Osteosarcoma: Clinical practice and the expanding role of biology

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Introduction

Before the identification of effective chemotherapy, amputation of an affected extremity led to less than 20% of patients with apparently localized osteosarcoma being cured. Metastases almost invariably appeared in the lungs. The introduction of effective chemotherapy in the 1960s immediately led to improvements in clinical outcome. These improvements were confirmed by randomized trials in which adjuvant chemotherapy was compared to no chemotherapy. Such rigorous testing to definitively prove the value of adjuvant chemotherapy, has rarely been conducted in oncology. Over the next several decades, advances were made in the treatment regimens. Induction chemotherapy prior to definitive surgery (neo-adjuvant) and combined with adjuvant therapy became standard. Amputation has mostly been replaced by limb salvage techniques as the treatment of choice. Currently, approximately 60 to 80% of patients presenting with localized osteosarcoma will go on to long-term disease free survival.

Despite these dramatic advances, progress is at a plateau. Recent clinical trials, which will be reviewed, attempting to improve outcome through intensification of therapy or incorporation of novel agents have generally not been successful. This is particularly apparent for the 20% of osteosarcoma patients who present with metastatic disease. Long-term disease free survival for this group of patients generally remains less than 20%.

Increasing focus has been placed on obtaining a greater understanding of the basic biology of osteosarcoma, with the goal of using that information to improve treatment. Translational laboratory research has focused on the clinical goals of identifying biological alterations that can serve as prognostic factors and therapeutic targets. Prognostic factors can be used to develop modifications in risk-based therapy, to improve the curability of “high risk” patients and minimize toxicity to the “good risk” patients. New agents with activity in the treatment of osteosarcoma needing to be identified as escalating the dose of the agents of proven activity have failed to change patient outcome. The large number of new drugs under development must be prioritized as realistically few can be tested in human osteosarcoma clinical trials, given the rarity of the disease. Therapeutic targets need to be critically evaluated to prioritize trials of drugs already in testing and to guide new drug development.

Molecular genetic alterations as prognostic factors

There are two major classes of sarcomas: [1] sarcomas with balanced karyotypes (reciprocal translocations) and alterations of tumor suppressor gene pathways which occur in relatively few cases, such as Ewing Sarcoma and [2] sarcomas, such as osteosarcoma, with complex unbalanced karyotypes and alterations of p53 and the retinoblastoma pathways in many cases. Alterations in the retinoblastoma and the p53 tumor suppressor pathways have a clear role in the pathogenesis of osteosarcoma. Combined inactivation of the two pathways is nearly universal. Patients with germ-line p53 mutations (Li-Fraumeni syndrome) or retinoblastoma alterations (hereditary retinoblastoma) have an osteosarcoma predisposition. The major functions of these tumor suppressor genes are: modulation of cell cycle arrest at G1/S and induction of apoptosis. In cases in which retinoblastoma and p53 are wild-type, the pathways are inactivated through a variety of other alterations including amplification of the 12q13 region (containing MDM2 and CDK4) or INK4A deletion.

Osteosarcoma typically has complex unbalanced karyotypes. The cytogenetic abnormalities are both numerical and structural. As examples, common numerical abnormalities in osteosarcoma include: gain of chromosome 1, loss of...
chromosome 9, 10, 13, and/or 17, and partial or complete loss of long arm of chromosome 6. Karotypic complexity may reflect chromosomal fusion-bridge-breakage cycles. These occur due to advanced telomere erosion and implicate telomerase activation as a necessary step in pathogenesis.

Numerous other oncogenes and tumor suppressor genes are altered in osteosarcoma tumor samples. Alterations in many of these oncogenes and tumor suppressor genes have been investigated for their potential usefulness as prognostic markers in osteosarcoma. These efforts have been hindered by the fact that the timing, sequence and relative importance of each of these alterations are unclear. It is also not known if the changes are indicative of a primary or later event, or which stage determines a tumor’s clinical behavior. Because these tumors almost universally show evidence of different types of genetic alterations inactivating the retinoblastoma and p53 tumor suppressor pathways, individual alterations may be prognostic. Recent data suggest low prognostic significance of p53 mutations in sporadic osteosarcoma. Some alterations, such as overrepresentation of chromosome 12p are more frequent in low-grade as compared to high-grade tumors, suggesting this may represent a progressive pathway to aggressive osteosarcoma. However, high-grade tumors do not evolve from low-grade lesions. A clearer model of osteosarcoma pathogenesis would assist in determining which genetic alterations should be evaluated for value as potential prognostic factors.

Drug resistance genes as prognostic factor

As chemotherapy has had a tremendous impact on the outcome for osteosarcoma patients, determinants of chemotherapy response may serve as potential prognostic factors. One of the most important known predictors of outcome is the histologic response of tumors to preoperative chemotherapy. Because drug response/resistance is complicated and multifactorial, it is unclear which aspects are critical determinants. Factors which may influence drug response include extracellular and intracellular processes. Extracellular influences include drug delivery dependent on vascular supply and interstitial pressure, hypoxia and hormonal milieu among others. Intracellular factors include influx of drug, efflux of drug, intracellular metabolism and catabolism, interaction of drug with its target, alterations in the drug target and DNA damage repair and response pathways.

One factor extensively studied as a potential prognostic factor in osteosarcoma is p-glycoprotein expression. P-glycoprotein is a transmembrane ATP-dependent protein encoded by the multi-drug resistance (MDR1) gene. This protein, when expressed, can result in the efflux of numerous chemotherapeutic agents, including doxorubicin, rendering them ineffective. As doxorubicin is the single most effective agent in the treatment of osteosarcoma, rendering this drug ineffective would be expected to negatively influence prognosis. In a very influential study, expression of p-glycoprotein by immunohistochemistry was associated with a decreased probability of patient event free survival. Since that time, other studies, which will be reviewed, have produced supportive and conflicting results. Thus far, none of these factors has emerged as a reliable prognostic factor in osteosarcoma, suggesting that the critical determinants of drug response have still to be identified. Many additional chemotherapy resistance/response factors are being actively evaluated as potential prognostic factors in osteosarcoma; the status of those studies will be reviewed.

Drug resistance as a therapeutic target

Identifying determinants of chemotherapy response/resistance in osteosarcoma may identify new therapeutic agents or treatment strategies. High dose methotrexate is a standard component of osteosarcoma therapy. The efficacy of methotrexate can be limited by the presence of intrinsic and/or acquired resistance. In experimental systems, mechanisms of resistance to methotrexate include decreased drug uptake, alterations in the drug's target dihydrofolate reductase through amplification or mutation, or decreased methotrexate retention secondary to impaired polyglutamylation. Numerous studies, which will be reviewed in my talk, have identified impaired transport of methotrexate into cells secondary to alterations in its carrier, the reduced folate carrier, as the major mechanism of resistance in osteosarcoma. Trimetrexate, another folate antagonist, is effective against transport defective cells as this agent does not utilize the reduced folate carrier for cell entry. In addition, transport defective methotrexate-resistant cells are poorly rescued in vitro by leucovorin, a reduced folate which bypasses the enzyme inhibition but depends on the reduced folate carrier for cell entry. Administration of trimetrexate with simultaneous leucovorin may therefore widen the therapeutic index, which has been further tested in pre-clinical models and clinical trials. It is hoped that the use of this and other strategies targeted specifically to the molecular alterations observed in osteosarcoma will improve patient outcome.

Conclusion

Enhanced understanding of biology will change how osteosarcoma patients are treated, hopefully leading to an improvement in outcome beyond that currently achieved with standard therapy.

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