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Signal Transduction Molecules as Targets for Cancer Prevention

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Abstract

Environmental and life-style aspects are major contributors to human carcinogenesis and, therefore, many human cancers may be preventable. Cancer is the end result of defects in cellular signaling processes that play a key role in the control of cell growth, survival, division, and differentiation. Therefore, identifying molecular and cellular targets critical in cancer development and prevention is an area of intensive research, driving the development of highly specific small-molecule inhibitors. A major idea today is that cancer may be prevented or treated by targeting the products of specific cancer-related genes, frequently encoding signaling proteins or transcription factors. Participants in these joint conferences discussed their latest findings in the identification of promising molecular targets and the development of agents directed against these targets with the goal of effectively transitioning these into the clinical setting.

Because numerous epidemiological studies clearly indicate that environmental and lifestyle aspects are major contributors in the etiology of human cancer, many cancers may be preventable. The stage for the conferences, 3rd Hormel Institute Frontiers in Cancer Conference and 8th International Skin Carcinogenesis Conference, was set by Allan Conney (Department of Chemical Biology, Rutgers, The State University of New Jersey) in his keynote address entitled "It's better to prevent cancer than to treat it." Cancer is the end result of aberrant cellular signaling processes involved in the control of cell growth, survival, division, and differentiation. Innumerable genes and gene products, which are crucial in the regulation of numerous cellular functions, are involved in the complex, multistage process of carcinogenesis. Thus, the elucidation of molecular and cellular targets critical in cancer development and prevention is an area of intensive research and is driving the development of highly specific small-molecule inhibitors, which may either prevent carcinogenesis, curtail its progression, or even cure the disease (Fig. 1). Participants discussed their latest findings in the identification of promising molecular targets and the development of agents, especially natural compounds, against these targets, which may ultimately transition into the clinical setting.

Identification of Promising Molecular Targets for Cancer Prevention and Therapeutics

What criteria determine whether a molecule is a promising molecular target for cancer prevention or therapeutics? Probably one of the most-used determinants of whether a molecule is a potential target for cancer prevention is its expression or activity level in cancer tissues compared to normal tissues. Ann M. Bode (The Hormel Institute, University of Minnesota) discussed the idea that various proteins, especially certain kinases and their target substrates, appear to exhibit a distinctive or aberrant activity or expression in cancer tissues compared to normal tissues, and therefore might be excellent targets for anticancer agents. In particular, the

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T-LAK cell-originated protein kinase (TOPK) is overexpressed in highly proliferating tumors, such as leukemias and myelomas, and appears to play a key role in tumorigenesis or metastasis (1). Cell lines in which the abundance of TOPK is elevated are more resistant to arsenite-induced apoptosis than are cell lines with low amounts of TOPK (2). High amounts of the TOPK protein are present in human colorectal cancer cells and cancer tissues and appear to play an important role in the development of colorectal cancer. TOPK promotes transformation in vitro and in vivo, and knockdown of TOPK in HCT116 colorectal cancer cells reduces this cell line's tumorigenic properties in vitro and in vivo (3). The cannabinoid receptors 1 and 2 (CB1 and CB2, collectively referred to as CB1/2) are directly activated by ultraviolet (UV) irradiation, and the absence of the CB1/2 receptors in mice resulted in a dramatic resistance to UVB-induced inflammation and a marked decrease in UVB-induced skin carcinogenesis (4). Anomalous activation of the phosphoinositide-3 kinase, PTEN, and Akt pathway leads to increased proliferation and decreased apoptosis in cancer pathology. John Digiovanni (University of Texas M. D. Anderson Cancer Center) reported that overexpression of the Akt protein can transform keratinocytes and, in transgenic mice, causes substantial changes in epidermal proliferation and differentiation, which, with age, can lead to spontaneous epithelial tumors in multiple organs (5). Furthermore, in these mice, Akt was activated in the skin in response to various chemically diverse skin tumor-promoting agents, and that enhanced downstream signaling contributes substantially to skin tumor promotion (6). G. Tim Bowden (Arizona Cancer Center, University of Arizona) identified adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) as an upstream regulator of *cox-2* mRNA stability and showed that the tumor suppressor LKB1 (also known as STK11 or serine-threonine kinase 11) phosphorylates AMPK (Thr¹⁷) (7). Bowden also reported new findings regarding the regulation of the HuR protein, an RNA-binding protein that stabilizes a number of RNA targets, including *cox-2*. HuR is highly abundant in numerous cancer tissues, and suppression of AMPK activity results in increased abundance of HuR, implicating AMPK in the regulation of HuR.

The importance of targeting oncogenic transcription and translation factors for cancer prevention was emphasized by Nancy H. Colburn (Laboratory of Cancer Prevention, National Cancer Institute, Frederick). The activation of the mitogen-activated protein kinase cascades can result in a multitude of cellular responses, including apoptosis, proliferation, inflammation, differentiation, and development, which are mediated through the transcription factors AP-1 and nuclear factor κ B (NF- κ B). Analysis of differentially expressed messenger RNAs (mRNAs) in tumor promoter-induced wild-type mice and in mice expressing dominant-negative Jun-TAM67, which blocks AP-1 activity, in the epidermis identified several functionally important AP-1 targets that are inhibited, including the gene encoding COX-2 (8). Pdc4, another potential tumor suppressor with loss of function in some human cancers, was identified as an inhibitor of transformation, AP-1-dependent transcription, and translation initiation (9). The role of AP-1, NF- κ B, COX-2, and prostaglandins (PGs) as endogenous tumor promoters was further highlighted by Susan Fischer (University of Texas M. D. Anderson Cancer Center). COX-2 is a key enzyme in the PG biosynthetic pathway (10), and COX-2 protein deficiency is associated with decreased UV-induced skin cancer, whereas elevated COX-2 protein and activity correlate with increased tumor development (11). Several studies suggested that the mechanism of COX-2 action was related to the endogenous tumor-promoting activity of PGs (12).

Although most of the potential molecular targets discussed so far appear to play a clear oncogenic role, many potential protein targets seem to behave paradoxically. For example, Adam Glick (Department Veterinary/Biomedical Sciences, Pennsylvania State University) suggested that the regulatory cytokine, transforming growth factor- β 1 (TGF- β 1), acts in a stage-specific manner to exhibit both stimulatory and suppressive actions in cancer development. Glick's group reported that TGF- β 1 has the ability to both promote and inhibit inflammatory responses, which might be related to its seemingly paradoxical function in cancer

development (13). Xiao-Jing Wang (Portland VA Cancer Center, Oregon Health and Sciences University) described evidence that Smads, which mediate the intracellular signals in response to TGF- β , have roles in tumor suppression and promotion. Based on their data from human cancer samples and from experimental models, Wang concluded that Smad2 and Smad4 mainly function as tumor suppressors in skin carcinogenesis in vivo, whereas Smad3 and Smad7 may have dual roles in the promotion and suppression of skin cancer (14,15).

The doubled-edged role of the activation of the oncogenic activating transcription factor 2 (ATF2) was discussed by Ze'ev Ronai (Bunham Institute). ATF2 is involved in the development of melanoma, and suppressing ATF2 activity impedes melanoma development. In contrast, in other tumor types, including breast and other types of skin cancers, ATF2 exhibits a tumor suppressor function, which suggests tissue- and tumor-specific functions of this transcription factor (16). The SAG/ROC2/ Rbx2-SCF E3 ubiquitin ligase was reported by Yi Sun (Comprehensive Cancer Center, University of Michigan) to have tumor-suppressing activity at an early stage of carcinogenesis, acting to promote c-Jun degradation and, thus, inhibit AP-1 activity. In contrast, this ligase exhibits a tumor growth-enhancing activity at later stages of carcinogenesis by promoting degradation of inhibitor of NF- κ B α (I κ B α) to activate NF- κ B and reduce apoptosis (17).

Protein kinase C (PKC) comprises a heterogeneous family of protein kinases that have different biological effects in normal and neoplastic melanocytes. For example, loss of PKC δ activity was reported in human squamous cell carcinoma (SCC) (18). Mitchell F. Denning (Cardinal Bernardin Cancer Center, Loyola University Medical Center) reported that PKC β is consistently lost and PKC ζ activity is increased in immortalized melanocytes and melanoma lines and that the loss of PKC β in melanoma appears to be important for melanoma growth. In contrast, Ajit K. Verma (Department of Human Oncology, University of Wisconsin) suggested that PKC ϵ is a master switch for the UV radiation-induced signaling network, because its overexpression sensitizes mouse skin for development of UV radiation-induced SCC by shifting the balance between apoptosis and proliferation (19).

Stem cells also seem to have an important role in oncogenesis. Irina Budunova (Department of Dermatology, Northwestern University) presented her group's findings using transgenic animals expressing the glucocorticoid receptor under the control of the *keratin5* promoter (K5.GR), which limits expression to epidermal cells of the skin. The K5.GR transgenic animals are resistant to skin carcinogenesis and exhibit diminished numbers of follicular epithelial stem cells, with reductions in their proliferative and survival potential and modification of the expression of stem cell "signature" genes (20). Duanqing Pei (Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences) discussed his work on the induction of pluripotent stem cells and the characterization of several transcription factors—Oct4, Sox2, Nanog, Klf4, and Myc—as master regulators of stem cell pluripotency (21). Rebecca Morris (The Hormel Institute, University of Minnesota) continued the stem cell discussion by focusing on the idea of a stem cell origin of tumors, especially in skin cancer. She indicated that the stem cell compartment within the cutaneous epithelium may be a major target of carcinogens and suggested that the permanent nature of the neoplastic lesion argues for a long-lived, slowly cycling population of cells that can persist throughout the lifetime of the animal despite the continual renewal of the epidermis and cycling of the hair follicles (22). Jingwu Xie (Department of Pharmacology and Toxicology, University of Texas) discussed the role of hedgehog signaling and its activation in the early stages of carcinogenesis (23). He hypothesized that the hedgehog signaling pathway functions to maintain the "stemness" of cancer cells and indicated that if the determination can be made as to whether hedgehog signaling is required for maintaining cancer stem cell population in a given tumor, then the ability to target that tumor with specific hedgehog inhibitors for treatment is highly possible.

Changes in cellular redox status have emerged as pivotal and proximal events in cancer. Young-Joon Surh (National Research Laboratory Molecular Carcinogenesis-Chemoprevention, Seoul National University) discussed the role of nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) in regulating phase-2 detoxifying and antioxidant gene induction to protect against oxidative insult (24). Nrf2 is found in a complex with Keap1 in the cytoplasm, and dissociation of Nrf2 from Keap1 by, for example, oxidative stress induces translocation of Nrf2 into the nucleus. Nrf2 then forms a heterodimer with a small Maf protein and binds to antioxidant-responsive elements in the promoter/enhancer regions of genes encoding many antioxidant and detoxifying enzymes (24). This topic was further developed by Basil Rigas (Pharmacological Sciences, State University of New York at Stony Brook), who emphasized that redox signaling and oxidative stress are not the same and that reactive oxygen and nitrogen species appear to have “multiple biological personalities” (25).

Finally, in the group of talks focusing on the identification of molecular targets, Ya Cao (Cancer Research Institute, Central South University, Changsha, Hunan) reported on the Epstein-Barr virus-encoded latent membrane protein 1 (LMP1)-mediated signal transduction pathway in nasopharyngeal carcinoma (NPC) (26,27). Cao's work focused on the construction of the entire signaling network triggered by LMP1 in NPC and suggested that the study of signaling transduction has shifted from studying single signaling pathways to studying signaling transduction networks, which are perceived as central to biological processes in carcinogenesis.

Cancer Prevention Strategies, Agents, and Progress

The prevailing thought today is that cancer can be prevented either by changes in lifestyle or by specific small-molecular inhibitors that target one or more proteins, such as those described above. Conney presented evidence that dietary phytochemicals, drugs, and exercise inhibit carcinogenesis in animal models (28,29). He suggested the use of combinations of low doses of compounds with different mechanisms of action alone or together with exercise as an approach for enhancing the effectiveness of cancer prevention and decreasing drug toxicity. In mice, Conney's group observed that tea administration, caffeine administration, or voluntary exercise inhibited UVB-induced carcinogenesis, decreased tissue fat, enhanced UVB-induced apoptosis, and potentiated apoptosis in tumors. Along these same lines of life-style changes and cancer prevention, Margot Cleary (The Hormel Institute, University of Minnesota) described the effectiveness of chronic calorie restriction (CCR) and intermittent calorie restriction (ICR) in preventing spontaneous and carcinogen-induced cancers in experimental animal models (30). The incidence of mammary tumors of ad libitum-fed mice was 77%, compared to 44% for CCR and only 3% for ICR mice. Similar findings were reported for the MMTV-neu mammary tumor mouse model and for the TRAMP (transgenic adenocarcinoma of the mouse prostate model) prostate cancer model.

Diet has attracted a great deal of interest in cancer prevention because it has been suggested to play a major role in cancer risk (31). In addition to being professed as generally safe, some dietary factors appear to have efficacy as anticancer agents by preventing or reversing premalignant lesions, as well as by reducing the risk of developing a second primary tumor (32). Some evidence suggests that certain dietary components might be used in combination with traditional chemotherapeutic agents to treat cancer (33).

Tea (*Camellia sinensis*, Theaceae) polyphenols suppress tumorigenesis in various animal models of cancer, although the primary targets for the cancer preventive actions remain unknown. Chung S. Yang (Department of Chemical Biology, Rutgers, The State University of New Jersey) reported that (-)-epigallocatechin-3-gallate and tea polyphenol preparations (polyphenol E) inhibited lung and intestinal tumorigenesis in two mouse models and colon

cancer in a mouse and rat model (34). Furthermore, feeding mice a diet containing a mixed tocopherol preparation rich in γ -tocopherol inhibited inflammation and colon carcinogenesis.

Flavonoids, including myricetin, quercetin, kaempferol, luteolin, and apigenin, are compounds found in edible tropical plants. Yong-Yeon Cho (The Hormel Institute, University of Minnesota) showed that kaempferol binds the N-terminal kinase domain of ribosomal S6 kinase 2 (RSK2) and specifically inhibits RSK2 activity, resulting in decreased proliferation of various cancer cell lines. He further demonstrated that RSK2-mediated phosphorylation of histone H3 at Ser¹⁰ is indispensable for cell transformation (35) and that kaempferol suppresses this phosphorylation by RSK2. Jill Pelling (Robert H. Lurie Comprehensive Cancer Center, Northwestern University) used various keratinocyte models (36) to show that apigenin treatment enhanced UVB-induced apoptosis, which was associated with changes in Bax localization and release of cytochrome c from the mitochondria.

Data from both in vitro and in vivo cancer models indicate that silibinin, a flavonolignan isolated from milk thistle extract, might be a promising anticancer agent. Rajesh Agarwal (Department of Pharmaceutical Sciences, University of Colorado Denver) (37,38) reported that silibinin protects against photocarcinogenesis in a strain of hairless mice. In UVB-irradiated epidermis, silibinin increased the abundance of p53 and its phosphorylation (Ser¹⁵) and delayed the transition from G₁ to S and from S to G₂/M phases of the cell cycle.

Nonsteroidal anti-inflammatory drugs, including the nonselective cyclooxygenase (COX) inhibitors aspirin and ibuprofen and the COX-2-specific inhibitor celecoxib, reduce the risk of cancer, but long-term exposure to these compounds has unwanted side effects, such as gastrointestinal toxicity and cardiovascular problems. The lipoxygenase (5-LOX) protein is also abundant in a number of epithelial cancers, and Chinthalapally V. Rao (Oklahoma University Cancer Institute, University of Oklahoma) presented results indicating that licofelone, an inhibitor of both 5-LOX and COX, suppressed chemically induced colonic aberrant crypt foci, as well as intestinal tumors formed due to loss of adenomatous polyposis coli (39). Dietary licofelone was also effective in suppressing breast tumor and pancreatic cancer xenograft growth, and the effect corresponded with inhibition of COX-2 and 5-LOX activities.

Even though smoking is a major risk factor for developing lung cancer, millions of people still smoke. The lab of Stephen Hecht (Masonic Cancer Center, University of Minnesota) is developing chemopreventive agents for smokers and ex-smokers (40) by targeting carcinogens in cigarette smoke. He discussed the two types of tobacco-specific nitrosamines, typified by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and polycyclic aromatic hydrocarbons, typified by benzo[a]pyrene, which are among the chief contributors to lung cancer. He reported that 2-phenethyl isothiocyanate (PEITC) and its major metabolite, *N*-acetyl-*S*-(*N*-2-phenethylthiocarbamoyl)-L-cysteine (PEITC-NAC), act as inhibitors of lung cancer induction by NNK in rats and mice. Furthermore, indole-3-carbinol (I3C) or combinations of PEITC-NAC and *myo*-inositol were also effective in reducing lung cancers in mice exposed to tobacco smoke carcinogens.

Nuclear hormone signaling is also an important target for cancer prevention and treatment. Junxuan Lü's group (The Hormel Institute, University of Minnesota) identified the major pyranocoumarin compounds decursin and decursinol angelate (DA) from the plant Korean Angelica Gigas Nakai (AGN) as members of a new class of compounds that block cellular responses to androgens, which has implications for prostate cancer prevention and treatment (41). In vitro studies showed that decursin and DA inhibited the growth and survival of leukemia, prostate, and breast cancer cells. An AGN extract reduced the growth of human androgen-independent prostate cancers in mice. Peter Ruvolo (The Hormel Institute,

University of Minnesota) presented evidence that the retinoid derivative fenretinide may be an effective agent for killing pre-B leukemia cells by a mechanism involving endoplasmic reticulum stress, and thus this compound may prove useful for preventing pre-B acute lymphoblastic leukemia, which is a common malignancy in children. Budunova discussed a nonsteroidal GR modulator called Compound A that has cytostatic and cytotoxic effects in numerous malignant epithelial and lymphoid cancer cells (42).

Transitioning Basic Science to the Clinic

Despite ongoing advances in drug discovery and preclinical testing, anticancer drug development remains a laborious, time-consuming process with limited success, which indicates a need to differentiate earlier between promising candidates and less likely effective compounds. Despite progress made in identifying important molecular targets and potential nontoxic anticancer agents, the challenge of transitioning these preclinical results into the clinic is daunting. Disappointingly, very few compounds show real promise based on clinical trial results. Ronald Lubet (Division of Cancer Prevention, National Cancer Institute) suggested that the best approach to developing chemopreventive agents is testing in animals for agents that produce results that parallel definitive clinical trial end points (for example, advanced preinvasive lesions or incipient cancers). Instead of administering the agent starting at the same time as the carcinogen and continuing administration of the preventive agent over the period of the study, one should consider initiating interventions when earlier lesions already exist. Although many agents are less effective when administered late, effective agents are the most likely candidates for Phase II and III trials because this better reflects the situation in humans. Paul Limburg (Mayo Clinic College of Medicine, Mayo Clinic Rochester) discussed the concept of Phase 0 clinical trials. Early in 2006 (43), the U.S. Food and Drug Administration issued an Exploratory Investigational New Drug guidance, which was designed to evaluate targeted anticancer agents in small, early-phase human clinical trials, referred to as “Phase 0” trials (Fig. 2). These trials are intended to expedite the clinical evaluation of new molecules by allowing reduced requirements for manufacturing and toxicologic assessment (44,45). Realistic goals for Phase 0 trials include (i) replication of preclinical mechanism(s) of action in a human intervention trial; (ii) characterization of initial pharmacokinetic and pharmacodynamic profiles; and (iii) evaluation of biodistribution patterns, based on imaging technologies. Success in a Phase 0 trial might permit a better selection of effective compounds for further development. Limburg further indicated that natural products represent an attractive source for chemoprevention agent discovery and, given their often-demonstrated favorable safety profile at standard doses, provide an excellent opportunity to explore potential benefits gained through the Phase 0 trial paradigm.

Gary Stoner (College of Medicine, Ohio State University) reported on a small Phase I trial in which a 10% black raspberry (BRB) bioadhesive gel applied topically to normal oral mucosa (10 patients) and to oral dysplastic lesions (17 patients) had no adverse effects and led to histologic regression of dysplastic lesions (46). Furthermore, short-term oral administration of BRB to patients with diagnosed colon cancer reduced cell proliferation and angiogenesis and increased apoptosis in colon tumor tissues. In patients with familial adenomatous polyposis, the administration of BRB in the form of rectal suppositories, with or without oral administration of BRB, resulted in a ~50% regression rate of rectal polyps in 9 months.

Steven Stratton (Arizona Cancer Center, University of Arizona) discussed the epidemic of skin cancer and their group's work in elucidating molecular signaling pathways and anticancer drug development. Chronic exposure to UV radiation is the predominant cause of nonmelanoma skin cancer, but the primary means of preventing this form of skin cancer, including reducing UV exposure and application of sunscreen, have had limited success. Stratton discussed the goal of initiating appropriate clinical trials of agents aimed at UV-specific signaling pathways.

Eventually, skin cancer treatment with topically administered chemopreventive agents could be customized to individuals based on the specific targets present in the individual's tumor.

Douglas Yee (Masonic Cancer Center, University of Minnesota) discussed the insulin-like growth factor (IGF) signaling system, the up-regulation of which is associated with increased incidence of malignancy. Yee indicated that strategies to reduce IGF signaling could be used to reduce the risk of cancer clinically and might include ligand down-regulation, neutralization of ligand, receptor down-regulation, or inhibition of receptor tyrosine kinase function. Hopefully, identification of relevant molecular targets, along with verification of effective agents in appropriate animal models and testing of compounds in Phase 0 clinical trials, will lead to a more rapid translation of preclinical results to successful clinical efficacy.

Technologies for Drug Development and Modalities for Early Detection

In the postgenomic era, computational modeling and simulations play a crucial role in cancer research. Computational biology is an interdisciplinary field that uses various techniques, including computer science, applied mathematics, and statistics, to address biological problems. Carlos Sosa (IBM, Rochester and Super Computing Institute, University of Minnesota) and Zigang Dong (The Hormel Institute, University of Minnesota) discussed the role of bioinformatics and virtual screening in cancer research. The supercomputer has been instrumental in mapping protein interaction networks in cancer cells—for example, the network of protein kinase interactions with substrates and regulatory molecules (47). High-speed computing has enhanced the ability to use computational screening of available chemical and natural-compound libraries against target proteins based on crystal structure or homology screening. In contrast, reverse docking and screening of protein structures against a known chemical can identify a chemical's molecular target(s) (48–51). Screening by computer analysis (in silico) uses molecular docking programs that “place” molecules noncovalently into the active site, a regulatory site, a protein-interaction site, or other biologically relevant site of a protein and then ranks candidate molecules by their ability to interact with the target protein. Such computer-identified drug targets can be validated in vitro and in vivo using biochemical assays, site mutagenesis, and animal studies. Finally, on a cellular or organismal level, modeling carcinogenesis to study cancer promotion, progression, and therapeutic effectiveness or the potential for drug resistance is promising.

Yuan-Ping Pang (Mayo Clinic Rochester) presented a specific example of successful in silico drug screening. Small-molecule inhibitors of members of the family of c-Jun N-terminal kinases (JNKs) have been proposed for potential treatment of cancer, asthma, and Parkinson's disease. However, indiscriminately suppressing all JNK activity is likely not the appropriate strategy, because each JNK appears to have a distinct function. The development of selective inhibitors of JNK1, JNK2, or JNK3 should aid in the differentiation of specific functions of each kinase, and these may also have application as specific therapeutic agents. With supercomputer technology, small molecules have been developed that show (i) selective inhibition of JNK1, (ii) inhibition of both JNK1 and JNK2, or (iii) selective inhibition of JNK3 (52).

New imaging modalities are facilitating successful therapy and early detection of disease. Stephen Lam (British Columbia Cancer Agency) presented optical coherence tomography (OCT) as an optical imaging method that can offer microscopic resolution for visualizing cellular and extracellular structures, for example, at and below the bronchial surface. Bronchoalveolar lavage (BAL), performed in the same bronchoscopic procedure, also allows measurement of biomarkers associated with regression or progression of preneoplastic lesions. OCT is a promising tool that does not require sample biopsy for in vivo imaging of preneoplastic lesions. In the case of bronchial lesions, this approach can permit analysis of the

lesion's history, its progression, and the effect of therapeutic intervention. Combining OCT with measurement of constituents in BAL fluid can be informative regarding the effects of therapeutic or chemopreventive agents (53).

Second harmonic signal generated (SHG) imaging is a second-order nonlinear optical imaging process that can be used to view hyperpolarizable structural proteins like collagen and myosin (54). The degree of alignment detected by the overlap of radially aligned collagen fibrils at the interface between a tumor and the stroma, using multi-photon laser scanning microscopy, is indicative of the growth status of the tumor mass. Marna Ericson (Department of Dermatology, University of Minnesota) showed that optical sectioning of thick tissue tumor samples revealed that the SHG signal of the tumor-associated collagen matrix of an actively growing tumor has a distinctive linear organization and alignment, whereas drug treatment caused a more disordered collagen matrix. SHG shows promise as a technique for monitoring the response to chemotherapeutic treatment in order to tailor treatment to individual patients.

Summary and Conclusions

Clearly, preventing cancer is better than having to treat this devastating disease. The research presented at two meetings highlighted advances in the identification of appropriate molecular targets, as well as the development of drugs or the identification of natural products that may be effective either as chemopreventives or as cancer therapeutics. Advances in computational, targeted drug design and imaging should enable rapid development and testing of candidate agents in the clinic.

References

1. Oh SM, Zhu F, Cho YY, Lee KW, Kang BS, Kim HG, Zykova T, Bode AM, Dong Z. T-lymphokine-activated killer cell-originated protein kinase functions as a positive regulator of c-Jun-NH₂-kinase 1 signaling and H-Ras-induced cell transformation. *Cancer Res* 2007;67:5186–5194. [PubMed: 17545598]
2. Zykova TA, Zhu F, Lu C, Higgins L, Tatsumi Y, Abe Y, Bode AM, Dong Z. Lymphokine-activated killer T-cell-originated protein kinase phosphorylation of histone H2AX prevents arsenite-induced apoptosis in RPMI7951 melanoma cells. *Clin Cancer Res* 2006;12:6884–6893. [PubMed: 17145805]
3. Zhu F, Zykova TA, Kang BS, Wang Z, Ebeling MC, Abe Y, Ma WY, Bode AM, Dong Z. Bidirectional signals transduced by TOPK-ERK interaction increase tumorigenesis of HCT116 colorectal cancer cells. *Gastroenterology* 2007;133:219–231. [PubMed: 17631144]
4. Zheng D, Bode AM, Zhao Q, Cho YY, Zhu F, Ma WY, Dong Z. The cannabinoid receptors are required for ultraviolet-induced inflammation and skin cancer development. *Cancer Res* 2008;68:3992–3998. [PubMed: 18483286]
5. Segrelles C, Lu J, Hammann B, Santos M, Moral M, Cascallana JL, Lara MF, Rho O, Car-bajal S, Traag J, Beltran L, Martinez-Cruz AB, Garcia-Escudero R, Lorz C, Ruiz S, Bravo A, Paramio JM, DiGiovanni J. Deregulated activity of Akt in epithelial basal cells induces spontaneous tumors and heightened sensitivity to skin carcinogenesis. *Cancer Res* 2007;67:10879–10888. [PubMed: 18006833]
6. Lu J, Rho O, Wilker E, Beltran L, Digiovanni J. Activation of epidermal akt by diverse mouse skin tumor promoters. *Mol Cancer Res* 2007;5:1342–1352. [PubMed: 18171992]
7. Zhang J, Bowden GT. UVB irradiation regulates Cox-2 mRNA stability through AMPK and HuR in human keratinocytes. *Mol Carcinog* 2008;47:974–983. [PubMed: 18449856]
8. Matthews CP, Birkholz AM, Baker AR, Perella CM, Beck GR Jr, Young MR, Colburn NH. Dominant-negative activator protein 1 (TAM67) targets cyclooxygenase-2 and osteopontin under conditions in which it specifically inhibits tumorigenesis. *Cancer Res* 2007;67:2430–2438. [PubMed: 17363560]
9. LaRonde-LeBlanc N, Santhanam AN, Baker AR, Wlodawer A, Colburn NH. Structural basis for inhibition of translation by the tumor suppressor Pdc4. *Mol Cell Biol* 2007;27:147–156. [PubMed: 17060447]

10. Fischer SM, Pavone A, Mikulec C, Langenbach R, Rundhaug JE. Cyclooxygenase-2 expression is critical for chronic UV-induced murine skin carcinogenesis. *Mol Carcinog* 2007;46:363–371. [PubMed: 17219415]
11. Rundhaug JE, Fischer SM. Cyclo-oxygenase-2 plays a critical role in UV-induced skin carcinogenesis. *Photochem Photobiol* 2008;84:322–329. [PubMed: 18194346]
12. Rundhaug JE, Pavone A, Kim E, Fischer SM. The effect of cyclooxygenase-2 overexpression on skin carcinogenesis is context dependent. *Mol Carcinog* 2007;46:981–992. [PubMed: 17583568]
13. Glick AB, Perez-Lorenzo R, Mohammed J. Context-dependent regulation of cutaneous immunological responses by TGF β 1 and its role in skin carcinogenesis. *Carcinogenesis* 2008;29:9–14. [PubMed: 17893229]
14. Bornstein S, Hoot K, Han GW, Lu SL, Wang XJ. Distinct roles of individual Smads in skin carcinogenesis. *Mol Carcinog* 2007;46:660–664. [PubMed: 17477360]
15. Owens P, Han G, Li AG, Wang XJ. The role of Smads in skin development. *J Invest Dermatol* 2008;128:783–790. [PubMed: 18337711]
16. Bhoumik A, Ronai Z. ATF2: A transcription factor that elicits oncogenic or tumor suppressor activities. *Cell Cycle* 2008;7:2341–2345. [PubMed: 18677098]
17. Gu Q, Bowden GT, Normolle D, Sun Y. SAG/ROC2 E3 ligase regulates skin carcinogenesis by stage-dependent targeting of c-Jun/AP1 and I κ B- α /NF- κ B. *J Cell Biol* 2007;178:1009–1023. [PubMed: 17846172]
18. D'Costa AM, Robinson JK, Maududi T, Chaturvedi V, Nickoloff BJ, Denning MF. The proapoptotic tumor suppressor protein kinase C- δ is lost in human squamous cell carcinomas. *Oncogene* 2006;25:378–386. [PubMed: 16158048]
19. Aziz MH, Manoharan HT, Verma AK. Protein kinase C ϵ , which sensitizes skin to sun's UV radiation-induced cutaneous damage and development of squamous cell carcinomas, associates with Stat3. *Cancer Res* 2007;67:1385–1394. [PubMed: 17283176]
20. Chebotaev D, Yemelyanov A, Zhu L, Lavker RM, Budunova I. The tumor suppressor effect of the glucocorticoid receptor in skin is mediated via its effect on follicular epithelial stem cells. *Oncogene* 2007;26:3060–3068. [PubMed: 17146443]
21. Pei D. The magic continues for the iPS strategy. *Cell Res* 2008;18:221–223. [PubMed: 18246087]
22. Morris RJ. Stem cells in the hair follicle and inter-follicular epidermis of mice following topical application of fluocinolone acetonide. *J Invest Dermatol* 2007;127:2707–2708. [PubMed: 18007689]
23. Xie J. Hedgehog signaling pathway: Development of antagonists for cancer therapy. *Curr Oncol Rep* 2008;10:107–113. [PubMed: 18377823]
24. Surh YJ, Na HK. NF- κ B and Nrf2 as prime molecular targets for chemoprevention and cytoprotection with anti-inflammatory and antioxidant phytochemicals. *Genes Nutr* 2008;2:313–317. [PubMed: 18850223]
25. Rigas B, Williams JL. NO-donating NSAIDs and cancer: An overview with a note on whether NO is required for their action. *Nitric Oxide* 2008;19:199–204. [PubMed: 18486630]
26. Li L, Guo L, Tao Y, Zhou S, Wang Z, Luo W, Hu D, Li Z, Xiao L, Tang M, Yi W, Tsao SW, Cao Y. Latent membrane protein 1 of Epstein-Barr virus regulates p53 phosphorylation through MAP kinases. *Cancer Lett* 2007;255:219–231. [PubMed: 17582679]
27. Liu YP, Tan YN, Wang ZL, Zeng L, Lu ZX, Li LL, Luo W, Tang M, Cao Y. Phosphorylation and nuclear translocation of STAT3 regulated by the Epstein-Barr virus latent membrane protein 1 in nasopharyngeal carcinoma. *Int J Mol Med* 2008;21:153–162. [PubMed: 18204781]
28. Conney AH, Zhou S, Lee MJ, Xie JG, Yang CS, Lou YR, Lu Y. Stimulatory effect of oral administration of tea, coffee or caffeine on UVB-induced apoptosis in the epidermis of SKH-1 mice. *Toxicol Appl Pharmacol* 2007;224:209–213. [PubMed: 17188726]
29. Zheng X, Cui XX, Huang MT, Liu Y, Shih WJ, Lin Y, Lu YP, Wagner GC, Conney AH. Inhibitory effect of voluntary running wheel exercise on the growth of human pancreatic Panc-1 and prostate PC-3 xenograft tumors in immunodeficient mice. *Oncol Rep* 2008;19:1583–1588. [PubMed: 18497969]
30. Bonorden MJ, Rogozina OP, Kluczny CM, Grossmann ME, Grande JP, Lokshin A, Cleary MP. Cross-sectional analysis of intermittent versus chronic caloric restriction in the TRAMP mouse. *Prostate* 2008;69:317–326. [PubMed: 19016490]

31. McMichael AJ. Food, nutrition, physical activity and cancer prevention. Authoritative report from World Cancer Research Fund provides global update. *Public Health Nutr* 2008;11:762–763. [PubMed: 18462560]
32. Hong WK. General keynote: The impact of cancer chemoprevention. *Gynecol Oncol* 2003;88:S56–S58. [PubMed: 12586087]
33. Ermakova SP, Kang BS, Choi BY, Choi HS, Schuster TF, Ma WY, Bode AM, Dong Z. (-)-Epigallocatechin gallate overcomes resistance to etoposide-induced cell death by targeting the molecular chaperone glucose-regulated protein 78. *Cancer Res* 2006;66:9260–9269. [PubMed: 16982771]
34. Xiao H, Hao X, Simi B, Ju J, Jiang H, Reddy BS, Yang CS. Green tea polyphenols inhibit colorectal aberrant crypt foci (ACF) formation and prevent oncogenic changes in dysplastic ACF in azoxymethane-treated F344 rats. *Carcinogenesis* 2008;29:113–119. [PubMed: 17893236]
35. Cho YY, Yao K, Kim HG, Kang BS, Zheng D, Bode AM, Dong Z. Ribosomal S6 kinase 2 is a key regulator in tumor promoter induced cell transformation. *Cancer Res* 2007;67:8104–8112. [PubMed: 17804722]
36. Abu-Yousif AO, Smith KA, Getsios S, Green KJ, Van Dross RT, Pelling JC. Enhancement of UVB-induced apoptosis by apigenin in human keratinocytes and organotypic keratinocyte cultures. *Cancer Res* 2008;68:3057–3065. [PubMed: 18413777]
37. Gu M, Singh RP, Dhanalakshmi S, Agarwal C, Agarwal R. Silibinin inhibits inflammatory and angiogenic attributes in photocarcinogenesis in SKH-1 hairless mice. *Cancer Res* 2007;67:3483–3491. [PubMed: 17409458]
38. Ramasamy K, Agarwal R. Multitargeted therapy of cancer by silymarin. *Cancer Lett* 2008;269:352–362. [PubMed: 18472213]
39. Rao CV. Regulation of COX and LOX by curcumin. *Adv Exp Med Biol* 2007;595:213–226. [PubMed: 17569213]
40. Hecht SS. Progress and challenges in selected areas of tobacco carcinogenesis. *Chem Res Toxicol* 2008;21:160–171. [PubMed: 18052103]
41. Guo J, Jiang C, Wang Z, Lee HJ, Hu H, Malewicz B, Lee JH, Baek NI, Jeong JH, Kim DK, Kang KS, Kim SH, Lu J. A novel class of pyranocoumarin anti-androgen receptor signaling compounds. *Mol Cancer Ther* 2007;6:907–917. [PubMed: 17363485]
42. Yemelyanov A, Czwarnog J, Gera L, Joshi S, Chatterton RT Jr, Budunova I. Novel steroid receptor phyto-modulator compound a inhibits growth and survival of prostate cancer cells. *Cancer Res* 2008;68:4763–4773. [PubMed: 18559523]
43. U.S. Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for industry, investigators, and reviewers. Exploratory IND studies. U.S. Department of Health and Human Services; Washington, DC: 2006.
44. Doroshow JH, Parchment RE. Oncologic phase 0 trials incorporating clinical pharmacodynamics: From concept to patient. *Clin Cancer Res* 2008;14:3658–3663. [PubMed: 18559579]
45. Kummar S, Rubinstein L, Kinders R, Parchment RE, Gutierrez ME, Murgu AJ, Ji J, Mroczkowski B, Pickeral OK, Simpson M, Hollingshead M, Yang SX, Helman L, Wiltrout R, Collins J, Tomaszewski JE, Doroshow JH. Phase 0 clinical trials: Conceptions and misconceptions. *Cancer J* 2008;14:133–137. [PubMed: 18536551]
46. Shumway BS, Kresty LA, Larsen PE, Zwick JC, Lu B, Fields HW, Mumper RJ, Stoner GD, Mallery SR. Effects of a topically applied bioadhesive berry gel on loss of heterozygosity indices in premalignant oral lesions. *Clin Cancer Res* 2008;14:2421–2430. [PubMed: 18413833]
47. Choi BY, Choi HS, Ko K, Cho YY, Zhu F, Kang BS, Ermakova SP, Ma WY, Bode AM, Dong Z. The tumor suppressor p16(INK4a) prevents cell transformation through inhibition of c-Jun phosphorylation and AP-1 activity. *Nat Struct Mol Biol* 2005;12:699–707. [PubMed: 16007099]
48. Lee KW, Kang NJ, Heo YS, Rogozin EA, Pugliese A, Hwang MK, Bowden GT, Bode AM, Lee HJ, Dong Z. Raf and MEK protein kinases are direct molecular targets for the chemopreventive effect of quercetin, a major flavonol in red wine. *Cancer Res* 2008;68:946–955. [PubMed: 18245498]
49. Lee KW, Kang NJ, Rogozin EA, Oh SM, Heo YS, Pugliese A, Bode AM, Lee HJ, Dong Z. The resveratrol analogue 3,5,3',4',5'-pentahydroxy-trans-stilbene inhibits cell transformation via MEK. *Int J Cancer* 2008;123:2487–2496. [PubMed: 18767048]

50. Li M, He Z, Ermakova S, Zheng D, Tang F, Cho YY, Zhu F, Ma WY, Sham Y, Rogozin EA, Bode AM, Cao Y, Dong Z. Direct inhibition of insulin-like growth factor-I receptor kinase activity by (-)-epigallocatechin-3-gallate regulates cell transformation. *Cancer Epidemiol Biomarkers Prev* 2007;16:598–605. [PubMed: 17372258]
51. Shim JH, Choi HS, Pugliese A, Lee SY, Chae JI, Choi BY, Bode AM, Dong Z. (-)-Epigallo-catechin gallate regulates CD3-mediated T cell receptor signaling in leukemia through the inhibition of ZAP-70 kinase. *J Biol Chem* 2008;283:28370–28379. [PubMed: 18687687]
52. Pang, YP.; Vummenthala, A.; Park, JG.; Wang, SH.; Dong, Z.; Bode, AM.; Cho, YY. U.S. provisional patent application 61/102,089. 2008.
53. Lam S, Standish B, Baldwin C, McWilliams A, leRiche J, Gazdar A, Vitkin AI, Yang V, Ikeda N, MacAulay C. In vivo optical coherence tomography imaging of preinvasive bronchial lesions. *Clin Cancer Res* 2008;14:2006–2011. [PubMed: 18381938]
54. Hompland T, Erikson A, Lindgren M, Lindmo T, de Lange Davies C. Second-harmonic generation in collagen as a potential cancer diagnostic parameter. *J Biomed Opt* 2008;13:054050. [PubMed: 19021430]

Fig. 1. General schematic of effective cancer prevention by targeting signaling proteins and transcription factors. Cells receive extracellular signals through membrane receptors, and the signal is amplified through protein kinase cascades. Frequently, the signal initiates changes in the transcriptional activity of various transcription factors that bind to target gene sequences, resulting in diverse cellular responses.

Fig. 2.

Transitioning basic science to the clinic. The goal is to clearly identify promising molecular targets and develop effective anticancer agents that specifically target these molecules and that then can be rapidly transitioned from preclinical findings to the patient.