Conclusions: In this study, we evaluated the effect of MCT1 inhibition in cervical cancer cells and observed a significant decrease in total cell biomass which may be a result of inhibition of cell viability, cell proliferation and/or induction of apoptosis. Further studies are needed to better comprehend the mechanisms by which MCT inhibition exerts its effect on cervical cancer cells, however, by the results herein presented a promising therapeutic target can be anticipated for this type of tumours.

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Involvement of Grb2 adaptor protein in nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) mediated signaling and anaplastic large cell lymphoma growth

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Background: Most Anaplastic Large Cell Lymphoma (ALCL) express oncogenic fusion proteins derived from chromosomal translocations or inversions of the Anaplastic Lymphoma Kinase (ALK) gene. Frequently ALCL carry the t(2;5) translocation that fuses ALK gene to Nucleophosmin (NPM1) gene. NPM-ALK mediated transforming activity induces different pathways that control lymphoma cells proliferation and survival. Grb2 adaptor protein is thought to play an important role in ALK-mediated transformation, but its interaction with NPM-ALK and its functions in ALCL is still unclear.
In this work we focused on Grb2 binding to NPM-ALK, its phosphorylation by the fusion protein and its role in regulating signaling pathways and proliferation of ALCL cells.
Methods: Human embryonal kidney cells HEK-293T were transfected with different Grb2 and/or NPM-ALK constructs and immunoprecipitation and immunoblot analysis were performed. All Grb2 and all kinase mutants were generated by PCR-based mutagenesis. Inducible ALK and Grb2 shRNA SU-DHL-1 and TS cells were obtained by co-transduction with pLV-tTRKRAB (TTA) vector and pLVTHM vector containing the H1 promoter shRNA cassette. NPM-ALK or Grb2 silencing is achieved when 1μg/mL of doxycyclin is added to the medium for 72 hours. To generate Grb2 shRNA-resistant constructs, wild-type or Y160F Grb2 were mutated in 4 bases in the sequence corresponding to the shRNA (Grb2INT3/4). Co-culture and proliferation experiments were performed.
Results: In this study we demonstrate that Grb2 binds to active NPM-ALK and is phosphorylated in human ALCL cells. We identified Y160 as major phosphorylation site of Grb2 by NPM-ALK. We found that Y160 of Grb2 is phosphorylated also by other oncogenic fusion tyrosine kinases such as TPR-MET, BCR-ABL and TEL-JAK2, as well as by wild-type receptor tyrosine kinases, such as ALK and MET. Further, we show that NPM-ALK combined mutations in Y152–156, Y567 and P415–417 almost completely abrogated Grb2 binding and Y160 phosphorylation. Finally, shRNA knock-down experiments showed that Grb2 is essential for SHP2 activation in ALCL and is required for sustained ALCL cell growth.
Conclusions: Grb2 silencing in ALCL cells strongly impaired cell proliferation, suggesting that Grb2 is fundamental for the full activation of a signaling cascade that involves Shc and SHP2 and assures lymphoma cells proliferation. Thus, Grb2 could represent a potential target to control cell proliferation in NPM-ALK mediated lymphomas.

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Integrating alternative splicing studies as a tool for innovative therapeutical interventions: focus on novel drug targets and novel epitopes

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Alternative RNA splicing is a key molecular mechanism for the generation of functional protein diversity. Abnormal alternative splicing can occur in cancer, resulting in the production of novel transcript variants or in an imbalance between mRNA isoforms. In both cases, it can affect the global pattern of protein expression within a cell, sustain tumour growth or affect drug response. Therefore, identification of cancer-associated alternative splicing variants may represent a significant step forward and potential source of new clinical diagnostic, prognostic and therapeutic strategies.
Results: ExonHit has generated discovery engines aimed at studying alternative splicing and is currently building libraries of alternative splicing events that are deregulated in cancer and in cases of therapy resistance. Here, using ExonHit's Genome Wide SpliceArray™ microarray, we investigated patterns of alternative splicing in diverse human cancers as well as in drug resistance models. Distinct splicing patterns were evidenced using principal component analysis and through statistical analysis of differential splicing. We show that a number of already targeted genes, as well as drug resistance genes, in fact undergo alternative splicing, which can ultimately affect drug response. We also implemented bioinformatic processes and selectivity filters that allowed to identify (1) alternatively splicing variants with altered druggable domains and (2) splicing variants that generate novel cell surface epitopes. Using this strategy, we show for example how the small GTPase Rac1 which is subjected to alternative splicing to generate the self-activated variant Rac1b, can be targeted through isoform-selective medicinal chemistry programs. In addition, we illustrate how such strategy can help to identify a number of other alternatively spliced transcripts containing novel amino acid sequences that can be used as novel epitopes. These novel epitopes-containing variants are now being used to target monoclonal antibodies for therapy.
Conclusion: Alternative RNA splicing offers a currently underexploited source of novel disease targets. Our results demonstrate a significant contribution of splicing to cancer development and drug response. Platforms dedicated to studying alternative splicing can be integrated into discovery processes to allow identification of novel targets for drug discovery which can then be subjected to innovative therapeutical interventions based on splicing variant-selective drug design, antisense- or antibody-based therapies.

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Investigations on organic anion-transporting polypeptides 1A2, 1B1 and 1B3 in colon cancer as potential targets for cancer therapy

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Background: Organic Anion-Transporting Polypeptides (OATP) selectively facilitate the uptake of endogenous substrates and drugs into cells. Previous studies suggest that certain OATPs are expressed in several cancers, which profiles them as potential target candidates for novel cancer therapeutics (I. Sainis *et al.*, *Mar Drugs* **8**, 629, 2010).
Materials and Methods: We investigated the expression of OATPs 1A2, 1B1, and 1B3 in human colon cancer. We studied their expression at the mRNA level by TaqMan real-time RT-PCR and at protein level by immunohistochemistry, in human colon cancer material and in four colon cancer cell lines (Caco, C205, HT29 and L174T). For immunohistochemistry we used the following antibodies: polyclonal rabbit SSV anti-OATP1A2, monoclonal mouse ESL anti-OATP1B1 and monoclonal mouse MDQ anti-OATP1B1/1B3.
Results: Using frozen samples from cancerous and adjacent non-cancerous colon tissues, we found OATP1B3 mRNA significantly expressed in 18/20 cancerous samples (where it reached 8.3-fold levels over control), as was also in the HT-29 and L174T cell line (4.7-fold enrichment). It normal colon OATP1B3 mRNA levels were undetected. Interestingly, OATP1A2 mRNA expression was also detected in the CaCo cell line, while HT29 expressed OATP1B1. The immunohistochemical study revealed that OATP1A2 and 1B1 were expressed in all studied cases while1B3 (by using the mMDQ antibody which also recognizes an epitope shared by 1B1) was expressed in 27/30 samples. Interestingly in positive cases, almost all cancer cells were stained positive. Furthermore, OATP1A2 protein expression was intense in 3/4 of the cases studied while 1B1 and 1B1/1B3 expression was weak in 40% and 63.3% respectively. Investigation of cancer-associated mutations of 1B3 is under way and will be presented.
Conclusions: Organic Anion-Transporting Polypeptides 1A1, 1B1 and 1B3 are differentially expressed in colon cancer. We suggest that further investigation of OATPs in colon and other cancers is warranted, in search of new cancer targets that may offer perspectives for the development of novel targeted cancer therapies.

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Identification of an inhibitor for melanoma cell migration through the inhibition of Pirin

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Background: Bcl3 was originally identified as a putative proto-oncogene which frequently rearranged in chronic B cell lymphocytic leukemia. Bcl3 interacts with NFkB by binding to p50 and p52. The overexpression of Bcl3 was reported to enhance cell survival, proliferation and tumor malignancy. Pirin is known to be bound to Bcl3, however, the exact roles of Pirin in tumor cells have not been clarified.
Materials and Methods: To discover the Pirin ligands, we have carried out a screening with the aid of a chemical array. In this screening system, small-molecules are covalently immobilized on the glass slides through a