Laboratory of Cell Signalling and Apoptosis

Death receptors, TRAIL, Daxx, cancer, cell death

Ladislav Anděra

ladislav.andera@img.cas.cz

www.img.cas.cz/research-groups/ladislav-andera

Research in our group is mainly focused on the analysis and characterization of signalling pathways leading to programmed death of cancer cells including cancer stem cells and uncovering mechanisms participating in the regulation of this signalling. In our major interest stands TRAIL (TNFα-Related Apoptosis Inducing Ligand), a ligand from the TNFα family capable of inducing apoptosis of transformed cells and not being harmful to the normal ones. TRAIL-induced apoptosis of sensitive cells is triggered by the interaction with its pro-death receptors TRAIL-R1/DR4 and/or TRAIL-R2/DR5. These receptors contain an α-helical protein-protein interaction domain called the death domain and together with Fas/CD95 or TNFR1 belong to the death receptor subfamily of TNFR receptors. Currently we deal with several aspects of death receptor-induced signalling such as i/ evaluation of the role of proximal signalling processes (endocytosis or endosomal acidification) in TRAIL ligand-receptor(s) Death-Inducing Signalling Complex (DISC) formation and activation of the initiator caspase-8, ii/ expression and activation of death receptors in human embryonic stem cells (hESCs) and from them derived somatic progenitors, or iii/ assessment of the effect of overexpressed/activated oncogenes such as c-myc on TRAIL- or FasL-induced apoptosis. In our collaborative projects we uncovered two novel drugs that sensitize resistant cancer cells to TRAIL-induced apoptosis. We also participate in the analysis of multiple aspects of TRAIL-induced signalling in leukaemia and colon carcinoma cells and characterization of cell death modalities in senescent normal and cancer cells. Among other death receptors we characterized posttranslational modifications and regulation of expression of the Death Receptor 6 (DR6), which is known to participate in the regulation of T- and B-cell activation and neurogenesis. We discovered that DR6 is a highly glycosylated and palmitoylated receptor, and its expression is induced upon activation of human or mouse T cells. Besides death receptors-related projects we also deal with some aspects of apoptosis- and transcription-regulating activities of chaperone adapter protein Daxx. We uncovered and characterized its functional interaction with the chromatin-remodelling ATPase Brg1, and in collaboration we investigate its possible role in the DNA damage response.

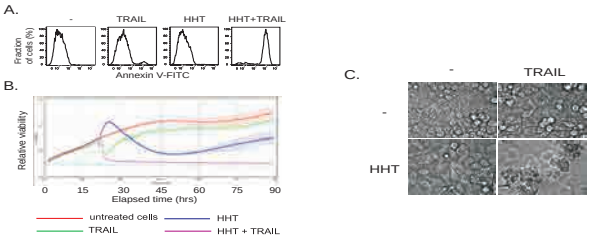

Fig. 1. Hommoharingtonine (HHT) sensitizes colorectal cancer cells to TRAIL-induced apoptosis
A. RKO cells were treated with TRAIL (20 ng/ml), 50 nM HHT and their combination for 5 hrs and apoptotic cells were quantified by Annexin-V-FITC staining and flow cytometry. B. Long-term viability of treated RKO cells analysed by xCELLigence assay. C. RKO cells treated with TRAIL, HHT and HHT+TRAIL viewed by phase-contrast microscopy.

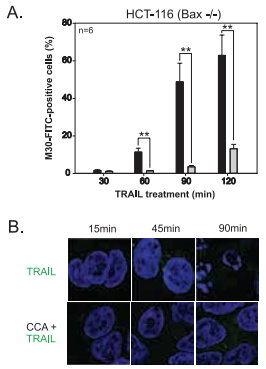

Fig. 2. Blocking endosomal acidification significantly attenuates TRAIL-induced apoptosis
A. Colorectal Bax-deficient HCT-116 cancer cells were either mock-treated (black bars) or pre-treated with 20 nM bafilomycin A1 (BafA1, grey bars), then treated with TRAIL (100 ng/ml) for indicated time periods, stained with M30-FITC (cleaved cytokeratine 18 - reflects activity of caspase-3) and analysed by flow cytometry. B. HCT-116 cells were treated with Alexa 647-labelled TRAIL (green) for indicated time periods, fixed, counter-stained with DAPI (nuclei) and visualized by confocal microscopy. BafA1 delays both aggregation of TRAIL-containing complexes in MVB and onset of apoptosis (condensed nuclei).

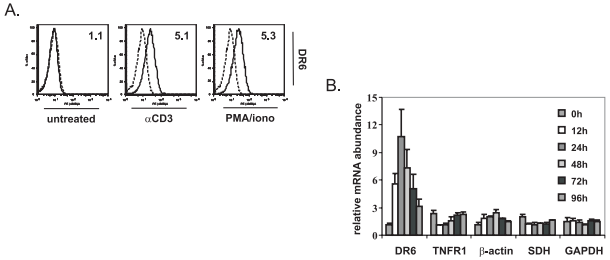

Fig. 3. Death receptor 6 (DR6) expression is induced in activated human T cells
A. Isolated human T cells were either activated by anti-CD3 crosslinking or PMA+ionomycin treatment for 48 hrs and the cell surface expression of DR6 was determined by its staining with monoclonal antibody and flow cytometry. B. Real-time qPCR analysis of the time-course of DR6 mRNA expression in PMA/ionomycin-treated human T cells and comparison with TNFR1 expression; b-actin, SDH and GAPDH serve as **housekeeping genes.**

- AS CR, KAN200520703 – The use of ultrasound in nanomedicine, 2007-2011, L. Anděra
- Ministry of Health of the Czech Republic, NS10287 - Experimental therapy of mantle cell lymphoma (MCL), 2009–2011 , L. Anděra
- GA CR, GAP301/10/1971 - Expression, signaling and function of Death Receptors in human embryonic stem cells., 2010-2012, L. Anděra GA CR, GAP301/10/1971 – Expression, signaling and function of Death Receptors in human embryonic stem cells, 2010-2012, L. Anděra
- Ministry of Education, Youth and Sports of the Czech Republic, LH12202 LH-KONTAKT - Analysis and suppression of mechanisms leading to the resistance of cancer cells to TRAIL., 2012-2014, L. Anděra
- Ministry of Health of the Czech Republic, NT13201 - Role of angiogenesis in mantle cell lymphoma (MCL) survival, growth, spread and response to therapy, 2012-2015, L. Anděra

1. Molinsky J, Klanova M, Koc M, Beranova L, Andera L, Ludvikova Z, Bohmova M, Gasova Z, Strnad M, Ivanek R, Trneny M, Necas E, Zivny J, Klener P. Roscovitine sensitizes leukemia and lymphoma cells to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. **Leuk Lymphoma 2012 Epub** Sep 8.
2. Klíma M, Broučková A, Koc M, Anděra L. T-cell activation triggers death receptor-6 expression in a NF-κB and NF-AT dependent manner. **Mol Immunol 2011** 48(12-13): 1439-1447.
3. Makrodouli E, Oikonomou E, Koc M, Andera L, Sasazuki T, Shirasawa S, Pintzas A. BRAF and RAS oncogenes regulate Rho GTPase pathways to mediate migration and invasion properties in human colon cancer cells: a comparative study. **Mol Cancer 2011** 10: 118.
4. Oikonomou E, Koc M, Sourkova V, Andera L, Pintzas A. Selective BRAFV600E inhibitor PLX4720, requires TRAIL assistance to overcome oncogenic PIK3CA resistance. **PLoS One 2011** 6(6): e21632.
5. Vondálová Blanárová O, Jelínková I, Szöor A, Skender B, Soucek K, Horváth V, Vaculová A, Andera L, Sova P, Szöllosi J, Hofmanová J, Vereb G, Kozubík A. Cisplatin and a potent platinum(IV) complex-mediated enhancement of TRAIL-induced cancer cells killing is associated with modulation of upstream events in the extrinsic apoptotic pathway. **Carcinogenesis 2011** 32(1): 42-51.

From the left:
Zuzana Naháčká, MSc / PhD Student (since 2011) · Jarmila Špegárová, PhD (since 2011) / Postdoctoral Fellow · Simona Benešová / Technician · Jan Bražina, MSc / PhD Student · František Pešina / Diploma Student (since 2012) · Ladislav Anděra, PhD /Head of Laboratory ·Gita Nováková / Diploma Student (since 2011) · Martin Peterka / Diploma Student

Not in the picture:
Michal Koc, PhD / Postdoctoral Fellow (until 2011) · Lenka Beranová, MSc / PhD Student (maternity leave) · Vladimíra Horová, MSc / PhD Student (maternity leave) · Jan Švadlenka, MSc / PhD Student · Naďa Hradilová / Diploma Student (until 2012)