P10-09
Nocodazole Induced suicidal death of human erythrocytes

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Background: The microtubule assembly inhibitor nocodazole has been shown to trigger caspase-independent mitotic death and caspase dependent apoptosis. Similar to apoptosis of nucleated cells, erythrocytes may undergo eryptosis, the suicidal erythrocyte death characterized by cell shrinkage and cell membrane scrambling with phosphatidylserine translocation to the erythrocyte surface. Stimulators of eryptosis include increase of cytosolic Ca^{2+} activity ($[Ca^{2+}]_i$), oxidative stress and ceramide. The present study explored, whether and how nocodazole induces eryptosis. **Methods:** Flow cytometry was employed to determine phosphatidylserine exposure at the cell surface from annexin-V-binding, cell volume from forward scatter, $[Ca^{2+}]_i$ from Fluo3-fluorescence, the abundance of reactive oxygen species (ROS) from 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) dependent fluorescence and the ceramide surface abundance utilizing specific antibodies. **Results:** A 48 hours exposure of human erythrocytes to nocodazole (≥ 30 µg/ml) significantly increased the percentage of annexin-V-binding cells without significantly modifying average forward scatter, significantly increased Fluo3-fluorescence, significantly increased DCFDA fluorescence and significantly increased ceramide surface abundance. The effect of nocodazole on annexin-V-binding was significantly blunted, but not abolished by removal of extracellular Ca^{2+}. **Conclusions:** Nocodazole triggers cell shrinkage and phospholipid scrambling of the erythrocyte cell membrane, an effect in part due to stimulation of Ca^{2+} entry, oxidative stress and ceramide.

P10-10
Effects of retinoid-X receptor- (RXR-) and retinoic acid receptor (RAR) isoform-selective synthetic retinoids on cultures of stem-like glioma cells

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Retinoic acid (RA) is required for development and homeostasis of the normal mammalian brain and may play a role in the initiation and progression of malignant gliomas. The subpopulation of stem-like glioma cells (SLGCs) was shown to resist standard glioma therapy and to propagate tumor regrowth. Recently, we have shown that all-trans retinoic acid (atRA) induced differentiation in one (T1338) out of 10 SLGC lines and that a synthetic RAR-pan agonist had a weaker effect than atRA.
In order to study the molecular basis for these observations we treated SLGC lines with a combination of RAR isotype-selective agonists and antagonists in the absence and presence of RXR-selective ligands. The effects on proliferation, vitality and differentiation were determined. Moreover, we used MSP (methylation specific PCR) and ChIP (chromatin immune precipitation) as well as expression studies to identify the molecular basis for the specific behavior of T1338.
Firstly, our data indicated that the atRA signal is transduced by the RARa and RARg isotype rather than RARb. Co-application of RAR- and RXR-selective synthetic ligands suggested a contribution of RXR to RA-responsiveness of the SLGC lines. The degree of *rar*b promoter methylation differed largely between distinct SLGC cultures. Although the promoter of the rarb gene contains a RA-responsive element (RARE) and rarb mRNA expression was stimulated by atRA promoter methylation remained largely unchanged after RA treatment. ChIP revealed particular high differences between the methylation and acetylation levels of the lysyl residue 9 in histone H3. Chromatin modifications were affected by atRA in T1338 cultures. Clones derived from the T1338 mother culture displayed some differences in their atRA responsiveness. The respective T1338 clones were studied in more detail in order to decipher the molecular basis for the specific behavior of the T1338 cell line.
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P10-11
Cisplatin (CDDP) induces modification of the intracellular calcium signaling in SH-SY5Y neuroblastoma cells: the role of calcium regulating proteins

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Neuroblastoma is an early childhood cancer. Treatment involves chemotherapy with anti-cancer agents: platinum complexes (CDDP, carboplatin), DNA alkylating agents and topoisomerase I inhibitors (e.g. topotecan (TOPO)). Using the human neuroblastoma SH-SY5Y cells we investigated the effect of CDDP on neuroblastoma cell death (cytotoxicity, apoptosis); but also on calcium homeostasis (using calcium sensitive fluorescent dye Fluo-4AM) and changes in the gene expression of calcium regulating proteins IP3Rs, RYRs, S100A6 using RT-PCR. Changes of the intracellular calcium ($[Ca^{2+}]_i$) concentration were recorded using fluorescence microscopy.
The application of CDDP (1nM-1µM) for 3h to 4h increased $[Ca^{2+}]_i$ concentration over time. The effects observed on $[Ca^{2+}]_i$ were concentration dependent. In addition, mRNA expression of selected $[Ca^{2+}]_i$-regulating genes was modified upon exposure to CDDP after 12h, 24h, 48h and 72h. The differentially regulated genes included those encoding the calcium-binding protein S100A6 and the calcium receptors ITPR1, ITPR3, RYR1 and RYR3. Down regulation of ITPR1, ITPR3 were observed at 24h and 72h and increased transiently at 48h following the CDDP treatment. Both RyR1 and RyR3 mRNA expression was modified with CDDP 10 µM. CDDP increased the mRNA expression of S100A6 concentration (at 48h and at 72h of 10µM of CDDP exposure).
Furthermore, a time and concentration dependent decrease in neuroblastoma cell viability was observed using the automatic cell counter ViCell and the Trypan Blue cell viability assay upon treatment 24-72h with CDDP (10nM-1µM). Furthermore, the detailed analysis of apoptosis was studied using the flow cytometry in combination with two fluorescent dyes SR FLICA for staining apoptotic cells and 7-AAD for necrotic cells. Our results showed an increase in the apoptotic population after CDDP treatment. While the 1µM CDDP revealed significant decrease of cell viability after 72 h of exposure (68.6%), the 10 µM CDDP was more efficient in killing SH-SY5Y cells: the 24h treatment resulted in 50% SH-SY5Y cell viability and 72h of exposure resulted in 13% SH-SY5Y cell viability. The decrease of cell viability correlates with the of $[Ca^{2+}]_i$ rise.
Overall, the treatment of SH-SY5Y cells with CDDP induces cell death by apoptosis that is at least in part mediated by changes in calcium signaling. More studies are required to understand the calcium signaling regulatory physiological and pathological processes related to neuroblastoma cell death upon chemotherapy with CDDP.

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Combinatory treatment with anticancer drugs (cisplatin (CDDP), topotecan (TOPO)) and pharmacological modulators of calcium signaling enhances cytotoxic effects in human neuroblastoma cells

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Neuroblastoma (NB) is a pediatric cancer that is treated with platin derivatives (e.g. cisplatin (CDDP)) and topoisomerase inhibitors (e.g. topotecan (TOPO)). CDDP and TOPO interfere with calcium signaling of NB cells that in turn is responsible for apoptosis. Here we tested the cytotoxicity of combinatory treatments of CDDP or TOPO with pharmacological modulators of specific molecules involved in calcium signaling in human NB cells.
Human NB cells SH-SY5Y, IMR32, NLF were analyzed using the ViCell XR automatic counter after a short time exposure (24h-72h) to low concentrations of CDDP or TOPO: 0.1nM-10µM with or without specific calcium signaling modulators: thapsigargin (THAPS), 2-Aminoethoxydiphenylborane (2APB), verapamil (VERA), nifedipin (NIFE), cyclosporine A (CYCLA), and cyclopiazonic acid (CYPZ). Treatment schemes included: untreated control (72h), cells treated with each calcium modulator alone (72h), cells treated with CDDP or TOPO (72h), and cells treated with combinations of calcium modulators and CDDP or TOPO (cells were first exposed to the calcium modulator for 30 min. followed by CDDP or TOPO for 72h).
THAPS had the highest cytotoxic effect followed by CYCLA, and 2APB in all cell lines tested; CPZ was cytotoxic in 2 cell lines, VERA and NIFE showed week cytotoxicity in IMR32. The combinatory treatment with 0.2µM THAPS and 10µM CDDP or 10nM TOPO had an additive cytotoxic effect in IMR32 for CDDP and TOPO while in NLF cells an additive effect was observed only for THAPS and CDDP. In SH-SY5Y, the combinatory treatment did not show additive effects, including combinations with a higher THAPS concentration (2µM). Additive effects were also seen with CPZ (20µM) treatment: an additive cytotoxic effect was observed in IMR32 for CDDP (10µM) and TOPO (10nM), while in NLF, the additive effect was restricted to the combination of CPZ and CDDP. In SH-SY5Y, this combinatory treatment had no clear additive effect. When 2APB was applied with CDDP an enhanced cytotoxicity was observed in IMR32 and NLF, but not in SH-SY5Y. Combination of 2APB with TOPO showed enhanced cytotoxicity in IMR32. Enhanced cytotoxic effects were observed when NIFE (50µM) was applied in combination with TOPO but only in IMR32 and NLF. Regarding VERA combinations, an additive cytotoxic effect was observed for VERA+CDDP in NLF while enhanced cytotoxicity of IMR32 and NLF was detected for VERA+TOPO.
Taken together, our results indicate additive cytotoxic effects when calcium signaling modulators are combined with conventional chemotherapy in neuroblastoma models, which in part were cell line dependent.

P10-13
The LncRNA HIF1α-AS1 mediates the response to acriflavine in human endothelial cells

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Acriflavine is known as a drug with anti-cancer activities. Mechanistically, it binds directly to HIF-1α and HIF-2α thereby inhibiting HIF-1 dimerization and transcriptional activity leading to the abrogation of all HIF1α downstream targets. The role of the long non-coding RNA (lncRNA) HIF1α-AS1 in this context is unknown. The lncRNA is located genomically at the transcriptional start site of HIF1α, but being transcribed in antisense direction along the HIF1α promoter. It has been shown to function pro-apoptotic and anti-proliferative in vascular smooth muscle and endothelial cells. We sought to identify the role of this lncRNA in the acriflavine context in human umbilical vein endothelial cells (HUVECs).
LNA-Gapmer mediated knockdown of the lncRNA did not influence HIF1a transcription, but resulted in increased HIF1α promoter activity during luciferase reporter assays. Gene Array analysis after knockdown suggested that HIF1α-AS1 is important for endothelial cell migration. HIF1α-AS1 depletion in HUVEC showed that HIF1α-AS1 alone has no effect during tube formation, whereas acriflavine treatment strongly inhibited network formation, which was expected as an anti-cancer drug. Expression of HIF1α-AS1 was massively increased after acriflavine treatment. The action of network formation inhibition by acriflavine strongly depends on the