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Wednesday 8 November 10:15–12:00

WORKSHOP 5

Chemoprevention and biomarkers

18 INVITED

Angiogenesis as a target for chemoprevention

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Angiogenesis is necessary for solid tumor growth and dissemination, a promising target not only in cancer therapy but also in prevention. We have shown that various molecules, such as flavonoids, antioxidants and retinoids, act in the tumor micro-environment inhibiting the recruitment and/or activation of endothelial cells and phagocytes of innate immunity. N-acetyl-cysteine, the green tea flavonoid epigallocatechin-3-gallate (EGCG), and Alpha lipoic acid (ALA) all prevent angiogenesis in the Matrigel sponge angiogenic assay in vivo and inhibit the growth of the highly angiogenic Kaposi's sarcoma tumor cells (KS-Imm) in nude mice. The synthetic retinoid 4-hydroxyfenretinide (4HPR) also showed anti-angiogenic effects. Taken together, these data indicate that angiogenesis is a common and key target of most chemopreventive molecules, where they most likely suppress the angiogenic switch in pre-malignant tumors, a concept we termed "Angioprevention". Functional genomics analyses of gene expression regulation by anti-angiogenic chemoprevention compounds in primary human umbilical endothelial cells (HUVEC) in culture through Affymetrix GeneChip arrays identified overlapping sets of genes regulated by the anti-oxidants. In contrast, the ROS-producing 4HPR induced members of the TGFβ-ligand superfamily, which, at least in part, explains its anti-angiogenic activity. NAC and the flavonoids all suppressed the IkB/NF-kB signalling pathway even in the presence of NF-kB stimulation by TNFα, and showed reduced expression of many NF-kB target genes. A selective apoptotic effect on transformed cells, but not on endothelial cells, of the anti-oxidants may be related to the reduced expression of the NF-kB dependent survival factors Bcl2 and Birc5/survivin, that are selectively over-expressed in transformed cells, by these factors. Inflammation is increasingly recognized as an angiogenic staimuilus in cancer, the repression of the NF-kB pathway suggests anti-inflammatory effects for the anti-oxidant compounds that may also have an indirect role in angiogenesis inhibition. For example, the green tea flavonoid EGCG inhibits inflammation-associated angiogenesis by targeting inflammatory cells, in particular neutrophils.

19 INVITED

"Integrative epidemiology" – from risk assessment to outcome prediction

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We propose an integrative epidemiologic approach to studying the entire cancer spectrum from exposure, to predisposition, early diagnosis and ending with cancer outcome, by applying the principles and perspective of epidemiology to the notable advances in molecular biology. This approach begins with epidemiologic case-control studies in which biologically intensive studies can be carried out usually on surrogate tissues (lymphocytes, plasma or serum). Common genetic variants that modulate behavior (eg CYP2A6) may also modify predisposition through altered metabolism of carcinogenic exposures. Tumor DNA can be isolated from serum or plasma, as a useful source for screening specific transcripts or mutations in mitochondrial or nuclear-DNA sequences. Identification of protein patterns in serum using high throughput proteomics linked to novel bioinformatics approaches can be useful for early detection. As we move along the continuum from surrogate to intermediate and target tissues, the procedures become more invasive, the size of the study more restricted, and we apply the case series approach. Pharmacogenetic profiles can be used to individualize therapy and to understand the functional consequences of chemoprevention, chemotherapy, or radiotherapy response. Again, common genetic variants may affect both risk and outcome, eg, matrix metalloproteases. At this level we can also correlate the genetic and epigenetic spectrum of changes in tumor tissue with epidemiologic data, and with surrogate tissue phenotype and genotype data. The converse direction also applies in that genes demonstrated to contribute to tumorigenesis provide a rich source of candidates for analysis related to exposure, to predisposition, early diagnosis. For example, polymorphisms in the cyclin D1 protooncogene, which is frequently elevated in tumors, are predictors of risk of development of lung cancer. This allows leveraging of the power of approaches arising from the completion of the human genome project that facilitate analysis of genetic changes in tumors on a global basis. This is a powerful new approach that cannot be successfully accomplished by any discipline independently. Integrating these new approaches requires a combination of the rigor of data and sample collection and validation inherent in epidemiologic research with the ability to perform global, unbiased analysis of genetic aberrations in tumors.

20 INVITED

The cancer preventive agent identification and early development program at the NCI

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Increasing knowledge of the molecular biology of cancer and improving methods of screening and early detection provide opportunities to prevent cancer initiation and to reverse or delay premalignant progression. To this end the Chemopreventive Agent Development Program of the Division of Cancer Prevention, U.S. National Cancer Institute has established a systematic research and development program to identify, develop, and qualify potential cancer preventive agents for clinical trials. The preclinical stages of the program encompass processes to identify potential agents and molecular targets, in vitro mechanistic and in vivo efficacy screening assays, in vivo intermediate endpoint and cancer incidence/multiplicity testing, and pharmacology and toxicology assessments. With chemical synthesis, manufacturing, and formulation data, also generated by the program, the preclinical in vitro and in vivo results are assembled into Investigational New Drug applications to the U.S. Food and Drug Administration for clinical studies. Clinical studies are initiated as single and repeat-dose phase 1 safety and pharmacokinetic studies and move forward to phase 2 biomarker modulation and efficacy studies. In this presentation, the processes of agent identification and development will be reviewed, particularly emphasizing newer computational approaches to agent identification using QSAR tools to mine chemical libraries for potential leads, toxicities, and molecular targets and to pharmacological evaluations using systems biology to study combinations of agents. Examples of agents currently in development will be presented to illustrate the challenges presented by complex botanical mixtures, biological peptide vaccines, single chemical entities, and combinations of agents. Experimental data illustrating the established methodologies for efficacy and toxicity assessment will be cited. Whether implemented as medical interventions in high risk subjects, dietary and supplement recommendations to population subsets, or changes in screening algorithms, the continued governmental commitment to cancer prevention promises to reduce significantly the economic and medical burden of cancer.

21 INVITED

Development of cancer chemopreventive agents post-coxibs

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The realisation that the long term use of the selective COX-2 inhibitors such as celecoxib can be associated with cardiac complications has led to the re-consideration of the risk-benefit assessment associated with their potential use in cancer chemoprevention. The coxib experience has engendered a subtle re-orientation of cancer chemoprevention drug development activities. There are indications according to which the adverse effects of coxibs are dose-dependent, so pre-clinical drug discovery and development efforts have been increasingly directed at the exploration of combinations of low dose coxibs with other drugs e.g. atorvastatin. An alternative development strategy is based on the expectation that diet-derived agents and age-old herbal remedies may have *a priori* a favorable safety record. Thus the characterisation of mechanisms of action and efficacy of naturally occurring agents with potential cancer chemopreventive properties has gained considerable momentum. Our group explores the chemopreventive efficacy, mechanisms, pharmacokinetics and pharmacodynamics of naturally occurring phytochemicals exemplified by silibinin from milk thistle, anthocyanins contained in fruits and berries and tricin from rice bran in the Apc^{Min+} mouse model of gastrointestinal carcinogenesis. All three agents reduced Apc^{Min+} mouse adenoma multiplicity. Measurement in clinical pilot studies of agent levels and biochemical changes germane to anticarcinogenesis in blood and tissues of humans who ingest these agents helps to ascertain whether the levels achieved in the preclinical

model are clinically achievable. In the case of silibinin, levels measured in the gut of colorectal cancer patients after daily consumption of formulated silibinin (silipide Indena; dose 1.44 g) for one week were about a fifth of the efficacious level of silibinin determined in the gut of *Apc*$^{Min+}$ mice, which had received silipide with their diet (0.2% silibinin) for their lifetime. Ultimately this type of work helps to identify promising efficacious and safe cancer chemopreventive substances.

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WORKSHOP 6

Overcoming critical barriers in immunotherapy

22 INVITED

CTLA-1 and PD-1 abrogation as targets for therapy

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CTLA-4 is a negative regulator of T cell activity and inducer of T cell tolerance. It is highly up-regulated on activated CD4 T cells, particularly CD25+ T regulatory cells. It promotes the anti-tumor activity of GM-CSF transduced tumor cell vaccines in mouse tumor models (van Elsas et al *JEM 2001*, Hurwitz et al *Cancer Res 2000*. CTLA-4 gene knock-out mice die of profound lymphoid proliferation and infiltrative myocarditis. CTLA-4 antibody has been shown to induce clinically meaningful responses in metastatic melanoma and RCC. The response rates range from 5% to 22%; most sustained over >12 months. Immune breakthrough events (IBEs) are associated with response and time to relapse (clinical benefit). The IBEs are principally colitis, hypophysitis and skin related, and are generally self-limited or medically manageable. There are rare cases of colitis requiring colectomy, but no infusion reactions or immunogenicity have been noted with the molecule. In a recent trial of CTLA-4 antibody at 3 mg/kg given intravenously every eight months for a year and a peptide vaccine administered subcutaneously 12 times in a year in patients with very high-risk resected melanoma, high levels of immunity were seen to MART-1 and gp100 by ELISPOT assay, and IBEs were clearly associated with time to progression, with $p > 0.03$. Elevated levels of antibodies to OmpC, and E. Coli gut antigen, were observed, and the presence of a single CTLA-4 nucleotide polymorphism called AG49 was associated to relapse and onset of autoimmunity ($p > 0.04$). 24/25 patients with resected stage IV disease (15) or stage IIIB/C disease (10, with a mean of 7 positive lymph nodes) are alive with a median of 17 months of follow-up. These promising data will be followed up with a confirmatory trial with a higher dose of CTLA-4 antibody.

Another way to utilize T regulatory pathways to increase tumor specific effector T cell activity is via modulation of PD-1 (programmed death-1), a molecule up-regulated on activated T cells, both CD8 and CD4 which binds B7-H1/2 and is a *negative* T cell regulator. PD-1 pathways block TcR signal transduction, and PD-1 knockouts show lymphocytic myocardial infiltration, as well as arthritis and glomerulonephritis. An anti-PD-1 human antibody has been generated that abrogates the activity of PD-1. A variety of experiments on the use of an anti-PD-1 abrogating antibody in vitro show that:anti-PD-1 increases proliferation of and enriches for functional tumor-antigen specific CTL and Th cells. Post-vaccine and endogenous pre-vaccine CTL specific for antigens MART-1 and gp100 from melanoma patients are increased in function and avidity as well as in number by 3–30 fold after exposure to PD-1 antibody in vitro. No changes in T cell phenotype were observed, but by Ki-67 staining and by CFSE staining, significant increases in levels of proliferationwere observed. PD-1 abrogation does not appear to impact on programmed cell death *in vitro*. Anti-PD-1 antibody may have an important role in rescuing and amplifying anti-tumor immunity *in vivo* and *in vitro*. We feel that clinical trials of PD-1 abrogating antibody are warranted, alone and with a cancer vaccine approach.

23 INVITED

T regs in cancer

T. Curiel[1,2], B. Barnett[1], W. Zou[1,3], I. Kryczek[1,3], M. Brumlik[1,2], J. Cheng[1], J. Rueter[1]. [1]*Tulane Medical School, Medicine, New Orleans, USA;* [2]*San Antonio Cancer Institute, Medicine, San Antonio, USA;* [3]*University of Michigan, Surgery, Ann Arbor, USA*

Cancers actively evade immunity through a variety of mechanisms. Recent evidence implicates CD4+CD25+ regulatory T cells (Tregs) in tumor immune evasion. The origins of tumor Treg remain poorly defined, as they may arise from naturally-occurring Tregs following tumor microenvironmental conditioning, or they may arise through a distinct differentiation pathway. Likewise, their mechanism(s) of action are not clearly defined *in vivo* and may be through contact-dependent or –independent pathways. Substantial evidence from mouse models for cancer demonstrates that depletion of Tregs improves endogenous and vaccine-induced tumor-specific immunity with improved tumor clearance and host survival. Tregs appear to help defeat immunity in some human cancers as well. To test the hypothesis that Treg depletion would improve immunity in human cancer, we undertook a phase I study that demonstrated that denileukin diftitox (Ontak, a fusion toxin consisting of interleukin-2 genetically fused to diphtheria toxin, approved to treat cutaneous T cell leukemia/lymphoma) depleted Tregs in ovarian, breast, bladder and lung cancer with concomitant improvement in general measures of immunity. Metastatic ovarian cancer largely regressed in one patient, prompting a phase II efficacy trial. This trial enrolled patients with epithelial ovarian cancer, stages III or IV failing first-line therapy with optimal surgical debulking and platinum-based chemotherapy, who were treated with Ontak 12 micrograms/kilogram once monthly. Six patients have been treated to date with one partial response, three disease stabilizations and two progressions. Immune analyzes remain incomplete post-Katrina, but existing data confirm that Ontak treatment is associated with reductions in phenotypic CD4+CD25+ blood Tregs and increased CD3+IFN-γ T cells. These data suggest that Ontak depletes Tregs in ovarian cancer. The link between Treg depletion and any observed immune changes or clinical effects is under active investigation, and remains to be determined. In a mouse model for ovarian cancer we demonstrated that the IL-2 moiety of Ontak does not mediate clinical effects; preliminary data suggest cytotoxicity for tumor cells is not a major mode of Ontak action. Future work will test Treg depletion with vaccination. Other regulatory cells (CD8+ Tregs and B7-H4+ myeloid cells) are also under investigation.

24 INVITED

Novel ideas in DC therapy and DC crosstalk

L. Zitvogel. *France*

Abstract not received.

25 INVITED

Spotlight on IDO: an ancestral metabolic enzyme turns immune regulator

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Indoleamine 2,3-dioxygenase (IDO) degrades the indole moiety of tryptophan and initiates the production of neuroactive and immunoregulatory metabolites, collectively known as kynurenines. The functional expression of IDO by dendritic cells has emerged in recent years as a major mechanism of peripheral tolerance. IDO contributes to maternal tolerance in pregnancy, control of allograft rejection, and protection against autoimmunity, inflammatory pathology and allergy. In both humans and mice, IDO-expressing tolerogenic dendritic cells are found in tumor-draining lymph nodes, possibly resulting in antigen-specific anergy. The wide spectrum of physiopathologic conditions in which IDO appears at work suggests that multiple mechanisms are used by this effector system to down-regulate T cell and inflammatory responses. Two theories have been proposed to account for tolerance induction via tryptophan catabolism. One theory posits that tryptophan breakdown suppresses T cell proliferation by critically reducing availability of this indispensable amino acid in local tissue microenvironments. The other theory assumes that kynurenines act to suppress immune reactivity, probably through direct interaction with effector T cells. Recent data obtained in our laboratory suggest that both effects are required for an efficient tolerogenic crosstalk of dendritic T cells and T lymphocytes.