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Topoisomerases and transcriptional regulation

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DNA topoisomerase inhibitors are widespread in nature and are among the most widely used anticancer agents. These compounds are able to stabilize reversible covalent DNA-topoisomerase complexes which over time will be converted into single- and double-strand DNA breaks due to collision with the replication fork or transcription machinery (for review, see 1). Although DNA topoisomerase inhibitors in many ways can be considered as classical DNA damaging agents, clustering analysis of a database linking gene expression with drug sensitivity clearly shows that topoisomerase inhibitors are different from pure DNA damaging agents (2). This is likely because DNA topoisomerases also play important roles as transcriptional regulators and participate in the cellular response to DNA damage (3).
Topoisomerase inhibitors may influence gene transcription via stress-induced signaling resulting in activation of p53 or NF kappaB. Alternatively, topoisomerase-mediated cleavage may selectively target certain transcription factors, as has been shown to be the case for amplified c-myc (4,5). Recent results indicate that the influence of topoisomerase inhibitors is not restricted to genes involved in growth and survival. Treatment with the topoisomerase II inhibitor etoposide stimulated the expression of interferon regulatory factor-7 (IRF-2) which plays an important role in the development and maturation of the immune system (6). Similarly, treatment with the topoisomerase I inhibitor camptothecin was associated with repression of target genes for the hypoxia inducible factor-1alpha (HIF-1alpha) such as vascular endothelial growth factor (VEGF) and inducible nitric oxide synthase (iNOS) (7,8). These exciting results suggest that the clinical activity of topoisomerase inhibitors may not only be due to their cytotoxic activity but also be associated with their capacity to modulate the expression of important factors controlling tumor invasion and tumor immunity.
The influence of topoisomerase inhibitors on gene expression could be due to several mechanisms. First, topoisomerases are able to modulate the superhelical density of DNA. Transcriptional initiation in eukaryotic cells rely on ATP-dependent chromatin remodeling complexes such as SWI/SNF to create negative supercoiling. This is accompanied by nucleosome sliding, altered DNA-histone interactions and the formation of alternative DNA structures such as DNA bends, cruciforms, Z-DNA and partially unwound DNA (9,10). Since the remodeling depends on the creation of superhelical torsion, the activity of the different chromatin remodeling complexes is strongly influenced by topoisomerases, in particular topoisomerase I. The second activity required for transcriptional initiation is histone acetyltransferases (HATs) which are in dynamic interplay with the histone deacetyltransferases (HDACs). Both HDAC1 and HDAC2 associate with topoisomerase II under normal physiological conditions and the two classes of enzymes are able to modify each other's activity in vitro as in vivo (11,12). Third, topoisomerases directly interact with a number of transcription factors including the TATA-box binding protein, Rb and p53 (13-15).
Taken together, these results provide new and exciting clues to the long-lasting clinical success of topoisomerase inhibitors and suggest alternative ways to select active compounds in the future.

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Combinatorial blockade of cancer targets using inhibitors of the HSP90 molecular chaperone

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The sequencing of the human genome and the elucidation of the molecular pathology of human cancers provides the basis for the systematic identification of new therapeutic targets. Use of appropriate molecular assays facilitates the development of personalised therapies targeted to the precise genomic profile of individual patients and their malignancies. Of both conceptual and practical importance, it is not yet clear whether successful treatment of human cancers harbouring several oncogenic abnormalities will necessitate correction of only a single molecular defect (house of cards effect and oncogene addiction model) or whether several or all of the oncogenic lesions must be modulated simultaneously (combinatorial therapy model). The Hsp90 molecular chaperone is responsible for the correct folding, function and stability of multiple oncogenic client proteins including ErbB2, Raf-1, Akt, CdK4, Met, steroid hormone receptors and mutant p53. Treatment with Hsp90 inhibitors results in depletion of client proteins through the ubiquitin-proteasome pathway. Combinatorial effects such as, for example, Raf-1 and Akt depletion result in simultaneous inhibition of the Ras®Erk1/2 and PI3 kinase pathways, leading to cell-type dependent cycle arrest in both G1 and G2M phase and also to apoptosis, together with effects on other aspects of the malignant phenotype, including invasion and angiogenesis. The geldanamycin analogue 17AAG inhibits the essential ATPase activity of Hsp90, shows promising effects in preclinical models and is now undergoing clinical trial in our institution and other centres in the US. Pharmacokinetic studies demonstrated the achievement of active plasma concentrations and pharmacodynamic endpoints (elevation of Hsp70 and depletion of Raf-1, CdK4 and LcK) provide proof of principle for Hsp90 inhibition in peripheral blood lymphocytes and tumour biopsies. Elevations in liver enzymes represent the main side-effect. An update on clinical and mechanistic studies with 17AAG will be provided. It is desirable to develop alternative Hsp90 inhibitors with potential advantages over the first-in-class compound. We have used high throughput screening and x-ray crystallography to identify and develop new inhibitors of Hsp90.

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Poster Sessions

Angiogenesis and metastasis inhibitors

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The antitumor activities of celecoxib are mediated primarily through inhibition of pge2 production

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We have demonstrated in a number of models that celecoxib treatment of established tumors produces tumor growth inhibition, reduced tumor angiogenesis via 2 fold increase in apoptosis and 50% reduction in proliferation of both tumor and stromal cells. Celecoxib treatment of a human head and neck 1483 xenograft model reduced tumor growth by 79%. Importantly, 2B5, a neutralizing PGE2 antibody, yielded similar tumor control, indicating that the antitumor effect is primarily attributable to reduction of COX-2-mediated prostaglandin (PGE2) production. We also observed that celecoxib treatment prevented hypercalcemia (14.2±0.8 mg/dl vs 9.4±1.7 mg/dl, vehicle vs celecoxib) and net weight loss throughout the study (-4.3±0.3 g vs +3.5±0.4 g, vehicle vs celecoxib treated). This observation was further tested when celecoxib was added to a colon 26 murine syngeneic colon tumor model of cachexia. This aggressive tumor induces significant weight loss (15-20%) with concurrent elevations in serum IL-6 (~7 fold, to >70 pg/ml) and serum Ca++ (16.5±1.3 mg/dl), two commonly used cachexia markers. Treatment of cachexic, tumor-bearing animals with celecoxib resulted in a 5-fold reduction in intra-tumoral PGE2, a marked and rapid weight gain (3.5±0.4g in 96hr), a reduction in serum IL-6 (<20 pg/ml after 24h celecoxib treatment) and a reduction of serum Ca++ levels (to 12.5±0.5 mg/dl). These data suggest that inhibition of COX-2 may have additional clinical utility in the reduction of tumor-induced cachexia. While preclinical studies suggest that celecoxib is efficacious in tumor prevention and at slowing tumor growth as a monotherapy, its antiangiogenic activity and safety profile make in an ideal add-on with current standard-of-care cytotoxic therapies including CPT-11, 5-FU and radiation. Because cytotoxics have been demonstrated to induce expression of COX-2, it has been suggested that COX-2-derived prostaglandins may be survival factors that ultimately limit efficacy; therefore, there may be additional benefit from combination therapy with cytotoxics and a COX-2-selective drug. We have also observed enhanced antitumor efficacy when celecoxib is combined with various cytotoxic therapies, particularly radiation where it is clearly synergistic in preclinical models. The mechanism of celecoxib radiosensitisation does not involve cell cycle redistribution, as observed with most other radiosensitising agents, and is currently under investigation.