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Review Article

Molecular pathogenesis and therapeutic strategies of human osteosarcoma

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Abstract

Osteosarcoma (OS) is a devastating illness with rapid rates of dissemination and a poor overall prognosis, despite aggressive standard-of-care surgical techniques and combination chemotherapy regimens. Identifying the molecular mechanisms involved in disease pathogenesis and progression may offer insight into new therapeutic targets. Defects in mesenchymal stem cell differentiation, abnormal expression of oncogenes and tumor suppressors, and dysregulation within various important signaling pathways have all been implicated in development of various disease phenotypes. As such, a variety of basic science and translational studies have shown promise in identifying novel markers and modulators of these disease-specific aberrancies. Born out of these and similar investigations, a variety of emerging therapies are now undergoing various phases of OS clinical testing. They broadly include angiogenesis inhibitors, drugs that act on the bone microenvironment, receptor tyrosine kinase inhibitors, immune system modulators, and other radio- or chemo-sensitizing agents. As new forms of drug delivery are being developed simultaneously, the possibility of targeting tumors locally while minimizing systemic toxicityis is seemingly more achievable now than ever. In this review, we not only summarize our current understanding of OS disease processes, but also shed light on the multitude of potential therapeutic strategies the scientific community can use to make long-term improvements in patient prognosis.

Keywords: osteosarcoma, soft tissue tumors, bone tumors, cancer therapy, osteogenic tumors, osteogenic differentiation

Introduction

Osteogenic sarcoma (osteosarcoma, OS) is the most common primary malignancy of bone in children, with a distinct correlation between periods of rapid bone growth and development of disease[1-2]. More commonly affecting males, primary tumors often arise in the metaphyses of long bones such as the femur or tibia[1,3-4]. OS disseminates rapidly throughout the body, with 20% of patients noted to have secondary involvement at the time of diagnosis; 90% of such metastases are found in the lungs[5-7]. Though treatment approaches can vary considerably, the standard of care generally involves wide surgical resection with either neoadjuvant

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or adjuvant chemotherapy regimen[8]. With one of the lowest survival rates among pediatric cancers, OS imparts a 5-year survivorship of 70% in patients treated for localized disease, but only 30% when metastases are present[6,9]. Therefore, there is a critical need to better understand the underlying mechanisms of disease development and progression. This review seeks to provide a concise and useful synthesis of findings from recent, promising research efforts into these topics so as to encourage future investigations aimed at improving OS therapy and patient outcomes.

Molecular basis of OS and potential targets for therapy

Defects in osteogenic differentiation leading to OS development

OS commonly develops during the pubertal growth spurt, when bone turnover is high and defects in differentiation and signaling have the potential to be amplified[10-11]. Osteoblasts arise from mesenchymal stem cells (MSCs), undifferentiated bone marrow stromal cells with the potential to self-renew and proliferate into bone, muscle, tendon, and fat[12-13]. Milieus of endogenous and exogenous factors are involved in driving the osteogenic pathway from MSC to osteoblast. Dysregulation of these markers, or new exposure to non-native stimuli (*e.g.* pro-tumor inflammatory cytokines), causes an imbalance between cellular differentiation and proliferation, ultimately contributing to a malignant phenotype[14-16].

There are thought to be various similarities between early osteoprogenitors and OS cells, including a highly proliferative nature, resistance to apoptosis, and similar expression profiles of genes such as alkaline phosphatase (ALP) and connective tissue growth factor (CTGF)[17-18]. Furthermore, it is widely held that the earlier these defects occur in the osteoblastic lineage, the more undifferentiated or aggressive the cancer cells[15,19-20]. Accordingly, more invasive OS cells are noted to have minimal expression of osteocalcin (OCN) and osteopontin (OPN), both of which are observed at higher levels in mature osteoblasts[21-23]. Another notable difference between late osteoprogenitors and OS tumor cells is the ability of the latter to evade senescence through an alternative lengthening of telomere (ALT) pathway[24]. Unlike more terminally differentiated osteoblasts with shortened telomeres resulting from many replication cycles, ALT allows OS cells to remain in a stem cell-like state and responsive to exogenous stimuli[25].

Bone morphogenetic proteins (BMPs) represent one such group of factors involved in OS stimulation[15]. Normally involved in carrying MSCs along an osteogenic lineage, BMPs are not only unable to induce differentiation of OS cells but may actually promote a more aggressive phenotype[26-27]. This is due to an intrinsic underexpression of Runx2, a transcription factor which usually serves as a master regulator of BMP activity by causing exit from the cell cycle and promoting terminal differentiation[15,28]. However, *RUNX2* overexpression is also correlated with poor prognosis of OS tumors, indicating that its expression is likely tightly controlled in normal osteogenesis[29].

Select BMPs additionally exert effects through the Wnt glycoprotein pathway, a signaling network that has been extensively implicated in suppressing osteoblastic differentiation[30-33]. Aberrant signaling by Wnt can also result in increased cell proliferation and migration through both the canonical β-catenin and non-canonical pathways[34-35]. Accordingly, many research efforts have shown promise for OS therapy through inhibition of Wnt and downstream proto-oncogenes[36-38]. However, similar to Runx2, it appears that normal Wnt signaling is also finely tuned, with other studies demonstrating a correlation between decreased pathway activity and hypoxic chemoresistance in OS[39].

Beyond understanding the roles of BMPs, Runx2, and Wnt, researchers have identified additional proteins of interest that may promote differentiation of OS cells, thereby inhibiting proliferation and increasing susceptibility to apoptosis. These include super proteins of the nuclear receptor family, such as PPARγ, retinoids, and estrogens[20,40-44]. 1,25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$], another nuclear receptor agonist, has shown promise by increasing expression levels of p21, a pro-apoptotic cell cycle regulator which drives osteogenic differentiation and senescence[45-48]. Finally, parathyroid hormone-related peptide appears to promote differentiation of OS cells, observed through upregulation of osteoprogenitor markers ALP and collagen type I[49]. Ultimately, it appears that loss of differentiation plays a critical role in osteosarcoma genesis, but numerous molecular targets involved in the osteoblastic lineage may offer significant promise in developing new treatments.

Abnormal expression of oncogenes and tumor suppressors

As observed in most cancers, abnormal activity of oncogenes and tumor suppressors is a key molecular underpinning of osteosarcoma[50]. c-Myc, perhaps one of the most researched and well-understood oncogenes in OS pathogenesis, is overexpressed in over 10% of tumors and is correlated with increased tumor

recurrence. Specifically, it increases invasiveness of cells through activation of the MEK-ERK pathway and decreases apoptotic potential[51-52]. Recent studies have shown that inhibition of c-Myc activity results in decreased proliferation, invasion, and viability of OS cells, demonstrating its considerable value as a therapeutic target[53-56]. Similar to c-Myc, c-Fos is another oncogene that correlates with a higher rate of metastasis when upregulated in a primary tumor[57]. Sorafenib, a kinase inhibitor commonly used in treatment of hepatocellular and renal cell carcinomas, causes a favorable response in OS cells by downregulating c-Fos and S100A4, another oncogene implicated in regulating the cell cycle, decreasing apoptosis, and inhibiting osteogenic differentiation of OS cells[58-59].

MDM2, a protein that marks the tumor suppressor p53 (see below) for degradation, is amplified in at least 1 out of 10 patients[60,61]. Furthermore, higher co-expression levels of MDM2 and CDK4, which promotes cell cycle progression, can be used reliably to distinguish low-grade OS from benign masses and correlates with further dedifferentiation into high-grade lesions[62-64]. The transcription factor MEF2D is overexpressed in clinical specimens from OS patients with poor prognoses, and silencing the protein using a miRNA suppresses cell proliferation by triggering G2-M cell cycle arrest[65]. *AURKA* (coding for Aurora-A kinase) is an oncogene and an important regulator of mitosis that has undergone much recent investigation[66]. AURKA silencers and inhibitors of Aurora-A kinase have shown promising results in OS by not only causing hyperploidy and apoptosis, but also by working synergistically with traditional chemotherapeutics in cell lines that have become resistant to single-drug treatment[67-68]. Indicating the true complexity of OS molecular biology, additional oncogenes have also been recently attributed to disease progression, including those that code for p21-activated kinase 7 (PAK7), E2F transcription factor 2 (E2F2), special AT-rich sequence-binding protein-1 (SATB1), and several microRNAs such as 301a[69-72]. These proteins are of high interest to researchers as potential targets for therapy in the future.

Deficient tumor suppressor activity appears to play an equally important role as dysregulation of oncogenes in OS pathogenesis. Rb, a regulator of the G1/S cell cycle transition, is found to be insufficient in about 70% of all sporadic cases of OS, not to mention the nearly 1000-fold increased risk for developing OS in individuals who inherit an inactivated copy of the gene[73-75]. Similarly, mutations in tumor suppressor p53 are commonly found in OS cells and contribute to disease progression by permitting cells with damaged DNA repair mechanisms to evade checkpoints and apoptosis[74,76-77]. In fact, patients with type 2 neurofibromatosis actually have a higher incidence of OS due to increased activity of MDM2 and destabilization of p53[78]. Finally, $p16^{INK4A}$ is another tumor suppressor that normally inactivates CDK4 and has undergone much recent investigation as a biomarker that is positively correlated with patient survival in OS[79].

Signaling pathway dysregulation

Aberrant cell signaling is an equally important piece in the molecular biology puzzle underlying osteosarcoma development and progression. Several ubiquitous pathways have been implicated in the disease, providing numerous potential therapeutic targets for researchers moving forward. The insulin-like growth factor (IGF) signaling axis is one that ties in closely with the development of disease during periods of significant bone growth, such as in adolescence. The IGF-1 receptor (IGF-1R) is a member of the tyrosine kinase family and is most commonly activated by the IGF-1 ligand, ultimately stimulating proliferation, protein synthesis, and glucose metabolism while inhibiting apoptosis[80]. Normal functioning of this pathway is integral to both tissue homeostasis and growth, but loss of regulation has been extensively implicated in tumorigenesis and spread of disease[81-82]. Specifically, increased expression levels of IGF-1and IGF-1R are associated with worse prognosis in patients with OS[83-84]. Furthermore, IGF-2 mediates chemoresistance through a state of autophagic dormancy that preserves cell survival[85]. A downstream mediator involved in both IGF and insulin signaling, insulin receptor substrate 1 (IRS-1) is critically important for MSC differentiation. Its deregulation appears to be involved in malignant transformation of OS cells[86]. The IGF binding proteins (IGFBPs), which modulate signaling through both IGF-dependent and IGF-independent mechanisms, have recently been implicated in OS[87]. Notably, IGFBP-5 expression is significantly downregulated in various cell lines, and exogenous administration of the protein has been shown to suppress tumor growth and metastasis by multiple mechanisms[88-89].

Downstream of IGF-1R, signaling is propagated through the PI3K/AKT and Ras/MAPK/ERK pathways[80]. Upregulation of the former has been significantly implicated in OS pathogenesis, resulting in increased proliferation, increased invasion, and decreased apoptosis of tumor cells[90]. Researchers have shown that various molecules cause this activation, including the long noncoding RNA metastasis-associated

lung adenocarcinoma transcript 1 (MALAT1), tumor necrosis factor receptor-associated factor 4 (TRAF 4), and autophagy related protein 6 (Beclin-1)[91-93]. Furthermore, suppressing such activation may not only be involved in decreasing the aggressiveness of tumors, but may also be involved in overcoming chemoresistance, further demonstrating the importance of PI3K/AKT signaling as a therapeutic target[91,94-97]. Discussed earlier, Aurora-A (and -B) kinase inhibitors appear to also suppress this pathway as a means of halting tumor progression, representing a class of potential drugs seeking to match the complexity of the underlying pathophysiology[98-99].

Finally, inflammation and cytokine signaling have been heavily implicated in the tumorigenesis of OS[100]. For example, transforming growth factor β (TGF-β) is linked to the dedifferentiation of osteosarcoma cells into cancer stem cells, a dynamic population associated with tumor invasion, radio- and chemoresistance, and poor prognosis[101]. Often found to be involved in autocrine signaling by cancer cells, TGF-β increases the migration potential of OS cells through MAPK activation[102]. Similarly, tumor necrosis factor α (TNF-α) is strongly correlated with disease spread, though researchers have recently shown that infliximab, a monoclonal antibody (mAb) to TNF-α, can decrease OS cell motility and pulmonary metastases in a mouse model[103].

Interleukins represent another important class of cytokines with similar roles in disease progression. A pro-inflammatory cytokine, interleukin 32 has a dose dependent effect on promoting invasion and migration of OS cells via activation of the AKT pathway and upregulation of matrix metalloproteinase 13[104]. Regulated by TNF-α and IL-1β, interleukin 34 is expressed by OS cells and similarly promotes tumor spread through neo-angiogenesis and recruitment of tumor-associated M2 macrophages, which further produce TGF-β and promote tumor growth[105-106]. Finally, interleukin 11 receptor α, a marker of poor long-term prognosis in various cancers, has been found to be overexpressed in OS and can actually serve in the development of improved noninvasive imaging and targeted therapy[107].

Inflammation maintains tumors in an aggressive state due to the milieu of molecules released by macrophages, many of which further recruit other inflammatory cells. Monocyte chemoattractant protein 1 (MCP-1, or CCL2) is an example of this type of chemokine, involved in the critical migration of monocytes across the vascular endothelium and into tissues[108]. Additionally in OS, MCP-1 expression is significantly upregulated and activates AKT signaling, with knockdown inhibiting both the proliferation and invasion of tumor cells[109]. Downstream of cytokines such as interleukins, the JAK2/STAT3 pathway also represents a notable target for potential therapeutics[110]. Recent studies involving the use JAK2/STAT3 inhibitors delayed OS growth *in vitro* and *in vivo*, with similar results seen through short hairpin RNA knockdown of STAT3[111-112]. Overall, it has become increasingly clear that modulating several notable signaling pathways and quelling the inflammatory response to tumors may lead to profound therapeutic response in OS.

Emerging therapies and clinical trials

Angiogenesis inhibitors

As in most types of cancer, the ability of an OS tumor to acquire a robust blood supply has significant implications for growth, metastasis, and ultimately prognosis[113-115]. Therefore, drugs aimed at limiting angiogenesis have become increasingly studied in the treatment of various malignancies, including OS[116-117]. Vascular endothelial growth factor (VEGF) is one of the key regulators in angiogenesis and a well-studied marker associated with decreased disease-free survival in OS[118-121]. VEGF inhibitors have demonstrated considerable success in basic science and translational studies by reducing growth and metastatic potential of OS tumors, with the potential to sensitize cells to chemotherapy[122-126]. There are also several early clinical trials that are investigating bevacizumab, a mAb to VEGF, in patients with OS. However, there is concern that this drug may cause adverse events, particularlyin the pediatric population, including lymphopenia, pneumothorax, and increased wound dehiscence[127-131]. Finally, sorafenib is a tyrosine kinase inhibitor of the VEGF receptor family that has also undergone clinical investigation[132]. In a phase II trial that studied 35 patients with unresectable OS previously unresponsive to standard therapy, sorafenib resulted in a 46% progression-free survival at 4 months and a reduction in tumor density in those with stable disease (34% of all patients)[133]. Therefore, such targeted therapy warrants further investigation. Results from other well-designed clinical trials are needed to shed light on outcomes, feasibility of combination therapy, and appropriate dosing of VEGF inhibitors in the treatment of OS.

Part of the PI3K/AKT pathway, mammalian target of rapamycin (mTOR) is another signaling molecule involved in angiogenesis, and mTOR inhibitors are under clinical investigation for use in mesenchymal tumors, including OS[134-136]. A phase II clinical study published in 2015 reported that combination treatment

with sorafenib and everolimus, an mTOR mAb, resulted in progression-free survival of unresectable OS at 6 months in 17 of 38 patients who had previously failed standard therapy[134]. However, another phase II trial involving an IGF-1R inhibitor, cituxumumab, and mTOR mAb temsirolimus found no objective effect in 11 pediatric patients with OS[137]. Since both of these studies involved the use of combination therapy, the role of mTOR inhibition alone in OS still remains unclear in the clinical setting, warranting the need for future studies addressing this issue.

Bisphosphonates

Bisphosphonates are used in the treatment of osteoporosis and can reduce skeletal-related events in adult cancers, but have also shown promise in specifically treating OS[138-139]. Pre-clinical studies demonstrated that these agents inhibit the proliferation and metastasis of OS through activating apoptosis, suppressing tumor-induced angiogenesis, augmenting T-cell-mediated cytotoxicity, and sensitizing to chemotherapy[140-147]. Due to the potential for adverse craniofacial effects-such as osteonecrosis of the jaw, there have been clinical trials seeking to understand the feasibility and dosing of bisphosphonates in OS treatment[139,148]. In 2011, Meyers *et al.* demonstratedthat pamidronate could be added to chemotherapy regimens without any increase in toxicity, resulting in 5-year event-free survival (EFS) rates of 72% and 45% for patients with localized and metastatic disease, respectively. Though the authors did not incorporate a no-treatment arm with regard to bisphosphonate, they commented that the agent might improve durability of limb reconstruction[149]. Now that bisphosphonates have been deemed potentially safe, there are ongoing phase II/III clinical trials that may shed light on the role of the more-potent zoledronic acid in treating high-grade osteosarcoma, both alone and in conjunction with combination chemotherapy (NCT00691236, NCT00470223).

Receptor tyrosine kinase inhibitors

As previously mentioned, signaling pathway aberrancies are heavily involved in the aggressiveness of OS. Receptor tyrosine kinases (RTK) represent a class of molecules involved in propagating extracellular signals from a variety of sources, often growth factors, resulting in increased gene transcription, protein synthesis, and cell proliferation[150]. VEGF receptors, blocked by sorafenib as discussed above, are members of the RTK family[151]. Another important RTK is the human epidermal growth factor receptor 2 (HER2/neu), extensively studied in the pathogenesis and treatment of breast cancer, but also of considerable interest in OS research[152-153]. Pre-clinical trials have shown that both direct and indirect HER2 inhibition can have significant effects on decreasing OS proliferation, inhibiting migration, and promoting apoptosis[154-155]. A recent phase II clinical trial found that trastuzumab, a mAb to HER2, can be safely dosed in conjunction with a chemotherapy regimen, but doesnot offer any improvement in outcome. However, the study did not randomize patients into treatment groups, so the trastuzumab-specific effects still remain to be identified. Of note, two recently completed clinical trials may offer some insight into the use of trastuzumab, but are yet to be published. A group from Memorial Sloan Kettering is studying the drug as a single neoadjuvant agent before surgery in recurrent OS, whereas a study from the National Cancer Institute is comparing patients receiving standard-of-care chemotherapy with and without trastuzumab (NCT00005033 and NCT00023998).

Discussed earlier, the IGF-1 receptor is a tyrosine kinase and an important therapeutic target in OS, especially for adolescent patients with increased serum levels of growth factors seen during the pubertal growth spurt[156]. IGF-1R inhibitors, including antibodies, have shown promising results *in vitro* and *in vivo* using animal models[157-159]. Some clinical studies have addressed the use of an IGF-1R mAb in patients with various soft tissue and bone tumors, reporting that it is well tolerated but may have limited or no response in terms of outcomes[137,160-163]. Another large, multi-center trial is evaluating the combination of cixutumumab, an IGF-1R mAb, and a VEGF-R inhibitor in patients with bone and soft tissue sarcoma. Of 54 patients with IGF-1R-positive bone sarcoma (18 with OS), 19 were progression-free at 12 weeks. Of the 54 patients with IGF-1R-negative soft tissue and bone tumors (6 with OS) who were also followed, 12 were progression-free at 12 weeks. Furthermore, based on histology, 13% of the patients with OS showed a partial response to therapy. As this trial is ongoing, the median overall survival for OS has not yet been reached[164]. Though promising, this study is evaluating two different drugs on a variety of tumors as classified by IGF-1R expression, only a small subset of which is OS. Therefore, there is a need to design clinical trials intended to understand the effects of IGF-1R inhibition on osteosarcoma specifically.

Immunotherapy

The immune system can be a valuable tool for targeting and destroying tumor cells, a topic which has

received considerable attention in the popular press[165-166]. Recent *in vitro* and *in vivo* work has shown the ability of a variety of immune cells, including natural killer cells, genetically modified T-cells, and viruses, to effectively kill OS tumor cells, making immunotherapy an intriguing prospect[167-170]. Mifamurtide, or liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE, Mepact), is an agent that causes recruitment of inflammatory macrophages and has been under clinical investigation with promising results[171,172]. The drug is of particular interest to researchers because of its favorable safety profile and ability to target metastases, such as in the lung[173-174].

In Europe, mifamurtide has already been approved for use in children, adolescents, and young adults for non-metastatic osteosarcoma after tumor excision[175]. This was in response to the results of a large multi-center, randomized phase III trial, known as the Intergroup Study 0133, demonstrating the drug's ability to improve overall survival in conjunction with 3- or 4-agent combination chemotherapy in newly diagnosed, high-grade, non-metastatic, resectable osteosarcoma[9]. Though a similar trend was seen in a smaller cohort of patients presenting with metastatic disease, the result was not of statistical significance[176]. A recent Markov process model using data from the Intergroup 0133 trial found that mifamurtide improved the lifetime effectiveness of chemotherapy in both metastatic and nonmetastatic disease[177]. Currently, in the United States, mifamurtide remains an orphaned drug, but additional well-designed, prospective, randomized controlled trials may offer a path for this promising drug to be re-introduced into the market as adjuvant therapy.

Granulocyte macrophage-colony stimulating factor (GM-CSF) exhibits similar macrophage-stimulating properties, but may also extend its reach to CD4 T-cells, natural killer cells, and dendritic cells[178]. In laboratory studies, GM-CSF has shown an ability to induce osteoblastic differentiation and apoptosis in OS cells[179-180]. An inhaled form of this factor was studied in a phase I trial evaluating patients with first isolated pulmonary recurrence of OS, with results showing that it had low toxicity but no discernible effects on immunostimulation or outcomes[181]. The same group recently completed a phase II study looking at inhaled recombinant GM-CSF, sargramostim, on a similar group of patients with first pulmonary recurrence of the disease, but the results are yet to be published (NCT00066365). Overall, immune system modulators represent an important class of anti-neoplastic agents that may undergo considerable growth and development in the near future.

Chemo- and radio-sensitizing agents

As tumor resistance remains one of the most significant barriers to improving patient prognosis, numerous research efforts have recently been directed towards increasing OS response to existing chemotherapy and radiotherapy regimens[80,182-188]. In addition to studies looking at various anti-neoplastic agents discussed above in combination with chemotherapy, a few researchers have also aimed to repurpose existing non-cancer drugs for use in OS[189]. The most notable example is metformin, an insulin-sensitizer considered to be the first-line treatment for type II diabetes mellitus[190]. An *in vitro* study found that metformin not only inhibits tumor cell growth but also sensitizes three different cisplatin-resistant cell lines to the drug, demonstrating a synergistic effect[191]. This effect might be mediated through crosstalk that exists between insulin- and IGF-signaling[192]. Similarly, proton pump inhibitors (PPIs) normally used for dyspepsia have shown promise in OS. A recent translational study studied two cell lines in culture as well as a murine xenograft model, finding that in both settings pre-treatment with esomeprazole sensitized tumor cells to cisplatin. The same study then evaluated 98 patients aged 40 years or younger with resectable nonmetastatic OS of the extremities who received esomeprazole in the two days before each round of neoadjuvant chemotherapy. When compared to a historical study that used the same chemotherapy regimen, the authors found that PPI pre-treatment increased local cytotoxicity of the drugs as evidenced by histologic tumor necrosis[193]. Though the study did not randomize patients or look at survival rates as an outcome, this represents an exciting starting point for researchers to design future clinical studies involving the potential chemosensitizing effects of PPIs.

Radiotherapy plays a considerable role in treating incompletely- or un-resectable primary tumors and chemoresisant metastases to the lung and axial skeleton, though not a standard OS treatment modality due to resistance[8,194-195]. Therefore, finding agents capable of sensitizing tumors to radiation may improve outcomes in patients who may need it as an end-of-the-line treatment option. Though most of the work to date has taken place in a laboratory setting, the findings do appear promising. A small molecule inhibitor of WEE1 kinase, found in many OS tissue samples, has been shown to bypass the G2 cell cycle checkpoint following radiation exposure, resulting in mitotic catastrophe[196]. Similarly, radiation combined with parthenolide, a naturally occurring molecule that interferes with NF-κB cell survival signaling, has a

synergistic effect on previously radio-resistant OS tumor cells, including cancer stem cells[197]. Previously discussed, the ALT pathway allows cancer cells to evade senescence and remain in a stem cell-like state, but also appears to play a role in resistance to irradiation. Suppression of Ku80, a protein involved in DNA repair via nonhomologous end joining, radio sensitizes an ALT OS line *in vitro*, with affected cells demonstrating shortened telomeres[198]. Results such as this should spur more investigations to overcome resistance to radiation, a treatment modality that has been underutilized in OS.

Novel drug delivery mechanisms

Though the most effective treatment modality to date in OS, chemotherapy also carries with it toxic effects, several of which can promote the development of a secondary malignancy or cause significant morbidity[199-201]. In response, as has been the approach in other cancers, researchers have studied the use of vectors for delivering OS therapy to a localized area, hoping to minimize unintended consequences. Stem cells represent such a promising option moving forward. Using bone-marrow-derived MSCs expressing the cytosine deaminase/5-fluorocytosine prodrug, a recent study found that the MSCs were able to migrate toward OS cells *in vitro*, resulting in cytotoxicity, and also inhibit subcutaneous tumor growth when injected locally into mice[202]. Furthermore, another study showed that RFP-labeled human MSCs could be injected into the tail vein of athymic nude mice and effectively localize to OS tumors. The MSCs, which were carrying the osteoprotegerin (OPG) gene, caused expression of the protein at the tumor site and resulted in decreased tumor growth and bone destruction[203].

Using nanocarriers may also be effective for delivering drugs to specific locations with sustained release[204-205]. A recent study demonstrated that nanoparticles loaded with paclitaxel and etoposide demonstrated increased cytotoxic effects on OS cell lines when compared to a combination of the drugs in native form[206]. Similarly, nanoparticles carrying the antibiotic salinomycin were designed to target CD133+ osteosarcoma cancer stem cells and caused pronounced *in vitro* and *in vivo* cell death. A new technology involves nanotubes made from halloysite, which can be mined from natural deposits, and has been shown to inhibit OS cell proliferation when delivering methotrexate[207]. Finally, organic molecules, such as liposomes and micelles, loaded with existing and experimental anti-neoplastic agents have been used with promising results in suppressing OS tumor cells in culture and in a xenograft model[208-210]. This approach may also be useful for potential drugs, such as curcumin, that previously could not be properly formulated due to their chemical properties (e.g. water insoluble)[211,212].

Conclusions and future directions

In conclusion, a considerable body of recent, cutting-edge research provides an optimistic view of osteosarcoma treatment and patient prognosis in the future. With continued investigation into the molecular underpinnings of this aggressive disease, we can hope to better understand the interplay between various signaling and differentiation pathways, identifying the most critical molecular targets for therapy. An effort to design meaningful translational studies can then allow for a bench-to-bedside approach involving potential therapeutics. Finally, there is a pivotal need to implement more high-quality randomized clinical trials, focusing on just patients with osteosarcoma, as it has become increasingly clear that the unique properties of this malignancy make it difficult to predict drug response in comparison to other bone or soft tissue tumors. Ultimately, there may exist in some combination of surgery, chemotherapy, and localized molecular therapy that can significantly improve outcomes and quality of life for those suffering from osteosarcoma.

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