An AAOS-ORS Symposium

Molecular Biology and Therapeutics in Musculoskeletal Oncology*

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Musculoskeletal oncology encompasses a broad array of diseases and treatment challenges. The most important issue facing a patient with a sarcoma is cure. Traditional cytotoxic chemotherapy has evolved empirically over the last several decades. While substantial improvements have been made in cure rates for pediatric patients with sarcoma, cure rates have plateaued at considerably less than 100% and chemotherapy for adult patients is far less effective. Starting with cytogenetic analysis and, more recently, the molecular dissection of tumors, it has become obvious that "sarcoma" is not a diagnosis per se but a group of diseases. Adult soft-tissue sarcoma alone comprises many different histologic subtypes. A better understanding of the biology of tumors at the molecular level has brought forth the possibility of targeted therapy, prompting the American Academy of Orthopaedic Surgeons (AAOS) and the Orthopaedic Research Society (ORS) to hold the Molecular Biology and Therapeutics in Musculoskeletal Oncology Research Symposium in September 2008.

In contrast to the broad-spectrum drugs that are used in traditional chemotherapy, targeted therapy is biologically based and attempts to counteract the exact abnormality of the tumor cell. The types of abnormalities include overactive cell-surface receptors, which are part of the signaling cascades that drive growth; the secretion of proteins that stimulate angiogenesis; and the loss of function of tumor-suppressor genes that normally restrain growth. The types of therapeutics used include monoclonal antibodies, drugs that block overactive receptors, and gene-therapy techniques. The concept is that instead of using the same drugs for all patients with a particular stage of disease, the treatment would be personalized on the basis of the biologic abnormalities. The difficulties include the fact that there are many molecular abnormalities in any one tumor, with heterogeneity from one cell to the next; genetic instability leading to additional abnormalities over time; an overlap of biochemical pathways between normal physiologic processes and tumor growth; and a lack of therapeutics capable of reversing or blocking many of the molecular abnormalities found in tumors. Another limitation of the strategy is that blocking the effect of a molecular abnormality does not usually cure the patient in the traditional sense but can suppress tumor growth for some period of time. Fortunately, sometimes there are synergies when targeted, biologically based therapy is combined with cytotoxic chemotherapy, leading to a higher chance of cure.

The AAOS-ORS Molecular Biology and Therapeutics in Musculoskeletal Oncology Research Symposium was held in Salt Lake City, Utah, in September 2008, to address these issues. In addition to the AAOS and ORS sponsorship, the symposium received grant support from the Orthopaedic Research

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and Education Foundation, the National Cancer Institute, the National Institute of Arthritis and Musculoskeletal and Skin Diseases, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development; collaborative sponsorship from the Musculoskeletal Tumor Society and the WWWW Foundation, Inc. (QuadW); and industry support from the Musculoskeletal Transplant Foundation, Stryker, and Biomet. The organizers cast a wide net so as to include leaders in the field representing academia, the National Institutes of Health, the U.S. Food and Drug Administration, industry, leading cancer institutions, and cancer-related organizations, both from within and outside the traditional orthopaedic boundaries. A complete list of participants and their affiliations is included in the Appendix. The objectives of the symposium were to establish the state of the art of molecular biology and therapeutics in musculoskeletal oncology, identify barriers and potential solutions to advance the field, establish research priorities, and provide an educational forum that could foster collaborations for new and established investigators. The meeting was organized around the major diseases in musculoskeletal oncology; current topics in cancer biology, including genomic screening; and novel therapies. The following summary of the presentations and deliberations of the breakout sessions is provided as a record of the meeting and to guide prioritization of research funding.

Keynote Address

The address was presented by Mario Capecchi, PhD, of the University of Utah.

Dr. Mario Capecchi, winner of the 2007 Nobel Prize in Medicine and Physiology for his work in transgenic mice, gave the keynote address. Capecchi initially used transgenic mice to study developmental biology but more recently turned his attention to sarcoma. Two mouse models of sarcoma were developed by introducing the pathognomonic translocations of synovial sarcoma and rhabdomyosarcoma into the mouse[1,2]. Both mice develop metastatic tumors, with all of the respective histopathologic and cytogenetic markers of the two tumors. These mouse models are an invaluable tool to study the pathophysiology of these sarcomas and to test new treatments. They may help investigators to identify the cell of origin in sarcoma, determine common molecular linchpins in sarcoma development, and/or show that each type of sarcoma is truly a different entity.

Tumor Biology

The presenters were Catherine O'Brien, MD, MSc, of University Health Network; Maurice Zauderer, PhD, of Vaccinex, Inc.; Roopali Roy, PhD, of Children's Hospital Boston; Roman Eliseev, MD, PhD, of the University of Rochester; and Sean Scully, MD, PhD, of the University of Miami.

Updates of new areas in tumor biology were presented in the first session. Cancer stem cells, immunotherapy with an emphasis on therapeutic antibody development, angiogenesis, apoptosis, and the cell cycle were all discussed and provided a backdrop to subsequent discussion of these topics as they relate to sarcoma[3-6].

Osteosarcoma

The presenters were Francis Hornicek, MD, PhD, of Massachusetts General Hospital; Marc F. Hansen, PhD, of the University of Connecticut Health Center; Bang H. Hoang, MD, of the University of California, Irvine; Rex Haydon, MD, PhD, of The University of Chicago; Ching C. Lau, MD, PhD, of Texas Children's Hospital; Stephen J. Withrow, DVM, of Colorado State University; Eugenie S. Kleinerman, MD, of the M.D. Anderson Cancer Center; Chand Khanna, DVM, PhD, of the National Cancer Institute; and Neyssa Marina, MD, of Stanford University Medical Center.

The treatment and cure rate for osteosarcoma have been stable for decades. One could argue that at least 50% of patients are not being treated optimally. Before chemotherapy, the cure rate was 20%. If it could be determined which 20% of the patients would be cured with surgery alone at the time of biopsy, these patients could be spared the side effects of chemotherapy. Additional patients with complete tumor necrosis after preoperative chemotherapy might also be spared postoperative chemotherapy and its morbidity. Thirty percent of patients will not be cured by current chemotherapy regimens[7]. If these patients could be identified, experimental agents could be added to the current regimen in the hope that effective new agents will be identified. Current research is attempting to solve these problems with use of molecular biology techniques.

In contrast to tumors in which there are reproducible cytogenetic abnormalities that might be effective therapeutic targets, osteosarcoma is a cytogenetic quagmire, making it difficult to know which pathways to target. Nevertheless, the chromosomal aberrations are not random. Loss of heterozygosity on the distal portion of chromosome band 18q21.33 results in loss of expression of the VPS4B gene, which regulates the processing of activated, internalized growth-factor receptors. This results in overexpression of growth-factor receptors on the cell surface and resistance to cytotoxic chemotherapy. Growth-factor receptors are targets for small molecule inhibitors and antibodies; however, in osteosarcoma, these agents would need to block intracellular receptors as well[8,9].

In the past, much attention was devoted to the multidrug resistance gene as a mechanism of chemoresistance. Expression of this gene does not predict relevant clinical outcomes, and resistance to chemotherapy is multifactorial. In a preliminary analysis of tumor samples from patients with osteosarcoma, obtained before and after neoadjuvant chemotherapy, gene expression profiling identified gene signatures (clusters of relatively small numbers of genes that are either underexpressed or overexpressed) that can identify patients who will have a poor response to chemotherapy and subsequently have metastatic disease develop[10]. These gene signatures will allow the identification of high-risk patients who could benefit from trials of

experimental agents, and they may include new therapeutic targets.

Recapitulation of developmental pathways that provide a growth advantage is yet another phenomenon that occurs in cancer. Epithelial-to-mesenchymal transition occurs during embryogenesis and is associated with cell migration. When epithelial-to-mesenchymal transition-related pathways are reactivated in cancer, cells develop a metastatic phenotype. One such epithelial-to-mesenchymal transition pathway involves Wnt signaling. The inhibition of Wnt signaling with low-density lipoprotein receptor-related protein 5 (LRP5) as a soluble decoy receptor can slow tumor growth and metastases in mouse models of osteosarcoma[11].

One concept of cancer is that of a cell that has dedifferentiated to a more primitive state. Treating the cell with differentiating agents might induce the cells back toward a more differentiated state and more normal behavior. The question is whether cancer cells are resistant to the effects of growth factors and cytokines which cause differentiation during normal growth and development, and whether the degree of differentiation that can be achieved will make the cells less aggressive in the face of the multitude of molecular derangements that frequently are present in cancer cells. Bone morphogenetic proteins (BMPs) induce differentiation of osteoblast precursor cells to osteoblasts through downregulation of the Id and CNN families of genes, whose overexpression in osteosarcoma leads to resistance to differentiation. This resistance to differentiation by BMPs may be overcome by forcing expression of transcription factors related to osteoblast differentiation, such as Runx2[12,13]. Translating these observations into treatment strategies will require gene therapy techniques that are yet to be developed.

The major difficulties in running clinical trials for osteosarcoma include the rarity of the disease, the long time line and expense involved in the development of new oncology drugs, and the limitation of studying one new drug or agent at a time. The strategies being used to work around these obstacles are collaboration by the European and American Osteosarcoma Study Group, composed of the North American Children's Oncology Group, German Austrian Swiss Cooperative Osteosarcoma Study Group, European Osteosarcoma Intergroup, and Scandinavian Sarcoma Group; collaboration with canine osteosarcoma researchers; and a biology protocol in which tumor samples are collected for tissue banking from patients with newly diagnosed disease to further understand the molecular pathways important in osteosarcoma development[14].

Spontaneous canine osteosarcoma is the best animal model for human osteosarcoma[15]. Eighty-five percent of canine patients present with stage-IIB disease, and cure with amputation alone is 10%. Metastases are to lung and bone. All of these features are similar to human disease. Limb salvage with allografts has also been studied in dogs. The higher rate of survival in the dogs that had a postoperative infection[16] has led to trials of immunotherapy utilizing liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE), initially in dogs and subsequently in humans[17]. MTP-PE is taken up by pulmonary macrophages, which kill tumor cells by utilizing tumor necrosis factor-alpha (TNF-α) and nitric oxide. When MTP-PE is given with cytotoxic chemotherapy, survival is increased by 10%, illustrating how manipulation of the pulmonary microenvironment and immune system can affect the development of metastases. Since osteosarcoma develops in 10,000 dogs annually, the possibility of rapidly testing new agents is feasible in the canine population[18].

One agent that has reemerged in cancer trials is rapamycin. Rapamycin is a macrolide with antifungal properties that inhibits the mammalian target of rapamycin (mTOR). mTOR is in the phosphoinositide 3-kinase-related kinase (PIKK) family of kinases, with serine and threonine activity. It mediates diverse cellular pathways including nutrient and growth factor response, mRNA transcription and protein translation, ribosome biogenesis, organization of the actin cytoskeleton, membrane trafficking, and protein degradation. Inhibition of these pathways with rapamycin and other rapalogs (rapamycin analogs) can inhibit tumor metastasis but is frequently associated with compensatory activation of alternative pathways, suggesting that combinations of targeted therapies will be necessary when attempts are made to block critical pathways[19].

The Fas-Fas ligand pathway induces apoptosis (cell death). Utilizing knockout mice, gene transfections, and gene knockdown, it has been shown that osteosarcoma cells lacking Fas develop pulmonary metastases, whereas upregulating Fas in tumor cells and Fas ligand in lung cells with inhalable agents such as liposome-encapsulated 9-nitrocamptothecin (L-9NC), gemcitabine, or interleukin-12 can prevent or help to eliminate pulmonary metastases in animal models[20].

Ewing Sarcoma

The presenters were Stephen Lessnick, MD, PhD, of the Huntsman Cancer Institute; Lee J. Helman, MD, of the National Cancer Institute; and Howard Chansky, MD, of the University of Washington School of Medicine.

Ewing sarcoma is characterized by a translocation between chromosomes 11 and 22, resulting in an aberrantly expressed transcription factor from the fusion of the EWS and FLI1 genes. In the three presentations, aspects of this and other molecular abnormalities in Ewing sarcoma were discussed and three questions were addressed. First, are there new approaches to molecular diagnostics? Although at the cytogenetic level, the vast majority of Ewing tumors have a translocation between chromosomes 11 and 22, there is considerable variability in the exact splice sites, leading to a variety of fusion products with differing downstream effects. This makes comprehensive molecular genetics testing mandatory if progress in diagnostics and therapeutics is to be made. Second, how can the transformation pathway be comprehensively

defined? When different cells are transfected with an identical Ewing fusion protein, different downstream effects and phenotypes are observed, suggesting that not only is the exact translocation splice site important but the context is also; therefore, identifying the cell of origin is critical[21]. Gene array analysis after knockdown of EWS/FLI1 with RNA interference can identify the downstream targets. There are 320 genes upregulated and approximately 1150 genes downregulated by EWS/FLI1 in Ewing sarcoma cells. Promising targets include NKX2.2, a transcription factor involved in neuronal differentiation; NR0B1, hypothesized to be a corepressor in adrenal development; CAV1, a membrane protein whose role in signaling is not yet well defined; and CD99, a membrane protein that is a classic immunohistochemical marker for Ewing sarcoma and whose function remains a mystery[22]. Third, how can we identify therapeutic targets in Ewing sarcoma? The paradigm for molecularly targeted therapies was discussed by considering the insulin-like growth factor (IGF) pathway. Insulin-like growth factor-1 receptor (IGF-1R) is required for oncogenic transformation in Ewing sarcoma. Insulin-like growth factor binding protein-3 (IGFBP-3) binds IGF and blocks its activity. EWS/FLI1 inhibits expression of IGFBP-3, leading to increased activity of IGF-1. Inhibition of IGF-1 signaling has antitumor effects in Ewing sarcoma model systems, lending itself to antibody and/or small molecule inhibitors.

Studies have demonstrated that IGF provides a survival signal that contributes to tumor cell resistance to DNA-damage-induced cell death[23,24]. This resistance is associated with mTOR signaling and can be reversed with agents that block mTOR. Aggressive, metastatic behavior in sarcomas is associated with activation of mTOR[25], and mTOR blockade with rapamycin or analogs inhibits rhabdomyosarcoma experimental pulmonary metastases[19]. Rapalog treatment of rhabdomyosarcoma leads to compensatory activation of Akt in vivo; this activation is IGF-dependent and can be blocked with IGF-1R blockade[26]. The preclinical studies with the human IGF-1R antibody have been favorable. Thus, there appears to be early evidence to suggest the beneficial combination of mTOR inhibition with IGF-1R inhibition. Specific to Ewing sarcoma, the humanized IGF-1R monoclonal antibody has shown clinical responses in Phase-I and II studies. An investigation is planned with use of an mTOR inhibitor and IGF-1R monoclonal antibody 1507 in a randomized study of relapsed Ewing sarcoma.

EWS/FLI1 blocks senescence in the Ewing precursor cell, as evidenced by changes in cell morphology after treatment with short interfering RNA (siRNA) directed against EWS/FLI1, expression array data, and decreased proliferation. Depletion of EWS/FLI1 in Ewing cell lines leads to activation of pRb through inhibition of hyperphosphorylation, reduction in activity of the cyclin and cyclin-dependent kinase pairs CDK4/cyclin D and CDK2/cyclin E mediated by increased levels of p27 and p57, and a reactivation of senescence[27]. Viral vectors can modulate pRb to affect the Ewing phenotype in vitro.

Chondrosarcoma

The presenters were Benjamin A. Alman, MD, FRCSC, of the University of Toronto; James A. Martin, PhD, of the University of Iowa; and Sean Scully, MD, PhD, of the University of Miami.

Defects in the negative feedback loop found in the growth plate involving parathyroid hormone-related protein (PTHrP), its receptor PTHR, and Indian hedgehog (IHH) can reproduce the syndrome of enchondromatosis in transgenic mice with defective PTHR or overexpression of Gli1 and Ptch1, the downstream signaling molecules for IHH. Both result in overactive IHH signaling. Although PTHR mutations are found in only 3% of human enchondromas, the fidelity of these transgenic models suggests that these or other abnormalities in this pathway may underlie the disease. When Gli2-overexpressing mice are crossed with a heterozygotic p53-mutant mouse, the mice get what appears to be soft-tissue chondrosarcoma. When human chondrosarcomas are grown in mice as xenografts, tumor growth can be inhibited with agents that block IHH signaling (triparanol)[28].

Telomerase maintains the terminal sequences of chromosomes that are normally deleted with each cell division and, in normal cells, lead to senescence. In chondrosarcoma, telomerase expression is correlated with grade, loss of senescence, invasion, and survival[29]. The mechanisms of cell-cycle dysregulation in chondrosarcoma remain largely unknown[30].

Soft-Tissue Sarcoma

The presenters were Jonathan Fletcher, MD, of Brigham and Women's Hospital; William H. Meyer, MD, of The University of Oklahoma Health Sciences Center; and Timothy J. Triche, MD, PhD, of Children's Hospital Los Angeles.

An overview of the cytogenetics and molecular classification of sarcomas, with use of Ewing sarcoma, neuroblastoma, and alveolar rhabdomyosarcoma as examples, was presented[31]. Methodologies such as fluorescent in situ hybridization (FISH) can detect specific translocations and oncogene amplifications. In the gastrointestinal stromal tumor (GIST) molecular paradigm, specific exonal mutations in cKIT and PDGFRA result in overactivity of these tyrosine kinases, which can be blocked with the drug imatinib. The dramatic results seen in some patients fuel much of the hope for targeted therapy. Unfortunately, secondary mutations of the KIT activation loop are able to confer resistance to both imatinib and the second-line agent sunitinib.

The Intergroup Rhabdomyosarcoma Study-IV survival data show a poor prognosis for intermediate and high-risk groups in rhabdomyosarcoma[32]. The paradigm for new drug development in rhabdomyosarcoma has utilized the testing of new agents first in xenograft models and then in phase-III clinical trials. Unfortunately, trials in intermediate and high-risk groups have

demonstrated that adding the agent topotecan to conventional backbone therapy (vincristine, actinomycin D, and cyclophosphamide) did not improve outcome. The current controversies include an approach to local control vis-à-vis dosing of radiation therapy and stratification by biology compared with histology, since the presence or absence of the PAX-FKHR translocation seems more important than the traditional histologic classification of embryonal (better prognosis) compared with alveolar rhabdomyosarcoma (worse prognosis)[33]. Pilot studies with the IGF-1R antibody and/or temozolomide that will include gene-fusion and gene-expression analysis are under way.

An update on genome-wide RNA transcriptional profiling of sarcomas, emphasizing the potential for an enhanced understanding of sarcomas by genomic methods, was also presented[34]. As cancer is fundamentally a genetic disease, genetic information about a given sarcoma or group can add fundamental new knowledge about pathogenesis, diagnosis, prognosis, treatment response, and potential for targeted therapy. Genome-wide profiling of DNA, RNA, methylation patterns, regulatory networks, and refinements thereof are likely to be increasingly applied to cancer diagnosis and treatment. The Strategic Partnering to Evaluate Cancer Signatures (SPECS) program has shown by gene expression profiling that forty-eight genes can predict survival as well as the composite of six clinical and histologic covariates in rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, and nonmyogenic sarcoma. Traditional gene-chip arrays utilize short nucleotide probes to analyze expression. Such probes are not sensitive to all of the variants of gene expression that can affect biology, such as splicing variants, mutations, and changes in expression of regulatory RNA. Chips with more and more probes are being used to detect some of these alterations; however, the ultimate solution will be chip-based sequencing of the genome. The SPECS program will integrate three complementary initiatives involving the National Cancer Institute, the Department of Defense, and the North American Children's Oncology Group in an attempt to identify prognostic and diagnostic gene signatures and new therapeutic targets.

Genomic Screening Techniques

The presenters were Paul Meltzer, MD, PhD, of the National Cancer Institute; Jay Wunder, MD, MSc, FRCSC, of Mount Sinai Hospital, Toronto; and Torsten O. Nielsen, MD, PhD, FRCPC, of the Genetic Pathology Evaluation Centre, BC Cancer Agency, Vancouver.

The approach to cancer genetics is dual: (1) identify inherited variants in the genome that increase cancer risk (genetic association) and (2) identify differences between the tumor genome and the normal genome (tumor profiling)[35]. Expression microarray profiling has exploded from 2000 spots in 1996 to 10,000,000 probes in 2006 and is at the point where sequencing technologies may supplant arrays. Two issues still stand in the way—complexity and "the last mile problem," which refers to bridging locally shared information and costs with their widely shared equivalents to span the complexity of the functioning genome, effectively getting beyond gene lists to a true understanding of the interactions among expressed genes and proteins and their ultimate effect on biology. Gene expression, copy number, chromatin modification, sequence modification, DNA methylation, and transcription-factor activity all need to be accounted for. The ecosystem for cancer genomics research, therefore, must include computational scientists as well as bench investigators and clinician scientists.

Genome-wide association studies with use of single nucleotide polymorphisms (SNPs) and copy number variations can identify new sarcoma-related genes. Single nucleotide polymorphism analysis is useful to identify genetic abnormalities with low penetrance. Since sarcomas are rare, their genetic basis could be a rare mutation in a critical gene or one or more mutations of the right combination of genes with low penetrance in the right environmental setting. Tag SNPs are representative SNPs in a region of the genome with a high nonrandom association of alleles at two or more loci (linkage disequilibrium). Single nucleotide polymorphism microarrays are an improvement over comparative genomic hybridization because of the higher resolution and the improved signal-to-noise ratio. Single nucleotide polymorphism arrays simultaneously measure both DNA copy number and allelic ratios. Copy number variations can indicate amplification of oncogenes and loss of tumor-suppressor genes and can confer risk to complex disease states[36]. There are substantive statistical issues and risks of false positives, and this technology only provides information about the linkage of a disease to a particular chromosomal region. One has to analyze this region in more detail to identify the gene(s) associated with the SNP. Efforts at studying sarcomas with this technology have been encouraging[37].

Soft-tissue sarcomas with consistent chromosomal translocations can be distinguished from those with complex karyotypes. Expression data for the former show consistent expression profiles that have yielded important pathophysiologic clues, diagnostic markers, and therapeutic targets[38]. Data were presented for GIST, synovial sarcoma, dermatofibrosarcoma protuberans (DFSP), and pigmented villonodular synovitis (PVNS) and included discussion of colony-stimulating factor 1 (CSF1) in PVNS[39] and TLE, an immunohistochemical marker relatively specific for synovial sarcoma[40]. It was emphasized that public access to primary-expression profile data is critical as it enables external validation. Future directions may include massive parallel sequencing with ChIP (chromatin immunoprecipitation)-sequencing to survey transcription-factor binding across the genome, expression profiling direct from cDNA,

and microRNA profiling[41]. Costs are high for equipment and data processing, analysis is complex, and standards for tissue banking need to be developed so as to not introduce artifacts into the system.

Novel Therapies and Regulatory Issues

The presenters were James H. Doroshow, MD, of the National Cancer Institute; Mathew T. Thomas, MD, of the U.S. Food and Drug Administration, Office of Orphan Products Development; and Spyro Mousses, PhD, of TGen.

Considering the complexity of intracellular signaling pathways, their redundancy, and cross-talk, it is apparent that inhibiting a single target in a complex signaling pathway is unlikely to provide sufficient therapeutic activity for the treatment of most genetically unstable human cancers, which leads one to the conclusion that combination therapies will be necessary[42,43]. The challenges raised by this conclusion include our incomplete understanding of the mechanisms of action of some biologics; our inability to assess the target effect (due to the lack of assays, standardization of assays, imaging tools, and commercially available agents formulated for in vitro use); and the lack of preclinical models to evaluate efficacy, schedule effects, and biomarker utility. Challenges in clinical trials methodology include the need to screen large numbers of patients, the need for tumor biopsies for analysis, the relevance of histologic homogeneity, the great variety of pharmacokinetic interactions, and the growing number of available agents for clinical trial. All of these issues are exacerbated by daunting financial, regulatory, and intellectual property challenges. Nevertheless, in studies utilizing two compounds to target a single pathway, parallel pathways, a single target, or multiple intracellular processes, synergies have been demonstrated. In ovarian and renal cell carcinoma, bevacizumab and sorafenib, which target vascular endothelial growth factor (VEGF) and its receptor, respectively, have demonstrated synergy. The creation of a facile route to the clinical testing of multiple targeted agents in combination is one of the highest strategic priorities of the National Cancer Institute's drug development program. Additional information can found at http://CTEP.cancer.gov.

In order to identify the most effective combination of targets, the Translational Genomics Research Institute (TGen, Phoenix, Arizona) uses the strategy of high-throughput functional genomic screening. The platform utilizes combinations of RNA interference in primary tumor cells or cell lines to identify critical genes and pathways related to cell growth[42,44]. Combinations of agents can then be selected, targeting either these specific genes or pathways in which these genes are a part. When this technique was used on three Ewing sarcoma cell lines, IGF-1, and its receptor, a series of kinases and fibroblast growth-factor receptor 4 were identified as critical for cell growth. Similar experiments can be performed in combination with cytotoxic chemotherapy to identify maximal synergies and genes related to chemoresistance[41]. Validation in primary tumor specimens, data mining, identification of biomarkers that can be used to tailor therapy to the individual patient and tumor and to monitor the response to treatment, and, ultimately, clinical trials are all part of the process. Utilizing functional genomics, it is hoped that this process will be more efficient than empiric trials[45].

Orphan diseases are defined as those diseases with a prevalence of less than 200,000 people in the United States. All of the pediatric and adult bone and soft-tissue sarcomas combined yield less than this number. The U.S. Food and Drug Administration (FDA) Office of Orphan Products Development, formed in response to the Orphan Drug Act of 1983, manages the mechanisms and incentives for the development of drugs and devices to treat orphan diseases. More information can be found at http://www.fda.gov/orphan.

Metastatic Disease

The presenters were Theresa A. Guise, MD, of the University of Virginia Health System; Yibin Kang, PhD, of Princeton University; and David Thomas, PhD, of the Ian Potter Center for Cancer Genomics and Predictive Medicine, Australia.

The interactions between cancer cells and osteoclasts or osteoblasts result in positive feedback loops involving transforming growth factor-beta (TGF-β) (by means of the Smad pathway), interleukin-11, PTHrP with osteoclasts, and endothelin 1 (ET1) with osteoblasts, resulting in either osteolytic or osteoblastic metastases[46,47]. Hypoxia by means of hypoxia-inducible factor-1 alpha (Hif-1α) has a synergistic effect on these signaling pathways and can potentiate tumor growth in bone. The increased understanding of the pathophysiology of metastatic bone disease has resulted in the ability to inhibit these pathways and their downstream effects at multiple points. The most widely used agents are bisphosphonates (the current FDA-approved therapy), but receptor activator of nuclear factor κB ligand (RANKL) antibodies and small molecule inhibitors of TGF-β, histone deacetylase (HDAC), endothelins, and Wnt are all on the horizon for future therapy. These treatments have resulted in measurable decreases in morbidity from metastatic bone disease, but no therapy has been shown to cause regression of established disease.

Paracrine signaling in osteolytic breast cancer metastases to bone can also be mediated by overexpression of matrix metalloproteinase-1 (MMP-1), ADAMTS1 (a disintegrin-like and metalloprotease domain [reprolysin-type] with thrombospondin type-I motifs), and endothelial growth factor-like ligands, which alters the ratio of RANKL/osteoprotegerin, resulting in osteoclastogenesis and osteoclast activity. The identification of pathways resulting in osteoclast activity may provide additional strategies to block osteoclast-mediated bone resorption in metastatic disease[48,49].

Although giant-cell tumor of bone is benign and usually can be treated with curettage, an adjuvant, and either cementation or bone-grafting, the local recurrence rate is 10% to 25%; some tumors are in locations making complete treatment impossible, such as the spine, and 3% of patients develop pulmonary metastases. Similar to metastatic bone disease, the RANK/RANKL pathway mediates osteoclast-like giant-cell formation[50]. Inhibition of this pathway with a humanized antibody against RANKL, denosumab, is undergoing phase-II testing in patients with unresectable or recurrent giant-cell tumors (clinical trials.gov identifier: NCT00396279). Preliminary clinical, histologic, and radiographic results are encouraging.

Tissue Engineering

The presenters were Lisa Capriotti, PhD, of Johns Hopkins University, and Johnny Huard, PhD, of the University of Pittsburgh.

One of the challenges of tumor therapeutics is the restoration of function following surgical resection. This is particularly true for malignant sarcomas of bone and soft tissue. While current methods of reconstruction with use of allograft or endoprosthetic replacement, and sometimes with soft-tissue transfers, are effective and permit limb salvage in the majority of patients, advances in tissue engineering are needed to enhance the function and longevity of these reconstructions.

Tissue engineering is a discipline that aims to restore lost or damaged tissue. In contrast to metallic implants or allograft bone grafts that remain inert, engineered tissue is a living tissue that incorporates with the surrounding host tissues, is alive and responsive to local and systemic signals, and takes on the structure and function of the lost tissues. Three basic components of tissue engineering are a synthetic matrix, cells, and genes or proteins, and they may be used singly or in various combinations. Because musculoskeletal tissues have mechanical function, the matrix needs to have the structural integrity and mechanical properties necessary to withstand the forces normally occurring in the replaced tissue. Other important properties of the matrix are biocompatibility, integration with host tissues, a porous structure that permits the attachment or ingrowth of cells, and kinetics of replacement or dissolution compatible with the transition to host replacement tissues. The biocompatibility and the interactions with the cellular components can be modified or enhanced with surface modifications that include the addition of matrix proteins. For example, the combination of hydrogel and stem cells may be effective for cartilage regeneration[51].

One particular type of stem cell is the muscle-derived stem cell that shows the tremendous potential of cellular therapies to facilitate tissue repair and regeneration. Muscle-derived stem cells behave in a manner compatible with multipotent stem cells, including a sustained ability to proliferate, enhanced rates of self-renewal, resistance to stress, and the ability to differentiate down multiple cell lineages. Muscle-derived stem cells can undergo differentiation into cartilage, bone, endothelial and neural tissues, and cardiac and skeletal muscle[52]. Interestingly, recent work has shown that muscle-derived stem cells are likely perivascular cells that are part of the vascular network present in all tissues[53]. One concern with any tissue-engineering approach that involves ex vivo culture of cells or growth-factor stimulation is malignant transformation, and the conversion of normal stem cells to cancer stem cells is an area yet to be studied.

The final aspect of tissue engineering involves the addition of genes or proteins. Genes and proteins provide signals that can stimulate cells to undergo proliferation and differentiation. The use of BMP-2 or osteogenic protein-1 to stimulate bone formation in nonunions is an example of this approach. Advances in developmental biology have identified additional genes and proteins involved in skeletal tissue formation. Other factors that play a role in bone, joint, and soft-tissue development include members of the TGF-β family (including the BMPs and the growth differentiation factors), the fibroblast growth factors, the hedgehogs, Wnts, parathyroid hormone-related protein, the insulin-like growth factors, and VEGF. One of the concerns with the use of growth factors and/or genes in patients with sarcoma is the possibility that these factors could increase the risk of recurrence. The concern arises as most of these factors have the ability to stimulate cells to undergo combinations of proliferation and differentiation, and thus one or more of these factors could increase the risk that nascent tumor cells (cancer stem cells) remaining in the surgical site could become activated and form a new tumor mass. Therefore, the safety of growth factors in engineering bone or soft tissues in the setting of cancer needs to be established.

A final concept of tissue engineering involves the development of a composite tissue. To date, this has been an elusive goal. A composite tissue would involve a tissue-engineered substrate that includes bone, cartilage, tendon, muscle, vessels, nerve, and skin, with all of the transitions and attachments. Although we are still at a stage where the engineering of single tissues has been challenging, it is envisioned that these composite tissue approaches someday will be feasible and will markedly reduce the morbidity of cancer and other conditions that result in loss of musculoskeletal tissues.

Recommendations

The rarity of sarcoma is both an opportunity and a barrier to advancement. The relatively small number of patients affected and the physicians involved with their care should make collaboration, participation in clinical trials, a focused effort, and availability of tissue specimens feasible. The small size of the patient population creates a limitation with the accrual of enough patients to generate statistically meaningful results in a timely manner. Relevant cell lines and animal models can

help to expedite the development of targeted therapies. The number of physicians and scientists is proportionately small relative to the patient population, but one can ask if there is a critical mass of established and new investigators to move the field forward. The Molecular Biology and Therapeutics in Musculoskeletal Oncology Research Symposium had six breakout sessions charged with addressing these concerns. The common theme for primary sarcoma was first to ensure adequate personnel to do the work. This can be accomplished by supporting early investigators through foundation grants to help to cultivate individuals who will be competitive for National Institutes of Health funding and capable of conducting the necessary work, which involves collaboration with clinical oncology societies, pharmaceutical companies, and academia. The generation of foundation support requires working with patient advocacy groups and private foundations that share a commitment to this initiative. Advocacy at the oncologic organizational and national level is necessary to help to ensure that adequate resources are allocated to sarcoma research. Ultimately, extramural funding targeted to sarcoma may be necessary to develop and sustain the critical mass of individuals and associated infrastructure to crystallize the field. Periodic meetings similar to this symposium would also facilitate these initiatives.

Those entering the field of orthopaedic oncology should strongly consider spending one or more years in basic-science training and seeking a mentored early faculty position. Clinician scientists devoted to sarcoma research will be a critical part of the workforce.

An understanding of the molecular basis of sarcoma and its response to treatment is critical. Our knowledge about sarcoma with consistent translocations is rapidly advancing; however, our knowledge about nonmyogenic soft-tissue sarcoma and chondrosarcoma seems to be particularly lacking. Future research should focus on basic biology linked to annotated tissue banks to identify therapeutic targets and pathways. The validation of cell culture and animal models in primary tumor tissue and the identification of molecular targets for tumors of individual patients will increase in importance. Annotated tissue banks are expensive, labor-intensive undertakings, but they are a critical resource for molecular oncology research. They have proven their value in the osteosarcoma research undertaken by the North American Children's Oncology Group. The single most important component to a sarcoma research strategy will be annotated tissue banks[54]. The procurement of tumor tissue from adult patients has lagged behind that from pediatric patients. This requires the development of tissue-procurement protocols and the requirement for clinical trials to include a biologic component that includes work on primary tissue. Operational issues that must be addressed include the ethics of mandatory tissue procurement, expense, institutional rules regarding who controls the tissue and determines the point in the process when the tissue goes into the tumor bank, and the role of open compared with needle biopsy to ensure an adequate amount of tissue. The criteria for being a cancer center could include participation in tissue procurement. Collaboration among physicians running clinical trials, surgeons and pathologists, and third-party tissue banks or a national tissue repository, as well as an adequate supply of basic scientists, will be necessary. Financing, input on trial design, and academic credit are all important ingredients in making these collaborations sustainable. Statements by funding agencies, patient advocacy groups, and clinical oncology societies about the importance of annotated tissue banks and tissue procurement may help to streamline the institutional review board review process and increase participation. Finally, communication with patients, clinicians, and researchers about tissue banking is necessary to ensure that this resource is developed and utilized.

Appendix

eA A list of all symposium participants is available with the electronic versions of this article, on our web site at jbjs.org (go to the article citation and click on "Supplementary Material") and on our quarterly CD/DVD (call our subscription department, at 781-449-9780, to order the CD or DVD).

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