Derepression in the Desert: The Third Workshop on Clinical Translation of Epigenetics in Cancer Therapeutics

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Introduction

In the past decade, the scientific and medical community has witnessed dramatic progress in epigenetics and has begun to understand some of the epigenetic mechanisms that contribute to human disease, particularly cancer. In January 2007, the third biannual workshop on Clinical Translation of Epigenetics in Cancer Therapeutics was held in Phoenix, Arizona. Its mission was to discuss the basic epigenetic mechanisms of transcriptional regulation and deregulation in cancer and evaluate the progress in translating new research findings in this field into effective anticancer therapies.

Cancer Epigenetics and New Drug Development

Epigenetics can be defined as the study of heritable changes in chromatin function that occur without alterations in DNA sequence. A growing body of evidence suggests that epigenetic changes may provide a greater contribution to cancer development and progression than genetic causes, and in some cases may be solely responsible for tumorigenesis. From a clinical perspective, this is potentially good news as the reversibility of epigenetic changes makes these processes attractive therapeutic targets. However, despite a growing number of recently identified chromatin modifying enzymes (see Supplementary Table S1 for histone methylation enzymes; cancer cell–specific DNMT3B transcripts encode truncated isoforms lacking the COOH-terminal catalytic domain (Lucy Godley, University of Chicago Pritzker School of Medicine, Chicago, IL). When the most frequently expressed aberrant transcript, DNMT3B7, was expressed in cells in change in gene expression were observed, which in some cases correlated with altered DNA methylation of corresponding CpG islands.

New drug development targeting epigenetics has included pharmacokinetic progress in DNMT inhibition. Currently available DNMT inhibitors have extremely brief half-lives due to instability in aqeous solution and rapid metabolism by cytidine deaminase. These pharmacokinetic limitations may contribute to the difficulty of successful application of these drugs in nonhematologic neoplasms. Peter Jones (Norris Cancer Center, University of Southern California, Los Angeles, CA) described S110, an acetylosine dinucleotide with a dramatic decrease in deamination by cytidine deaminase (Supplementary Fig. S1). Newer approaches to drug development use genomic techniques. Kimberly Stegmaier (Dana-Farber Cancer Center, Boston, MA) described the application of DNA microarray gene expression signatures to drug discovery and has developed "connectivity maps" for various Food and Drug Administration (FDA)–approved drugs, diseases, and gene expression profiles. The use of such connectivity maps, which are available via internet-based resources,5 can indicate a potential therapeutic agent or combination for a given disease. Based on differences between the gene expression profiles for normal and acute myelogenous leukemia (AML) stem cells, Monica Guzman (University of Rochester School of Medicine, Rochester, NY) identified parthenolide, an inhibitor of NFκB that works by targeting IkB kinase. Parthenolide was selective for AML and chronic myelogenous leukemia stem cells and with little effect on normal progenitor/stem cells in vitro and in vivo assays. The histone deacetylase (HDAC) inhibitor valproic acid (Supplementary Fig. S1) enhanced the maintenance and clonogenic capacity of both normal and leukemic progenitor cells in vitro (Gesine Bug,

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Klinikum der J.W. Goethe-Universitas, Frankfurt (Germany). Although these data remain to be evaluated in vivo and the study has yet to be extended to other HDAC inhibitors (HDACi), this issue raises concerns about the treatment of AML with HDACi. Some AML patients treated with valproic acid display effects that are consistent with the results reported by Bug. However, some patients also show short-lived hematopoietic improvement. This may indicate that valproic acid has heterogeneous effects on distinct leukemic cell populations; leukemic progenitor cells may be expanded while more mature leukemic cells may be killed (Janice Gabrilove, Mt. Sinai School of Medicine, New York, NY). These data highlight the importance of defining cancer stem cells and studying epigenetically targeted drugs specifically on these populations.

**HDAC Inhibitors: Clinical Studies**

A variety of protein deacetylase inhibitors may affect gene expression through modification of chromatin conformation. These drugs function as HDACi, thereby enabling the reacetylation of histone lysine residues required for transcriptionally active chromatin by histone acetyltransferases. These compounds have undergone intensive early-stage clinical development in recent years. Key questions include the molecular mechanisms underpinning clinical activity, whether such drugs can exhibit molecular specificity, and whether such drugs should be targeted to specific HDAC enzymes or specific classes of HDAC enzymes, rather than acting as pan-HDACi. The FDA approval of vorinostat (Suplementary Fig. S1) for treatment of cutaneous T-cell lymphoma set a first clinical standard for HDACi performance. The meeting focused on emerging clinical data on this and other HDACi. Romidepsin (FK228, depsipeptide; Supplementary Fig. S1) is also active in T-cell lymphomas; however, it remains unclear whether romidepsin depsipeptide offers advantages over vorinostat (Susan Bates, National Cancer Institute, Bethesda, MD). Previous studies had raised concerns about cardiac toxicity of this drug; current data suggest that those concerns were likely overstated. Other newer HDACi discussed included the hydroxamic acid belinostat (PXD101; Supplementary Fig. S1) and the benzamides MGC0103 (Supplementary Fig. S1) and MS-275 (now SDX-275; see Supplementary Fig. S1). The latter two structurally similar drugs putatively possess class I HDAC selectivity. It is not yet clear whether any of these agents offer clinical advantages over vorinostat (Johann deBono, Royal Marsden Hospital, Sutton, United Kingdom; Guillermo Garcia-Manero, MD Anderson Cancer Center, Houston, TX; Steven Gore, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD).

Because fusion genes that involve core binding factor recruit HDACs as part of a transcriptional co-repression complex, AML with core binding factor gene rearrangements have been speculated to be particularly good targets for HDACi. Preliminary data suggest that such patients may be more sensitive to romidepsin than patients with normal karyotypes (Toyosi Odénike, University of Chicago Pritzker School of Medicine, Chicago, IL). Whereas these leukemias represent a minority of cases of AML and are more likely to be cured by cytarabine-based chemotherapy than other subsets of AML, these early data suggest that core binding factor leukemias represent a fertile area for proof-of-concept studies targeting such transcriptional repression complexes.

In advanced breast cancer, treatment with vorinostat resulted in disease stabilization in 4 of 14 cases. Taking the view that administering the drug in the preoperative stage would allow investigation of pharmacodynamic effects in postresection specimens, Véronique Stearns (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD) described plans to evaluate the safety and tolerability of vorinostat in women with primary breast cancer before surgery. Such novel clinical trial designs focus on the procurement of posttreatment tissue to help elucidate the mechanisms of action of epigenetically targeted drugs.

**HDAC Inhibitors: Mechanisms of Action**

Whereas the focus of the meeting was the translation of epigenetic science to the treatment of cancer, the "off-target" effects of drugs may be as important to study as the putative "specific" drug target. Steven Grant (Virginia Commonwealth University, Richmond, VA) emphasized that the so-called HDACs in fact lead to the acetylation of many other proteins in addition to histones. Killing of neoplastic cells by HDACi may require generation of reactive oxygen species, activation of proapoptotic genes, and inhibition of proteasome function. Inhibition of IκB kinase via flavopiridol enhances cytotoxic effects of vorinostat by inhibiting NFκB activity (Supplementary Fig. S2). Other inhibitors of the NFκB pathway could also be useful in enhancing the proapoptotic effects of vorinostat. Brian Gabrielli (University of Queensland, Brisbane, Australia) reported that tumor cell sensitivity to another hydroxamate HDACi, LAQ824, was associated with the loss of G2 checkpoint. He also reported that HDACi-mediated induction of p21WAF1/CIP1 protected cells from apoptosis, and inhibition of p21 potentiated the proapoptotic effects of HDACi thereby enhancing HDACi antitumor activity, consistent with Grant's work on inhibition of p21 with flavopiridol. Many of these studies are carried out using established cell lines and the results remain to be validated in primary tumor cells or in vivo models (Supplementary Fig. S2).

A number of studies over the past decade have indicated that HDACi may act in a highly cell context–dependent manner. Ricky Johnstone (The Peter MacCallum Cancer Institute, Melbourne, Australia) addressed the requirement of tumor necrosis factor (TNF)–related apoptosis-inducing ligand and death receptor signaling for apoptosis induction by vorinostat in a c-myc–driven murine model of lymphoma. TNF-related apoptosis-inducing ligand and death receptor signaling were not required for vorinostat-induced apoptosis; rather, the intrinsic mitochondrial pathway was important because BID- and BIM-deficient mice had lower response to HDACi. However, studies by Reuben Lotan's group (MD Anderson Cancer Center, Houston, TX) indicated that both Fas death receptor–mediated and mitochondrial apoptotic pathways are involved in mediating the effects of vorinostat and valproic acid. Mechanism-based combinations entering clinical trials based on in vitro synergy or gene expression data include combinations of HDACi with proteasome inhibitors (Gail Eckhardt, University of Colorado Cancer Center, Denver, CO) and HDACi with all trans-retinoic acid and vascular endothelial growth factor antagonists (Roberto Pili, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD).

Kapil Bhalla (Medical College of Georgia, Augusta, GA) discussed the mechanisms of tumor cell death due to inhibition of HDAC6, a deacetylase whose primary substrates are nonhistone cytoplasmic proteins such as α-tubulin and heat shock protein 90 (Supplementary Fig. S3). By interfering with the proper balance of acetylation of these proteins, inhibition of HDAC6 has been shown to interfere with proteasomal function and aggresome formation. Thus, HDACi
have been shown to synergize with both heat shock protein 90 and proteasome inhibitors in cancers such as multiple myeloma that accumulate a large amount of misfolded proteins. Use of these drugs leads to cancer cell death due to unresolved misfolded protein response (Supplementary Fig. S3).

HDACi may cause apoptosis by increasing DNA damage (Feyruz Rassool, University of Maryland Greenebaum Cancer Center, Baltimore, MD). HDACi may selectively kill leukemic cells by enhancing misrepair of DNA double-stranded breaks and thus indirectly causing apoptosis through activation of cellular response to DNA damage. These results are consistent with data from Scott Hiebert’s laboratory (Vanderbilt University School of Medicine, Nashville, TN) showing that HDAC3-null cells accumulate DNA damage and have impaired DNA repair and delayed cell cycle progression. Thus, some of the effects observed with HDACi may be specifically due to the inhibition of ubiquitously expressed HDAC3. HDACi also lead to normalization of heterochromatin surrounding translocation breakpoints (Mary Callanan, Institut Albert Bonnoit, Université Joseph Fourier, Grenoble, France).

The data discussed confirm that HDACi constitute a class of complex drugs with multiple molecular targets. Ongoing efforts in in vitro and animal models as well as the ex vivo study of tissue from treated patients will help identify the critical targets and enable more rational development of this class of active anticancer agents.

DNA Methylation

DNMT inhibitors are clinically active in patients with hematologic malignancy. Both azacytosine analogues 5-azacytidine (aza-CR) and 5-aza-2'-deoxycytidine (decitabine) are approved in the United States for the treatment of myelodysplastic syndrome. As previously noted, both drugs have short half-lives and are currently only available as parenteral formulations. The extent to which their clinical activity depends on the reversal of promoter methylation remains an area of scientific uncertainty. A variety of phase I trials have combined or sequenced DNMT inhibitors with HDACi in an attempt to achieve synergistic expression of epigenetically silenced genes. The DNMT inhibitor decitabine induces hematologic recovery in AML and myelodysplasia patients, particularly those with a normal karyotype (Michael Lübbert, University Medical Center, Freiberg, Germany); continuous treatment may be superior to interrupted treatment. Trials of decitabine and aza-CR with valproic acid (and in one study, all-trans-retinoic acid as a third drug) have been encouraging but have not suggested synergy comparable to that seen in vitro for gene expression with these drugs, potentially limited by the low potency of valproic acid as a HDACi (Garcia-Manero). Sequential treatment of AML cells with decitabine and HDACi leads to synergistic apoptosis, suggesting that the synergy of this class of drugs may not be limited to reexpression of epigenetically silenced genes (Gore). In a phase I trial combining aza-CR and the HDACi MS-275, the best responses developed in patients receiving low doses of the HDACi. After 3 days of aza-CR administration, some patients already showed an induction of DNA damage–associated H2AX (Gore). This combination has entered a randomized phase II trial in the U.S. Intergroup; this trial randomly assigns patients to a 10-day schedule of aza-CR with or without the HDACi. This will be the first trial directly comparing the effects of a DNMT inhibitor alone and in combination with a HDACi.

Robert Brown (Glasgow University, Glasgow, United Kingdom) observed that early trials with epigenetic therapy in solid tumors have been disappointing. It has been difficult in these studies to establish the relationship between pharmacokinetics and target pharmacodynamics, as well as biological and clinical response (particularly in the tumor tissue). Decitabine has caused a dose-dependent decrease in DNA methylation and an increase in fetal hemoglobin expression. In ovarian cancer, patients treated with chemotherapy acquire resistance, which correlates with promoter hypermethylation of hMLH1. Reversal of this hypermethylation using DNMT inhibitors leads to the reestablishment of chemosensitive disease. The pace of clinical investigation of DNMT inhibitors in nonhematologic malignancies has lagged behind that in hematologic malignancies, in part due to the known activity of the azacytosine nucleoside analogues in myeloid malignancies, first observed in 1989.

Detection of Biomarkers

Current translational approaches to epigenetically targeted therapy have been hampered by the lack of reliable molecular markers that predict response (Jonathan Licht, Lurie Cancer Center, Northwestern University, Chicago, IL). Bjorn Hackanson (James Cancer Center, Ohio State University, Columbus, OH) discussed Epityper and Bio-COBRA methods to accurately monitor changes in DNA methylation. COBRA, a methylation-specific PCR–based approach, is limited by specific primers. In contrast, Epityper is based on mass spectrometry of bisulfite–converted DNA and can provide quantitative assessment of multiple CpGs within DNA sequences of up to 600 bp. A genomics-based methylation assay (“HELP”) was discussed by Maria Eugenia Figueroa (Albert Einstein College of Medicine, Bronx, NY), which uses comparative isoschizomer digestion of the genome with the HpaII and MspI enzymes to create a quantitative representation of the methylation level of CpGs throughout the genome in combination with customized high-density oligonucleotide microarrays. Using HELP, they could discriminate a finer classification of AML than with gene expression arrays alone, possibly due to a better sensitivity for detecting locus-specific changes that result in mRNA levels too small to be detected by expression arrays. The effective application of (epi-)genomics-based assays will potentially facilitate the identification of molecular targets associated with clinical response, which will in turn guide the next generation of clinical development of these approaches.

Targeting Protein-Protein Interactions

Current drugs that target epigenetic changes inhibit effector proteins that are widely used in the epigenome. Such drugs may have broad application but suboptimal specificity. Better specificity could potentially be achieved by targeting interactions between specific epigenetic marks and adaptor proteins. However, protein–protein interactions have traditionally been considered to be difficult, if not impossible, to affect pharmacologically. A number of investigators discussed the targeting of protein–protein interactions as a therapeutic approach to inhibiting the function of transcription factor oncogenes. Ari Melnick’s laboratory (Albert Einstein College of Medicine, Bronx, NY) disrupted the interaction between the SMRT corepressor and the BTB/POZ domain of BCL6, thus inhibiting transcriptionally repressive BCL6 activity. Peptide inhibitors of BCL6 potently and specifically kill B-cell lymphoma cells. This interaction is also the target of a peptide aptamer approach (Paul Ko-Ferrigno, Hutchison/MRC Research Centre, Cambridge, United Kingdom). John Bushweller (University of
Virginia School of Medicine, Charlottesville, VA) described the development of small-molecule inhibitors that disrupt the interaction between AML1 (Runx1) and CBFβ. These inhibitors restore the ability of AML1-ETO–containing Kasumi cells to differentiate in response to all trans-retinoic acid treatment. Additionally, Jay Hess (University of Michigan School of Medicine, Ann Arbor, MI) described peptides that interfere with the interaction of MLL with menin, which is required for the leukemogenic activity of MLL. Anders Näär (Harvard Medical School and Massachusetts General Hospital Cancer Center, Charlestown, MA) described the use of a high-throughput fluorescence polarization screen for compounds that disrupt KIX domain–mediated interactions; however, the KIX domain is present in many coactivators and it may be difficult to generate specificity. This is a potential problem with other small-molecule drugs directed at disrupting protein-protein interactions. Overall, this session showed that such protein-protein interactions may represent “druggable” targets, which could lead to treatments with a high degree of specificity.

Summary of Findings and Recommendations for Future Research

This meeting has reflected well the current status of epigenetic cancer therapy and highlighted its promising future, which has been underscored by the clinical success with FDA-approved vorinostat, aza-CR, and decitabine, as well as encouraging results from the use of nucleoside analogues in therapeutic trials of AML. Nevertheless, there remain many major challenges for the future basic and clinical research in this field.

Although much knowledge has been accumulated in clinical epigenetics, particularly relating to the role of DNA methylation patterns in cancer, the precise array and extent of epigenetic modifications that distinguish particular cancer cell types (as well as cancer stem cells), together with the mechanisms that are responsible for their establishment, remain poorly understood. There is a clear need for identification and validation of novel therapeutic targets and development of drugs to modulate their activities. Appropriate in vitro and in vivo model systems also need to be developed for preclinical studies. Future clinical trials should be mechanism driven and supported by strong preclinical data. Therefore, effective communication and collaboration between basic and clinical work is essential for rapid translation of acquired knowledge into effective anticancer therapies. At present, there is a wide range of HDACi in clinical trials despite a lack of clear understanding of their mechanisms of action in a particular disease. It is highly unlikely that these drugs solely act via inhibition of histone acetylation, and potential off-target effects should be investigated using appropriate in vitro or in vivo disease models and gene knockout and/or knockdown technologies. The expansion of structurally diverse HDACi and their use in a variety of clinical trials without understanding their exact mechanisms of action in a given disease have led to confusion in the field about the suitability of these agents in a particular therapeutic strategy, the most effective dosing, and schedules of administration. Exploring all potential mechanisms of action of these compounds (epigenetic and nonepigenetic), particularly in phase II trials when clinical responses are expected, must remain a high priority to further the rational development of this class of drugs and, to lead to further rational development of epigenetic strategies.

In contrast to HDACi, there is a lack of drugs that would more specifically act on other cancer-causing epigenetic modifications such as DNA and histone methylation. Nucleoside analogues are unstable and act through incorporation to DNA rather than directly targeting DNMT enzymatic activities. Currently, there are no drugs that inhibit histone methyltransferases or histone demethylases, although there are some in development. The role of specific histone methyltransferases and histone demethylases in cancer also remains to be better explored. Additionally, clearly defined clinical and molecular outcomes need to be established for each drug or drug combinations to evaluate if these agents exert desirable molecular effects in target cancer cells and whether these effects correlate with clinical response. It is clear that rapid progress in this highly promising field will not be accomplished without a concerted effort by both the basic and clinical scientific community. In this respect, this meeting has been invaluable in bringing together leading investigators in this field. An important take-home message is that clinical trials with these agents should be based on strong molecular data and positive preclinical results in appropriate animal cancer models, and, most importantly, it is this basic and clinical work that should influence and guide the pharmacologic development of “epi-drugs”.

Disclosure of Potential Conflicts of Interest

S.D. Gore: honoraria, Pharmion, Celgene; consultant as chair of a Data Safety Monitoring Board for a clinical trial, Gloucester; consultant on clinical trial design, Syndax; consultant on scientific advisory board regarding trial design, MGI Pharma; consultant as central reviewer of responses on a clinical trial, Gloucester. The other authors disclosed no potential conflicts of interests.

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