimed on testing three different widely used cytostatics and inhibitors (TRAIL, 17-AAG and PLX4720) in resistant colon cancer model cell lines. We analyzed the combinatory effect on TRAIL induced apoptosis and molecular mechanisms in various genotype backgrounds.

Results presented in this thesis thus should contribute to the better understanding of TRAIL-triggered cell signaling and hopefully also to its rational application in the clinical practice.