Review Article

Resistance mechanisms in the radiation therapy of osteosarcoma: a brief review

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Abstract

Osteosarcoma is the most common primary malignancy of bone, typically presenting in the first or second decade of life. Osteosarcoma is a very aggressive type of tumor, which has devastating effects on the patient. Especially, pediatric patients suffering from osteosarcoma, are very vulnerable to its side-effects. Most patients suffering from osteosarcoma have a very poor prognosis and thus the understanding of its mechanisms, both oncogenetic as well ontogenetic are of crucial importance. The stagnancy of clinical outcomes may is explained by heterogeneity and complexity as well as genetic background. Not many studies have dealt with the molecular mechanisms of resistance to radiation therapy in osteosarcoma, yet the main genes proposed for their participation include p53, p21, NF-κB, RAS, Rb and GRIM-19. In addition, recent studies have highlighted the role of epigenetic mechanisms in osteosarcoma resistance to therapy and in particular the role of miRNAs. Due to the devastating effect of the disease to the suffering patients, more studies are required in order to unravel the radiation-induced cell death resistance.

Keywords: Osteosarcoma, Radiological features, Resistance mechanisms, Genomics

Introduction

Osteosarcoma (OS) or Osteogenic Sarcoma is a malignancy of the bone. It is a very aggressive type of tumor derived from mesenchymal cells exhibiting osteoblastic differentiation[1]. OS is the most common primary bone cancer, which is characterized by high-grade malignancy of the mesenchymal cells that produce osteoid and immature bone tissue. OS occur mainly at the sites of bone growth, which is linked to osteoblast proliferation and thus it is probable that cells acquire genomic aberrations as well as epigenetic changes. They are the second most common primary bone tumor after multiple myeloma, the most common of mesenchymal tumors and the most common primary malignancy of bone in children and adolescents.

Epidemiology

Osteosarcomas can be either primary or secondary and these have differing epidemiological data. In particular, *Primary Osteosarcoma* (POS), occurs in young patients (10-20 years) with 75% taking place before the age of 20 because the growth centers of the bone are more active during adolescence with male prevalence[2]. In addition, *Secondary Osteosarcoma* (SOS) occurs in elderly patients, usually secondary with preexisting *Paget's* disease, post-radiotherapy for other conditions, extensive bone infarcts, osteochondroma and osteoblastoma[2].

Location

Osteosarcoma's location with regard to primary tumors is typically unique to intramedullary, metaphyseal regions of

long bones, and has an inclination to the knee joint, with a percentage up to 60% occurring there. On the other hand, secondary tumors have a higher frequency in flat bones, especially the pelvis, as in Paget's disease. Specifically, common regions include *femur, tibia, humerus* and other less common regions include *fibula, innominate bine, mandible, maxilla* and *vertebrae.*

Pathology

According to the degree of differentiation, location and histological variants, osteosarcomas can be divided in subtypes. These subtypes diversify in x-ray imaging findings, epidemiological data and biological behavior, and are ranked as follows: The first category is the *Intramedullary* OS, which consists of 80% of all OS cases. It is a high-grade OS and includes the *Telangiectatic Osteosarcoma* (TOS) and *Low Grade Osteosarcoma* (LGOS). The second category is the *Surface* or *Juxtacortical* OS (JOS) which makes up to 10-15% of all cases and can be further subdivided to the subcategories of *Intracortical* OS (IOS), *Parosteal* OS (PAOS) and *Periosteal* OS (PEOS). The third and final category is the *Extraskeletal* OS (EOS), which is rarer and consists of 5% the total OS cases[3].

Histology

Osteosarcomas are related to increased cellularity and intense cellular atypia with insignificant production of osteoid (radiologically as osteolytic lesions). Other histological findings are the most differentiated spindle-shaped cells and production of osteoid or immature bone tissue (radiologically as osteosclerotic lesions).

The clinical findings are based on recent onset of progressively aggravated pain and tumescence of affected area. It is possible to be observed pathological fracture, restricted mobility of the proximal joint, low-grade fever and elevated levels of alkaline phosphatase.

Radiological features

Plain radiograph

The findings in plain radiograph are intramedullary osteolytic lesion with partial osteosclerosis, possible infiltration of cortex and extension to soft tissue. The matrix is not observed in plain radiograph at 10%. The limits of the damage are unclear and the periosteal reaction is characterized by aggressive morphology, usually *Godman* triangle.

Computed tomography

The role of computed tomography adds little to plain radiograph, with regard to long bones.

Magnetic resonance imaging

The magnetic resonance imaging is a complementary method for showing intramedullary extension (skip lesions), extension to the pineal gland and joint, as well as possible infiltration of neovascular structures at extraosseous level.

Differential diagnosis

The differential considerations include Ewing sarcoma, cortical desmoid, aggressive osteoblastoma, osteomyelitis, metastatic lesion to bone and aneurysmal bone cyst.

Treatment

The treatment of osteosarcoma is complex and it constitutes a challenge for therapeutic decisions. The factors that render this type of tumor complicated are the variance in primary tumor locations, extent of metastatic disease, surgical possibility, growth rate, molecular features and existence or not of relapse. The types of treatment include surgery, chemotherapy and radiation therapy. The necessity that turns out is conclusive resection of the primary tumor and this is accomplished in an array of patients. Furthermore, it is attainable the combination of neoadjuvant chemotherapy and complete surgical resection, followed by adjuvant chemotherapy. Consequently, progress establishes imperative need the association of osteosarcoma and genetics.

Genomic mechanisms of resistance to radiation-induced apoptosis in osteosarcoma

Early studies of osteosarcoma resistance

The mechanics of radiation-induced apoptosis resistance has been a subject of investigation for the last 50 years. Early studies have attempted to identify resistance mechanisms to radiation in OS through the acquisition of immunity against radiation[4,5]. These studies have hypothesized that murine models of OS could be sensitized to radiation therapies if immunized with antibodies against OS. Further studies have found that sarcomas and the subsequent radiation therapy, not only produce resistant sarcoma tissue but also peripheral lymphocytes acquired resistance to co-administrated chemotherapeutics[6]. Similarly, an older study has highlighted that osteosarcoma, along with melanoma, manifested one of the most resistant cell type against radiation in an *in vitro* cell culture system[7]. A similar report has investigated the resistance mechanisms of sarcoma in *in vitro* cell culture systems, where it was found that one of the main problems of radiation therapies was the acquisition of resistance and most importantly this resistance was acquired very fast[8]. These early studies have outlined the very problem with radiation therapy in OS that is the resistance of the tumor to the complementary radiation. One of the main reasons suspected for sensitivity to radiation therapy was the formation of Reactive Oxygen Species (ROS) due to the application of radiation[9-11]. This was considered to be one of the main cytotoxic agents in radiation-induced apoptosis, yet it was later suggested that apoptosis was also occurring due to the DNA damage produced.

The Role of RB and TP53

The high degree of heterogeneity explains the difficulty that prevents the understanding of molecular pathogenesis of osteosarcoma. Osteosarcoma, is deprived a standard translocation or genetic mutations. Several genes have been investigated for the mechanisms of radiation-induced resistance in osteosarcoma. Examples of such genomic mechanisms include the tumor suppressor gene Rb, or the retinoblastoma protein[12]. Rb is a tumor suppressor protein that is dysfunctional in several major tumors. The retinoblastoma plays an important role in cell cycle progression and participates in restricting the G1-S transition. The ability of Rb is to prevent excessive cell growth and when Rb is phosphorylated to pRb inactivating the protein which allows the cell cycle progression. Essentially, osteosarcoma is characterised by inactivation of both Rb and p53 (TP53)[13].

TP53, or tumor protein 53, is a transcription factor that regulates critical genes in DNA damage response, cell cycle progression and apoptosis pathways[14]. TP53 acts as a tumor suppressor in all tumor types. In normal cells, the TP53 levels are low[15]. The cellular concentration of TP53 must be regulated. As opposed to the ability to suppress tumors, high level of TP53 may accelerate the process by excessive apoptosis. The major regulator of TP53 is MDM2 that acts as a negative regulator and can trigger the degradation of p53 ubiquitin system[16]. If the TP53 gene is damaged, tumor suppression is severely reduced. People who receive germline mutation of TP53 will probably develop osteosarcoma in early childhood, a syndrome known as *Li-Fraumeni* syndrome.

GRIM-19

The gene associated with retinoid-interferon mortality (GRIM-19) is a tumor suppressor that mediates cell apoptosis in multiple cancer types. The role and the mechanism of GRIM-19 in osteosarcoma remains unclear. However, it is recently reported that GRIM-19 was found to be downregulated in OS, while radiation-induced apoptosis in OS is tightly linked to TP53 upregulation, whose down-regulation infers radiation-induced apoptosis resistance[17]. Thus, it has been shown that GRIM-19 upregulation alleviates the resistance of OS to radiation therapy *in vitro*. Interestingly, previous reports have highlighted the role of GRIM-19 in the mechanisms of glucocorticoid-induced apoptosis resistance, in acute lymphoblastic leukemia[18].

The Role of P21/RAS

Later on, the concept of radiation resistance has become more concise as it has become more evident that several genes were responsible for the acquired resistance to radiation therapy. In particular, older studies have found that the RAS family of genes participate in radiation therapy resistance. In particular, it was found that RAS overexpression and p21 were observed in resistant osteosarcoma cells. This finding was supported by the fact that the inhibition of p21 through levostatin, lead osteosarcoma cells to radiation therapy sensitization[19]. A similar study manifested that RAS overexpression was directly linked to radiation resistance[20]. Another report suggested that radiation resistance is accompanied by chemo-resistance in osteosarcoma, but also it has shown that different sarcomas manifested different levels of chemo- and radiation-resistance[21]. In concordance with these studies, it was again confirmed that lovastatin functioned as a p21/RAS membrane association inhibitor and restored the sensitivity to chemotherapeutics such as doxorubicin, a very potent chemotherapeutic, and hydrogen peroxide as well as to radiation[22].

The role of TP53

One of the first studies in osteosarcoma cells included the role of p53. In this study, human osteosarcoma SAOS-2 cells, which have a deletion in p53 gene, were transfected with a human p53 cDNA[22]. Among the produced clones, three clones (SAOS-MC10, SAOS-MC11 and SAOS-MC43) manbifested p53 mRNA expression[22]. Interestingly, the p53 protein in SAOS-MC43 was lower than that in SAOS-MC11, which suggested that they expressed a mutated p53 variation. Thus, consequently it was observed that SAOS-MC11 and SAOS-MC43 were more sensitive and more resistant, respectively, to radiation-induced apoptosis than the parental SAOS-2 cell line. The findings of this study suggested that the exogenous p53 protein was responsible for the observed radio-sensitivity in the OS cells[22,23]. In more recent reports, it has been found that evasion of p53 function and in particular through the loss of ASPP1 and ASPP2, two proteins that function as p53 enhancers. It has been found that loss of those two proteins conferred resistance to radiation and chemotherapy in breast cancer and sarcomas[24]. The interesting finding was that loss of ASPP proteins could function as a resistance mechanism only in wild-type p53 proteins and not mutants, indicating that ASPP protein loss is an alternative pathway through which cancer cells acquire resistance. These findings brought about the significance of p53 and further studies have attempted to look deeper into its role. These studies confirmed that p53 mutations contribute to the cancer cells' resistance mechanisms[25,26]. Further on, in previous studies it was found that it is not solely the presence of mutations on p53 that played a role in its anti-tumor property alleviation but also its post-transcriptional modifications such phosphorylation. Thus, it has been shown that both the wild-type as well as the mutant p53 can be post-transcriptionally phosphorylated and hence modified as such not to function properly as tumor-suppressor protein[27]. In this same study it was shown that Ser(392) non-phosphorylatable p53 mutants p53H175A392 and p53W248A392 more potently transformed rat embryo fibroblasts in cooperation with the ras oncogene than p53H175S392 and p53W248S392. p53H175A392 also had an enhanced ability to confer cellular resistance to the cytotoxic effect of cisplatin and UV radiation[24]. Finally, more

recent studies have shown that albeit p53 is a necessary element in tumor-suppression and thus its malfunction a basic mechanism for radiation therapy resistance, as well as chemotherapy resistance, in osteosarcoma[28-30].

The role of NF-κB

NF-κB is one of the most studied transcription factors for its inflammatory properties but also for its participation in tumor-resistance phenomena. Yet, a previous report has shown that Ultra-Violet (UV) radiation activates NF-κB towards its DNA-binding function but it is transcriptionally inactive, suggesting that this could be taking place through p53 activation and subsequent p3O0/CREB co-activation[31]. However, more recent studies have highlighted that NF-κB activation is equivalent to tumor resistance in both chemotherapy as well as radiation therapy[32,33]. These findings was confirmed by very recent reports, where it was found that osteosarcoma acquired resistance to therapy through the simultaneous activation of the Akt/NF-κB pathway[34,35]. In a very recent report, an explanation was given for that effect which included the evidence that osteosarcoma resistance comes about due to BMI-1 over-activation and its inhibition alters the resistant phenotype and turns osteosarcoma cells to pro-apoptotic and anti-proliferative behavior[36].

miRNAs and osteosarcoma resistance

One of the latest discoveries in the mechanics of osteosarcoma-resistance mechanisms is the role of miRNAs. The first reports on osteosarcoma biology and miRNAs concerned their role in metastatic mechanisms as for example, its ability to metastasise to the brain[37]. A very known miRNA, miR-34a, has been reported to play a role in many tumor types and in the case of osteosarcoma it was found that it promotes multi-drug resistance as well as radiation resistance by binding the CD117 gene[38]. A similar finding suggested that miR-184 promotes Doxorubicin resistance by binding to BCL2L1 gene, a pro-apoptotic gene. In this study, it was shown that miR-184 over-expression leads to poor response to Doxorubicin and probably radiation therapy[39]. Further on, it has been found that miRNAs play a role in the microenvironment of mesenchymal and stromal cells, participating in osteosarcoma migration and metastasis[40]. Similarly to the previous studies, the most recent one suggested that miR-590 mediates chemoresistance and radiation resistance by inhibiting the p65 protein[29].

Conclusions

Osteosarcoma is a very aggressive type of tumor, which has devastating effects on the patient. Especially, pediatric patients suffering from osteosarcoma, are very vulnerable to its side-effects. Most patients suffering from osteosarcoma have a very poor prognosis and thus the understanding of its mechanisms, both oncogenetic as well ontogenetic are of crucial importance. The molecular mechanisms concerning osteosarcoma progression, prognosis, diagnosis and

therapy are still largely unknown. More studies are needed towards that direction, in order for us to further understand how this type of tumor functions. Radiation therapy is a very important part of osteosarcoma therapeutic protocol and unfortunately resistance to radiation therapy is very easily acquired by these cells. It is still unknown why this happens, yet more studies are required towards that direction.

Authors' contributions

GK: Reviewed literature, drafted manuscript, reviewed manuscript, proof-edited the manuscript. GIL: drafted the manuscript, proof-read the manuscript and gave final permission for publication.

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