Presently, we describe the parallel synthesis of a library containing 108 analogues of deglycobleomycin and the results of an ongoing biochemical evaluation of that library. Also described is the solid phase synthesis of bleomycin A5 itself, as well as three bleomycin analogues altered within the carbohydrate moiety. The ability of these species to mediate DNA and RNA cleavage will be discussed.

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Natural product-based phosphatase and tubulin-polymerisation inhibitors

P. Wipf, *University of Pittsburgh, Department of Chemistry, Pittsburgh, PA, USA*

A major goal of our work is to demonstrate the potential of complex natural products to serve as biological probes of cell cycle events and as lead structures for anticancer drug development. Complex natural products-derived targets pose significant challenges for analog synthesis due to their structural diversity and the requirement for multi-step syntheses. In a collaborative effort, we have made significant progress in the application of combined solid phase - solution phase synthetic strategies for the development of biologically relevant Cdc25 dual-specificity phosphatase inhibitors and antimitotic agents. After several stages of iterative optimizations, we have identified submicromolar inhibitors in each series that exceed the potency and selectivity of the natural product lead structures that inspired the combinatorial chemistry library development. This talk will present our interdisciplinary approach in both areas with a focus on synthetic methods and summarize the new perspectives that we have gained in the attempt to condense distinct functionalities of the structurally diverse natural product leads.

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A combinatorial chemistry approach to gene targeting agents

D.E. Thurston, *University of London School of Pharmacy, CRC GTDD Research GRP, London, United Kingdom*

The ability to modify gene expression using small molecules should lead to novel therapeutic agents as well as providing the tools to carry out carry out functional genomics studies. For example, the down-regulation of key genes or families of genes in cancer, bacterial, viral or parasite cells should lead to novel anticancer, antibacterial, antiviral and antiparasitics agents.
In principle, gene down-regulation can be achieved by intervening at the DNA, RNA or protein level, and many marketed drugs work by interacting with a specific protein. More recently, effort has been put into targeting RNA with macromolecules, and some success has been achieved with antisense, ribozyme and RNAi approaches. However, there are significant difficulties in translating macromolecules with in vitro activity into therapeutic agents. For these reasons attention has been given to targeting the DNA template itself.
Targeting DNA to regulate gene expression has a number of advantages. The most significant advantage is that most cells contain only two copies of a given gene and successful blocking of transcription ensures that no further RNA transcripts are produced. This is an inherently more sensitive and efficient approach compared to antisense-type technologies where drug molecules and RNA transcripts need to be present in stoichiometric amounts for maximum down-regulation efficiency. Furthermore, the DNA template is left intact and is capable of producing more RNA transcripts.
Some success with gene targeting at the DNA level has been achieved with nucleic acids, proteins and small molecules. The development of small molecules for gene targeting has created much interest because, unlike macromolecules, they can have favourable cellular permeation and pharmacokinetic properties and can be developed as therapeutic agents.
This presentation will review recent advances in targeting DNA using small molecules and will include recent data from the author's own laboratory which uses a combinatorial chemistry approach to produce libraries of DNA-interactive agents.

Wednesday 20 November

WORKSHOP

Altering the threshold of apoptosis

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Targeting mitochondria for apoptosis induction

G. Kroemer, *Institut Gustave Roussy, CNRS-UMR1599, Villejuif, France*

The supreme goal of anti-neoplastic chemotherapy is the selective eradication of cancer cells, which appears to depend on the induction of apoptosis, the cell's intrinsic death program. One major critical event (checkpoint) intergrating several apoptosis pathways is mitochondrial membrane permeabilization (MMP). MMP largely determines the point-of-no-return of the death process, and is triggered by chemotherapy, both *in vitro* and *in vivo*. MMP is subject to a complex regulation, and local alterations in the composition of mitochondrial membranes, as well as alterations in pre-mitochondrial signal-transducing events, can determine chemotherapy resistance. Detecting MMP may be useful for detecting chmotherapy responses *in vivo*. Moroever, chemotherapeutic agents may be designed to induce MMP by local effects on mitochondria. An alternative strategy for cell death induction consists in misdirecting apoptosis effectors normally sequestered in mitochondria (and normally only release after MMP) to the extra-mitochondrial compartment. Thus, for instance overexpression of AIF (apoptosis inducing factor) can enforce the induction of apoptosis in cells which are resistant to MMP.

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Novel small molecule inhibitors of Bcl-xL anti-apoptotic proteins

D. Hockenbery, *USA*

Abstract not received.

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Regulation of Bcl-2 family members during drug-induced apoptosis

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Proteins of the Bcl-2 family are critical determinants of the cellular threshold for apoptosis [1]. The importance of the pro-apoptotic proteins Bak and Bax to the engagement of apoptosis after drug-induced damage was demonstrated by the drug resistance of Bak/Bax double knock out cells [2]. Bax and Bak proteins require activation by cell damage-induced signals to trigger apoptosis. This process is thought to involve the participation of BH-3 only members of the Bcl-2 family such as Bid, Bim and Bad and is countered by the anti-apoptotic proteins of the family such as Bcl-2 and Bcl-xL.
We examined Bax activation following drug-induced damage in SH-EP 1 (glial-like) and SH-SY5Y (neurone-like) neuroblastoma (NB) cells are derived from the parental line SK-N-SH. In a clonogenic assay, both cell lines are sensitive to cisplatin, but only SH-EP1 cells are sensitive to Taxol. In SH-EP1 cells, Bax undergoes three changes prior to cytochrome c release and apoptosis induced by either cisplatin or Taxol. Step 1 is a conformational change at the N-terminus of Bax, Step 2 is the translocation of Bax from cytosol to mitochondria and Step 3 is Bax dimerisation at the mitochondrial surface [3]. Steps 1-3 of Bax activation also occur in SH-SY5Y cells after either drug yet cytochrome c is released from mitochondria only after cisplatin treatment and not after Taxol. We are currently investigating why Taxol resistant SH-SY5Y neuroblastoma (NB) cells fail to fully activate the pro-apoptotic protein Bax after Taxol treatment and which of the BH-3 only proteins play a role in Taxol and cisplatin induced apoptosis.
Bcl-2 family proteins also respond to signals derived within the cellular microenvironment. Our recent data show that 5 pro-apoptotic Bcl-2 family proteins (Bax, Bid, Bad, Nip3 and Bim) are down-regulated in several cancer cell lines under conditions of tumour hypoxia and that this correlates with

resistance to several of anticancer drugs with differing mechanisms of action.
Finally, the role of Bid as a lipid transfer protein [4] and its relationship to the pro-apoptotic function of Bid will be discussed.

References

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Roles of oncogenes and tumor suppressor genes in apoptosis

S. Lowe, *Cold Spring Harbor Laboratory, NY, USA*

p53 was initially identified as the "guardian of the genome" based on its ability to mediate a G1 arrest following DNA damage. However, p53 can participate in many processes involved in maintaining cellular integrity following stress, including cell-cycle checkpoints, DNA repair, senescence, apoptosis, angiogenesis, and the surveillance of genomic integrity. The relative contribution of each of these processes to tumor suppression is not known. We hypothesized that complete ablation of crucial p53 effector functions may produce tumors that are phenotypically identical to those with p53 mutations yet retain wild-type p53. To this end, we examined the ability of dominant-acting genes that completely disable apoptosis downstream of p53 to phenocopy the effects of p53 mutations during tumor development and treatment responses in the Em-myc transgenic mouse. This system provides an ideal setting in which to study p53 action during tumor development an therapy, since loss of p53 function dramatically accelerates tumor development and produces profound drug resistance to conventional chemotherapy. Using this system, we show that disruption of apoptosis provides the sole advantage to developing lymphoma cells that lose p53 function, whereas disruption of cell cycle checkpoints and anueploidy are mere byproducts of p53 loss. In contrast, disruption of both apoptosis and cellular senescence account for the impact of p53 loss on drug resistance. The implications of these results for understanding p53 action and the heterogeneity of treatment responses in human tumors will be discussed, as well as new insights into the program of cellular senescence and its role in treatment outcome.

Wednesday 20 November

PLENARY SESSION 2

Proffered Papers 1

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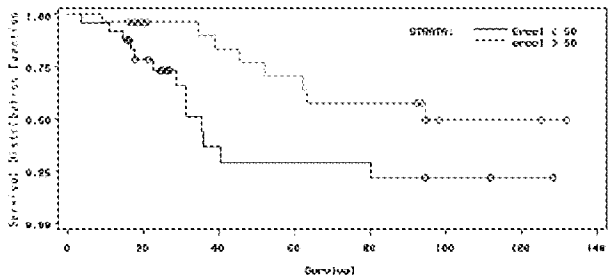
Increased ERCC1 expression predicts for improved survival in resected patients with non-small cell lung cancer (NSCLC)

G. Simon, S. Sharma, P. Smith, G. Bepler. *H. Lee Moffitt Cancer Center, Thoracic Oncology Program, Tampa, USA*

ERCC1 expression has been previously reported to predict for cisplatin resistance in patients with gastric carcinomas and NSCLCs (Metzger et al JCO 1998, Pg 309). Consequently gastric and NSCLCs treated with a cisplatin based chemotherapy had superior overall survival if their ERCC1 expressions were low. We evaluated the effect of ERCC1 expression on overall survival in 49 patients with Stage IA to IIIB NSCLC who underwent surgical resection. One of the 49 patients received postoperative adjuvant chemotherapy and radiation therapy. Five patients received post-operative adjuvant radiation therapy alone. Forty-three patients received no adjuvant therapy. Tissue specimens from these patients were collected and immediately frozen in liquid nitrogen. Total RNA was extracted, reverse transcribed, and used for real-time quantitative PCR (ABI Prism 7700). Gene expression was normalized using 18S rRNA as reference. ERCC1 expression ranged from 4.96 to 13,160.20. Median value for the entire group was 54.76. When we used 50 as the cut off there was a statistically significant difference in survival for patients with ERCC1 expression more than 50 (94.6 months) vs. less than 50 (35.5 months)(P=.01)(Wicoxon Rank Sum Test). See Graph 1.



Graph 1

Additionally, when we divided the entire cohort on the basis or ERCC1 expression to <30, 30 to 100 and >100. There was again a statistical significant survival between the three groups. Median Survival was 94.6 months for >100, 62.1 months for 30 to 100 and 35.5 months for <30. These differences were statistically significant (P value = 0.03). On the basis of our results we conclude that patients with an efficient DNA repair mechanism (High ERCC1 expression (>50)) have a better survival than patients in whom this mechanism is impaired (Low ERCC1 Expression (<50)). However patients with high ERCC1 expression also respond poorly to chemotherapy. Since patients with Low ERCC1 expression have a poorer prognosis but respond better to chemotherapy, they are likely to benefit from chemotherapy. Hence we propose that future adjuvant and neoadjuvant chemotherapy trials, should stratify patients according to their ERCC1 status. Updated results and multivariate analyses will be presented at the meeting.

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Mechanisms of cisplatin resistance - role of yeast SKY1 and its human homologue SRPK1

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The therapeutic potential of cisplatin, one of the most active and widely used anticancer drugs, is severely limited by the occurrence of cellular resistance. We used the budding yeast Saccharomyces cerevisiae as a model organism to identify and characterize novel genes involved in cisplatin-induced cell kill, and found several candidate players. Most strikingly, we identified SKY1 (serine/arginine-rich-protein-specific kinase from budding yeast) as a cisplatin sensitivity gene, whose disruption conferred a 4-fold cisplatin resistance. In cross-resistance studies, we observed resistance of yeast sky1del cells (i.e., cells from which the SKY1 gene had been disrupted) to cisplatin, carboplatin (but not oxaliplatin), doxorubicin and daunorubicin, and hypersensitivity to cadmium chloride and 5-fluorouracil. Furthermore, these cells did not display reduced platinum accumulation, DNA platination or doxorubicin accumulation, indicating that the resistance is unrelated to decreased drug import or increased drug export. Based on the modification of the anticancer drug sensitivity profile and our finding that sky1del cells display a mutator phenotype, we propose that the Sky1p protein might play a significant role in specific repair and/or tolerance pathways. Heterologous expression of the human SKY1 homologue SRPK1 (SR-protein-specific kinase) in yeast sky1del cells restored cisplatin sensitivity, suggesting that SRPK1 is also a cisplatin sensitivity gene, inactivation of which could lead to cisplatin resistance. Treatment of human ovarian carcinoma A2780 cells with antisense oligodeoxynucleotides directed against the translation initiation site of SRPK1, led to downregulation of SRPK1 protein and conferred a 4-fold resistance to cisplatin. Our findings strongly suggest that SRPK1 is involved in cisplatin-induced cell kill and indicate that SRPK1 might potentially be of importance for studying clinical drug resistance. Therefore, we have recently set out to screen clinical samples for SRPK1 expression and correlation with responsiveness to platinum-based chemotherapy.
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References

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