Osteosarcoma is the most common primary tumor of bone. It accounts for approximately 19% of all malignant tumors of bone. Osteosarcoma affects individuals in the general population at a frequency of approximately 1 in 100,000 with males slightly more commonly affected than females.

**Molecular genetics of osteosarcoma**

Osteosarcoma tumorigenesis has been linked to alterations in several genes. The first association of osteosarcoma with an inherited predisposition was the observation by Kitchin and Ellsworth that patients with bilateral retinoblastoma had an unusually high incidence of osteosarcomas regardless of whether the patient had been treated with radiation. They concluded that as patients with bilateral disease had the inherited form of retinoblastoma that there must be a pleiotropic effect of the gene for retinoblastoma that resulted in an increased predisposition for secondary osteosarcomas. This predisposition was further demonstrated by the observation that osteosarcoma tumors from patients with bilateral retinoblastoma underwent tumor-specific loss of constitutional heterozygosity (LoH) for the same region of chromosome 13 that occurred in the retinoblastoma tumors. This association was confirmed by the identification of the retinoblastoma susceptibility gene (RB1) on human chromosome 13 which permitted several groups to demonstrate that mutations in the RB1 gene occurred in a high percentage of osteosarcomas.

The second gene associated with osteosarcoma was the p53 gene. Li and Fraumeni had identified a familial cancer syndrome based on rhabdomyosarcoma and associated with breast cancer and other neoplasms. Osteosarcoma was later confirmed as a part of the constellation of tumors within the Li-Fraumeni Syndrome. Mutations in the p53 gene were first observed in sporadic osteosarcoma, and then followed by the discovery of germline mutations in Li-Fraumeni Syndrome families. Subsequently it was shown that p53 is commonly mutated in sporadic osteosarcoma as well.

Both RB1 and p53 are considered prototypic tumor suppressor genes in that complete loss of function is required before tumorigenesis can occur. Both genes are involved in cell cycle regulation. The RB1 gene functions as a tumor suppressor by acting as the major regulator of the G1 to S phase progression in the cell cycle. The RB1 protein accomplishes this by binding to and suppressing the function of the E2F transcription factor. The ability of the RB1 protein to bind E2F is controlled by phosphorylation, which is mediated primarily by the cyclin D1/CDK4 complex. The p16 protein product of the INK4A gene in turn inhibits CDK4. Thus, RB1 and p16 suppress cell proliferation while cyclin D1 and CDK4 promote proliferation. Several studies have suggested that the gene encoding the p16 protein (CDKN2A) is inactivated in osteosarcomas that lack RB mutations and that the p16-pRb cell-cycle control pathway is deregulated in the majority of osteosarcomas.

p53 also plays a role in cell cycle control by regulating DNA repair. p53 is believed to function as a cell cycle checkpoint after DNA damage following irradiation with cells appearing to enter a sustained arrest in the G2 phase of the cell cycle. p53 also has a critical role in regulating apoptosis. Thus, expression of mutant forms of p53 likely alter cellular resistance to the DNA damage.

As with RB1, mutations in genes that regulate p53 have been identified in osteosarcoma. The MDM2 gene, located on chromosome 12q13 along with CDK4, encodes a protein that binds p53 and blocks the activity of the p53 by directing it to the ubiquitination pathway. Overexpression of MDM2 in osteosarcomas provides an alternative means to disrupt the normal p53 pathway. Another protein involved in this pathway is the p14 product of the INK4A gene, which is transcribed from the same gene producing the p16 protein involved in the RB1 pathway. The p14 protein exerts a protective effect on p53 by binding to the MDM2 gene product. Alterations consistent with inactivation of p14 have been
found in osteosarcoma tumors and cell lines\textsuperscript{39,40}.

Genes other than p53 and RB1 have also been associated with osteosarcoma. High frequencies of allelic loss have been detected at 3q and 18q, suggesting that at least two other tumor suppressor genes important in osteosarcoma may exist\textsuperscript{41-43}. HER2/neu (c-erbB-2) overexpression has been observed in approximately 40\% of cases and has been associated with early pulmonary metastases and decreased survival\textsuperscript{44,45}. Bone morphogenetic proteins are important in the induction of cartilage and bone formation and patterning of skeletal elements\textsuperscript{46}. Expression of bone morphogenetic protein type II receptor was found to correlate with metastasis in osteosarcomas\textsuperscript{47}.

Rothmund-Thomson Syndrome has also been linked to osteosarcoma\textsuperscript{48-52}. Mutations in the RECQL4 gene have been implicated in at least a subset of Rothmund-Thomson Syndrome patients\textsuperscript{51,53}. The RECQL4 gene product has homology to the E. coli DNA helicase RecQ, which has been implicated in double-strand break repair and suppression of illegitimate recombination\textsuperscript{53,54}. DNA helicases function in all processes in which access to single-stranded DNA is required, including DNA replication, DNA repair and recombination, and transcription of RNA. The functions of the RecQ-like genes are unknown; however, a growing body of evidence points to a function in restarting DNA replication after the replication fork has become stalled and in suppressing genetic recombination and in ensuring accurate chromosome segregation\textsuperscript{55-58}.

Paget’s Disease of bone has been linked to osteosarcoma as well. As early as 1889, Sir James Paget observed sarcomas arising in five of his 23 patients with osteitis deformans\textsuperscript{59}. Various reports have placed the incidence of osteosarcoma in Paget’s Disease between 0.7-5\% of Paget’s patients\textsuperscript{60-63}. Osteosarcoma secondary to Paget’s Disease is uncommon in patients with monostotic disease but may occur in up to 10 percent of patients with severe, polyostotic involvement\textsuperscript{64}. Although the incidence of osteosarcoma in Paget’s Disease is relatively low, it contributes significantly to the mortality and morbidity because of the high incidence of Paget’s Disease in the population\textsuperscript{65}; osteosarcoma related to Paget’s Disease account for about 3\% of all osteosarcomas\textsuperscript{66}, 20\% of the patients with osteosarcoma who are older than 40 years of age\textsuperscript{67} and as high as 50\% of the patients with osteosarcoma over the age of 60\textsuperscript{67}. Analysis of LoH in 96 sporadic osteosarcomas identified a putative tumor suppressor locus that mapped to chromosome 18q\textsuperscript{68}. Analysis of osteosarcomas from patients with Paget’s Disease revealed that these tumors also underwent LoH in this region\textsuperscript{69}. This region has also been implicated in predisposition to familial Paget’s Disease of bone\textsuperscript{68,70}, suggesting that the association between Paget’s Disease and osteosarcoma may be due to common underlying genetic origins. Of interest, within this region lies the gene for the Receptor Activator of NF-kB (RANK), one of the major genes involved in regulation of bone remodeling\textsuperscript{70-72}. However, although germline mutations in RANK have been discovered in Familial Expansile Osteolysis\textsuperscript{73,74} and Expansile Skeletal Hyperphosphatasia\textsuperscript{75}, two skeletal hyperplasia syndromes similar to Paget’s Disease of bone, and although osteoclasts in pagetic patients have been shown to be hypersensitive to RANKL suggesting that there may be an alteration in RANK signaling\textsuperscript{76-79}, examination of the TNFRSF11A locus for mutations in both Paget’s patients and osteosarcoma tumor cell lines, has thus far failed to reveal mutations in the coding region of the gene\textsuperscript{80,81}.

Other genetic questions in osteosarcoma

Osteosarcoma is a highly variable disease which likely reflects the cell of origin of the tumor in the mesenchymal-osteoblastic lineage. Osteosarcomas have traditionally been divided into two broad groups based on cell morphology: conventional histology comprising the osteoblastic, chondroblastic, fibroblastic and small cell forms of osteosarcoma and the atypical histology osteosarcoma including parosteal, periosteal, telangiectatic, high-grade surface, giant cell and well-differentiated intraosseus osteosarcomas\textsuperscript{82,83}. The molecular genetic bases of these variations in histology have yet to be systematically explored.

The age of onset of osteosarcoma can be divided into three distinct peaks. The first peak is from 10 to 25 years of age with a peak occurrence between ages 10 and 18 coinciding with active skeletal growth during the post-pubescent growth spurt and primarily occurs at appendicular skeletal locations with the majority of tumors occurring at the distal femur/proximal tibia\textsuperscript{84}. The second peak occurs between 30 and 40 years old and affects primarily the head and neck, most commonly the mandible\textsuperscript{89-101}. The third peak takes place after the sixth decade of life and primarily affects the axial skeleton and is almost exclusively related to Paget’s disease of bone.

Six to 13\% of osteosarcomas occur in the head and neck with the most common site being the mandible, followed by the maxilla and the other bones of the skull\textsuperscript{101,102}. Based on degree of cellular atypia, frequency of local versus distant metastases, time until metastases, and median age of onset, there is strong evidence that osteosarcoma of the long bones and osteosarcomas of the head and neck represent separate diseases\textsuperscript{103,104,105,106,107}. One of the possible reasons for this difference in phenotype may be that the bones of the head and neck undergo a different program of development from those of the long bones of the skeleton\textsuperscript{107}. In the precursors of the bones of the head and neck (the calvaria of the skull, the maxilla and the mandible), mesenchymal cells differentiate directly into osteoblasts in areas of membranous ossification in a process known as intramembranous ossification. In the remaining portions of the skeleton, mesenchymal cells differentiate into chondrocytes which secrete the characteristic extracellular matrix of hyaline cartilage. Cartilage models of bones (anlagen) are formed and subsequently replaced by bone in a process called endochondral ossification. Examination of the process of endochondral and intramembranous ossification has demonstrated that there are genes...
which are expressed in common to both pathways as well as genes that appear to be unique to each of the pathways. Telomere maintenance is regarded as a key mechanism in overcoming cellular senescence in tumor cells and is frequently achieved by the activation of telomerase. However, there is an alternative mechanism of telomere lengthening which is characterized by an absence of telomerase activity. In osteosarcomas, the majority of tumors appear to maintain their telomeres through this ALT pathway. This is interesting in light of the discovery that osteosarcomas, unlike most childhood tumors, show a significant degree of aneuploidy. Osteosarcoma tumor karyotypes range from near-diploid to near-hexaploid with many specimens showing multiple clones with different degrees of ploidy. The underlying basis for this variation is also unclear at present.

Conclusion - the future of osteosarcoma research

Thus the genetics of osteosarcoma has many opportunities for new discoveries. Microarray analysis, comparative genome hybridization, spectral karyotyping, mass spectroscopy proteomics and transgenic mouse models all hold promise for new discoveries in the molecular genetics of this fascinating disease. All could lead to significant expansions of biomedical research horizons in this understudied disease, precipitate a paradigm shift in research, and lead to substantial improvements in the treatment of this serious disease.

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