Molecular Target-Based Treatment of Human Cancer: Summary of the 10th International Conference on Differentiation Therapy

Arthur Zelent,1 Kevin Petrie,1 Zhu Chen,2 Reuben Lotan,3 Michael Lübbert,4 Martin S. Tallman,5 Ryuozo Ohno,6 Laurent Degos,7 and Samuel Waxman8

1Section of Haematological Oncology, Institute of Cancer Research, London, United Kingdom; 2Shanghai Institute of Hematology, Rui-Jin Hospital, Shanghai, China; 3University of Texas M. D. Anderson Cancer Center, Houston, Texas; University of Freiburg Medical Center, Freiburg, Germany; Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; 4Aichi Cancer Center Hospital, Nagoya, Japan; 5Institut d’Hematologie, Hopital St. Louis, Paris, France; and 6Department of Medicine, Mount Sinai School of Medicine, New York, New York

Abstract

The 10th International Conference on Differentiation Therapy was held between April 29 and May 3, 2004, in Shanghai, China. In the tradition of previous conferences from this series, which have been held biannually since the first meeting organized 20 years ago by Samuel Waxman and Giovanni Rossi in Sardinia, the organizers of the 10th International Conference on Differentiation Therapy aimed to gather basic and clinical cancer investigators in a setting of plenary sessions, workshops, and poster presentations to maximize the effective exchange of information and foster the establishment of collaborative interactions. Approximately 300 scientists attended the meeting with a mission to discuss targeted approaches to cancer treatment, which stem from our understanding of basic biological processes and the mechanisms of their deregulation during tumorigenesis. (Cancer Res 2005; 65(4): 1117-23)

Introduction

The last century witnessed dramatic advances in cancer therapeutics. One of the most promising novel strategies has been the so-called targeted anticancer therapy based on compounds that interfere with cellular targets directly connected with pathogenic events. Such therapies are expected to target specifically tumor cells, thus allowing for strong anticancer effects and minimal toxicities. A number of such target-based anticancer therapies are now successfully used in routine clinical practice. For example, in chronic myelogenous leukemia, the Abelson tyrosine kinase inhibitor Imatinib (Gleevec) targets the activity of BCR-ABL oncoprotein; in acute promyelocytic leukemia (APL), all-trans-retinoic acid (ATRA) or arsenic trioxide (As2O3) targets PML-RARα fusion. The introduction of ATRA or Imatinib in the treatment of APL or chronic myelogenous leukemia patients has significantly improved the management of these diseases.

The 10th International Conference on Differentiation Therapy focused on addressing how normal cellular regulatory processes are corrupted during tumorigenesis and what potential targets for therapeutic intervention are highlighted by results of basic studies of normal cell function and carcinogenesis. Preclinical and clinical trials addressing effectiveness of single agent or combinatorial therapies in solid and hematologic tumors were also discussed.

The meeting began with a Keynote Lecture on "Molecular Mechanisms of Protein Degradation," which was delivered by Robert Huber (Max-Plank Institute for Biochemistry, Martinsried, Germany), Nobel laureate for Chemistry, who described similarities and differences between several multil protein protease complexes while drawing some general conclusions as to the role of protein degradation in cell function and anticancer therapy. The fact that malignant cells are more susceptible to some proteasome inhibitors has been well recognized and a number of such drugs are currently in clinical trials. Recent Food and Drug Administration approval of the proteasome inhibitor bortezomib (PS-341) for treatment of relapsed and refractory multiple myeloma provides proof of principle that the proteasome is a valid target for anticancer therapy.

Epigenetic Mechanisms in Cancer Pathogenesis and Therapy

Although genetics have played a dominant role in cancer research, over the recent years, epigenetics have arguably become equally important in this field. The potential reversibility of epigenetic modifications (e.g., those that direct chromatin organization) renders the enzymes that are responsible for tumorigenic epigenetic changes important targets for drug development. Tony Kouzarides (Wellcome/Cancer Research UK Institute of Cancer and Developmental Biology, Cambridge, United Kingdom) and Frank J. Rauscher III (The Wistar Institute, Philadelphia, PA) reviewed the relationship between different epigenetic modifications in regulating gene expression. In mammalian cells, for example, methylation of Lys9 on histone H3 and hypoacetylation of histones are usually associated with methylated DNA, heterochromatin, and gene silencing. Histone hyperacetylation and methylation of Lys4 on histone H3, on the other hand, are associated with unmethylated DNA, euchromatin, and gene expression (Fig. 1). Using unmodified H3-tail peptide, Kouzarides’ laboratory characterized inhibitor of acetyltransferase complex containing SET/TAF1β and pp32. Consistent with its role in gene silencing, inhibitor of acetyltransferase bound preferentially to unphosphorylated and hypoacetylated histone H3 tail. Kouzarides also described studies of lysine methyltransferases in budding and fission yeast that led to identification of histone H4 Lys20 methylase SET9. Unlike methylation of other lysines, methylation of H4 Lys20 did not seem to be associated with regulation of chromatin structure and gene expression; however, functional...
SET9 was required for proper DNA damage response, thus corroborating views that deregulation of methyltransferase activities may play a role in carcinogenesis.

Rauscher’s presentation shed light on how a Krüppel-associated box (KRAB)–associated protein 1 [KAP-1; also called transcriptional intermediary factor 1β (TIF-1β)] corepressor coordinates gene silencing following recruitment to a specific DNA site by a KRAB domain zinc finger transcriptional repressor. Using a hormone regulated system, Rauscher’s team has shown that KAP-1 serves as a molecular scaffold that recruits and coordinates the entire machinery required for stable gene silencing including histone deacetylation, H3 Lys9 methylation, and deposition of heterochromatin proteins. These studies also showed that the silencing effects established through KAP-1 exist over a short chromatin region (four to five nucleosomes) and are stably maintained over many population doublings even in the absence of hormone and thus discontinuation of KAP1 targeting to chromatin. Silencing induced by KAP-1 complex was followed by regional DNA hypermethylation, perhaps reflecting potential importance of DNA methylation in maintenance of histone-directed silencing machinery at a given locus. The system established by Rauscher’s laboratory should play an important role in further studies of the hierarchy and coordinated interplay between DNA methylation and the histone code (differential array of covalent histone modifications).

Rauscher also stressed the importance of developing small-molecule drugs that can inhibit enzymatic activities of and disrupt interactions between specific components of chromatin-modifying complexes.

In analogy to the KRAB domain–containing proteins, the poxvirus and zinc finger domain zinc finger transcriptional repressors PLZF and BCL-6, which are involved in the pathogenesis of APL and non-Hodgkin lymphoma, act through recruitment of multiprotein complexes with chromatin remodeling activities. Pier Paolo Pandolfi (Memorial Sloan-Kettering Cancer Center, New York, NY) presented evidence that PLZF–related protein POKEMON (also called LRF, FBI-1, and OSZF) may also play a role in the pathogenesis of human cancer. Aberrant expression of POKEMON was found to be associated with lymphoid tumors, such as follicular and diffuse large B cell lymphoma, and its overexpression induced lymphoid tumors in transgenic mice. Coexpression of both BCL-6 and POKEMON in human tumors was associated with higher proliferative index and was a predictor of a better response to chemotherapy.

Stephen Baylin (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD) discussed the contribution of abnormal gene promoter DNA hypermethylation to tumor development. Baylin pointed out that acquisition of methylation in promoter regions leading to silencing occurs very early in cancer progression at a premalignant stage. On the basis of this observation, he proposed that epigenetic changes provide a precancerous milieu for genetic mutations to occur and presented results that are consistent with this hypothesis. For example, in 90% of colorectal cancers, mutations of APC, CTNNB1 (β-catenin), or AXIN2 lead to aberrant Wnt pathway signaling. Baylin’s laboratory has shown that genes encoding secreted frizzled-related proteins, which can inhibit Wnt signaling, are silenced by DNA methylation very early in tumorigenesis before APC or CTNNB1 gene mutations that can already be detected in premalignant lesions. Restoration of secreted frizzled-related proteins function in colorectal cancer cells where DNA methylation activity has been abolished by genetic deactivation of DNA methyltransferase 1 (DNMT1) and DNMT3b leads to down-regulation of Wnt signaling despite persistence of downstream activating mutations (Fig. 2). Such results suggest that use of direct or indirect (5-azacytidine) DNMT inhibitors may be therapeutic and/or chemopreventive in colorectal cancer and underscore the general importance of therapeutic approaches targeting epigenetic silencing of gene expression in neoplastic diseases.

The role of epigenetics in acute myeloid leukemia (AML), particularly the APL subtype, was discussed by Pier Giuseppe Pelicci (European Institute of Oncology, Milan, Italy) who described results indicating that transcriptional deregulation of gene expression by APL–associated PML-RARα fusion oncoprotein is both direct, through recruitment of nuclear receptor corepressors, histone deacetylases (HDAC), DNA, and histone methyltransferases to ATRA-regulated genes, and indirect through relief of transcriptional repression of the Rb-E2F target gene, CyclinE, due to titration of Suv39H1 histone methyltransferase. The latter role
seems to enhance specifically the self-renewal of stem cells as overexpression of Suv39H1 reversed this phenotype. Enhancement of stem cell self-renewal by PML-RARαs is consistent with a view that acute leukemia–associated fusion proteins induce a preleukemic state and other genetic events, mutations of tyrosine kinase receptors, for example, are required for the development of the full leukemic phenotype. Pelicci also presented data demonstrating that antileukemic action of the HDAC inhibitor valproic acid is due, at least in part, to induction of proapoptotic molecules such as the tumor necrosis factor–related apoptosis-inducing ligand and its receptor DR5. It remains to be seen whether these molecular events are valproic acid specific; however, because preleukemic cells lack sensitivity to valproic acid, it is unlikely that use of HDAC inhibitor alone will produce lasting therapeutic effects in AML.

Paul Marks (Memorial Sloan-Kettering Cancer Center, New York, NY) discussed the results of phase I clinical studies with suberoylanilide hydroxamic acid (SAHA), a nonspecific class I and II HDAC inhibitor that his group developed. In vitro, SAHA displays selectivity for tumor cells and its mechanism of action involves cell growth arrest, induction of apoptosis, and, in some cases, induction of differentiation. In clinical trials, encouraging results have been obtained in a number of solid and hematologic malignancies, including cutaneous T cell lymphoma, diffuse large B cell lymphoma, mesothelioma, and laryngeal carcinoma. Clinically stable disease has been observed in some patients treated with SAHA and correlated with an increase in histone acetylation. Both i.v. and p.o. preparations of SAHA have been developed for clinical use and following administration lead to rapid accumulation of the drug, which is well tolerated. Reversible, mostly nonhematologic adverse effects were observed, such as anorexia, fatigue, nausea, and dehydration. Phase II studies are currently in progress. Preclinical studies have indicated that SAHA synergizes with other anticancer agents such as radiation, cytostatic agents, kinase inhibitors, and differentiation inducers. Studies with other HDAC inhibitors suggest that optimal effects on relieving transcriptional repression may be achieved when used with inhibitors of DNA methylation (e.g., 5-azacytidine), suggesting combination use of these agents in therapy.

In a workshop on “Therapeutic Targeting of Aberrant Transcription,” Michael Lübbert (University of Freiburg Medical Center, Freiburg, Germany) discussed therapeutic implications of low-dose treatment with demethylating agents in myeloid malignancies. In development parallel to studies carried out by Silverman and colleagues of the Cancer and Leukemia Group B in the United States, who used low doses of 5-azacytidine to treat myelodysplasia, Lübbert and his European colleagues developed a deoxycytidine analogue of 5-azacytidine (i.e., decitabine) for the treatment of high-risk myelodysplasia. This drug showed an overall efficacy of 50% in these patients, with 30% of patients achieving hematologic and cytogenetic remissions. Clinical administration of decitabine and patient response were reflected by demethylation of the hypermethylated p15 gene promoter and concomitant up-regulation of protein expression in the bone marrow cells of these patients. However, compared with outgrowth of normal hematopoietic cells in vivo, the differentiation of the abnormal clone was not a major effect. Thus, growth inhibition, possibly due to reactivated p15 expression, may underlie the therapeutic effects of DNA demethylating agents in myelodysplasia. Recently, low-dose decitabine approach plus ATRA was adapted to older patients with full-blown AML, and preliminary results indicate achievement of complete hematologic and cytogenetic remission in some patients.

In a parallel workshop on “Cancer Pathogenesis and Lessons from APL,” Arthur Zelent (Institute of Cancer Research, London, United Kingdom) described results indicating cross-talk between ATRA and myelomonocytic growth factors in AML cell differentiation, which involves activation of mitogen-activated protein kinase signaling downstream of growth factor receptor and synergistic activation of retinoic acid receptors by ATRA and mitogen-activated protein kinase–dependent phosphorylation. Similarly, Christine Chomienne (Hôpital St. Louis, Paris, France) reported that ATRA and granulocyte colony-stimulating factor acting through a phosphoinositide 3 kinase–dependent pathway could synergistically induce differentiation of ATRA-resistant APL cells. Zelent indicated further that in vitro pretreatment of AML cells with 5-azacytidine resulted in synergistic effects on ATRA-mediated gene expression and differentiation induction. These studies suggest that combinatorial application of agents that relieve transcriptional repression followed by differentiation inducers seems a promising and pertinent approach to anti-AML therapy.

Cancer Therapy Based on Inductions of Differentiation and Molecular Targeting

The application of differentiation therapy to AML has remained an important topic for discussion at this conference. Martin S. Tallman (Feinberg School of Medicine, Northwestern University, Chicago, IL) reviewed and discussed current clinical therapeutic strategies in APL, the most curable AML in adults. With current approaches, which include ATRA- and anthracycline-based chemotherapy and maintenance, ~70% to ~80% of patients seem to be cured. The early death rate remains ~10% and its major cause remains hemorrhage (~50-60% of patients). Arsenic trioxide is the treatment of choice for patients with relapsed and refractory APL. After one 25-day course of arsenic trioxide, the molecular remission rate is ~55%; after two courses, it was ~80%. Now that the majority of patients seem to be cured of their disease, issues of long-term complications, such as extramedullary relapse (previously uncommon in APL), secondary malignancies (primarily myelodysplasia and AML), and delayed cardiomyopathy have emerged. Clearly, reduction or elimination of chemotherapy from APL treatment protocols would greatly reduce such complications. Clinical studies to evaluate effectiveness of arsenic trioxide, either alone or in combination with other nonchemotherapeutic agents, in induction, consolidation, and maintenance therapy of APL are in progress.
progress. Arsenic trioxide therapy as a single agent in untreated patients induces a complete remission (CR) rate of ~90% with a high incidence of PCR negativity. Zhi-Xiang Shen (Shanghai Institute of Hematology, Shanghai, China) reported that the combination of ATRA plus arsenic trioxide is more effective in newly diagnosed patients compared with either ATRA or arsenic alone. The benefits seem to be a shorter time to CR, as well as a more profound reduction in the degree of residual disease and a lower relapse rate. Overall, 95% of patients achieved CR with no relapse after a 2-year follow up. The toxic effects of this combination treatment were the same as with ATRA or arsenic trioxide therapy alone.

In a workshop entitled “Novel Agents and Therapeutic Approaches to Non-APL AML,” Ryuozo Ohno (Aichi Cancer Center, Nagoya, Japan) discussed the limitation of cytotoxic therapy and the need of new therapeutic approaches for the cure of non-APL AML. According to the results of the Japan Adult Leukemia Study Group, there seems to be no improvement in CR and cure rates for adult AML within their past five consecutive studies (covering the past 12 years) using cytotoxic chemotherapy and stem cell transplantation. APL, which has been treated with ATRA regimens since 1992, was the only exception. This fact definitely underscores the need for development of targeted therapies against pathogenic molecules that are specifically responsible for other AMLs. Aside from fusion oncoproteins, the FLT3 receptor tyrosine kinase emerged as an important target in AML. Tomoki Naoe (Aichi Cancer Center, Nagoya, Japan) discussed that internal tandem duplication or point mutations of FLT3 are observed in ~30% of AML, and its presence indicates poorer prognosis. Therefore, targeting drugs on abnormal FLT3 should abrogate the progress of AML and possibly, in combination with currently available cytotoxic or other targeting drugs, will contribute to higher cure rates. Masahiro Kizaki and Keisuke Ito (Keio University School of Medicine, Tokyo, Japan) presented a new approach for the treatment of AML with natural compounds, namely capsaicin (a homovanillic acid derivative) from the seeds, and 1′-acetoxychavicol acetate from the rhizomes of Languas galanga, better known as Siamese ginger.

A potentially novel therapeutic agent (SIH-10) targeting activity of M2 AML–associated AML1-ETO fusion oncoprotein was described in a Plenary Presentation by Zhen-Yi Wang (Shanghai Institute of Hematology, Shanghai, China) who pioneered use of ATRA in APL. SIH-10 was derived from a Chinese medicinal herb and at 1 μmol/L concentrations can degrade the oncogenic fusion protein and induce apoptosis of AML1-ETO-positive leukemic cells. Wang also stressed the importance of using combinatorial approaches to the treatment of hematologic malignancies with agents that target multiple pathways, thus leading more effectively and at less toxic doses to differentiation and apoptosis of malignant cells.

Combination-targeted approaches to cancer treatment were discussed in greater detail by Samuel Waxman (Mount Sinai School of Medicine, New York, NY) whose early studies on differentiation induction of mouse erythroleukemia cells have lead to a concept of using combinatorial cytotoxic differentiation therapy in leukemia. Waxman described that in addition to HDAC inhibitors, which potentiate ATRA effects in APL, compounds acting through other mechanisms can exert the same effects. His studies focus on dithiophene derivatives, which at nanomolar concentrations can potentiate differentiation of APL with ATRA to a degree obtained with clinically used HDAC inhibitors. At these low concentrations, dithiophenes alone have no effects, but at micromolar levels they exert proapoptotic activities on a variety of tumor cells in vitro.

The potential of using rexinoids [retinoid X receptor (RXR) agonists] for therapy of AML has been addressed by Michele Lanotte (Hôpital Saint Louis, Paris, France) who presented results indicating that primary effect of rexinoids on APL cells is to induce apoptosis without differentiation. However, the addition of either cyclic AMP (cAMP) or ATRA rescues differentiation, indicating dominant effects. Related studies from Jian-Hua Tong’s laboratory (Shanghai Institute of Hematology, Shanghai, China) indicate that ATRA induction of APL cell differentiation involves activation of adenylate cyclase leading to an increase in cellular cAMP levels and protein kinase A activity. In ATRA-resistant APL cells, cAMP cooperates with rexinoid signaling for growth arrest and differentiation induction, suggesting that such combinations of agents may be useful in differentiation therapy of non-APL AML.

Hugues de Thé (Hôpital St. Louis, Paris, France) described studies addressing molecular basis for synergistic action of rexinoids and cAMP in APL cell differentiation. He showed that APL-associated oncoprotein PML-RARα binds in vitro to DNA elements other than those recognized by RXR-RAR heterodimers as PML-RARα/RXR tetramer, rather than a homodimer. Nevertheless, results presented by Scott Kogan (University of California, San Francisco, San Francisco, CA) indicate that homodimerization function of PML-RARα (due to the PML moiety) is important for development of APL in a transgenic mouse model. The data presented by de Thé indicated, however, that RXR was present in the PML-RARα oncoprotein complex, as with cAMP rexinoids readily induced differentiation of APL cells and activated transcription from PML-RARα-specific response elements. The requirement of cAMP for these rexinoid activities de Thé ascribed to the ability of cAMP-activated protein kinase A to uncouple (presumably by phosphorylating the RARα moiety of the fusion protein) dependents of RXR activation from the RAR (Fig. 3). This argues against the existence of RXR-specific differentiation pathway for APL cells and suggests that the view of APL cell differentiation depends on activation of specific gene expression by PML-RARα.

Limited clinical trials of epigenetic therapy using combinations of ATRA, valproic acid, and a cyclic AMP–elevating agent (pentoxifylline) in older patients with AML or myelodysplastic syndromes were described by Laurent Degos (Hôpital St. Louis, Paris, France). Of 10 patients, 2 achieved a CR and a near CR. This novel treatment approach that translates directly from results of basic in vitro and in vivo studies is promising and warrants further investigation. Substitution of a DNA demethylating agent, which is more potent at relieving transcriptional repression, for the HDAC inhibitor or use of both agents in succession may produce better results.

Given that AML is a stem cell disease, work aiming to identify targets for therapy in a small population of leukemic stem cells that drive the disease is critically important. Stephen Emerson (Abramson Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA) reported that the trimeric regulatory complex NF-Y activates several stem cell HOX genes such as HOXB4, C4, and D4. In normal bone marrow hematopoiesis, the regulatory subunit of the NF-Y trimer NF-Ya is preferentially expressed in hematopoietic stem cells and is markedly down-regulated with myelomonocytic differentiation, suggesting that NF-Y activity is important for stem cell maintenance and is temporally controlled through regulated expression of the NF-Ya subunit. Supraphysiologic induction of NF-Y activity in murine primitive bone marrow cells...
by retroviral transduction of NF-Ya induced HOXB4 transcription and blocked terminal differentiation of transplanted cells in vivo, whereas dominant-negative NF-Ya significantly biased the same cells toward granulopoiesis. NF-Ya was also highly expressed in AML blasts. These results suggest that NF-Ya is a potential therapeutic target for differentiation therapy in acute leukemia. However, it is not clear how the activity of such a factor could be specifically targeted in the leukemic stem cells without interfering with their normal counterparts and hematopoiesis in general.

Mechanisms of Apoptosis and New Frontiers in Cancer Therapy

Disruption of appropriate cell death programs plays an important role in cancer pathogenesis. Progress in identification of the molecular components that control cellular life-or-death decisions and play important roles in cancer cell survival continues to provide new targets for future anticancer therapy. Xia-Dong Wang (University of Texas, Southwestern Medical Center, Dallas, TX) described studies focusing on the role of inhibitor of apoptosis proteins in cancer and crystal structure–guided studies to design small molecular inhibitors for this family of proteins that block apoptosis initiated from both the cell surface and mitochondria in human cells. Inhibitor of apoptosis proteins are overexpressed in many human cancers, suggesting a means by which cancer cells evade apoptosis during tumorigenesis and become resistant to chemotherapy and radiation therapy. Wang and his coworkers solved the crystal structure of a functional domain of XIAP (BIR3) and its cellular antagonist protein, Smac/DIABLO. Based on this structure, they synthesized a small-molecule mimic for Smac, which effectively binds to several members of the inhibitor of apoptosis family of proteins including XIAP, cIAP1, and cIAP2. Serendipitously, they discovered that chimeric compounds were much more active in competition assays than monomers, results consistent with native Smac protein functioning as a homodimer. Antitumor activity for the dimeric compound was observed within the 50 to 100 nmol/L range, which was even better than for Smac itself. The dimeric molecule also synergized with both tumor necrosis factor–related apoptosis-inducing ligand and tumor necrosis factor-α to promote caspase activation and apoptosis in human cancer cells. Although the Smac mimicking compound has not yet been evaluated for nonspecific toxicities, Wang’s laboratory is moving forward with studies addressing its in vivo effects in tumor xenograft models.

In a workshop on "Translational Strategies for Induction of Differentiation and Selective Apoptosis in Solid Tumors,” Reuben Lotan (University of Texas M. D. Anderson Cancer Center, Houston, TX) described results of studies addressing mechanisms of action of several novel synthetic retinoids, including the heteroarotinoid ShetA2, the adamantyl-containing retinoids CD437, MX3350-1, ST1926, and phenylretinamides (e.g., 4-HPR) in human non–small cell lung cancer, as well as head and neck squamous cell carcinoma cell lines. These agents targeted the mitochondria, causing release of cytochrome c and activating caspases 9 and 3. CD437 and MX3350-1 decreased the levels of the antiapoptotic proteins Bcl-2 and Bcl-XL and increased the amounts of proapoptotic Bax, Fas ligand, Fas and other death receptors (e.g., DR4 and DR5), and cleaved Bid. The mechanisms of apoptosis induction seemed to be independent of activation of nuclear retinoid receptors and some of these retinoids acted additively or synergistically with the cyclooxygenase-2 inhibitor celecoxib and the farnesyltransferase inhibitor SCH66336, thus suggesting their potential application in cancer chemoprevention and therapy.

Yongkui Jing (Mount Sinai School of Medicine, New York, NY) described recent findings on the mechanism by which As2O3 induced apoptosis. He reported that the levels of glutathione peroxidase, catalase, and glutathione-S-transferase δ determined the cell sensitivity to As2O3 treatment among leukemia and lymphoma cells because they serve as the H2O2 and As2O3 detoxification system. The data presented indicates inverse relationship between the levels of glutathione-S-transferase δ expression and sensitivity to As2O3 treatment.

Yoshio Honma (Saitama Cancer Center, Saitama, Japan) described that Cotylenin A and IFN synergistically inhibited growth and induced the apoptosis of several human carcinoma cell lines, in part by increasing the levels of tumor necrosis factor–related apoptosis-inducing ligand and its receptor DR5. This treatment combination induced these genes early in lung carcinoma cells while sparing normal lung epithelium. Similar results were obtained from in vivo studies of human lung cancer cells as xenografts, suggesting that combination of cotylenin A and IFN may be a promising new anticancer therapy.

Shifting gears into the area of cancer profiling using high-throughput techniques, Tom Look (Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA) described studies that address the molecular pathogenesis of childhood T-cell leukemias. Using oligonucleotide microarrays, three distinct gene expression signatures were identified that indicated leukemic arrest at specific stages of normal thymocyte development: LYL1⁺ (pre-T), HOX11⁺ (early cortical thymocyte), and TAL1⁺ (late cortical thymocyte). HOX11 activation was found to be significantly associated with a favorable prognosis. These results show a previously undetected molecular pathway to leukemic survival.
heterogeneity among childhood T-cell leukemias and suggest the ability of gene expression profiling to stratify patients into clinically relevant subgroups. Additionally, Look described a model of clonally derived T-cell acute lymphoblastic leukemia in zebrafish transgenic for mouse c-myc, which provides a platform for drug and genetic screens aimed at identifying mutations that suppress or enhance c-myc-induced carcinogenesis.

Paul Fisher's laboratory (Columbia University, College of Physicians and Surgeons, New York, NY) has used an overlapping pathway screening strategy to identify a number of up-regulated genes during terminal differentiation of HO-1 melanoma cells and senescence in human fibroblast cells. Fisher gave a detailed characterization of one such gene, hPNPaseold-35, which encodes polynucleotide phosphorylase with 3',5' exo-RNase activity. Over-expression of hPNPaseold-35 inhibited HO-1 melanoma cell growth and induced senescence in normal human melanocytes, suggesting that hPNPaseold-35 plays an important role in cell growth control and may be useful in gene therapy of cancer.

Zhu Chen (Shanghai Institute of Hematology and State Key Laboratory for Medical Genomics, Shanghai, China) described a systems biology approach for the study of hematopoiesis and leukemia. Examination of current and potential therapeutic agents in APL at cellular, biochemical, and transcriptome levels have suggested that combination use of ATRA/As2O3 leads to distinct but synergistic therapeutic effects. In the chronic myelogenous leukemia model, gene expression network analysis revealed that in addition to the modulating pathways downstream of BCR-ABL, Imatinib (Gleevec) exerts effects on chromatin structure and activation of a mitochondria-mediated intrinsic apoptotic pathway. To further address molecular leukemogenesis in a systematic way, the Leukemia Genome Anatomy Project has been launched in Shanghai Institute of Hematology. This project aims to address specific interactions between abnormalities in cytosolic signaling pathways (particularly tyrosine kinases), which confer proliferation/survival advantage, and in nuclear signaling (transcription factors) that leads primarily to differentiation arrest. Zhu Chen anticipated that

<table>
<thead>
<tr>
<th>Presenting author</th>
<th>Tumor type</th>
<th>Molecular therapy</th>
<th>Associated therapies</th>
<th>Outcome/effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurent Degos</td>
<td>AML</td>
<td>ATRA + valproic acid + pentoxifyline (cAMP elevation)</td>
<td></td>
<td>20% remission</td>
</tr>
<tr>
<td>Yoshio Honma</td>
<td>Myelodysplasia</td>
<td>Cotylenin A + IFN-α</td>
<td>Celecoxib (cyclooxygenase-2 inhibition)</td>
<td>Specific apoptosis (cell lines)</td>
</tr>
<tr>
<td>Ruben Lotan</td>
<td>Lung cancer</td>
<td>Synthetic retinoids: ShetA2, CD437, MX3350-1, and ST1926</td>
<td>SCH66336 (farnesyltransferase inhibition)</td>
<td>Specific apoptosis (cell lines)</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>Phenylretinamides: 4HPR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micheal Lübbert</td>
<td>Myelodysplasia (high risk)</td>
<td>Decitabine</td>
<td>ATRA (in AML)</td>
<td>50% effective</td>
</tr>
<tr>
<td>Paul Marks</td>
<td>T cell lymphoma</td>
<td>SAHA</td>
<td>Radiotherapy</td>
<td>Disease stabilization</td>
</tr>
<tr>
<td>Diffuse large B cell lymphoma</td>
<td></td>
<td></td>
<td>Cytostatic agents</td>
<td></td>
</tr>
<tr>
<td>Mesothelioma</td>
<td></td>
<td></td>
<td>Kinase inhibitors</td>
<td></td>
</tr>
<tr>
<td>Laryngeal carcinoma</td>
<td></td>
<td></td>
<td>Differentiation inducers</td>
<td></td>
</tr>
<tr>
<td>Tomoki Naoe</td>
<td>AML (FLT3)</td>
<td>Kinase inhibition</td>
<td>Cytoxic therapy</td>
<td>80% remission (28 days)</td>
</tr>
<tr>
<td>Zhi-Xiang Shen</td>
<td>AML</td>
<td>As2O3 + ATRA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhen-Yi Wang</td>
<td>AML (AML1-ETO)</td>
<td>SIH-10</td>
<td>Tumor necrosis factor–related apoptosis-inducing ligand</td>
<td>Specific apoptosis (cell lines)</td>
</tr>
<tr>
<td>Xia-Dong Wang</td>
<td>Cancer cell lines</td>
<td>Smac/DIABLO mimic</td>
<td></td>
<td>Specific apoptosis (cell lines)</td>
</tr>
<tr>
<td>Samuel Waxman</td>
<td>APL</td>
<td>ATRA</td>
<td>Dithiophenes (cytotoxic therapy)</td>
<td>Differentiation/apoptosis (cell lines)</td>
</tr>
<tr>
<td>Arthur Zelent</td>
<td>AML</td>
<td>ATRA + granulocyte colony-stimulating factor/granulocyte macrophage colony-stimulating factor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
findings emerging from such studies might further strengthen the
drug discovery paradigm shift to targeted therapies of leukemias and
cancer in general.

Conclusions

Overall, this was a very successful and informative meeting that
strongly reflected the excitement and promise of a new frontier in
cancer research that targeted treatment strategies represent.
Consistent with recent progress in the understanding of epigenetic
mechanisms that control gene expression in normal and cancer
cells, much of the conference was devoted to cancer epigenetics
and potential therapeutic strategies that can revert the aberrant
epigenetic modifications that are responsible for establishment of
repressed chromatin and gene silencing in carcinogenesis.
Nevertheless, the drugs that target chromatin structure also face
some problems with specificities of action. For example, SAHA and
other HDAC inhibitors that are currently in clinical trials do not
discriminate between different deacetylases and are often thought
of as one drug family, although they are both structurally and
functionally diverse and possess different spectra of activities
toward a variety of tumors. It is likely that these differences reflect
additional mechanisms of action that are specific for a given
inhibitor, which need to be carefully and extensively examined
before these drugs can be used most effectively in therapy,
particularly in combination with other agents. Targeted combina-
torial therapies are likely to be more effective than single-agent
protocols and in APL may decrease the need for chemotherapy,
thus greatly reducing undesirable and long-lasting side effects that
are associated with the use of highly toxic chemotherapeutic
agents. Results discussed at this meeting indicate that the
development of successful targeted anticancer therapies for other
more common malignant diseases is not far behind (see Table 1
for examples). Given that most cancers seem to be driven by a
small proportion of cancer stem cells, these cells remain important
targets for both basic research of cancer pathogenesis and for
therapeutic intervention. There remain, however, considerable
challenges associated with characterization and isolation of such
cells from different tumor types and obtaining sufficient quantities
of cancer stem cells for molecular studies. Although this particular
area of cancer research and many targeted therapeutic strategies
are still in very early stages of development, the overall take-home
message from the 10th International Conference on Differentiation
Therapy is that the future of targeted anticancer therapies is highly
promising.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page
charges. This article must therefore be hereby marked advertisement in accordance
with 18 U.S.C. Section 1734 solely to indicate this fact.
We thank all the organizers, session chairs, speakers, and all participants for their
contribution to this successful meeting. The organizers thank the Ministry of Sciences
and Technology of China, National Natural Science Foundation of China, Chinese
Academy of Sciences, Shanghai Municipal Commission for Science and Technology,
and Samuel Waxman Cancer Research Foundation for support.
Molecular Target-Based Treatment of Human Cancer: Summary of the 10th International Conference on Differentiation Therapy


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/4/1117

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.