

## ABSTRACT

TRAIL ligand can trigger apoptosis of permissive human cells via engagement of its two pro-apoptotic receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5). Its ability to induce apoptosis independently on p53 status and to selectively kill cancer cells *in vitro* and *in vivo* made this ligand an attractive target in cancer research. However, acquired resistance of primary cancer cells, unsatisfactory outcome of clinical trials and recent studies arguing that TRAIL might under specific conditions promote cancer progression, opened new plethora of questions, which need to be addressed.

Though both receptors DR4 and DR5 are ubiquitously expressed, different types of tumours show preference for either of the receptors. The relative participation of DR4 and DR5 in TRAIL-induced signalling is still largely unknown. To analyse TRAIL receptor-specific signalling, I prepared Strep-tagged, trimerised variants of recombinant human TRAIL ligands with high affinity for either DR4 or DR5 receptor. Using these receptor-specific ligands, I examined a contribution of individual pro-apoptotic receptors to TRAIL-induced signalling pathways. I found that in TRAIL resistant colorectal HT-29 cells but not in pancreatic PANC-1 cancer cells, DISC formation and initial caspase-8 processing proceeded comparably in both DR4- and DR5-activated receptor complexes. However, TRAIL-induced apoptosis proceeded in both cell lines predominantly via DR5 receptor. ShRNA-mediated downregulation of DR4 or DR5 receptors in HT-29 cells also pointed to a stronger contribution of DR5 in TRAIL-induced apoptotic signalling. In contrast to TRAIL-induced apoptosis, I did not observed significant differences in necroptotic signalling activated either by DR4- or DR5-specific ligands. Activation of auxiliary signalling pathways involving NF- $\kappa$ B and stress kinases p38 and JNK proceeded under apoptotic conditions mainly in a DR5-dependent manner, while these kinase pathways were during necroptosis similarly activated.

In addition to TRAIL receptor-specific signalling this Thesis introduces Manumycin A (Man A) as an effective sensitizer of TRAIL-induced apoptosis in colorectal cancer cell lines RKO and SW-620. I documented that treatment of RKO and SW-620 with Manumycin A in combination with TRAIL ligand or ABT-199/737 led to statistical increased apoptosis and co-treatment of these cells with Man A and TRAIL WT led to stronger activation of caspases -8/ -9 and caspase-3 as

well as increased processing of caspase-3 targets PARP and Bid proteins. Importantly I found out that this sensitizing effect is most probably related to the enhanced production of likely mitochondrial ROS in ManA-treated cells.

Additionally, data from the collaborative project on human embryonic stem cells (hESC) and human-induced pluripotent stem cells (hiPSC) showed that despite expression of both DR4 and DR5 receptors in these cells, they were naturally resistant to TRAIL-induced apoptosis. However, their pre-treatment with homoharringtonine led to decrease of expression of two anti-apoptotic proteins Mcl-1 and cFLIP and to their sensitization to TRAIL-induced apoptosis. Similarly shRNA-mediated downregulation of cFLIP led to their enhanced apoptotic response to TRAIL and thus cFLIP likely represents the important regulatory node in TRAIL-induced apoptosis of human pluripotent stem cells.

In summary, this study provided several new options how to overcome cellular resistance to TRAIL-triggered apoptosis and proposed answers to several questions raised in the field of TRAIL-induced signalling. It also provided first systematic insight into DR4-/DR5-specific signalling in colorectal and pancreatic cancer cells, where using a number of approaches documented that apoptotic and auxiliary but not necroptotic signalling in these cells largely relies on DR5 receptor. Moreover, I proposed secondary metabolite of *Streptomyces* Manumycin A as a novel potent sensitizer of TRAIL-induced apoptosis and I provided the first evidence that, irrespective of their origin, human stem cells express canonical components of the extrinsic apoptotic system and upon stress can activate death receptor-mediated apoptosis.