CB-C04

INHIBITION OF TUMOR GROWTH, STEROIDOGENESIS, HORMONE AND DRUGS RESISTANCE BY AN ACYL-COA SYNTHETASE 4 NEW INHIBITOR

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Acyl-CoA synthetase 4 (ACSL4) is an isoenzyme of the fatty acid ligase-coenzyme A ligase family taking part in arachidonic acid metabolism. This enzyme is involved in cancer development, particularly in breast and prostate cancers, where a correlation between its high expression and tumor cell aggressiveness has been found. Functionally, it was shown that ACSL4 is part of the mechanism responsible for increased breast and prostate cancer cell proliferation, invasion and migration. ACSL4 is involved in the regulation of several signal transduction mechanisms including mTOR pathway and furthermore increase the expression of proteins involved in drugs resistance. Another relevant property of this enzyme is its participation as an essential protein in the activation of cholesterol transport from the external to the internal mitochondrial membrane, the regulatory and rate-limiting step in the syntheses of all steroids. The development of selective inhibitors for ACSL4, which may inhibit tumor growth and steroidogenesis, may be an important tool in the prevention and treatment of breast and prostate cancers expressing ACSL4. In this work, we have developed and characterized a new ACSL4 inhibitor, PRGL493. An ACSL4 homology model was generated by MODELLER and a docking based virtual screening was performed. The selected compound was modified to improve particularly solubility properties and fully characterized through nuclear magnetic resonance and mass spectroscopy. We demonstrated that PRGL493 was effective in reducing AA-CoA levels, product of ACSL4 activity, in a cell free assay system using recombinant protein and in intact cells using cells models for steroidogenesis and tumor growth. Our compound inhibited cell proliferation and migration of highly aggressive human breast and prostate cancer cell lines (MDA-MB-231 and PC-3 respectively) and *in vivo* using the Chick Embryo Chorioallantoic Membrane model assay. In addition, PRGL493 inhibited steroid synthesis both *in vitro* in Leydig, adrenal and PC-3 prostate cancer cells and *in vivo* in a mouse model, inhibiting cholesterol transport o the inner mitochondrial membrane and thus preventing steroid accumulation. Moreover, the inhibitor produced sensitization to hormonal and chemotherapeutic treatment. The combination of PRGL493 with tamoxifen, cisplatin, doxorubicin or paclitaxel in MDA-MB-231 cells, or with docetaxel in PC-3 cells showed a synergistic effect on the inhibition of cell proliferation. The results show that ACSL4 is an essential target for breast and prostate cancer therapy. These findings open an important route to treat these tumors that may lead to the development of rational cancer treatment and continue to lead to the introduction of the combination of chemotherapy to specific other agents.

CB-C05

MULTIPLE REACTION MONITORING (MRM): CHALLENGES IN MASS SPECTROMETRY BASED PROTEIN QUANTIFICATION

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Macrophages are essential cells of the innate immune system. Exposure to toxic substances such as methamphetamine (Meth) impairs their protective function. We used a targeted proteomics approach to determine the effects of Meth exposure on infected macrophages. Our model uses human monocyte derived macrophages (hMDM) *in vitro* infected with HIV-1 (CIC), exposed to Meth after infection (CIM), and exposed to Meth before and after infection (MIM). The trypsin digested samples were analyzed with UPLC-MS/MS using a multiple reaction monitoring (MRM) mode. Proteins selection was based on previous studies of the HIV-1 infected hMDMs proteome. Five replicate injections of three concentrations of whole-cell lysates were measured for three conditions CIC, CIM, and MIM. The spiked-in BSA peptides showed no interferences in the presence of the complex biological matrix in contrast to endogenous β-actin, galectin-1, and galectin-9. Galectin-1 expression increased in MIM at all tested concentrations when compared to either CIC or CIM. Experimental verification of *in silico* predefined peptides of two randomly selected proteins present in the investigated cell lysates, clathrin heavy chain 1 and chitinase-3-like protein 1, showed that only 19.4% and 8.3% were high-responding peptides, respectively. BSA peptides spiked-in to CIC, CIM and MIM-treated hMDM whole-cell lysates show concentration dependent linearity in highly complex biological matrix, unlike the peptides from endogenous proteins. Multiple concentrations injections are therefore needed to rule out false positives. Only few peptides are high-responding for MRM.

CB-C06

CHMP4B IS REQUIRED FOR THE EFFICIENT REPLICATION OF *TOXOPLASMA GONDII* IN DENDRITIC CELLS

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Toxoplasma gondii is an apicomplexan parasite, responsible of toxoplasmosis, a disease that can be lethal to immunocompromised individuals. *T. gondii* is an intracellular parasite that resides within a parasitophorous vacuole (PV), where it grows and replicates. It is thought that *T. gondii* is able to interact with the host cell cytoplasm through the intravacuolar tubulo-vesicular network, a membranous structure connected to the PV membrane. In this work, we focus on the interaction between the parasite and dendritic cells (DCs), the most potent antigen presenting cells capable of triggering CD4+ and CD8+ T cell responses. First, we found that DC treatment with U-18666A, an inhibitor of multivesicular bodies (MVBs) formation, significantly inhibited the antigen presentation ability of *T. gondii*-derived antigenic peptides in the context of both, MHC-I

and MHC-II molecules. Nevertheless, the treatment with this drug did not affect the infection rate of DCs. By indirect immunofluorescence and confocal microscopy, we observed a clear recruitment of different MVB components to the PV, although most strongly the protein CHMP4b, core of the ESCRT III complex. In addition, upon DC infection by *T. gondii*, we evidenced a complete redistribution of CHMP4b around the PV and an over-expression of this protein, as compared to uninfected DC. Indeed, by Western blot analysis we confirmed a gradual increase in CHMP4b expression along the progression of *T. gondii* infection, suggesting a critical demand for CHMP4b by the parasite. To test this possibility, we silenced CHMP4b expression in DCs and analyzed the proliferation of a fluorescent *T. gondii* strain. Accordingly to our hypothesis, we found that the parasite is not able to replicate correctly in DCs after CHMP4b depletion, especially at later time points post-infection. Given the major role of CHMP4b in the formation of intraluminal vesicles, we think that it might be necessary for the adequate development of the *T. gondii* intravacuolar tubulo-vesicular network. Ongoing electron microscopy-based experiments in this direction will allow as to determine the relevance of CHMP4b during the formation of these intravacuolar structures.

CB-C07

IDENTIFICATION OF A MINIMAL SEQUENCE OF P21 THAT SENSITIZES TUMOR CELLS TO DNA-DAMAGING AGENTS

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DNA-damaging agents used in traditional chemotherapy are cytotoxic by means of interfering with DNA replication. These agents target cells with high proliferation rate by generating lesions in the DNA that impair replication and cause cell death. The efficacy of anticancer treatments is, however, highly influenced by the cellular capacity to respond to DNA damage. One central mechanism that enables cancer cells to survive is Translesion DNA Synthesis (TLS). This process involves specialized DNA polymerases that synthetize a short patch of DNA across the lesion, a situation where replicative DNA polymerases would normally stall. Therefore, inhibiting TLS would be deleterious to these cells when used in combination with DNA-damaging agents. Our group has previously identified the cyclin-CDK inhibitor p21, as the first global inhibitor of TLS. A stabilized version of p21 (sp21) can inhibit the recruitment of TLS polymerases to replication factories after DNA damage without interfering with normal DNA replication, and this is dependent on its PCNA-binding domain. In this work, we have found that a smaller version of p21 which contains only its PCNA-Interacting Region (sPIR) is sufficient to robustly inhibit the recruitment of TLS polymerases to replication factories post ultraviolet (UV) radiation. By using a non-replicative lentivirus system as an overexpression tool, we have found that the sPIR increases cell death in the context of many DNA-damage inductors such as UV, cisplatin and hydroxyurea. Lately, we have also found that in many tumor cell lines co-inhibition of TLS and Chk1, a protein with a pivotal role in in the intra S-phase checkpoint, efficiently synergize to enhance replicative stress and cell death. Our data suggest that the PIR domain of p21 is a versatile tool with potential therapeutic utility.

CB-C08

GENETIC POLYMORPHISMS ON G-QUADRUPLEXES AS A CAUSE OF ONCOGENES TRANSCRIPTIONAL AND TRANSLATIONAL EXPRESSION VARIATIONS.

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G-quadruplexes (G4) are nucleic acid secondary structures that can be transiently folded within proximal promoter regions (PPRs) in G-rich single-stranded DNA regions exposed during transcription and in G-rich RNA sequences within 5' untranslated regions (5’ UTRs) or other mRNA regions involved in translational control. G4s have been proposed as novel transcriptional and translational regulatory elements originally and mainly described in oncogenes. On the other hand, genomic scale association studies by massive DNA sequencing revealed that single nucleotide polymorphisms (SPNs) associated with human diseases are present mainly near transcription start sites, within PPRs and 5' UTRs. The goal of this work was to identify SNPs overlapped with putative G4 forming sequences (PG4) described as transcriptional or translational regulators (located within PPRs or 5' UTRs, respectively) of oncogenes, that may affect G4 folding, hereafter called SNP-PG4. First we performed a bioinformatic analysis using Ensembl database to identify the SNPs (reported in COSMIC, ClinVar, dbSNP and HGMD genetic variation databases) overlapped with the PG4s (and their +/- 5 bp flanking sequences), described as transcriptional regulators for 10 oncogenes and as translational regulators for 15 oncogenes. For each reference sequence we generated a collection of variable sequences containing each polymorphism and a mutant sequence with no PG4 (unable to form G4). Then we used several DNA and RNA G4 folding predictors in order to identify those SNP-PG4 that may affect G4 folding or stability. Based on the results of this analysis, from 88 DNA and 256 RNA sequences corresponding to the SNP-PG4 of the analyzed oncogenes, we chose 41 and 15, respectively, for further analysis. The selected sequences correspond to the *c-MYC*, *BCL2*, *cKIT*, *RET* and *VEGF* oncogenes for G4s in PPRs and to the *CCND3*, *NRAS*, *HSAFY*, *ESR1*, *FGF2*, *ZIC1* and *TRF2* oncogenes for G4s in 5’ UTRs. Spectroscopic analyses by Circular Dichroism (CD) demonstrated that some SNPs cause quantitative or qualitative spectral changes. Moreover, qPCR stop assays and CD melting assays indicate that the same SNPs induce G4 stability changes. In agreement, 1D ^{1}H NMR spectroscopy confirmed that SNPs induce quantitative and qualitative changes for the SNP-PG4s identified for *c-MYC* and *NRAS*. Finally, SNP-PG4s that produced significant structural variations *in vitro* were cloned into pGL3 promoter vector (for PG4s controlling transcription) or into psiCHECK-2 vector (for RNA PG4 controlling translation) and were transfected into HEK293 cells, revealing that SNPs altered luciferase reporter activity. Results gathered in this work suggest that SNP-PG4s that alter G4 folding may be the cause of differential expression of oncogenes leading to tumor predisposition, establishment, progression or metastasis and should be considered as a novel molecular etiology mechanism for the predisposition or establishment of diseases.

CB-C09

CONVERGENT APPROACH TO THE STUDY OF LONGEVITY OF *CERATITIS CAPITATA* AND *DROSOPHILA MELANOGASTER* MALES.