



Review

Metallothionein: Potential therapeutic target for osteosarcoma

Salma Siddiqui^a, Ajai Singh^a, Sabir Ali^{a,*}, Manish Yadav^a, Vaishnavi Pandey^a, Dilutpal Sharma^b^a Department of Orthopaedic Surgery, King George's Medical University, Lucknow, U.P, India^b Department of Biochemistry, King George's Medical University, Lucknow, U.P, India

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ABSTRACT

The most aggressive and deadliest form of cancer for adolescence and young adults is osteosarcoma. Current treatment of osteosarcoma mainly consists of chemotherapy along with surgery has dramatically improved survival rate. However unfortunately, the survival-rate remained unchanged in its metastatic stages that might be due to chemoresistance against the treatment of osteosarcoma. Till date, in clinical applications, there is no precise diagnostic/prognostic marker of osteosarcoma. Metallothioneins are thiol rich intracellular proteins, which binds to several cytotoxic agents. Metallothionein isoforms participate actively in numerous physiological and patho-physiological processes like proliferation, apoptosis, angiogenesis, and the heavy metals detoxification. There are many evidences in literature which are suggestive of participation of these proteins in carcinogenesis and antitumor therapy. Furthermore, a number of studies also reveal the important role of metallothioneins in tumor cell defense mechanism against the radiotherapy by preventing apoptosis. Also in osteosarcoma patients in comparison to healthy controls, the higher level of metallothioneins levels further indicates their significant role. Moreover higher level of metallothionein-2A in chemotherapy resistance patients with osteosarcoma further becomes potential supportive evidence. According to the information available in the literature, one may accomplish that metallothioneins has a role in osteosarcoma progression and chemoresistance and may become a potential diagnostic marker as well as a reliable therapeutic target. However, further multicentric studies are needed in support. A better knowledge of the communication among metallothioneins in osteosarcoma as well as with chemotherapeutic agents is necessity and may exposed new perspectives in cancer treatment.

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1. Introduction

Osteosarcoma [OS] is the commonest and highly aggressive bone tumor with malignancy,¹ accounting for 30–80% of primary skeletal sarcomas.² Currently, it is measured to be the 2nd death agent of children and young adults having cancers.³ Their rate of incidence is moderate type with 10–26 per million new cases worldwide (Fig. 1⁴ each year.⁵ It arises from mesenchymal cells which produces immature cells.⁶ It can occur in any age but most prominent in young age population of age between 10–30 years.⁷ It may arise in any bony regions; however, metaphysical regions of long bones, such as proximal tibia (15%), distal femur (30%), and proximal humerus (15%) are the common areas (Fig. 2).^{8,9}

The metallothioneins was first discovered by kagi and vallee in

1957 and considered as the metal binding protein.¹⁰ Kagi and Vallee in 1960¹¹ and Kojima in 1976¹² reported this protein as a cysteine-rich (33 mol %), heat-resistant and 7 kDa protein with low molecular weight. In 2011 Duncan et al. used the “magic number” term to set up the metal for protein or metal for ratios based cysteine of available spectroscopic data which results in a thermodynamically stable compound-complex.¹³ In humans, there are four types of MT of similar biochemical structure.¹⁴ MTs are commonly synthesizable in body tissues mostly. Large amounts of metallothioneins are synthesized primarily in kidney and liver. Human MT is made up of eleven functional isoforms including MT1 (along with its 7 family members that are A,B, E, F, G, H, M & X) MT2 (also known as MT2A), MT3, & MT4. The MT1 as well as MT2A majorly depicted in several organ-tissues & cells, while MT3 & MT4 observed in certain epithelial tissues along with brain.¹⁵ Metallothioneins participates in various processes like heavy metal detoxification, Cu & Zn based and homeostasis protects the cell against oxidative stress as well as promotes cell survival, angiogenesis, apoptosis, proliferation etc. in

* Corresponding author.

E-mail address: sabir_6484@rediffmail.com (S. Ali).

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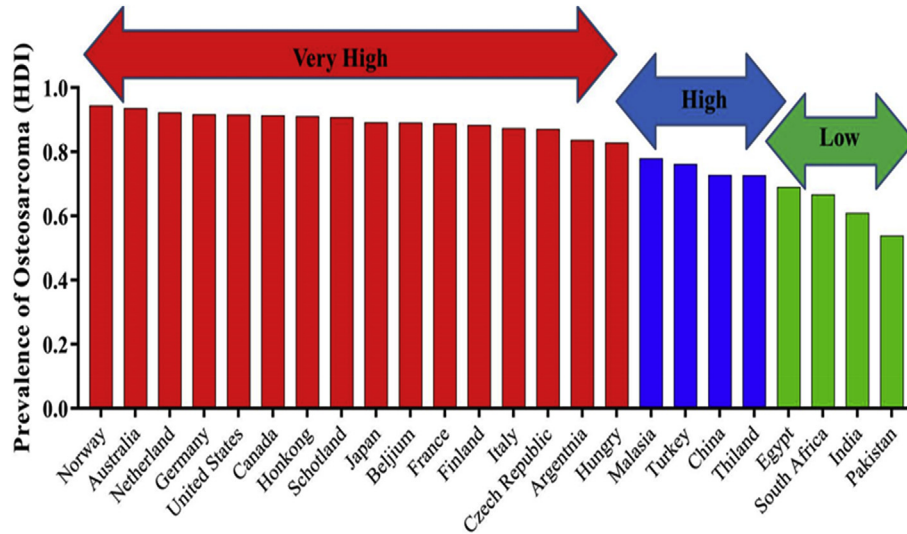


Fig-1. Prevalence of Osteosarcoma in different countries using analysis based on Human Development Index (HDI).

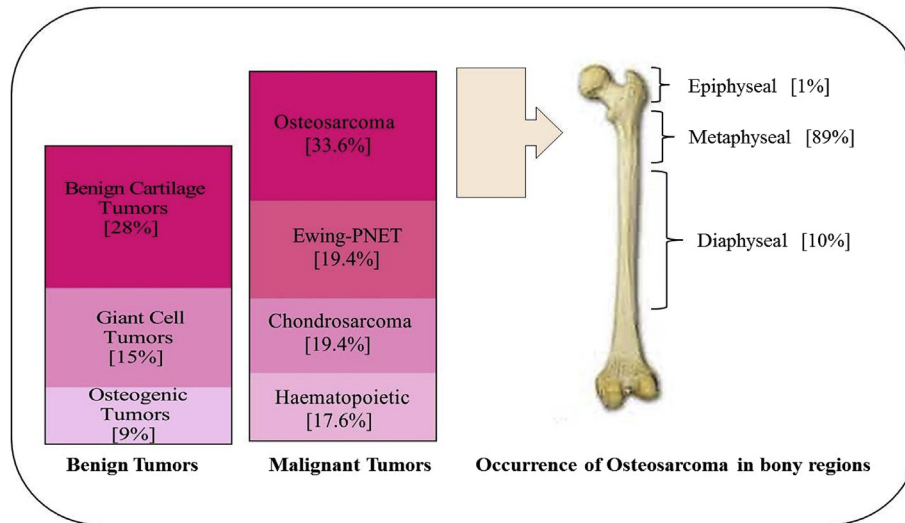


Fig-2. Prevalence of bone tumors with occurrence of Osteosarcoma in bony regions.

the body¹⁶ (Fig. 3). MTs re-present strong affinity of biological intracellular metals for example, zinc or copper, which contribute in the homeostasis of their ionized forms.^{17–19} Also they chelate the heavy metals with bivalency for example cadmium, mercury lead, or platinum using, thiol groups of cysteine filtrates which helps in the detoxification of cell.²⁰

This study comprehensively focused on the function of MTs in OS progression along with chemoresistance and shows evidences that we may use MTs as a potential prognostic/diagnostic biomarker which further open new approaches in the treatment of OS.

2. Etiology of osteosarcoma

There has been a lot of research into the etiology of OS but still, its exact cause is unknown. There are few risk factors that have been associated with OS like growth, somatic alterations, germline genetics and environmental factors. All these factors increase the risk of developing OS (Fig. 4).²¹

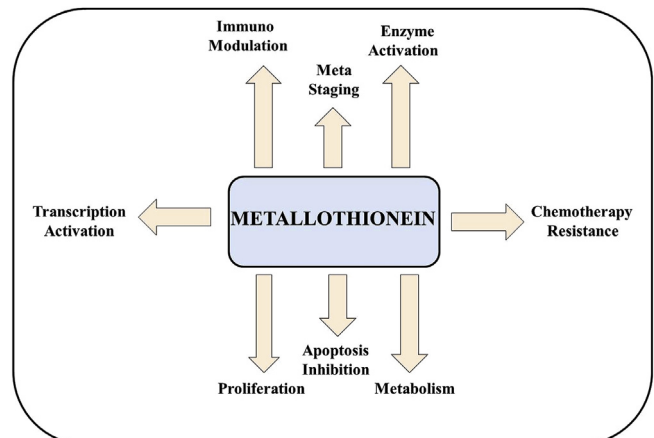


Fig-3. Metallothionein involvement in various carcinogenesis related processes.

3. Pathophysiology

The origin of OS is the mesenchymal cells and their histological hallmark is based on the production of malignant osteoid. Inactivation of P53 and RB pathways is a major event in OS genesis.²² Once mutation of DNA occurs, it activates the oncogene which leads to a deactivation of the suppressor gene and produces malignant osteoblasts that lead to a proliferation of abnormal osteoblasts. This causes the formation of malignant osteoid tissue. The osteoid tissue results in uncontrolled growth of the tumor that causes overgrowth of malignant osteoid tissue which causes suppression of red bone marrow.

Suppression of bone marrow causes decrease in blood cells which further weaken the immune system and make susceptible the body to severe infections as the body is unable to produce leukocytes against pathogens. A decrease in RBC leads to anemia, fatigue, weakness, and a decrease in platelets leads to bleeding and bruising. The overgrowth and crowding of osteoid cells generates pressure inside the bone which is responsible for the pain and pathological fractures (Fig. 5).²³

4. Metallothioneins in tumors

There are many evidences in existing literature in support that the MTs significantly involved in oncogenesis. Numerous studies has shown expression of MTs as a suitable prognostic indicator or marker for tumor and chemo resistance in a various malignant cancers like ovarian, breast, prostate, head, neck, melanoma, and other soft tissue sarcoma.²⁴ MTs expressions level varies from cancer to cancer like in human tumor of larynx, pancreas, kidney, uterus, and breast, the expression of MTs was higher whereas lower in case of liver tumors, further in case of thyroid, prostate, stomach, lung, and central nervous system tumors, variable MTs expression was observed.²⁵

5. Metallothioneins in nontumor condition

Krizkova et al. reported that MT genes and their isoforms are observed in persons with aging, obesity, and osteoporosis. After further study he also revealed that the same protein is also expressed in many other disorders too including, cardiovascular diseases, diabetes and etc.²⁵ Several recent findings on MT also have revealed their expression in neurodegenerative diseases other than Alziemer like Amyotrophic Lateral Sclerosis, Parkinson's Disease, brain trauma, brain ischemia, prion disease, and psychiatric

diseases.²⁶

6. Metallothioneins and osteosarcoma

The MTs are intracellular proteins. The participation of MTs in several physiological and patho-physiological processes like angiogenesis, apoptosis, proliferation along with detoxification of heavy metals emphasizes the involvement of these MT isoforms in carcinogenesis and tumor therapy. The MTs in tumor cell plays a vital role in defense mechanism against the effects of radiotherapy by inhibiting the apoptosis. The MTs are also known for their roles in the process of detoxification, drug-neutralization and favoring cell survival.²⁷ Furthermore, in bone tumors, increased level of MT was detected in serum of patients with OS and in comparison with normal healthy controls.²⁸ However, in biopsies of tumor same compared with non-malignant bone biopsies.²⁹ In addition, Habel et al. in their study straightly reveal the contribution of MT2A to chemotherapy resistance in OS patients.³⁰ Till now neither the metastasis of OS nor the chemoresistance in OS explained completely. However, the immunology based biological study of chemo resistant and metastatic lesions might be the reason of this.³¹

Chemoresistance having the multifactorial causes is the commonest reason for death in patients with OS (OS). Exclusion of cisplatin cells is the major cause of drug resistance.^{32,33} However, one another proposed mechanism having involvement of MT rich in thiol group. These MT are the proteins of cytoplasm bearing higher percentage of thiol groups and are rich in cysteine that binds to various heavy metals cytotoxicity, such as cisplatin.³⁴ Several studies had been suggested the role of MT in drug resistance^{35–37} and transcriptomic analysis of elevated MT1L as well as MTIG in weakly approachable OS tumors.³⁸

In 2013 a review by Martina et al. concluded all observations about polymorphic role of MT for person MT1-2-3 & MT4 along with relation to processes based on pathology, & application of MT-protein as a biological indicator for further pathological and analytical studies.³⁹ Krizkova et al.,²⁵ in 2010 showed a 5-fold increase in level of MT in the serum of patients having cancer disease in comparison with serum of normal healthy donors. This study indicates that the MT level in blood serum can be recognized as a potential bio-marker of diagnosis, prognosis, with estimation of efficient therapy of pediatric tumors. Furthermore, Habel et al.,³⁰ in 2013 observed over expression of MT2A that induces chemoresistance to drugs via chelating platinum based drugs. They showed the contribution of MT2A in resistance due to

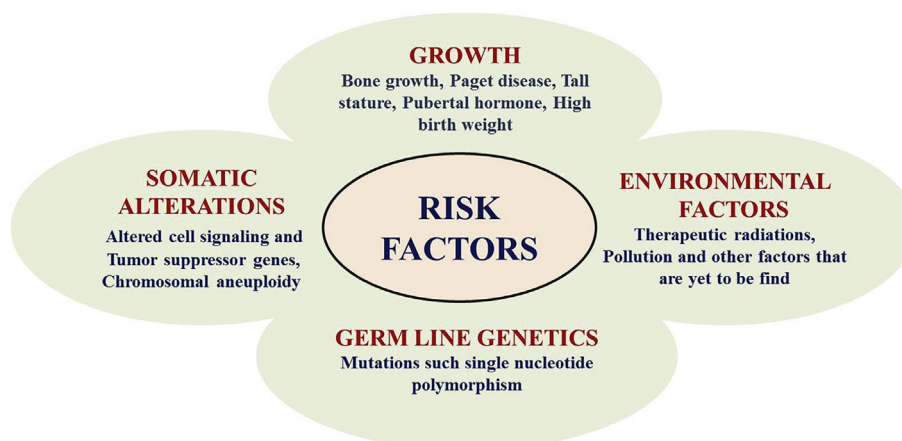


Fig-4. Different predisposing risk factors of Osteosarcoma.

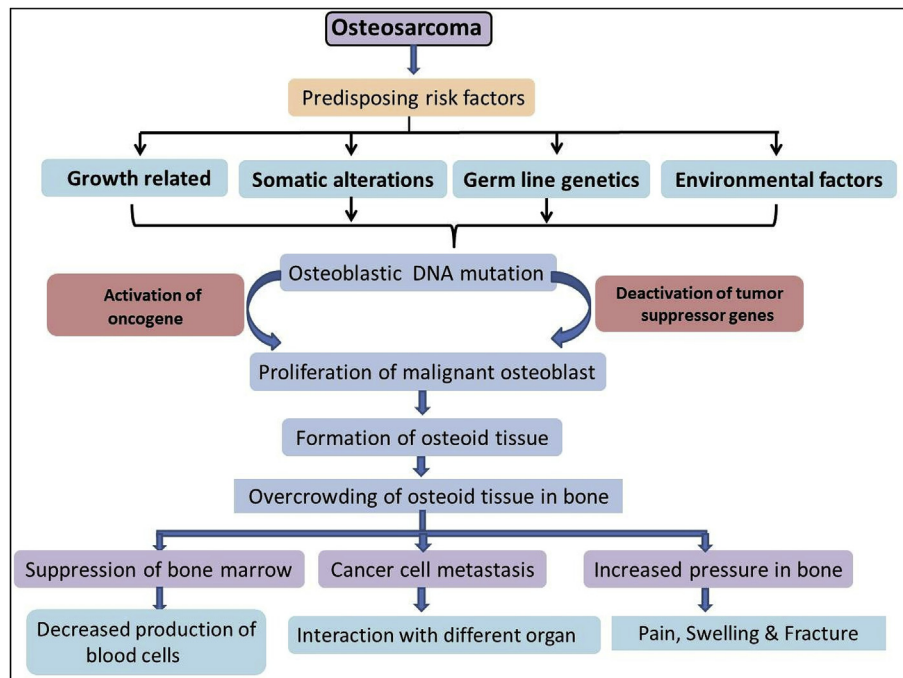


Fig-5. Pathophysiology of osteosarcoma.

chemotherapy in OS, and its effects are mediated by zinc chelation. In 2010 Endo-Munoz et al.,²⁹ observed up-regulation of MT family members, i.e. MT1-E, MT1-H, MT1-X, MT1-B, MT1-G, MT1-L and MT2 or MT2A, in samples of OS. Out of these few were common in ten highly up-regulated genes. However, there was no connection was observed among their response and expression.

In 1998 Shnyder et al.,⁴⁰ observed that there is no particular difference was observed in the survival rates of MTs & P-gp expressers or non-expressers, i.e. no relation among before chemotherapy expression of protein and survival of OS patients with both expression or non-expression of the MTs & P-gp phenotypes. In 1997 H. Uozaki et al.⁴¹ observed that four drug-resistant proteins (GST π , LRP, MTs and Hsp27) expressed immuno-histochemically to find an additional prognostic marker of OS.

As per the recorded data by him out of fifty-four biopsies, (43%) 23 cases were found with overexpression of MTs, (76%) 41 cases were found with overexpression of GST π , (24%) 13 cases were found with overexpression of Hsp27 and (17%) 9 cases were observed with LRP. However, the expression based analytical-comparison of protein levels during biopsies and surgical resections of specimens explained increases in expression of LRP protein ($p = 0.006$), whereas a significant decrease in GST π expression ($p = 0.017$) and insignificant alteration was noted in the expression of MTs and Hsp27.

The physiological involvement of MTs in the effects of anti-cancer drugs is not well explained yet, and therefore further analytical studies relating the MTs and chemotherapeutics of OS patients are in demand. However, there are few studies suggestive of the role MTs over-expression in tumors as well in sera of the OS patients contributes to chemotherapy resistance.

7. Establishing the Osteosarcoma's diagnosis

The diagnosis of primary OS is achieved via imaging (plain radiographs of the infected bone and adjacent joint). However, the ultimate conclusion depends on histo-pathological analysis via biopsy. OS promptly appears as a mixed radio dense and lytic lesion

arising in an eccentric manner from the metaphyseal bone. The cortical devastation and periosteal observation are common and typically manifest in a sunburst pattern.⁴² Approximately 75% of OS arise in the intramedullary cavity and are referred to as "classical" or "conventional" OS. Histological analysis of OS explained that the spindle or polyhedral mesenchymal cells with, scattered mitotic figures, pleomorphic nuclei and varying levels of anaplasia expresses malignancy. Pre-mature and disorganized production of osteoid is a characteristic hallmark and therefore must presented for diagnosis.

Conventional OS is dependent of the extracellular matrix. Further it is sub-divided into types of osteoblasts, chondroblasts, and fibroblasts.⁴³

8. Current trends in the management of osteosarcoma and their major challenges

8.1. Mechanism of chemotherapy

Chemotherapy (chemo) is a treatment based on combination of cancer drugs. The aim of chemo is to pause or slow-down the growth of cancer cells. Chemotherapeutic drugs interfere with a cancer cell's ability to reproduce. Treatment of OS is employing neo adjuvant chemotherapy followed by antagonistic and purposeful surgical resection in order to maintain the negative margins and a prolonged course of adjuvant chemotherapy.⁴⁴ In between the year 1960s–1980s the cure in OS was escalated from <10% to 60%–75%, by the introduction of chemotherapy. Chemotherapy for OS is usually given intravenously. Chemotherapy is usually given before surgery to reduce the tumor size and in case given after surgery usually to destroy the tumor cells that may left in the body. Standard chemotherapeutic agents in OS are cisplatin, doxorubicin, methotrexate, and ifosfamide.⁴⁵ Cisplatin binds to DNA of the tumor and inhibits the synthesis via the DNA crosslinking. Doxorubicin intercalates with the uncoiling of the DNA double helix and inhibits DNA and RNA synthesis.

Methotrexate is a folate anti metabolite which obstructs the

Table 1
Different Mechanism of Chemotherapy Resistance.^{57–66}

	MECHANISM	REFERENCES
CHEMOTHERAPY RESISTANCE	Drug Accumulation	Effective inhibition of dihydrofolatereductase due to alteration in transport of MTX. ⁵⁷
	Drug Inactivation	The over expression of GSTP1 protein is practically induced by chemotherapy that leads to chemoresistance and associated with a poor prognosis. ^{58,59}
	DNA repair	Knockdown of endonucleases XPF and XPG, the two NER factors, minimises the efficiency of cisplatin- and oxaliplatin-damaged DNA-repair in OS cells (U2OS) by up to fivefold. ⁶⁰
	Signal Transduction Pathway	The serine-threonine kinase, mTOR (rapamycin), plays a vital role in the regulation of protein translation, cell growth and metabolism. The mTOR signaling pathway is being used as a therapeutic target as variation of the mTOR pathway are usual in cancers, like OS. ⁶¹
	Apoptosis	P53 gene, which plays a significant role in cell cycle arrest and apoptosis, has been confirmed to be occupied in the modulation of anti-cancer drug based cytotoxicity. ⁶²
	Autophagy	Inhibition of autophagy by knockdown can effectively enhance tumor cell death induced by diverse anti-cancer drugs in pre-clinical models. ⁶³
	Drug resistance due to Cancer Stem Cell microRNA	Many studies had revealed the implications for Cancer Stem Cell (CSC) in the drug resistance and tumor metastasis of OS. ⁶⁴ Altered miRNA expression has been indicated to be associated with various cancers. ^{65,66}

synthesis of purine and pyrimidine (thymidylc) acid by binding dihydro-folate-reductase and ifosfamide resulting, crosslinking of DNA strands and blocked protein synthesis.

Within the cytoplasm, MTs contains the maximum amount of thiol-groups. The capacity for binding of these thiol groups is higher for various agents showing cell-cytotoxicity, such as compounds of Pt and other agents with alkylation bindings. Elevated MT level are machinery of anticancer drug resistances, as internal cytoplasmic MT-binding checked the interaction of dynamic molecules with their target and the intra-nuclear Deoxy Ribo Nucleic acid of tumor cells.⁴⁶ The observation of MTs has been linked to chemoresistance of carcinoma cells,⁴⁷ cancer cell lines⁴⁸, tongue squamous cell carcinoma cell lines,⁴⁹ gastric tumor cell lines⁵⁰ or ovarian carcinoma cell lines.⁵¹ All these studies suggest the importance of MT in the mechanisms of chemoresistance.

8.2. Osteosarcoma treatment and major challenges

OS is a systemic disease of bony region which affect whole body and got lethal if left untreated. Now a days the advancement and improvement in the chemotherapy, diagnosis, and surgery have made a important change from amputation to salvage surgery for tumor resection.^{52–54}

Presently standard and potential treatment involves both surgical treatment and chemotherapies. In the surgical part, limb-salvage surgery is a standard treatment for OS that majorly preserves a limb.⁵⁵ Moreover due to chemotherapy treatment, the survival rate of OS patients is constantly improving. However in few metastatic cases, survival rate of OS patients remained unchanged.⁵⁶ Chemoresistance in OS patients is might be due to several mechanisms like-decreased intra-cellular accumulation of drug, inactivation of drug, abnormalities of DNA repair, hindrance of signal transduction pathways, auto-phagy based chemo resistances, micro RNA off functionality and drug resistance based on cancer stem cell [Table-1].⁵⁶ However, amongst the above said mechanism, the resistance of drug is the major factor that limits the efficiency of chemotherapy, might acquire the resistance during treatment or may be interestedly resistant.⁶⁷

9. Future prospects

Till date, despite of using combination based on multi-chemotherapy, OS still remains, drug-resistant and recurrent tumor that affects the survival rates. Furthermore, real-time effect of chemotherapy to a patient is also lacking that might bias the treating surgeon treatment protocol by unable to choose the most potential chemo-agent drugs. Therefore, for a potential chemotherapy,

identification of biomarkers of chemosensitivity in OS patients is desperately required that also might reveals the impact each cycle of chemotherapy in real-time at molecular level. Many previous comprehensive studies have identified numerous genes that may possibly involve in the development or progression of OSs, and represent candidate biomarkers and/or drug targets. However, in clinical applications, presently, there is no specific marker available for predicting the prognosis and chemosensitivity of OSs. As the chemotherapeutic drugs generally contain the metals like cis-platin which inhibit the molecular pathway of targeted cell, the MT genes which are the major metal binding protein may have a potential role in OS chemoresistance and its analysis could provide a new method of selecting the treatment strategy and can provide novel therapeutic targets of OS.

10. Conclusion

It is believed that MTs actively participate in carcino-genesis and initiated chemo-resistance in tumor cells. The up-regulated level of MTs offer inhibition against apoptosis and promote cell proliferation, hence promoting tumourigenesis.

Pubmed, the established NCBI search engine was explored with search strategy: “Metallothionein osteosarcoma” for eligible studies published in English and the search resulted in astonishing twenty hits out of which five were included in this paper. In reference to the available literature, we may conclude that MT has a role in OS progression and chemoresistance. However further multicentric studies are required to prove, “MT as a prognostic marker of OS” as the existing literature are not sufficient and contradictory.

Conflicts of interest

There are no conflicts of interest to declare.

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