

Osteosarcoma in Dogs and Small Animals

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Abstract

Although osteosarcoma is the most common cancer that affects bone, it is extremely malignant, and current treatments include surgical resection of the affected area and multi-agent chemotherapy, although the survival rate is generally poor for those affected by metastases. Since osteosarcoma treatment has remained unchanged for the past few decades, there is a need for further advancements in understanding osteosarcoma biology and therapeutics, which means that reliable animal models that can accurately recapitulate the disease are required. Canine osteosarcoma has a dismal prediction, with one-year survival probability of fewer than 45% after evaluation and treatment. Respiratory metastasectomy is one option for treating canine individuals with metastases; however, metastatic disease therapy is only advised if the underlying tumor is completely remitted. Although rodents are the most common animal model of osteosarcoma, they may not be the best. Each of these alternatives has advantages over traditional rodent models for pre-clinical research, and research on these cross-species platforms imparts knowledge of metastases biology and potential new osteosarcoma treatments.

Keywords: Osteosarcoma, Canine, Dogs, Limb amputation, Surgery, Vaccine

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Introduction

Osteosarcoma is a kind of severe recurrent bone tumor that most commonly affects humans. It primarily happens throughout young adulthood, with an additional surge occurring in middle age (beyond 40) (Ottaviani & Jaffe, 2009; Moore & Luu, 2014). The tumor is distinguished by modified formation of osteoid (abnormal bone matrix) and unusually complicated karyotypes (Letson et al., 2001; Karlsson et al., 2013). While the exact target cell for malignant transformation in the development of osteosarcoma is unknown, it is believed to be a mesoderm stem cell (MSC) or an osteoblast-lineage cell (Mutsaers & Walkley, 2014). Evidence from mice models suggests that any of various cell phases may act as target cells for osteosarcoma development (Quist et al., 2015). Osteosarcoma is largely a disease of adult canines in canine patients; the average age of patients at assessment is around 8 years, with a slight surge in occurrence in puppies (under three years old) (Modiano et al., 2006; Morello et al., 2011). This is in contrast to the scenario in human patients, when the greatest occurrence occurs throughout puberty. Nonetheless, the disease's natural history is identical in dogs and humans with osteosarcoma (Scott et al., 2011). Furthermore, it is discovered that the genome-wide expression patterns of canine osteosarcoma are indistinguishable from human pediatric osteosarcoma, and more similar to human osteosarcoma than any other human malignancy (Thomas et al., 2009; Schiffman & Breen, 2015). In dogs, there is a high breed preference for cancer risk, such as osteosarcoma. Many large breed dogs have a higher risk of osteosarcoma than other breeds (McNeill et al., 2007; Dobson, 2013; Karlsson et al., 2013). The hereditary factors that determine osteosarcoma vulnerability in dogs are not well understood, and it is probable that the same variables will similarly influence human osteosarcoma vulnerability. The discovery of therapeutic procedures in dogs and humans has proved useful to both species (Paoloni & Khanna, 2008). In reality, existing therapeutic choices are comparable in both species. Canines account for 85% of appendicular skeleton malignancies and 75% of all osteosarcomas (Cristo et al., 2017). The majority of cases occur in medium to big male canines of all ages, with a greater incidence between 7 and 8 years old. Although it is not believed to be genetic, several breeds, such as Great Dane, German Shepherd, St. Bernard, Irish Setter, and Boxer, may exhibit this trait. Rottweilers and Greyhounds are known to be disease-prone (McNeill et al., 2007; Rosenberger et al., 2007).

History

The Norwegian manager survey data from 4 varieties (Labrador retriever, Newfoundland, Leonberger, and Irish wolfhound) indicated osteosarcoma (OSA) cases ranging from 0.2 to 8.9% (Anfinsen et al., 2011). The proportion of dogs positive for OSA at two Polish institutions was calculated to be 2.7 to 10.7%, according to breed (Sapierzyński & Czopowicz, 2017). From 1995 to 2002, 764 (0.19%) of the 394,061 insured

Swedish dogs acquired osteosarcoma. This represents a yearly prevalence of 27.2 dogs per 100,000 in the data set (Egenvall et al., 2007). Although the canine data only contains registered dogs, it is a huge sample and hence likely to represent the country's canine population as a whole. Thus, the prevalence of canine bone cancer is twenty-seven parts greater than in homo sapiens. The greater prevalence rate of canine osteosarcoma creates the canine individuals as an excellent model for disease in humans. Whereas any breed of dog may get osteosarcoma, certain types of bigger dog breeds are at a greater risk (Parkin et al., 2010).

Epidemiology

MET, FOS, IGF1R (Insulin-Like Growth Factor 1 Receptor), PVT1/MYC, RUNX2, and HER2 are among the cancer-causing genes that have been linked to osteosarcoma in dogs. A few of these modifications include adjustments to the copy number. There have been reports of consistent chromosomal and genome copy number alterations canine osteosarcoma (Boston et al., 2006).

Gross and Histological Features of Canine Osteosarcoma

Although osteosarcoma tumor cells are more likely to colonize the lung metastatically, it's interesting to note that different cell lines have different capacity for metastatic spread. Interestingly, in mice models of Li-Fraumeni, the kind of p53 mutant allele utilized and the period during the mouse's lifespan that Cre is employed to cause p53 gene mutation affect the rate of lung metastasis (Walkley et al., 2008). Histopathology revealed a non-limited embryonic malignant cancerous proliferation in several builds and a network of cigar shape to star shaped cells with a bony matrix, establishing an intricate design or disorganized islands, occasionally engorged by the red blood cells, and lined by an acceptable quantity of multinucleate large cells. Cells that were neoplastic exhibited open phase nuclei with one to three nucleoli, limitless cytoplasm, and slightly basophilic or coarsely eosinophilic granular material at the margins. Pleomorphism (shape variation) was enhanced, resulting in 4 mitotic forms per high-power field (40x). This cancer resembled osteoblastic osteosarcoma. The radiologic assessment revealed bone neoplasia characteristics such as Codman's triangle, osteolysis, and periosteal palisade response, as well as a rise in soft tissues similar to osteosarcoma (Parkin et al., 2010). Complete limb removal was done four days following the analysis. A white tumor of 15x20 cm occupied the whole cranial and diaphyseal portion of the humerus, extending to the humero-radio-ulnar junction.

Table.1: Molecular and Genetic Factors Linked with Dogs Osteosarcoma

Elements*	Roles in Canine OSA	References
<i>p53</i>	<ul style="list-style-type: none"> p53 is recasted and overemphasized in osteosarcoma cell lines and main tumors. Osteosarcoma tumors show an absence of p53 genetic variability. 	(Levine & Fleischli 2000; Kirpensteijn et al., 2008)
<i>RB</i>	<ul style="list-style-type: none"> RB1 protein expression is decreased or missing in osteosarcoma tumors. 	(Thomas et al., 2009)
<i>PTEN</i>	Phosphatase and tensin equivalent removed on chromosome 10 is removed and down-regulated in OSA cell lines. <i>PTEN</i> copy number absent in Osteosarcoma tumors	(Levine et al., 2002) (Thomas et al., 2009; Angstadt et al., 2011)
<i>MYC</i>	Myelocytomatosis oncogene copy number rises in osteosarcoma tumors Myelocytomatosis oncogene activation seen in a large number of Rottweiler osteosarcoma neoplastic growths.	(Thomas et al., 2009)
<i>CDKN2A/B</i>	Hereditary hazard gene locations (150 kb towards the 5' end CDKN2A/B) found in Osteosarcoma neoplastic growths from highly hazardous breeds	(Karlsson et al., 2013)
<i>ErbB-2/HER-2</i>	Human EGFR-2(HER2) mRNA overexpressed in OSA cell lines and tumor	(Flint et al., 2004)
<i>IGF-1/IGF-1R</i>	Osteosarcoma cell lines exhibit IGF-1/IGF-1R, which modifies attachment free development and attack in response to IGF-1	(MacEwen et al., 2004)
<i>RON/Met/EGFR</i>	Mesenchymal Epithelial Transition is revealed in Osteosarcoma cell lines; increased invasion/shifting in result to ligand activation (HGF). MET expressed in OSA tumors Estimated glomerular filtration rate EGFR and Research Octane 2009; McCleese et al., Number Ron are expressed in osteosarcoma cell lines and malignancies; Met is associated with Estimated glomerular filtration rate (EGFR) and Research Octane Number (Ron) in OSA cell lines.	(Liao et al., 2005; Fieten et al., 2009; Raffaella et al., 2013)
<i>STAT3</i>	Constitutive stimulation of signal transducer and stimulation of transcription 3 (STAT3) in bonny tumors and cell lines; increased viability and growth in osteosarcoma cell lines Osteosarcoma tumors produce Oncostatin M, which enhances signal transducer and activator of transcription 3 (STAT3 activation), VEGF production, and invasion in Osteosarcoma cell lines.	(Fossey et al., 2011)
<i>mTOR</i>	Mammalian target of rapamycin (mTOR) activity in dog osteosarcoma cell lines; blocking of the mTOR pathway reduces cell persistence.	(Gordon et al., 2008)
<i>ezrin</i>	High ezrin expression in osteosarcoma tumors is related with early development. Activation of ezrin by protein kinase C promotes cell migration	(Khanna et al., 2002; Hong et al., 2011)
<i>PDGFs/PDGFRs</i>	OSA tumors express platelet-derived growth factors (PDGF-A/B) and PDGFR α/β , while Osteosarcoma cell lines overexpress these proteins.	(Maniscalco et al., 2013)
<i>MMPs</i>	Osteosarcoma cell lines show raised levels of MMP-2/-9	(Loukopoulos et al., 2003)
<i>miR-134</i> <i>544</i>	miR- Reduced expression of miR-134 and miR-544 (syntenic to the human 14q32 miRNA cluster) in osteosarcoma neoplastic growth is related to lesser survival.	(Sarver et al., 2013)

Risk Factors

Environmental circumstances were identified as contributory factors for osteosarcoma. It is reported that there is a linear relationship between local radiation exposures and the danger of osteosarcoma. Arlen et al. found that inhabitants in locations with radiation exposures that fluctuated between 1,200 rads/week to 24,000 rads/2 years had a higher risk of developing osteosarcoma. Being exposed to radiation has been associated with osteosarcoma among radium dial operators. Bassin et al. also stated that discovery of fluoridated water was a major contributing hazard for neoplasms (Bassin et al., 2006). Gandhi et al. found that fluoride-induced oxidative and swelling stress contributes to the development of cancerous growth (Gandhi et al., 2017). Other chemical hazards consist of methylcholanthrene and chromium salts, beryllium oxide, zinc beryllium silicate, asbestos, and aniline colors (Tan et al., 2009). There is a large number of genes changed in human and canine Osteosarcoma, making it difficult to explore how they work in depth. Table 1 illustrates the biochemical and genetic variables involved in canine Osteosarcoma, highlighting those that are also dysregulated in human Osteosarcoma.

Diagnosis

The diagnosis depends on a medical exploration, radiography of the lesion, and a small fine needle aspiration (FNA) conducted to determine the kind of neoplastic growth (Mehl et al., 2001). Due to the high malignant risk, an X-ray of the thoracic region is indicated as an extra diagnostic. If limb-salvage surgery is being examined, a blood test, computed tomography scan, or magnetic resonance imaging should be ordered. The TNM system (T-tumor, N-lymph node, M-metastasis) allows for the differentiation of three disease phases. Stage I contains a low-grade (G1) lesion with no history of metastasis (M0); stage II comprises a high-grade (G2) lesion without metastasis (M0); and stage III is a lesion with metastatic abnormality (M1). Regardless of histologic grade, phases I and II are separated into two categories based on anatomic context (A and B). Group A is within a compartment (neoplastic growth has prevailed within the bone) (T1), while group B is within other compartments (neoplastic growth has spread outside the bone into other adjacent structures). Although ultrasonography is also available, it is operator-dependent and requires knowledge of orthopedic ultrasonography. Additionally, many bone neoplasms are superficial, therefore ultrasonography systems may not be suited for them in practice. Furthermore, the use of ultrasound is restricted to assessing soft tissues and the bone's surface because, without severe lysis, shadowing at the bone-soft tissue interface makes it impossible to assess the more interior structures within the bone. On the other hand, it might help with diagnostic samples (Liu et al., 2007). In numerous organizations, technetium-99m nuclear scintillation was formerly the gold standard for oncologic grading of bone cancers and metastasis. However, it lacks specificity because it shows regions of dynamic bone turnover and hyperthermia, along with other variations (Wenaden et al., 2005). How imaging can aid to distinguish benign from malignant tumors and osteosarcoma from other malignant skeletal neoplasms because the cytologic and histopathological features of distinct bone cancers may extend over. When changes in radiography are not conventional or a conclusive diagnosis is necessary to determine future treatment options, bone biopsy is most helpful. Bone biopsies should be minimized or their placement should be designed to allow the biopsy string to be withdrawn in one piece throughout the procedure if limb-sparing surgery is to be carried out (Liu et al., 2007). Radiographs (two views) and anatomic landmarks should be used as a guidance for healthcare professionals when arranging for bone biopsies. They can also use CT, MRI, ultrasound, or fluoroscopy directly. It is advised to take post-biopsy radiographs to make sure the biopsy needle is positioned correctly. An alternative method that could help the doctor to be certain that the right part of the tumor has been sampled is a biopsy guided by ultrasonography or CT (Le et al., 2010).

Treatment

Conventional approach to treating dogs is limb-salvage surgery and chemotherapy with adjunct therapy. Treatment options comprise surgery (limb amputation or limb-salvage surgery), radiation therapy, and chemotherapeutic treatment (Boston et al., 2006). Amputation is a primary operation that enhances their existence, provides pain alleviation, and thereby postpone euthanasia (Thompson & Pool, 2002). It entirely eliminates the original tumor, minimizes postsurgical complications, decreases the period of surgical anesthesia, and reduces the cost in contrast to the limb-salvage treatment (Jehn et al., 2017). Since the selection of chemotherapy medications does not appear to affect their existence, treatment selections are often influenced by toxicity, quality of life, and cost (Szewczyk et al., 2015). Chemotherapy is only advised after the original tumor has been eliminated, and carboplatin is now the preferred medication. Numerous carefully monitored trials demonstrate that the only way to get a clinical response for osteosarcoma is to follow the recognized standard of therapy. No alternative therapies have been proven to be effective or consistently give therapeutic benefit in controlled trials, and anecdotal advantages from botanical or "unorthodox" treatments have not been replicable. By one year, 90% of dogs have metastatic cancer of the bones, which is the leading cause of death or destruction. Respiratory metastasectomy is one option for treating canine individuals with metastases; however, metastatic disease therapy is only advised if the underlying tumor is completely remitted. After a respiratory metastasectomy, the average duration of survival can reach six months; however, survival is often less than two months in the absence of a routine surgical treatment.

Prognostic Factors for Osteosarcoma in Dogs

Diagnostic and prognostic tools are important aspects that influence therapy outcomes. Typical osteosarcoma disease development comprises of an ancient tumor, generally beginning inside the medullary cavity that develops, grows, and attacks, and if left untreated, commonly metastasizes to the airways (Bacci et al., 2008). Canine osteosarcoma has a dismal prediction, with one-year survival probability of fewer than 45% after evaluation and treatment (Moore et al., 2008; Frimberger et al., 2016). Approximately 54% of dogs that survive past a year acquire metastatic illness, with an average survival duration of 243 days (Culp et al., 2014). There is collecting confirmation in few diagnosis (despite restricted to study numbers) that additionally to comprehending that particular breeds are more susceptible to progressing osteosarcoma, there are also specific genetic variations inside the tumors came across among specific breeds that may have indicative significance (Thomas et al., 2009; Angstadt et al., 2011). A variety of variables have been diagnosed as having forecasting importance in canine

osteosarcoma. The prevalence of obesity and tumor sites have been thought to be particularly important, because these characteristics are readily accessible to all patients. Dogs who have less body weight have a longer life time (Lascelles et al., 2005; Amsellem et al., 2014), but dogs with proximate humeral tumors have a lesser survival time (Boerman et al., 2012). Serum indicators have also been investigated; such indicators are certainly required, especially if consistently tested, as they allow practitioners to initiate talks about outcome-related issues with clients without relying on sophisticated diagnostics. Higher plasma activity of alkaline phosphatase in canines has been linked to a limited free of illnesses period and length of survival (Garzotto et al., 2000).

Therapeutic Tumor Vaccines

In recent years, numerous tumor vaccination platforms have been examined as innovative therapy options for enhancing the supervision of a variety of carcinomas, including canine OS. In one study aimed at augmenting instinctive defenses created within the neoplastic niche, an IV, mitigated *Salmonella typhimurium* (VNP20009) that primarily restricts and expands within neoplastic tissues was tested in a small number of dogs with OS (n = 4) and demonstrated efficient anti-neoplastic activity, demonstrated by incomplete remission in canines. Despite some signs of anticancer immune activation, this study found significant side effects, restricting VNP20009's usage to largely a research context. A tumor lysing vaccinia virus (strain LIVP6.1.1) was also effectively examined in the laboratory for its capacity to destroy D-17 cells, in addition to a bacterial agent for boosting immune responses against OS cells. The experimental to clinical study of such oncolytic viral techniques in dogs with OS has not yet been described, despite the fact that the tumor lysing vaccinia virus technique generated strong site specific innate immune responses in mouse xenograft models.

In canines with osteosarcoma, advanced combination cytokine vaccination approaches have been tested and shown promising results. In one study, the effects of SC plus suicide-gene therapy with ganciclovir (GCV) administered combined with irradiation autologous or allogeneic OS tumor cells administered intratumorally or peritumorally, xenogeneic cytokine-producing cells (hGM-CSF and hIL-2), were assessed in dogs with appendicular or axial OS (n=5) (Gentshev et al., 2013). Clinically, the combined cytokine vaccine approach demonstrated mild antitumor effects; two dogs with axial OS maintained stable illness, while one canine with appendicular osteosarcoma experienced a partial response. The majority of the five OS canines lived less than a year, with survival times ranging from 96 to more than 386 days (Finocchiaro et al., 2011). A novel vaccination method has recently been published in a veterinary experiment that combines standard of treatment (surgery and chemotherapy) with an ammonia-based antineoplastic vaccine in canines with non-metastatic OSA (Marconato et al., 2022).

Future Innovations

The administration of bisphosphonate medication, particularly zoledronic acid and pamidronate, in conjunction with routine therapy for the cure of osteosarcoma has been examined in various laboratory-based studies and in animal osteosarcoma models, particularly dogs (Hoddinott et al., 2020; Suva et al., 2021). While their usefulness in the treatment of primary illness is unknown, more promising data has been revealed for the treatment of metastases, which is a significant accomplishment given the ferocity of osteosarcoma metastatic dissemination. Gemcitabine has been advocated in the last few years as a therapy for respiratory metastases. Gemcitabine, a deoxycytidine counterpart, is previously availed for the treatment of sanguine and human solid tumors, comprising recurrent and inoperable high-grade osteosarcoma (Nabhan et al., 2001; Gesto et al., 2012; Palmerini et al., 2016). The quest for novel molecular targets in osteosarcoma is continuing, and chondroitin sulfate proteoglycan 4 (CSPG4) has emerged as an attractive molecule. This integral membrane protein can be produced on the cell surface as a ~250 kDa N-linked glycoprotein or a ~450 kDa N-linked glycoprotein with a proteoglycan component. Chondroitin sulfate proteoglycan 4 (CSPG4) has tissue specific expression, primarily in a limited number of poorly developed precursor cells, such as marrow stromal cells microglial in the brain and spinal cord, and venous pericytes (Price et al., 2011; Beard et al., 2013; Nicolosi et al., 2015; Rolih et al., 2017).

Conclusion

One type of cancerous osteo tumor is dog breeds osteosarcoma that has a high metastatic potential. Currently, the most successful treatment regimen used for the treatment of OSA consists of a combination of surgical procedures, chemotherapy, and radiation. Veterinary physicians faced various hazards while treating the patients including hindrance in lowering metastasis and boosting anticancer effectiveness of cytostatic medicines while minimizing unfavorable side effects of the medication. While facing all these challenges, new techniques are being investigated for the OSA therapy, such as the application of nanoparticles. In vitro investigations on human osteosarcoma cell lines indicate that fucoidan or dioxide nanoparticles might be successful medications for treating initial tumors and preventing OSA recurrence. Encapsulating doxorubicin and siRNA in liposome-based dextran nanoparticles has an opportunity to produce innovative therapeutics. Dogs with OSA may benefit from medications used to treat cancer in human health, but further research in veterinary medicine is required.

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