multitarget tyrosine kinase receptor inhibitor including those important for angiogenesis (vascular endothelial growth factor receptors) with both antiangiogenic and antitumor properties. However, if targeting the function of VEGF leads primarily to significant tumor regression it could also be followed by tumor relapses. One possible strategy to overcome this therapeutic obstacle might be to overexpress anti-vascular factors to destroy tumor vasculature, rather than inhibiting the formation of new vessels. Recently, the angiopoietin (Ang) family has been identified to cooperate with VEGF to control angiogenesis. Among Angs, Angiopoietin-2 (Ang2) is involved in vessel remodelling leading to angiogenesis or vessel regression depending on VEGF activity. Accordingly, it is of interest to investigate whether an Ang2 overexpression may impair tumor growth, in particular in the brain. The aim of this study was to evaluate and compare the effect of an anti-angiogenic strategy (by the use of SU treatment), an anti-vascular strategy (by a tumoral overexpression of Ang2) or the combination of both Ang2/SU, on glioma vasculature, angiogenesis and growth.

Materials and methods: 9L glioma cells were implanted in the striatum of Fischer 344 rats. Ang2 was overexpressed by 9L cells from a stable transfection. SU treatment was administered daily 3 days after 9L cell implantation. Four groups of animals were studied: 9L (n=10); 9L-SU (n=7); 9L-Ang2 (n=13); 9L-Ang2/SU (n=11). Tumor volume was analysed by MRI (7T, Bruker). Tumor vascularization was assessed by immunohistochemistry using antibodies against RECA-1.

Results: MRI results showed that, at day 18, each treatment leads to a significant reduction of the tumor volume in comparison with non-treated animals, i.e 72% (9L-SU), 86% (9L-Ang-2), 95% (9L-Ang2/SU) suggesting that combined inhibition of VEGF and overexpression of Ang2 enforces glioma regression. Immunohistochemistry study showed a significant decrease in tumor vessel density and length in the 9L-SU group in comparison with the other groups. Interestingly, this anti-angiogenic effect of SU treatment totally disappeared in presence of an Ang2 overexpression.

Conclusions: Collectively, our data reinforce the interest to consider anti-vascular strategies for glioma treatment, in parallel to anti-angiogenic strategies.

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Role of E2F1 in sporadic Burkitt lymphoma formation

I. Molina Privado[1], P. Rebollo[1], D. Martín[2], M.J. Artiga[2], J. Menárguez[3], E.K. Flemington[4], M.A. Piris[2], M.R. Campanero[1]
[1]"Alberto Sols"Biomedical Research Center, Molecular and Cellular Cancer Biology, Madrid, Spain; [2] Spanish National Cancer Research Centre, Molecular Pathology, Madrid, Spain; [3] Gregorio Marañón Hospital, Pathological Anatomy, Madrid, Spain; [4] Tulane Health Sciences Center, Pathology, New Orleans, USA

Treatment of Burkitt Lymphoma(BL),an aggressive non-Hodgkin´s lymphoma,requires intensive combination chemotherapy that is associated with systemic toxicity.A better knowledge of the molecular basis of BL lymphomagenesis might help to develop less toxic treatments.The molecular mechanism underlying sporadic BL(sBL)formation still remains largely unknown.No single genetic lesion,besides c-myc translocation has been associated to the pathogenesis of most sBL cases to date.Patients would benefit from novel therapies aiming at specific molecular markers that have yet to be identified.To gain insight into the molecular basis of sBL tumor formation we looked for genes whose expression was deregulated in most sBL cases and may therefore have a role in lymphomagenesis. Because c-myc induces the expression of activators E2F family members and they can behave like oncogenes in mouse models,we compared their expression in BL and lymphoblastoid B-cell lines(LCLs)and found that both mRNA and protein E2F1 levels,but not those of E2F2 and E2F3,were higher in BL than in LCL cell lines.Using qPCR and immunohistochemistry we determined that the relative expression of E2F1 mRNA and protein was also much higher in tumor samples derived from sBL patients than in control tissues or in samples from other B cell lymphomas. The elevated E2F1 expression is not a consequence of the high proliferation rate of these cells,because other lymphoma samples with a similar proliferation index showed no increased E2F1 expression. To asses whether elevated E2F1 is required for tumorigenicity of sBL we reduced E2F1 expression by specific shRNAs in DG75 sBL cell line.Reduction of E2F1 expression greatly decreases their ability to form colonies in soft agar and their tumor formation capacity when inoculated in immunodeficient SCID mice,reduces their proliferation capacity,causes their accumulation in the G2/M phase of the cell cycle and leads to hyperploidy. These findings indicate that E2F1 is involved in sBL formation and suggest that it might collaborate with c-myc in sBL lymphomagenesis by eliciting progression through G2/M. Since E2F1 is not required for proliferation of most normal cells and,in fact, E2F1-/- mice are viable until old age,our results suggest that E2F1 is a promising target for developing novel and less toxic treatments of sBL. Moreover,our data show for the first time that over-expression of any E2F family member might play a positive role in the formation of a human tumor.

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SiRNA mediated downregulation of Bcl-2 in small cell lung cancer cell lines - impact on protein level and cell viability

A. Santos[1], J.P. Pereira[1], M.C. Pedroso de Lima[2], S. Simões[1], J.N. Moreira[1]
[1]University of Coimbra, Faculty of Pharmacy and Center for Neuroscience and Cell Biology, Coimbra, Portugal; [2] University of Coimbra, Faculty of Sciences and Thecnology and Center for Neuroscience and Cell Biology, Coimbra, Portugal

Background: The identification of activated proto-oncogenes has made possible to consider such genes as targets for gene silencing-based therapies. One of such targets is the BCL2 gene, known for its anti-apoptotic activity and overexpressed in most cases of small cell lung cancer (SCLC). The success of any gene silencing strategy depends on three main factors: the achievement of a suitable pharmacokinetic and biodistribution profile of the gene silencing drug; relevance of the target gene for tumor viability; and the potency and selectivity of the gene silencing drug. In this context, the objective of the present study was to evaluate the in vitro potency of different gene silencing molecules in terms of Bcl-2 downregulation and cytotoxicity against SCLC cells, as well as to evaluate the relevance of BCL2 as a molecular target in this type of tumor.

Materials and methods: An antisense oligonucleotide (ODN) and two small interfering RNA (siRNA) sequences targeting specifically the BCL2 mRNA at different positions, were transfected into SCLC cells that overexpress the Bcl-2 protein. The levels of Bcl-2 protein were determined through flow cytometry. Cell growth inhibition was evaluated by the resazurin reduction method, whereas cell viability was evaluated by flow cytometry with 7-aminoactinomycin D staining in direct correlation to Bcl-2 protein signal. Combined treatments of anti-BCL2 ODN or siRNA and the cytotoxic drug cisplatin were evaluated using both the Bliss Independence and Loewe criteria, and error propagation applied to infer about the uncertainty of the predictions of additivity.

Results: In experiments where tumor cells were submitted to 1 or 2 treatments with G3139 ODN or anti-BCL2 siRNAs, a significant sequence-specific downregulation of Bcl-2 protein levels was observed that, surprisingly, did not cause any sequence-specific inhibition of cell growth. Under treatment conditions resulting in the strongest Bcl-2 downregulation, anti-BCL2 siRNA-treated SW2 cells presented higher viability than cells treated with a non-targeted sequence. Combined treatments of anti-BCL2 ODN or siRNA and the cytotoxic drug cisplatin were merely additive on cell growth inhibition.

Conclusions: Overall, these results point out for the higher potency of siRNA, as compared to ODN, in silencing the BCL2 oncogene, but most importantly, question some previous established concepts on the role of Bcl-2 protein in SCLC cell lines.

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Towards combined treatments targeting Met and downstream survival signals: from cell biology to preclinical trials

A. Furlan[1], A. Hussain[1], D. Barila[2], S. Patané[1], B. Maigret[3], R. Dono[1], F. Maina[1]
[1]Institute of Developmental Biology of Marseille-Luminy, Team RTK signalling: From Mouse Development & Pathologies to New Molecular Therapies, Marseille, France; [2] Istituto Telethon Dulbecco, Università di Roma, Roma, Italy; [3] Université H. Poincaré, Nancy 1, Nancy, France

Studying the nature of Met oncogene signals and establishing therapeutic approaches to target Met-triggered neoplasia represent two major aims of our laboratory.

The Met oncogene plays a major role during tumorigenesis, by conferring to neoplasic cells growth advantage, invasive abilities and decreased sensitivity to therapeutic agents. It is hence not surprising that Met is a prognostic marker for a variety of tumours. More recently Met received further attention as it can confer resistance to EGFR inhibitors in lung, medulloblastoma multiforme and breast tumours, by substituting EGFR activity. We recently uncovered novel mechanisms involving well-known proto-oncogenes, which are activated by Met to promote hepatocyte survival in developing livers. First, Met acts through the PI3K-Akt pathway to induce the translation and nuclear translocation of Mdm2, which is an endogenous antagonist of p53 activity and cell death. Besides, Met triggers Mdm2 transcription through a novel molecular mechanism currently under investigation. Interestingly, we found that these pathways also contribute to the survival and the anchorage-independent growth of cancer cells with aberrant Met.

Based on these findings, we are developing new therapeutic approaches combining drugs targeting both Met and its survival signalling targets. With

this strategy, we intend to enhance the efficiency of cancer therapies, and cover up possible resistance to individual treatments. Studies are in progress to assess the in vitro efficacy of these combined treatments. In parallel, we perform preclinical trials to investigate the importance of these mechanisms for tumor formation and metastasis in vivo. The efficacy of these treatments is first investigated in chicken metastasis assays. Besides, we have generated transgenic mice over-expressing Met in a temporally and spatially regulated manner. Met-overexpressing cells also express the luciferase, for non-invasive monitoring of primary tumours and metastasis. These mice are precious tools to better understand Met-triggered tumorigenesis and validate the efficiency of combined treatment to un-favour cell survival in Met-triggered cancers.

Altogether, by targetting Met activity and its downstream survival signals, we will elucidate the efficacy of novel combined anticancer therapies.

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A standardized approach to animal study data collection, analysis and management practices expands possibilities for the sharing of results

E. Ibsen[1]
[1]*Studylog Systems Inc., Animal Study Management Software, South San Francisco, USA*

The lack of data digitization and a standardized approach to data collection, analysis, content, format and storage conventions makes animal study (AS) data access and cross-study comparisons impractical within and across research organizations. Current methods of AS data collection, analysis and management for animal studies in oncology are highly variable and primarily manually-based, using combinations of paper notebooks and electronic spreadsheets. As a consequence, data cannot be accessed and shared effectively because: 1) data recorded in lab notebooks remain un-indexed and inaccessible once collected; 2) spreadsheets often contain only primary measurement data and are stored in data silos, 3) AS conditions, methods and model details are recorded inconsistently, in insufficient detail, and reside in multiple locations and formats. The unintended consequence is a systematic, unintentional, and yet preventable waste of data, information, organizational knowledge, time and resources. Continued improvements in in vivo modeling result in an ever-increasing amount of information which is becoming increasingly difficult to manage. A growing number of scientists are finding that commercial AS management software applications provide a standardized approach to managing this information which reflects industry-wide best practices. These software systems also improve process efficiency, data integrity and security, and data accessibility as well as increase the transparency of study data and processes, facilitate personnel oversight, IACUC compliance, and inter-study comparability. Standardization of data into a single, digital form would enable the creation of a centralized, web-based study repository for the voluntary sharing and pooling of AS data from many labs, which would give investigators, journals, and institutions the opportunity to publish their both negative and positive non-proprietary results. By having the details on historical studies available, researchers could obviate needless repetition of studies, reduce the number of animals used, expedite model implementation projects, refine current animal models and build on existing work. The net result would be a significant improvement in information sharing, much more efficient use of limited research resources and ultimately a decreased time to identify, develop and market new therapeutics for cancer.

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ZNF23 induces apoptosis in human ovarian cancer cells

X. Ding[1], C. Huang[1], H. Sun[2], S. Yang[1], R. Ge[2], F. Shen[2], Y. Wang[1]
[1]*Institute of Neuroscience, Lab of Neural Signal Transduction, Shanghai, China;* [2] *Eastern Hepatobiliary Surgery Hospital Second Military Medical University, Division of Comprehensive Treatment, Shanghai, China*

The Krupple-associated box-containing zinc finger proteins (KRAB-ZFPs) constitute one of the largest families of transcription factors. Some members of this family play critical roles in regulation of development, cell proliferation and apoptosis. Many KRAB-ZFPs are implicated in malignancies. Previously, we cloned and characterized ZNF23, which was a member of this family. Its gene localized to the 16q22, a chromosomal region frequently altered in solid tumors. ZNF23 expression was reduced in human cancers and ectopic expression of ZNF23 inhibited cell growth by inducing cell cycle arrest. Here we showed that in ovarian cancer cells, ZNF23 expression also induced apoptosis. The protein level of ZNF23 was greatly down-regulated in 20 samples of ovarian tumors compared with that in 13 samples of normal ovarian tissues. In ovarian cancer cell line SKOV-3, restoration of ZNF23 expression led to apoptosis as demonstrated by activation of caspase-3, nuclear condensation and formation of a sub-G1 peak. This apoptotic process was accompanied by loss of mitochondrial membrane potential, cytochrome c release and caspase-9 activation. Furthermore, ZNF23-induced apoptosis was partially through down-regulation of Bcl-XL. Thus, our study suggested that ZNF23 also played an important role in the induction of apoptotic cell death, and pointed to the possibility that its down-regulation might render ovarian cancers to an increased survival capacity.

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Endothelial cell KIT expression in pediatric brain tumors

M. Puputti[1], P. Pernilä[2], O. Tynninen[3], A. Paetau[3], H. Sihto[2], H. Joensuu[4]
[1]*Helsinki University, Helsinki Biomedical Graduate School, Helsinki, Finland;* [2] *Helsinki University Central Hospital, Laboratory of Molecular Oncology, Helsinki, Finland;* [3] *Helsinki University Central Hospital (HUSLAB) and University of Helsinki, Department of Pathology, Helsinki, Finland;* [4] *Helsinki University Central Hospital, Department of Oncology, Helsinki, Finland*

Background: Receptor tyrosine kinases expressed in vascular endothelial cells are potential targets for therapy with tyrosine kinase inhibitors. KIT (receptor of the stem cell factor, SCF) has recently been found in tumor vessel endothelial cells of glioblastomas. It is not known whether KIT is expressed in endothelial cells of pediatric brain tumors, and its role in tumor angiogenesis is unknown.

Methods: We evaluated KIT, phosphorylated (p-KIT), SCF, and VEGFR-2 (vascular endothelial growth receptor factor-2) expression in a series of 69 pediatric brain tumors (35 pilocytic astrocytomas, 12 medulloblastomas, 11 ependymomas, 7 dysembryoblastic neuroepithelial tumors (DNTs), and 4 gangliogliomas) using immunohistochemistry. The median age at the time of the diagnosis was 9 yrs (range, from 0 to 20). PDGFRA (platelet derived growth factor receptor alpha), KIT, VEGFR2, and EGFR (epidermal growth factor receptor) gene copy numbers were determined using chromogenic in situ hybridization.

Results: Marked endothelial cell KIT expression was found in 13 (37%) of 35 pilocytic astrocytomas, 6 (55%) ependymomas, and 1 (8%) medulloblastoma, whereas none of the DNTs or gangliogliomas showed marked expression. Twelve (60%) of the 20 tumors with marked expression expressed also markedly p-KIT as compared to only 7 (14%) of the 49 tumors that lacked marked KIT expression (P =.0001, chi-square test). Endothelial cell KIT expression was associated with age at diagnosis in the largest histological subset (pilocytic astrocytoma), where 11 (69%) of the patents aged 8 or less had marked endothelial cell expression as compared to only 2 (11%) of those older than 8 (P =.0004). In the entire series 16 (53%) of patients < 8 at diagnosis had marked KIT expression in tumor endothelial vessels as compared to 4 (10%) among those > 8 (P <.0001). No KIT, PDGFRA, VEGFR2, or EGFR gene amplifications were detected in the endothelial cells of any of the tumors.

Conclusions: Endothelial cells of pediatric brain tumors often express KIT that is activated (phosphorylated), whereas marked VEGFR-2 expression is rare. Patients diagnosed with pilocytic astrocytoma at a young age often have a tumor with a marked endothelial cell expression of KIT, whereas such ex-pression is usually not found in tumor vessels of pilocytic astrocytomas detected at an older age. These findings suggests that angiogenesis of pilocytic astrocytomas of young children may differ from that of older ones.

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Heterocyclic thiosemicarbazones and their Zinc(II) complexes inhibited proliferation of four different neoplastic cell lines

T. Stanojkovic[1], A. Primikyri[2], Z. Kljajic[3], M. Dosi[2], Z. Juranic[1], M.A. Demertzis[2], D. Kovala-Demertzi[2]
[1]*Institute for Oncology and Radiology of Serbia, Experimental, Belgrade, Serbia;* [2] *Inorganic and Analytical Chemistry University of Ioannina, Department of Chemistry, Ioannina, Greece;* [3] *Institute of Marine Biology Kotor, Department of Chemistry, Kotor, Montenegro*

Background: It has been reported that thiosemicarbazones (TSCs) are potent antitumor agents. This class of compounds can suppress tumor growth by inhibiting the bioactivity of ribonucleotide reductase (RNR). RNR is the rate-limiting enzyme in DNA synthesis due to its low abundance in normal cells. In comparison with several other key enzymes, RNR shows the greatest increase in activity in tumor cells and, therefore, RNR is considered an important intracellular target for inhibiting cellular proliferation. The aim of this work was to asses the antiproliferative action of some newly synthesized heterocyclic thiosemicarbazones and their zinc (II) complexes, on four human neoplastic cell lines: human cervix carcinoma (HeLa), chronic myelogenous leukemia (K562), and breast carcinoma (MDA-MB-453, and MDA-MB-361).

Methods: A new series of thiosemicarbazones (TSCs) and Zinc(II) complexes were synthesized. The structures of all the compounds were determined by analytical and spectral (1H-NMR,13C-NMR, IR, MS and