A Review of Osteosarcoma Therapeutics

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ABSTRACT

Osteosarcoma is a rare but deadly cancer, predominantly affecting both adolescent and young adult populations. Osteosarcoma occurs when an aggressive malignant neoplasm arises from transformed cells of mesenchymal origin, which eventually produce a malignancy in the osteoid. Diagnosis of osteosarcoma typically results from symptoms of pain or swelling in the bone, which can be confirmed through laboratory testing of alkaline phosphatase and lactate dehydrogenase levels as well the detection of microscopic and macroscopic lesions. Pathogenesis of osteosarcoma is caused by a diverse set of factors including physical agents, radiation, chromosomal aberrations and viral infection which dysregulate cellular functions. Current research focuses on understanding how microRNAs play a role in osteosarcoma and other aggressive cancers. In this review, we discuss current treatments options including chemoresistant strategies and immunotherapies that show promise at combating osteosarcoma and other cancers.

Introduction

Osteosarcoma is a low incidence or uncommon cancer, which originates in the bones and is predominantly found in adolescents and young adults. Osteosarcoma usually occurs in individuals in the range of 10 to 30 years of age, although teens are the most commonly affected. Each year, 1,000 cases of osteosarcoma are diagnosed in the United States, and 45% of those cases occur in children and adolescents. Approximately seventy percent of patients with non-metastatic osteosarcoma can survive long-term with multidrug chemotherapy. However, patients with metastatic osteosarcoma rapidly develop lesions and become resistant to chemotherapy. The development of secondary tumors in these patients is a common cause of morbidity. New therapies for metastatic osteosarcoma are needed to help prevent morbidity in these patients. Below we review current mechanisms of pathogenesis, diagnosis, and treatment strategies including chemoresistant therapies, immunotherapies, and microRNA derived techniques.

Mechanisms of Pathogenesis

Numerous factors have been associated with osteosarcoma pathogenesis including age, gender, environmental agents, genetic background, and viral infection. Rapidly growing bones, especially during puberty, are an easy site for osteosarcoma tumorigenesis. Physical agents, ultraviolet light, and ionizing radiation are agents known to cause osteosarcoma in 2% of all cases but have not been demonstrated to have a large effect in pediatric cases.
Methylcholanthrene, chromium salts, beryllium oxide, zinc beryllium silicate, asbestos, and aniline dyes are chemical agents known to cause osteosarcoma. Chromium salts, with or without methylcholanthrene treatment, can transform non-tumorigenic osteoblast-like human osteosarcoma cells. Although the chromium salts alone were highly toxic to the cells, the cells that survived had a marked increase in anchorage independent growth when compared to controls. Cells treated with chromium salts and methylcholanthrene together had an even larger rate of anchorage independent growth. The cells themselves were not tumorigenic when tested in nude mice, but had altered phenotypes that demonstrated hallmarks of a stage in the carcinogenesis cascade. Beryllium oxide and calcined phosphor were shown to induce osteosarcoma as well as other neoplastic growths in a rabbit model. Rabbits were injected three times a week for six to eight weeks. Osteosarcomas developed in 6 of the 9 animals that lived over 1 year and the first tumor appeared 11.5 months after the start of the experiment. The data demonstrate that beryllium compounds not only induced osteosarcoma, but also metastatic tumors of the lung, liver, spleen, kidney, heart, and urinary tract. Earlier studies support this data demonstrating that injection of beryllium compounds induced osteosarcoma in the epiphysis, tibial, scapula, and femoral in mammals such as guinea pigs and rabbits.

Genetic changes such as chromosomal abnormalities or mutations in tumor suppressor genes and proto-oncogenes can also contribute to the onset of osteosarcoma. Chromosomal abnormalities in patients with Bloom syndrome, Werner syndrome, Li-Fraumeni syndrome, and hereditary retinoblastoma often have a higher risk of developing multiple malignancies such as osteosarcoma. Osteosarcoma has also been linked to amplification of chromosomes 6p21, 8q24, an 12q14, loss of heterozygous chromosome a 10q21.1, and changes in chromosomes 9, 10, 13, and 17.

The mutated forms of tumor suppressor genes p53 and retinoblastoma (Rb) lose their functions and are associated with various cancers. p53 and Rb genes are known to repair DNA damage or induce cell apoptosis. If they become mutated, these protective functions are compromised and can allow the cell to become neoplastic. Both of these genes have been indicated in the pathogenesis of osteosarcoma. Fifty percent of all cancers have a mutated p53 gene; this gene is also mutated in 22% of osteosarcomas, showing the importance of this gene mutation in cancer’s progression. Rb is important in cell cycle regulation by binding transcription factors of the E2F family until CDK4/cyclin D complex phosphorylation occurs. Mutation in Rb allows for E2Fs to allow the cell cycle to continue without regulation. An individual with Li-Fraumeni syndrome has a 70% chance of developing primary invasive cancer, including osteosarcoma but excluding skin cancer.

Proto-oncogenes such as c-fos, c-jun, myelocytomatosis proto-oncogene protein (c-myc) have been associated with osteosarcoma. Activator protein 1 complex (AP-1) is a heterodimeric complex composed of c-fos and c-jun. AP-1 controls bone metabolism, cell proliferation, and differentiation, whereas c-myc stimulates growth and division in the nucleus. Analysis of primary skeleton neoplasms via immunohistochemistry found c-fos and c-jun expression in bone-forming lesions. Further analysis demonstrated that high-grade osteosarcomas had elevated levels of c-fos and c-jun associating their expression with aggressive human osteosarcoma. Wu et al.’s data demonstrate that c-fos expression is elevated 150% in human osteosarcoma sections when compared to benign or normal tissues. Further supporting the conclusion, that c-fos is involved in the growth and spread of osteosarcoma tumor formation. In another study using osteosarcoma and lung metastases, c-myc and c-fos gene and protein expression was significantly elevated in relapsed tumors and was correlated with metastasis frequency and intensity. Myc overexpression has also been correlated with osteosarcoma pathogenesis and chemotherapeutic resistance. Shimizu et al. demonstrated that overexpression of c-myc in bone marrow stromal cells derived from Ink4a/Arf null mice could generate lethal osteosarcoma cells. In a different murine osteosarcoma study, TAM67 was used to conditionally inhibit AP-1 activity in highly metastatic K7M2 cells. AP-1 inhibition blocked the migration and invasion potential of these cells and increased mouse survival suggesting that AP-1 inhibition could be used a therapeutic tool to prevent invasion, metastasis, and migration of osteosarcoma tumors.

Diagnosis

Patients diagnosed with osteosarcoma will normally present with pain and swelling in the metaphyseal bone of the distal femur, proximal tibia, and proximal humerus; blunt force trauma to those regions have also been noted before diagnosis, although a scientific link to trauma and osteosarcoma is unknown. Pain is typically associated with activity and overtime the pain occurs during restful periods and is also attributed to growing pains in children. In a study involving osteosarcoma symptoms, pain in the knee joint was always the first reported and was more intense when bearing weight and at night. Two-thirds of patients had a limp, and only seven percent of patients had a pathological fracture. Among these patients, the study identified that the mean total delay for diagnosis was 17 weeks.

Noninvasive diagnostics methods have improved detection over the past decades and includes the use
of radiography, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) or a combination of these methods. Radiographs typically detect lesions with defects including osteolytic areas, periosteal reactions, or the development of soft tissue masses. CT scans are utilized at defining fracture sites or irregularities in mineralization, the cortices, or neurovascular development. Often MRIs are employed to assess soft tissue invasion, neurovascular damage, bordering joint damage, or to determine bone marrow replacement needs. Ongoing research is being conducted to determine ways PET can be used to determine metabolic rates of osteosarcoma, the response rate of neoadjuvant therapy and other post treatment changes which is thoroughly reviewed by Brennan et al. Still, biopsy and microscopic examination are required to confirm the diagnosis. These examinations carry additional prognostic implications such as subtype classification and histological response to neoadjuvant chemotherapy. Osteosarcoma subtypes can be divided into many groups including conventional osteosarcoma (subdivided into osteoblastic, chondroblastic or fibroblastic groups), telangiectatic, small-cell, low-grade, parosteal, periosteal, and high-grade surface osteosarcomas. These subtypes are classified based upon their histological appearances.

No official laboratory test exists as a diagnosis for OS. However, basic lab tests such as complete blood count, metabolic panels, and other functional tests can be useful pretreatment to assess a patients help before the onset of chemotherapy. Laboratory testing has shown that alkaline phosphatase levels can be elevated in osteosarcoma patients by approximately 40%. When patients had elevated levels of the alkaline phosphatase enzyme in the preoperative stage, their recurrence rate is found to be much higher, and they have a poorer prognosis. Lactate dehydrogenase levels can also be elevated in osteosarcoma patients. In a multi-institutional osteosarcoma study, elevated lactate dehydrogenase (LDH) levels were found to be the most predictive factor for a poorer prognosis. In another study that correlated LDH levels and prognostic value, researchers found that metastatic patients had a significantly higher level of LDH than patients who only had localized osteosarcoma. It is still unclear whether lactate dehydrogenase and alkaline phosphatase should be used as indicators for osteosarcoma, however approximately 80% of patients present with microscopic metastatic disease or have undetectable patterns. Therefore, utilizing LDH and alkaline phosphatase levels as indicators of osteosarcoma maybe useful in early stages or in conjugation with other diagnostic measures.

**Osteosarcoma Treatment Options**

Following clinical presentation and diagnosis, the next step is the treatment to remove and potentially eradicate the tumors. Surgery is commonly the first step in treatment and can include removal of just the tumor or potentially the limb itself. Surgical treatment requires the complete removal of the affected tissue including areas where biopsies occurred, drainage, and other potentially contaminated tissue. Chemotherapy following surgery is the next step to ensure eradication, although chemotherapy can be given before and after in some cases. Early chemotherapeutic agents bleomycin, cyclophosphamide, and actinomycin D were frequently used, but now doxorubicin and methotrexate are now more commonly used. Clinical trials relating to many osteosarcoma treatments have been completed or actively ongoing. A host of chemoresistant strategies, immunotherapies, and microRNA-derived techniques have been developed to help improve patient outcomes and is reviewed below.

**Chemoresistant Strategies**

Gene expression is often altered in osteosarcomas so that the tumor cells can continue to proliferate despite chemotherapeutic treatments. Downregulation of reduced folate carriers (RFCs) are often observed in chemoresistant osteosarcomas. RFCs are located at the cell membrane and are utilized by chemotherapeutics like methotrexate to enter the cytoplasm of the cell. Mutations seen in the RFC protein sequence include Leu291Pro, Ser46Asn, Ser4Pro, and Gly259Trp and prevent chemoresistant treatments from entering tumor cells. These mutations alter the structure of the enzyme so that drugs like methotrexate cannot associate with the protein and enter the cell membrane. An alternative to methotrexate is trimetrexate, a drug that has a similar chemotherapeutic function, but does not utilize RFC to enter the cell. Limited clinical studies exist using trimetrexate but transport defective tumor cells are sometimes more sensitive to this drug. Therefore, trimetrexate is a potential candidate to overcome the methotrexate transport resistance. Another cellular enzyme that prevents drugs from remaining in tumor cells is the P-glycoprotein pump (P-GP). The transcription factor, MDR1 (multidrug-resistant gene) can upregulate P-GP in chemoresistant tumor cells and promote the removal of chemotherapeutic drugs like doxorubicin from the cytoplasm of the cell. Other genes are often directly upregulated in osteosarcoma tissues to directly combat the presence of chemotherapeutic drugs. Glutathione S-transferase P1 (GTSP1), an enzyme that has detoxifying characteristics, inactivates various types of treatments and is often
overexpressed in osteosarcoma. Osteosarcoma patients with mutant variants of the GSTP1 protein have shown increased resistance to several therapeutic drugs including methotrexate, Adriamycin and cisplatin. To combat GSTP1-based resistance, an inhibitor of GSTP1, NBDHEX was developed. In vitro studies have observed that NBDHEX does not increase apoptosis in tumor cells but does prevent metastatic signaling.

Signaling pathways for cell proliferation and anti-apoptotic factors become overactive in several tumor cells including osteosarcoma. Chemotherapeutic drugs like rapamycin, cisplatin and doxorubicin that have inhibited these pathways in the past are now obsolete. New treatment strategies and pharmaceuticals are being developed to improve these key treatment types. For instance, rapamycin and its analog molecule, cell cycle inhibitor-779, are used in tandem in one treatment that has led to significant inhibition of the mammalian target of rapamycin (mTOR) pathway.

Doxorubicin treatment together with the insulin-like growth factor receptor 1 (IGF-R1) inhibitor, tyrphostin, increases cellular apoptosis much better than doxorubicin treatment on its own. New treatments cediranib and trastuzumab were generated to specifically target vascular endothelial growth factor (VEGF) and human epidermal growth factor receptor 2 (HER2) respectively, resulting in inhibition of tumor growth.

Advances in genetic manipulation within the genome of a lentivirus can generate an abundance of short-hairpin RNA (shRNA), miRNA and cDNA sequences to knockdown specific gene expression, such as IGF-R1, BCL-2 and BCL-xL, that allows for increased sensitivity to doxorubicin and cisplatin. Autophagy is a process that occurs in cells under harsh conditions where the cells’ organelles and proteins are degraded but prevents the cell from undergoing apoptosis. This process is observed frequently in tumor cells and promotes chemo-resistant characteristics, as observed in osteosarcoma cell lines treated with doxorubicin and roscovitine. Several treatments have been developed to push cancerous cells into apoptotic processes from the autophagy state. In several in vitro studies, the use of autophagy inhibitors chloroquine and 3-methoxyadenine (3-MA) treated simultaneously with 5-fluorouracil, paclitaxel (PCX) and cisplatin increased apoptotic events in known autophagy osteosarcoma cells.

Immunotherapies

Chemotherapeutic resistance has led to the development of new strategies in immunotherapeutics. Immunomodulation, adoptive T-cell immunotherapy, vaccines, immunologic checkpoint blockade, oncolytic virotherapy and targeted therapies have been developed. Immunomodulation adjusts the immune system in a way to target the cancer itself. For example, a synthetic lipophilic analogue of muramyl dipeptide, muramyl tripeptide phosphatidylethanolamine (MTP-PE), has been encapsulated into liposomes (L-MTP-PE) as a way for targeted therapy to allow monocytes and macrophages to induce tumoricidal qualities. Tumoricidal qualities help immune cells release factors such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β). The release of these proinflammatory factors promotes the removal of residual micrometastases not eliminated with surgery and chemotherapy. Induction of tumoricidal activity by macrophages induced with L-MTP-PE may be dependent on interferon-γ (IFN-γ), which enhances the liposome intake of the macrophage.

Interferons are pleiotropic cytokines that are involved in antitumor, antiangiogenic, apoptotic, antiviral, and cellular immune responses. Three subtypes of IFN, IFN-α, IFN-β, and IFN-γ, which have direct and indirect activation of T-cells and B-cells. IFN-α has been shown to inhibit osteosarcoma growth and arrest growth of tumors, therefore it is commonly used as the IFN treatment of choice. A small study of three patients with osteosarcoma related pulmonary metastases underwent treatment with human leukocyte interferon. Treatment reduced the tumor size temporarily 6-8 months after administration.

In a phase two clinical trial with 20 patients suffering from high-grade osteosarcomas, IFN-α2a treatment only caused partial tumor regression in three patients. Granulocyte macrophage-colony stimulating factor (GM-CSF) has also been shown to induce differentiation and apoptosis of osteosarcoma cells. In this study, treatment of SaOS-2 human osteosarcoma cell line with GM-CSF daily promoted the differentiation and function of these cells including extracellular matrix mineralization and collagen production. However, fourteen days post GM-CSF treatment, the SaOS-2 cell line was found to have high levels of apoptotic cell death when compared with the controls via flow cytometry. Interleukin-2 (IL-2) has been added to standard treatments to help increase the prognosis of patients with osteosarcoma. IL-2 can stimulate and upregulate T-cells and natural killer (NK) cells to activate lymphocytes to become lymphocyte-activated killer cells (LAK). LAK cells have the ability to target and kill tumor cells. Studies show that IL-2 treatment with complete surgical remission and can prevent recurrence and increase survival rate. This therapy has been shown to significantly increase white blood cells, decrease alkaline phosphatase, but can cause influenza-like symptoms and high fever. Despite these reversible severe side effects, heavily treated pediatric patients have a 50% better prognosis.

Adoptive T-cell therapy (ACT) uses T-lymphocytes exhibiting antitumor activity to mediate responses. Genetic engineering of T-cells, T-cell receptor (TCR)-modified T-cells, chimeric antigen receptor (CAR)-modified T-cells, and NK cells, have shown promise to target tumor cells and
have been extensively reviewed and therefore are briefly summarized below. T-lymphocytes that have been removed from a patient, expanded in vitro, genetically engineered and reintroduced into the patient have been found to be very effective at tumor regression. Genes for TCRs can be cloned into lentiviruses or retroviruses and used for infection of autologous T-cells. Synthetic receptors with extracellular single-chain variable fragments (scFv) derived from monoclonal antibodies, transmembrane domain, and intracellular domain with differentiation clusters known as CARs can also be used as effective tumor treatment. CAR T-cells have been developed against human epidermal growth factor receptor 2 (HER-2) and IL-11 receptor alpha (Ro). For example, a majority of osteosarcoma patients, express low levels of HER2 in their osteosarcoma cells. As a result, HER2 monoclonal antibodies used to treat tumor cells are ineffective. Ahmed et al. demonstrated that genetic modification of T cells for a specific antigen such as HER-2 can cause regression of established osteosarcoma lesions in a metastatic mouse model. Though CAR therapies have had serious side effects including severe respiratory distress and death, NK cells have an important role in tumor surveillance and recognition and can be important to the elimination of tumor cells. Recently, data demonstrated that the reduction of Killer Immunoglobulin-Like Receptor (KIR) receptor-ligand expression in osteosarcoma cells could increase the susceptibility of tumor cells to NK cell mediated. Other studies support this data by demonstrating that disrupting interactions of KIR with their ligands on tumor cells in vivo can elicit antitumor response. Potential treatments of the future might include inducing solidarity of tumors or activating NK cells to recognize specific oncogenic features in a similar manner to T-cells, leading to NK destruction of tumors.

Vaccines using tumor-associated factors to induce an antitumor immune response have been in development. Tumor-associated factors include gangliosides, heat shock proteins, autologous dendritic cells, tumor peptides or proteins, and autologous or allogeneic tumor cells. Adjuvants, IL-2 and GM-CSF, or other immunostimulants can be used in the vaccines to enhance the response. Immunologic checkpoint blockades, CTL antigen-4 (CTLA-4) and PD-1, are currently being researched in osteosarcoma immunotherapy. CTLA-4 is an immune regulatory molecule for attenuating antitumor responses downstream of T-cell activation. Ipilimumab, a monoclonal antibody, blocks CTLA-4 to enhance antitumor responses by inhibiting regulatory T-cells immunosuppressive capabilities. PD-1 is part of the CD28 family and is expressed on activated T-cells. When PD-1 and PD-ligand are activated T-cell are stimulated to undergo apoptosis, which contributes to a poor cancer prognosis. Nivolumab is an antibody that blocks PD-1 and can inhibit metastasis, enhance effector T cell function, and increase cytokine production in patients with melanoma, renal-cell cancer, and non-small-cell lung cancer, although its use has been limited in osteosarcoma.

Oncolytic virotherapy is a new treatment approach utilizing replication-competent viruses to selectively infect and damage cancer tissue without the harm to normal tissue, including osteosarcoma. Adenoviruses are double stranded DNA (dsDNA) viruses associated with mild respiratory infections, alimentary and conjunctiva infections. Adenoviruses infect cells using receptor-mediated endocytoses, releasing early genes to begin transcription, with these genes binding to Rb and p53 proteins. Attenuated adenovirus mutants have been shown to be capable of lysing p53-deficient tumor cells but not cells expressing functional tumor suppressor protein p53. Injection of this adenovirus into human cervical carcinomas in a nude mouse model was capable of reducing tumor size. A phase 1 study using Onyx-15, an adenovirus that targeted p53-deficient cells was well tolerated by patients with head and neck cancer. Five of the 22 patients were found to have some response to therapy and as a result further investigations were necessary. Another possible oncolytic virus utilized herpes simplex viruses (HSV). HSVs are neurotropic dsDNA viruses with two serotypes, HSV-1 and HSV-2, which infects the mucosa of the mouth, eyes, and anogenital tract. HSV natural infection causes the host to halt protein synthesis, therefore stopping HSV protein synthesis. The primary neuropathogenicity gene in HSV is γ-34.5 with its protein causing dephosphorylation of eIF-2 thereby removing the inhibition of protein synthesis. Ras signaling pathway is commonly mutated in cancer, which suppresses the protein kinase (PKR) that inhibits γ-34.5 thereby, allowing HSV to replicate in cancer cells. ICP-6 is a subunit of ribonucleotide reductase essential in DNA viral replication and is highly expressed in cancer cells. HSV ICP-6 is mutated causing it to only be able to divide in cancer cells and not normal cells. Therefore, a vaccine with HSV having ICP-6/γ-34.5 deletion may allow it to specifically target cancer cells.

MicroRNAs

Small non-coding RNA sequences are transcribed from the genomes of animal and plant cells as well as the genetic material of some viral families. Although they do not generate proteins of their own, some of these non-coding sequences are able to regulate protein expression. These sequences are known as microRNA (miRNA). Once transcribed, the pre-miRNA is modified by RNase III enzyme Drosha that forms secondary structures with the RNA sequence before entering the cytoplasm. The double stranded miRNA is then recognized by the RNase III endonuclease known as Dicer, which breaks the secondary
structures creating miRNA strands that have the capability of silencing gene expression\(^9\). The mature miRNA can either bind directly to a complementary messenger RNA (mRNA) or enter the active site of a ribosome, bind to and prevent movement of the complementary miRNA within. Both mechanisms inhibit protein synthesis of the mRNA and limit gene expression\(^8\). This process is a normal occurrence in healthy cells, miRNAs regulate the expression of necessary enzymes and transcription factors that if overexpressed may influence cellular issues. In fact, the study of miRNA over the past two decades has created hypotheses that the development of cancer may be due to irregular expression of miRNA in equal proportion to sporadic mutations of the particular genes\(^9\).

Several miRNAs have been observed specifically as chemo-resistant and radiation resistant factors in treating osteosarcoma by targeting tumor suppressor genes that allow apoptosis of the cell, inhibit cell proliferation and migration from the tissue\(^5\). Human apurinic/apyrimidinic (AP) endonuclease APE1 is an endonuclease responsible for repairing DNA damage after exposure to radiation treatment. miRNA-513a-5p is generated to maintain levels of APE1 expression. Osteosarcoma and other aggressive cancers have overexpression of APE1 producing unregulated repair of oncogenic genes as well as downregulation of the miRNA-513a-5p\(^2\). Reintroducing miRNA-513a-5p into osteosarcoma cell lines that have reduced expression of APE1 caused cells to undergo apoptosis after DNA damage to radiotherapy\(^8\). Natural upregulation of miRNA-224 inhibits Ras-related C3 botulinum toxin substrate 1 (Rac1), a GTPase that when overexpressed in tumor cells is involved in cell proliferation and metastasis. However, overexpression of miRNA-224 creates a negative feedback that allows Rac1 to inhibit the sequences’ production and allow Rac1 to perform its function unhindered by cisplatin treatment\(^4\). Osteosarcoma cell lines can undergo successful apoptosis if treated with exogenous miRNA-224 and cisplatin simultaneously\(^4\). Expression of miRNA-138 as well increases tumor sensitivity to cisplatin treatment. EZH2, an inhibitor of the caspase-3 enzyme, is inhibited by miRNA-138, which allows the cell to enter an apoptotic state after chemotherapy treatment\(^9\).

Some miRNA sequences are known to increase chemoresistance of osteosarcoma cell lines. Upregulation of miRNA-21 impedes sprouty homolog 1 (Spry1) and sprouty homolog 2 (Spry2) gene production, important inhibitors of tyrosine kinase receptor signaling from certain growth factors\(^6\). Spry1 and Spry2 function are a major part of cisplatin therapy to reduce tumor characteristics, therefore upregulation of miRNA-21 increases cisplatin resistance of bone tumor and promotes uncontrolled cell proliferation\(^7\). Upon treatment with cisplatin and doxorubicin, upregulation of miRNA-140-5p is observed to induce autophagy of the osteosarcoma cell lines and results in cell death\(^6\). However, another sequence is upregulated, miRNA-184, after treatment with doxorubicin that increases cell survival by blocking apoptosis inhibitor BCL2L1, contrary to the expression and function of miRNA-140-5p\(^6\). miRNA-367 can also prevent apoptosis of cancer cells. miRNA-367 specifically conquers apoptosis through the downregulation of BNIP3L/Nix and the upregulation of BCL-XL\(^10\). Other miRNAs that correlate with poor chemotherapeutic remediation in pediatric osteosarcoma include miRNA-221 and miRNA 210\(^10\). miRNA-221 prevents expression of NF-κB inhibitors which maintains cell proliferation despite treatments\(^3\). miRNA-210 promotes and maintains the initiation of the cell cycle by acting upon EZF3 cell cycle regulator, MNT the myc antagonist and homeobox proteins, despite signals from chemo-therapeutic conditions\(^5\). Repressing expression of certain oncolytic miRNA sequences is equally as important as maintaining expression of tumor suppressor genes in other miRNA sequences. This strategy will allow an unhindered chemotherapy treatment to successfully fight against osteosarcoma and other aggressive cancers.

**Conclusions**

Osteosarcoma is a rare but deadly cancer affecting pediatric patients and in some cases adults. With its rarity, treatment options are limited and require innovative solutions to find effective options, which eradicate tumor cells and tissue without the need for amputation. Emerging treatment options utilize or combine tools harnesses from the host tissue response, chemoresistant therapeutics, immunotherapies, and microRNA therapy to effectively combat and eliminate cancer cells with the ultimate goal of improving patient prognosis and survival rate.

**References**


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