Cancer is a disease of marked genetic instability with osteosarcoma as one of its most extreme forms. Osteosarcoma arises predominantly in children during the period of rapid skeletal growth. It is a rare cancer, occurring in about 600 humans per year in the US. The rarity of osteosarcoma makes a study of its sarcomagenesis and testing for new treatments challenging as there are just not enough patients to recruit to clinical trials. To complicate matters, the initial diagnosis and chemotherapy may be started before a patient is referred to a tertiary care center. This means that tissues available for research have already been exposed to a number of clinical manipulations, so interpretation of what is peculiar to osteosarcoma and what may be a consequence of chemotherapy and surgery is challenging. Two US organizations are making efforts to implement strong collaborations between clinical centers in the US and Europe, so that archives of clinical data and tissues to support cell and molecular biology studies can be established. The Children’s Oncology Group, COG, (http://www.childrensoncologygroup.org) sponsors the study and care of children with osteosarcoma while the Connective Tissue Oncology Society, CTOS, (www.ctos.org) sponsors the study and care of osteosarcoma in adults, predominantly sufferers of Paget’s disease.

Unlike carcinoma studies where normal cells have been genetically modified to study the cancers originating in those tissues, osteosarcoma cells have been used to understand normal bone biology. Since the introduction of the original osteosarcoma osteoblast-like cells, UMR 106 and ROS 17/2.8, as models for osteoblast biology nearly 30 years ago, there has been little cross-talk between those scientists who study bone biology and those who study osteosarcomagenesis. The availability of new genomic and imaging technologies and of new drugs for bone diseases such as osteoporosis, in addition to the plethora of new compounds to treat cancer, offer a rich opportunity for breakthrough discoveries that will benefit both fields.

The goals of this workshop are to provide a brief primer on the current state of the art and knowledge of osteosarcoma, hoping that this will facilitate new insights and foster new collaborations between the two scientific disciplines for bone biology and osteosarcoma.

Despite the significant contribution of osteosarcoma research to a general understanding of cancers, basic research on sarcomas has not been well funded, limiting interest and progress. However, interest in acquiring more insight into sarcomagenesis as a model for more common cancers was stimulated recently by the introduction of a new drug, Gleevec (imatinib mesylate, formerly known as STI571) for the treatment of patients with Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML). When tested in other sarcomas, specifically the rare gastrointestinal stromal cell tumor (GIST), the compound showed efficacy against specific mutations of the tyrosine growth factor receptors, c-kit, generating the broad hypothesis that efficacy of treatments for sarcomas would be improved by targeting their aberrant proteins and/or specific genetic mutations. It also stimulated an initiative to define sarcomas by their genetic abnormalities, using gene microarrays, to complement conventional tissue-based pathology staging, and facilitate development of more specific drugs.

Within this context, osteosarcoma presents a unique challenge as it represents the extreme member of a group of sarcomas in which multiple chromosomal abnormalities occur on many of the chromosomes. There are deletions as well as replicates of part or whole chromosomes and multiple areas of recombination within and between chromosomes. While it is widely recognized that many of the current models of osteosarcoma osteoblast-like cells synthesize greater quantities of many proteins and in different proportions compared to primary osteoblasts, the significance of this in terms of multiple or aberrant gene copies within each cell has rarely been considered.

Our first speaker is Richard Gorlick, MD, Sloane-Kettering Memorial Cancer Center, New York, one of the leading centers for studies of osteosarcoma in humans. He
will give us a perspective on the clinical aspects of osteosarcoma, the indications and limitations of the current chemotherapeutic agents, what is known of the biology of osteosarcoma and the need for more research to help prioritize the many newly available cancer drugs. In the past, clinical and basic research on osteosarcoma led to dramatic breakthroughs in patient care and insights into cancer. Prior to 1972, all patients with osteosarcoma died within 2-5 years of diagnosis. Doctors treating osteosarcoma established the first stringent controlled clinical cancer trial to test if a regimen, which included chemotherapeutic agents as adjuvant therapy, would improve survival. Surgeons successfully tested if limb-sparing surgery could replace amputation of affected sites, without increasing the likelihood of recurrence of sarcoma at the site. These two advances improved survival to 70%. The remaining cases encompass metastatic disease, primarily to the lungs and survival for more than 2-5 years is unlikely. Dr. Gorlick will review concepts and ideas derived from the various clinical trials to improve osteosarcoma treatment regimens, and discuss his perceptions of what research will be needed to predict and improve survival.

In the early 1900s, the risk of osteosarcoma in humans was increased by the widespread use of radio-isotopes, from workers using radium to paint the dials of a watch, and occasionally licking their paintbrushes to the use of radium to treat certain medical conditions. Much of the early research on bone was driven by a need to understand the risk such compounds posed to humans. One of the great research centers for this work was at the University of Utah, under the leadership of Webster Jee and Scott Miller. Their group developed the beagle dogs in the 1950s as a valid model in which to study the long-term effects of bone seeking radionuclides in skeletal biology. We are fortunate to have Scott Miller, Division of Radiobiology, University of Utah, a key investigator in these long-term studies, to present his perspective on the field. Basic research advances in understanding of DNA repair mechanisms and identification of the genes that are likely to mutate in response to irradiation have still to be integrated into our current concepts of the skeletal responses to bone-seeking radionuclides. Dr. Miller will review the radioisotopes that target the skeleton and explain the biology that underlies the various skeletal responses.

The next speaker, Dr. Marc Hansen from the University of Connecticut Health Center, will begin to link the clinical studies to the genetics and genes associated with osteosarcoma. In the past, studies on genes identified in osteosarcoma had a broad application to other cancers. The identification of p53 and Rb genes in human conditions at higher risk of osteosarcoma led to break throughs in understanding oncogenesis and how mutations or loss of these two genes perturb regulation of the cell cycle and orderly apoptosis in nearly every cancer. Some of the first studies to identify viral oncogenes that were the counterpart of cellular proto-oncogenes, and elucidate the key role they play in cancer, identified the fos oncogene as the transforming factor of the FBJ virus that caused osteosarcoma in animals. Dr. Hansen has been one of the pioneers in applying and developing microarray and bioinformatics technology to the study of osteosarcoma genetics. He will bring us up-to-date on how more recently discovered bone cytokines and genes that regulate osteoblast differentiation may have roles in osteosarcomagenesis. Osteosarcoma osteoblast-like cell lines were developed for the study of osteoblast differentiation. UMR 106 cells were derived from a clone isolated from an osteosarcoma induced in a rat by radioactive phosphate. ROS 17/2.8 cells were derived from a clone isolated from a spontaneous rat osteosarcoma. The criteria for selection of these cells as models of osteoblast differentiation and function were alkaline phosphatase-positivity, increased cAMP in response to PTH and PGE2, increased osteocalcin in response to 1,25-vitamin D3, and synthesis of a mineralized matrix when cells were implanted into immunodeficient mice. While many of the osteosarcoma osteoblast-like cell lines induce ectopic bone formation in immunodeficient mice, none are metastatic to the lungs suggesting none model the behavior needed to study osteosarcoma. Within an osteosarcoma, there are relatively few metastatic cells; currently, there are no techniques to specifically recognize and isolate those specific cells. In the future, in testing osteosarcoma osteoblast-like cells, we should be more critical in analyzing what biology belongs to osteosarcoma and what represents normal osteoblast physiology.

Aside from the use of bone-seeking radionuclides and radiation exposure of dogs, rats and mice to induce osteosarcoma, there is a dearth of animal models and cell lines to study this cancer. Dogs, especially large dogs, are 10 times more likely to suffer from osteosarcoma than humans, and all die within 2-5 years of treatment. Unlike humans, the onset of most dog osteosarcoma is usually not until middle age, but the response of dogs to treatment appears to closely mimic that of humans. As pets, the dogs are treated in veterinary clinics and interventions can be tested as they are in human clinical trials. Because of this, clinical trials on new drugs are often implemented first in dogs as the animal model of choice prior to their use in humans. Although spontaneous osteosarcoma occurs in mice and rats more commonly than in humans, there are no predictable signs that allow for selection of animals at risk. Hormones, such as parathyroid hormone, diethylstilbestrol, estrogen and glucocorticoids, may promote osteosarcoma in susceptible rats, but a near lifetime exposure of 18-24 months is required to increase the incidence from 0.1% to 10-50%. Large numbers of animals are required as we lack the tools to predict which animals will develop metastatic osteosarcoma. Genetically modified mice overexpressing c-fos and mice modified by deletion or inactivation of p53 by the SV40 Large T-antigen promoter transgene containing the promoter for whey acidic protein or amylin, or deletion of Nf2 encoding the protein, merlin, develop osteosarcoma within 5-12 months. The relevance of these single-gene modified models to the far more
genetically complex human and dog spontaneous osteosarcoma remains unknown. The more common animal model for osteosarcoma studies is the use of xenografts in which metastatic osteosarcoma cells are injected intravenously or implanted into the muscle or long bones of immunodeficient mice. Most freshly isolated osteosarcoma cells are not metastatic under these conditions. Cells must be transfected with an oncogene such as K-ras, and then passaged through several successive animal implants, harvesting metastases each time to clone the next generation of cells and repeating the cycle until cells are obtained which rapidly and predictably metastasize to bone. Under these conditions, genetically manipulated cell implants induce metastatic osteosarcoma with metastases in the lung in their immunodeficient mouse hosts within 3 months in 50-80% of mice.

Our last speaker Dr. Patrick Mantyh, University of Minneapolis, has used this type of mouse model to study bone pain in metastases to bone and in osteosarcoma. The symptom that often brings a patient to the clinic prior to the diagnosis of osteosarcoma is bone pain; in bone metastases from breast, prostate and other sites, bone pain is often chronic and intractable. Apart from data on anatomical pathways, we know little of the nerve pathways in bone and less of bone pain. Dr. Mantyh’s work offers us an exciting novel perspective in an aspect of bone research that has been under-studied. His work and the agents he is using to oppose pain offer novel insights not only into pain control, but also fundamental bone biology. This work suggests new uses for bisphosphonates and selective COX-2 inhibitors. To supplement the work that will be presented in the workshop, Dr. Mantyh’s students are exhibiting their data as posters.

In future work, we need to go beyond these models to make full use of the modern technologies at our disposal, and the current continuously changing concepts in the cancer field. The cancer hypothesis that there are progressive genetic breakdown points over time until finally cell cycle controls are breached and the cells are transformed, is challenged by a newer version that requires six essential alterations in cell physiology to occur collectively to permit malignant growth. The latter hypothesis states that there must be self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis and tissue invasion and metastases. It is remarkable that, with the exception of metastases, many of these same criteria are being used to define a regenerative adult stem cell. The dogma until now has been the originating cell in sarcoma is a primitive mesenchymal progenitor cell. In contradiction to this, a recent editorial (Nature Reviews: Cancer 2002 2:401) of an article on neural stem cells and the oncogenesis of glioma posited that appropriate genetic manipulations in a differentiated cell were more likely to induce dedifferentiation and oncogenesis, than were the same manipulations in the parent stem cell. Bone biology has repeatedly demonstrated the ability of cultured primary osteoblasts and bone marrow stromal cells to transdifferentiate and dedifferentiate in vitro, in the presence of specific local cytokine(s). We need to understand if such studies may have relevance to the development of osteosarcoma. In addition to developing a wider variety of metastatic osteosarcoma cells, we need to create technologies that will break down a normal cell to generate the “genome meltdown” that occurs in osteosarcoma. As professionals, we should educate ourselves and consider our societal role in public education and policies relevant to the fears and risks associated with not only new technologies using radio-isotopes that benefit society, but also those that are detrimental to all, such as “dirty radiation” bombs and inappropriate use and disposal of nuclear wastes. As scientists and clinicians deeply committed to improvement in health care through our research, we must continue to develop the hypotheses, data and experimental models that will improve the quality and expectation of life in children and adolescents affected by one of the most extreme forms of cancer.

References


