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Involvement of Giant Cells in the Development of Bone Tumor

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Abstract

Osteosarcoma (OS) or Osteogenic sarcoma is a commonly occurring primary bone tumor that occurs in young age including adolescence age group and contributes approximately about 20% of all the other sarcomas. Giant cell rich osteosarcoma (GCRO) is considered as a rare sub-type in the category of primary osteogenic sarcoma. GCRO sarcoma possesses giant cells that look like osteoclasts in abundance and insufficient amount of osteoids are also present. GCRO excludes several features related to classical radiographic aspects in conventional osteosarcomas. This results in its pivotal importance in recognition as a subspace of osteosarcoma and its distinction from other meticulously related tumor in bone. GCRO can be either benign or malignant osteolytic tumor that can be observed on the plain radiographs. Furthermore, giant cell tumor (GCT) whether benign or malignant, in case of histological differentiation is challenging and is highly important because of its aggressively fatal consequences. The GCT contributes nearly 5% of primary bone and the tumor bone is commonly found in the end points of the long bone. In this review, we attempted to recognize and summarize the involvement of giant cells in the development of bone tumor and describe some important gene expressions to get an insight about the strategies to control this type of bone tumor.

Keywords: Osteosarcoma; Giant cell tumor; Giant cell tumor of bone; Giant cell tumor stromal cells; *p63*; Histone H3.3; RANK ligand

Abbreviations: OS: Osteosarcoma; GCRO: Giant Cell Rich Osteosarcoma; GCT: Giant Cell Tumor; GCTSC: Giant Cell Tumor Stromal Cells; RANK: Receptor Activator of Nuclear Factor Kappa B; RANKL: RANK Ligand; MFR: Macrophage Fusion Receptor; IHC: Immunohistochemistry; DC-STAMP: Dendritic Cell Specific Trans-Membrane Protein; GCTB: Giant Cell Tumor of Bone; M-CSF: Macrophage-Colony Stimulating Factor; PMMA: Polymethylmethacrylate; OPG: Osteoprotegerin

Introduction

Osteosarcoma (OS)

Osteosarcoma is enlisted among the most familiar types of malignant tumor of bone in adolescents and young adults. U.S. has the highest number of cases approximately four per million per year [1]. A recent report highlights a case of dominant type of sarcoma that gets unfolded in a tumor in giant cells in a bone pinned down with the treatment of denosumab [2]. Primary neoplasms of bone are proved to be very much rare that involves 0.2% of overall human tumor fatigue. Osteosarcoma is mostly observed as primary non-hematopoietic malignant bone tumor that involves the production of osteoid matrix emerging through cancerous cells [3].

On the basis of demographics, osteosarcomas can be classified into two categories, primary and secondary. These are intramedullary or centrally positioned in long tubular bones and are of high grade mostly. **Primary osteosarcoma** is observed in highly young patients (10-20 years) and 75% cases occur before the age of 20 years due to some important locations called as the growth centers of the bone that are much more active during puberty/adolescence. These type of tumors are typically observed in the vital metaphyseal regions of long bones, showing an astonishing propensity for the knee involving around 60% of presence. **Secondary osteosarcoma usually** occurs in the old age and is commonly secondary to malignant declension in case of Paget disease, pervasive bone infarcts post-radiotherapy for other conditions, osteochondroma and osteoblastoma [4]. These tumors undergo a much wider dissemination including the combined scope of their underlying condition, and thus have a greater occurrence rate in flat bones, especially the pelvis that is the most suitable site for Paget disease. In case of clinical presentation, patients usually come up with bone pain and soft-tissue mass or swelling sometimes [5]. Osteosarcoma is diagnosed prominently once it shows up in its classic or conventional form. The emergence of osteoid directly by the tumor cells, called as tumor osteoid or malignant osteoid, is actually required for the high level diagnosis of osteosarcoma. The genetic contradictions are highly complex and variability is shown at a very high rate. The p53 analysis can be implemented to prognosticate the individual cases with the help of tumor tissue. Immunohistochemistry (IHC) cannot be applied in osteosarcoma due to insufficient proof in data as per the reports [6].

Giant cells (GCs)

The initial research studies consider the tumor to be much more cellular and reasonably vascularized and consisted of circular oval or somewhat spindle mono-nucleated cells containing huge conglomeration of giant cells with multiplex nuclei [7]. The stromal cells include faint cytoplasm possessing an indefinite extremity, a nucleus having an explicit nuclear membrane and a routinely protruding nucleolus. The multi-nucleated giant cells possess certain nuclei that are collateral to the nuclei in stromal cells, physically/biochemically and practicably resemble to osteoclasts. The giant cells of this type are usually observed in other several primary sarcomas related to bone, in conjunction with osteosarcoma, dedifferentiated chondrosarcoma, uniform pleomorphic sarcoma, fibrosarcoma and leiomyosarcoma [8]. Giant cells are mostly composed of polyploid complements of

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chromosomes that maintain the balance between chromosomes, irrespective of the changes in total chromosome numbers. If there are more than one chromosomes present in unequal numbers, there could be the possibility of a type of deformity known as aneuploidy, which is commonly observed in several types of cancerous diseases [9,10]. The inflation in the capacity/size of other cellular structures is because of enlargement of a nucleus of the cell. Thus, in order to perpetuate the orderly ratio of the cell size, the entire cell will be leveled accordingly. The nonappearance or incompleteness of mitotic phase leads to the formation of giant cells. These giant cells normally emerge either by one or a coalescence of three distinctive methods: (a) mitotic catastrophe, (b) cell fusion or (c) endo-replication process [11]. Giant cell is basically a multi-nucleated, large in size, formed as a result of a fusion of various cells such as macrophage, epithelioid cells, monocytes, etc. These types of cells are mostly found at the site of chronic inflammation and other granulomatous conditions. The phenotypic variation in multinucleated giant cell types is based on the confined ambiance and the physicochemical nature of the source, to which, the giant cell types and their monocyte/macrophage precursors signal back [12]. According to recent reports on foreign body giant cells and osteoclasts specifically, a number of common factors such as; macrophage fusion receptor (MFR), an adhesion protein, vitronectin, dendritic cell specific trans-membrane protein (DC-STAMP) and a fusion factor play an effective role in the formation and function of giant cells. These giant cells are pathologically a valuable asset and are used as important diagnostic tools in osteology [13]. The four peculiar types of multinucleated giant cells have been studied so far. These are; Mycobacterium-induced granulomas related giant cells, different giant cell tumors of bone, formation and function of osteoclasts, and giant cell formation and function of foreign body. These giant cells can be analyzed in different ways such as irradiation analysis and other methods based on gene mutations etc [14].

Giant cell tumor of bone (GCTB)

Giant cell tumor of bone (GCTB) being sectionally rapacious neoplasm contributes to about 5% of the overall primary bone tumors. This type of tumor plays a vital role in making an effective impact on radiographic and histopathologic demonstrations involving distinctive benign, malignant and metabolic abrasions rich in giant cells [15]. Giant cell rich osteosarcoma term was initially explained by Bathurst and Sanekin in 1986 [16]. An unusual case of osteosarcoma occurs that accounts for nearly 1-3% of overall cases in conventional osteosarcomas. Conventional osteosarcoma is largely found in young adults. It mostly shows up at the beginning of 20th year of life and almost about 60% of the patients are of 25 years of age. Conventional osteosarcoma shows a fervent tendency in the participation of elongated bones of the appendicular skeleton; especially, distal femur, proximal tibia, and proximal humerus [17]. Few reports elucidate malignancy in giant cell tumor as a sarcoma that emerges in tumor of giant cells. A sarcoma that emerges in a tumor linked to giant cells is considered as primary malignant giant cell tumor and the one that emerges at the site of the antecedently diagnosed tumor in giant cells is labeled as secondary malignant giant cell tumor. Sarcomas that emerge in primary giant cell tumor can be malignant fibrous histiocytoma or fibrosarcoma. These can be secondary malignant giant cell tumor as well [18]. A new category of secondary malignant giant cell tumor was reported which described carcinosarcoma to be a malignant abrasion in case of giant cell tumor. The tumor of giant cells of bone is usually seen in meta epiphyseal arena that gets developed post skeletal maturation [19]. In case of tumor formation at the starting point, mono-nuclear histiocytic cells effectively play an important role at the encampment of the tumor and fusion occurs to process multi-nucleated giant cells (MGCs) tumor. The stromal cells of neoplastic tumor of giant cells express the activator of receptor in nuclear factor κB ligand (RANKL) thus supporting the unification with macrophage colony stimulating factor (M-CSF) that acts as a cofactor [20].

Giant cell tumor in general consists of mononuclear histiocytic cells, giant cells with multiple nuclei enclosed in monocytic-histiocytic system and cells of neoplastic tumor that have high proliferation rate, also known as giant cell tumor stromal cells (GCTSC) with no involvement in the monocytic-histiocytic system. A case in which soluble factors from the GCTSCs help in the induction of multinucleated giant cell formation from monocytes was demonstrated by Nishimura et al. in 2005 [21]. These multinucleated giant cells possess characteristic biomarkers evocative of osteoclasts [22]. The chemo-attraction of mononuclear histiocytes as well as the development of multinuclear giant cells is signaled by giant cell tumor stromal cells. GCTSC gene expression indicates the early osteoblastic differentiation of stromal cells and discrimination features of mesenchymal stem cells are also shown in this case. GCT involves a lytic abrasion centralized in the pineal region but exclusively includes the metaphysial point and extends to the adjoining articular cortex. Less than 2% of this region can be seen in the metaphysis or diaphysis and all the lesions initiate in the intramedullary region in the major long bones such as the femur and tibia. These lesions become symmetric and centrally located with the growth and are mostly eccentric [23,24].

Giant cell tumor of bone (GCTB) is known for being a primary tumor of a bone as a special case possessing significant biological characteristics in the form of three histological different types of cells such as: Osteoclast like multi-nucleated giant cells, the spindle shaped, fibroblast-like mesenchymal stromal cell, and a discoid morphology called macrophage-like cells [25]. World Health Organization categorizes this type of tumor as a benign one but this tumor shows highly acute local aggressiveness, predisposition for local relapse especially in spine, and uncommon metastasis [26]. GCTB is also shown to exhibit malignant transformation like sarcomatous changes due to the process of irradiation in case of primary treatment or spontaneous malignant transformation irrespective of radiation therapy. This tumor was described by Cooper in 1818, since then, the understanding of GCTB has reached to suitable level, and till date, a number of attempts have been exercised in order to describe the prognostic parameters for GCTB [27]. A distinguished parallelism has been observed between generalized cell types in bone tumor and giant cells involved in bone tumor. This apparent difference shows a clear strategy in bone tumor based on different factors such as tumor loci, primary or secondary observation, based on the gender involved, whether benign or malignant etc. The table cited below (Table 1) gives a brief outlook about the normal cells as well as giant cells involved in the tumor of bone based on certain factors.

Development of Giant Cell Tumor of Bone (GCTB)

p63 gene expression in GCTB

p63 gene is considered as one of the potential members of the family of p53 tumor suppressor genes that is involved in the nuclear expression in several different cell types like myoepithelial cells, urothelium, squamous epithelium of mammary and salivary glands and basal cells in prostate. The alteration observed in the expression of a number of *p63* target genes due to mutant-p53 protein shows high level of intrusive conduct (Figure 1). The complex which consists of *p63*, mutant p53 along-with phosphor- SMAD2 alters the Cyclin G2 as

well as SHARP1 expression. Dicer expression arbitrated by p63 could get hampered if mutant p53 binds to Pin1, this leads to the enhanced metastases process in an *in vivo* model for tumor. The intervention shown by *p63* in the repression of several genes including DEPDC1 (DEP domain containing 1) could be resisted by Mutant p53. The actual mechanism by which the proteins such as; Cyclin G2, DEPDC1, SHARP1, Dicer are inculpated in the process of invasion and metastasis are still under study [28].

The expression of *p63* immuno-staining in the mononuclear cells of giant cell tumor was reported by Dickson et al. in 2008. This technique is highly useful to differentiate between giant cell tumor and other giant cell rich tumors, such as aneurysmal bone cysts, chrondroblastoma and reparative giant cell granuloma [29]. Furthermore, no significance shown in case of gender, age and dominance or radiologic findings was reported earlier [30-34]. Meanwhile, eloquent distinctions between specific tissue or elapse and recurrence were observed with the help of statistical analysis. In this case, positive correlation was performed to check the involvement of extensor tendon, flexor tendon and join capsule. In the method of identification of 20q11 amplifications in 55% of GCTB, genomic hybridization provides the data for various molecular models to help in the monoclonal neoplastic processes [35]. Other related data are helpful in suggesting the important role of p53 expression in case of 25% of GCTB. The osteoclastic giant cells possess the ability to enhance the bone resorption through cathepsin K and matrix metalloproteinase, thus, showing a potential evidence for the role of RANKL signaling in the pathogenesis of GCTB [36]. The *p63* expression prominence determined in giant cell tumor present in a bone in contrast with other lesions rich in giant cells supports the role of giant cells. In a cross-sectional view, *p63* immune-histochemical expression was checked in approximately about 100 giant cell rich lesions in bone in a sequential manner that involved 31 giant cell related tumors present in bone, 14 cases of osteosarcomas consisted of 3 variants rich in giant cells, 18 aneurysmal cysts present in bone (consisting of one solid variant), 17 chondroblastomas, 8 fibromas that do not get ossified, 8 tenosynovial giant cell tumor types and 4 brown tumors. The immuno- histochemical analysis performed for *p63* showed nuclear expression for *p63* in 96.8% of bone related giant cell tumor, 14.3% of osteosarcoma cases, 22.2% aneurysmal cysts of bone, 68.7% of chondroblastomas, 50% fibromas with non-ossifiying conditions, 75.0% of brown tumors and excluded the cases of tenosynovial giant cell tumors [29]. Observing the depth of staining, it was diagnosed that vigorous staining took place in 48.4% of giant cell tumors emerged in bone, 35.3% in case of chondroblastomas and 7.1% in case of osteosarcomas (in 2 cases, both including variants rich in giant cells). Voluminous staining was shown in case of 58.0% of bone related giant cell tumor in major proportion, 23.5% in chondroblastomas and 14.3% in case of osteosarcomas.

Giant cell tumors (GCTs) consist of nearly 15% to 20% of non-malignant bone lesions and can be described as stage 2 or 3 lesions, being benign but locally aggressive, possessing a typical relapse rate after curettage alone nearly 50%. In the past few decades, several different therapies have been suggested, including mechanical, chemical, thermal, biologic, injection and embolization treatments. The main purpose of these modalities is to curb over the microscopic disease in the reactive zone after the removal of gross tumor through curettage [37]. According to the previous reports, it has been shown that the giant cells express calcitonin receptors. It has been demonstrated that giant cells in chronic giant cell granulomas (CGCGs) are osteoclasts, which has also been observed in an immune-histochemical study using osteoclast specific monoclonal antibodies. Thus, calcitonin inhibits the function of giant cells directly causing an elevation in the influx of calcium into the bones and in this way functions antagonistically to parathormone [38].

According to the previous studies, GCTBs can be shown to exhibit secondary changes such as aneurysmal bone cyst (ABC) change, foamy histiocytic aggregates and reactive bone or osteoid formation. GCTB

Bone cells/Giant cell Bone Tumor	P63 gene expression	Histone H3.3 mutation	Receptor Activator of Nuclear factor Kappa-B Ligand(RANKL)	Soft tissue recurrence	References
Normal cells of Bone	i) Restricted to nucleus.	i) H3.3 is encoded by *H3F3A* gene and functions normally in the gene regulation.	i) Functions normally in bone resorption.	i) Itdoes not occur in normal bone cells.	[28,29], [30,31], [32], [33,34]
	ii) Stronger specific nuclear p63 immuno-reactivity.		ii) Essential for skeletal homeostasis.	ii) Does not occur at curettage site.	
	iii) Plays important role in skeletal development.	ii) No mutation occurs in normal bone cells	iii) RANKL is released by bone-forming cells called as osteoblasts.	iii) Soft tissues include, fibroblasts, tendons, synovial membranes, fats etc. present normally in the body.	
	iv) p63 helps in the regulation of several cell activities such as proliferation, cell maintenance, differentiation, cell adhesion and apoptosis.	iii) H3.3 is involved in differentiation, reprogramming.	iv) RANKL plays an important role as an inducer of osteoclastogenesis and a marker of bone remodeling.	iv) Soft tissue does not undergo any relapse process in normal body.	
		iv) H3.3 plays an important role in embryonic development.			
Giant cell Tumor of Bone(GCTB)	i) Over-expression occurs by switching off the tumor suppressing activity of p53.	i) H3.3 mutation occurs in GCTB.	i) Participates in activation and proliferation of giant cells in Bone tumor.	i) High risk of recurrence in GCTB.	[35,36], [37,38], [39,40], [41,42]
		ii) H3.3 acts as a diagnostic biomarker for GCTB.	ii) Inhibition of bone resorption occurs.	ii) Commonly occurs at the area adjacent to curettage site.	
	ii) It plays role in oncogenesis and progression.	iii) Linked to epigenetic dysregulation of KLLN/ PTEN and HIST1H2BB.	o iii) Osteolytic bone metastasis in GCTB is mediated by the RANK and RANKL pathway.	iii) Plain radiograph and MR imaging are commonly used techniques to detect soft tissue recurrence in GCTB currently.	
	iii) Almost 96.8% GCTBs are positive for P63.				
	iv) p63 acts as a prognostic biomarker in GCTB.	iv) GCTB shows recurrent mutations in H3.3 genes.	o iv) RANKL is produced directly by the cancer cells.	iv) Identified occasionally in the central portion and usually at periphery.	

Table 1: Normal bone cells and giant cell bone tumor separated on the basis of factors such as; p63 gene expression, H3.3 mutation, RANKL and soft tissue recurrence.

is quite different from various types of bone lesions with osteoclastic giant cells that include chondroblastoma, non-ossifying fibroma, brown tumor (hyperparathyroidism), giant cell reparative granuloma, primary ABC and osteosarcoma as well, and particularly the giant cell-rich type. The treatment result evaluation was mixed up with the fact that although most of the relapse occurs within 3 years of treatment, late recurrence of giant cell tumors, even after 15 years, has been shown to take place in the location where identified lesion had been surgically removed; others were shown to relapse as late as 42 years later [30].

Histone *H3.3* mutations in GCTB

Histone *H3.3* is encoded by two genes that are stationed in distinct loci. H3F3A is located on chromosome 1 and *H3F3B* is positioned on chromosome 17. The confinement of macroH2A to the managerial sites related to pluripotency genes has been reported recently, its diminution leads to the elevation of 25-fold related to the competence of reprogramming as well as escalation in the instigation regarding the genes that show pluripotency characteristics, which in-turn hints

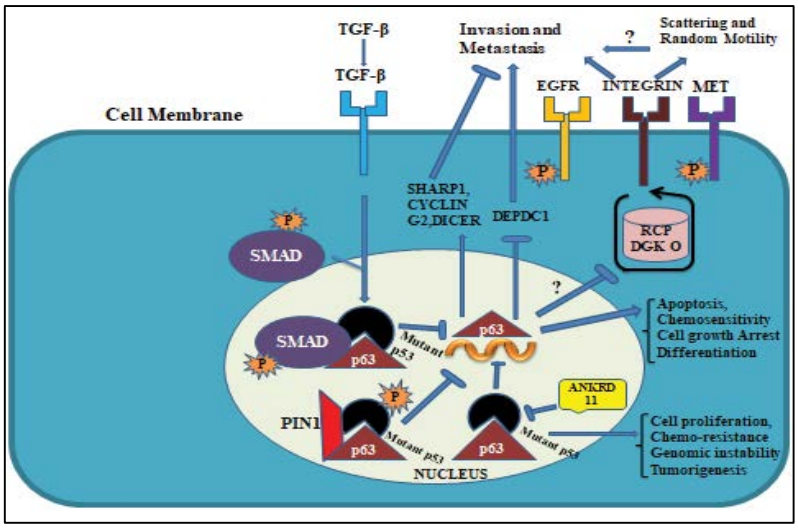

Figure 1: Interaction of different factors concomitantly and specifically highlighting the inhibition of the function of p63 by Mutant p53 that results in the alteration of gene expressions of several proteins including SHARP1, Cyclin G2, DEPDC1, Dicer and thus invasion and metastasis occur by the reprocessing of integrins and growth factor receptors.

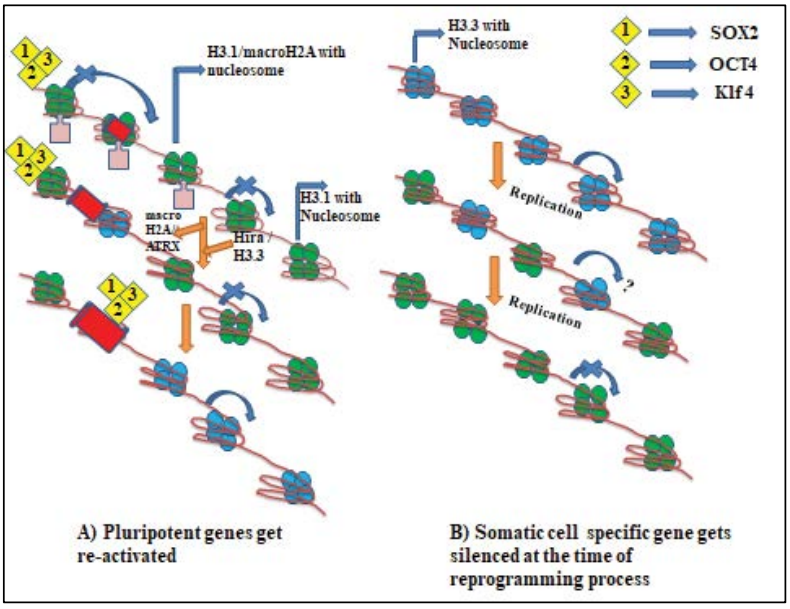

Figure 2: Opposing roles of histone H3.3 in reprogramming. (A) The genes exhibiting pluripotency are present in a repressive chromatin environment in somatic cells. With the help of transient binding that occurs by a reprogramming factor to its binding site gives rise to histone H3.3 incorporation, which in turn helps to develop a highly compact chromatin structure and improved access of the binding site. The possibility of co-binding of Oct4, Sox2 and Klf4 and activation of the target pluripotency gene are increased by these chromatin modifications. (B) Increased levels of histone H3.3 mark the active genes. H3.3 is wiped off by the replication-dependent intervention of histone H3.1 at the time of DNA replication. The gene re-activation or silencing process is dependent on the suitable signals being created. The gradual silencing of the tissue-specific genes occurs along-with reprogramming process. However, increase the probability of H3.3 incorporation is shown with the help of high levels of expression in H3.3 and thus the memory enrichment of the somatic cell gene expression pattern takes place.

towards the important idea linking macroH2A with its potential role related to repression [39]. The comprehensive process of re-instigation of silent genes is entirely based on the successive accumulation of *H2A.Z* by virtue of 5-AzaC treatment, which supports the *H2A.Z,* to exhibit an important role in reprogramming process [40-45]. The embodiment as well as misplacement of specific histone variants is highly important in case of the entire galvanization of pluripotency exhibiting gene meshwork (Figure 2A). The proper designation of the epigenetic memory provenance is unclear so far, in this case, several nuclear transfer experiments performed in *Xenopus* witnessed the over-expression in *H3.3* gene, which in turn results in the augmentation of the reminiscence of MyoD expression, thus showing the persistence for almost up to 12 embryonic demarcations, irrespective of paucity in transcription process during the prescribed time span [46-50]. The transcriptional recognition is elevated by the sudden escalation in the *H3.3* coverage present in the eggs [51]. The manifestation of the equilibrium related to reprogramming process is entirely dependent on the content of *H3.3* present, resulting in the resuscitation of the genes exhibiting pluripotency as well as the commemoration in the arrangement of somatic cell expression arrangement, this entire process is coordinated in the presence of elevated levels of *H3.3* (Figure 2B).

The establishment of mutations in H3F3A is one of the potential examples of operating diversifications, encoding histone variant H3.3, in pediatric tumors, exhibiting tremendously intermittent mutations that impinge the N-terminal tail of *H3.3* and kicks off the amino acid substitution of lysine 27 to methionine (p.Lys27Met) and of glycine 34 to arginine or valine (p.Gly34Arg or p.Gly34Val) [37]. Adrienne Flanagan [52] studied giant cell tumor of bone exhibiting periodical mutations in *H3.3* genes. This tumor has been reported mostly in children, adolescents and young adults, which advocate the fact that the established propulsive mutations are experimentally correlated. Despite being known to the finding that chaperones play a vital role to regulate the impeachment of *H3.3* for both active and repressive transcription process, the testimony of mutations in both H3F3A and *H3F3B* reported by Behjati [53], the proposition regarding the exponential expression as a substitute to the deposition process could be the reason for conclusive factor in case of tumorigenesis [38]. Various studies in the past came up with the suggestions that approximately 85 to 95% of GCTBs contain H3F3A pG34 W mutation and further a small subset (each less than 1 to 2%) hold the H3F3A p.G34 L, p.G34 M, p.G34R or p.G34 V mutation. Anti-*H3.3* G34W mutant antibody has been shown to possess the applicability for immune histochemical staining nowadays. Hence, the evidence of the diagnostic utility of the *H3.3* G34 W mutant antibody for GCTB and its variant was further studied [32].

According to the recent studies, a distinguishing genetic modulation of GCT was observed, which acts as a leading mutation in the H3F3A gene of the family of histone 3.3A, 3A that is stationed on chromosome 1q21. This family is very important in the regulation of transcription, DNA replication and chromosomal cohesion. The DNA of 6 patients was analyzed in chondroblastoma and was observed that most of the cases witnessed point mutations in the *H3F3B* gene and also at the later stage, out of 53 cases of patients stimulated with GCT, s that 49 (92%) possessed missense mutations in the H3F3A gene. In 48 cases, the substitution was caused by GCT mutation in a glycine (Gly at codon 35 with tryptophan (trp), thus the p.Gly35Trp modulation was discovered. However, the substitution was noticed to take place with leucine (Leu), p.Gly35Leu. in one case. Point mutations delineated in GCT so far came up with the H3F3A modifications such as: p.Gly35Trp, p.GlyLeu, p.Gly35Val, p.Gly35Met, p.Gly35Glu, while no mutation established in the *H3F3B* gene. This study is of diagnostic importance as GCT mainly consists of a huge group of multinucleated giant cells coordinated together in a fashionable manner according to the historical perspective and it can also be available at high level

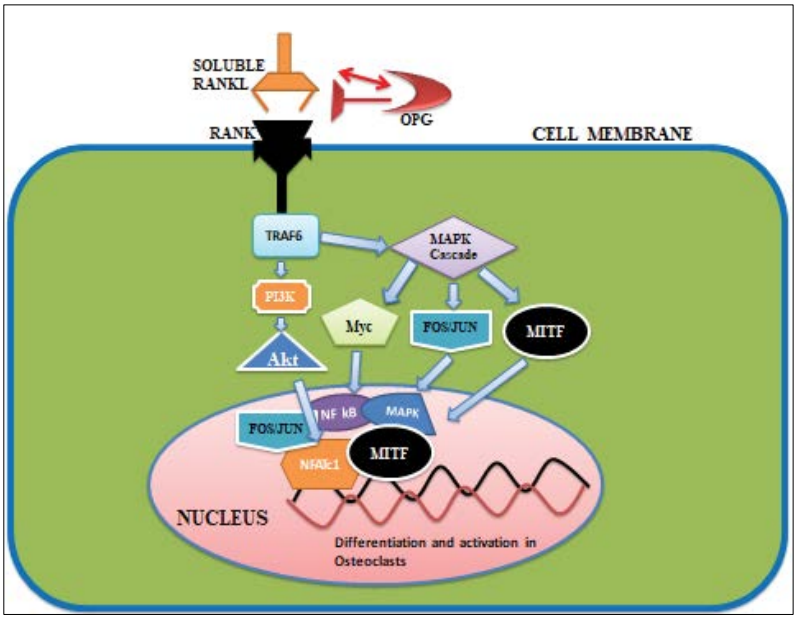

Figure 3: RANK/RANKL signaling exhibiting a huge network in cancer: RANKL being a trimeric mesh, is present in soluble form or it can also be released in a membrane. Also, Secreted RANKL can be obtained by the process of proteolysis of the membrane form or it can also be produced from a specific transcript. The interaction takes place between Trimeric RANKL and a trimeric receptor, RANK which helps in the triggering of a signaling cascade that controls the transcription of a number of effector genes. Other several protagonists also interact in order to help in the regulation of the complete binding between RANKL and RANK. Hence, OPG plays an important role in bridging the contact with RANKL.

in other bone related cancers. In case of highly accurate differential diagnosis, particularly in giant cell rich sarcomas, these alterations in the H3F3A gene, with a time saving and highly cognizant nature play an important role to extricate GCT from tumors with resemblance in their histology [39].

Receptor activator of Nuclear factor Kappa-B Ligand (RANKL) in GCTB

RANKL/RANK signaling is considered as the backbone in the regulation of osteoclast formation, activation and survival in the modeling and remodeling of bone as well as in divergency of cathartic circumstances described by elevated bone turnover. Tumor necrosis factor-α (TNFα) superfamily comprises of around 40 members, the number of membrane or soluble receptors is found to be the same. RANKL has been found to be one of the potential members in the TNF-α superfamily (TNFSF11), binding to the membrane receptor called as, receptor activator of nuclear factor-κB (RANK). The clearly defined intracellular signal transduction occurs due to the effective activity in between RANKL and RANK. This entire signaling process is managed with the help of an imitated receptor known as osteoprotegerin (OPG) [40] (Figure 3). OPG helps in the safeguarding of bone in case of exaggerated resorption by binding to RANKL and in turn intercepting the binding to RANK. In this way, the leading definitive of mass and strength in bone is credited to the contingent consolidation of RANKL and OPG [33]. Giant cell tumor mainly consists of neoplastic mononuclear stromal cells and reactive neoplastic multinucleated giant cells actively responsible for bone resorption. This process is mediated by interaction between receptor activator of nuclear factor-kB (RANK) expressed by giant cells and RANK ligand (RANKL) on stromal cells. The receptor activator of nuclear factor kappa B ligand (RANKL) discovery plays a marvelous role in the pathogenesis of GCTB [54].

It has been shown that the receptor activator of nuclear factor kappa-B ligand (RANKL) and its receptor RANK axis play a pivotal role in the development of GCTB. Mononuclear stromal cells express RANKL, which then binds to RANK on the surface of osteoclast- type multinucleated giant cells that results in the process of activation and proliferation of these giant cells. The exact role of RANKL is the result of the critical action of denosumab. Denosumab is a monoclonal antibody inhibitor of RANKL and is highly effective in limited clinical trials for blocking tumor progression in the patients which possess recurrent or unresectable giant cell tumors. It can be a potential anti-tumor agent for GCTB. The previous reports reveal that the post-denosumab GCTBs histological alterations come up with the depletion of osteoclastic giant cells, new bone formation and spindle cell proliferation. Such appearances are very much different from the histology of conventional GCTB and can be the reminiscent of osteosarcoma or malignancy in GCTB that actually leads to diagnostic difficulty to a greater extent [41].

Previous studies showed that multinucleated cells as well as several mononuclear cells expressed RANK all over GCTB. RANKL signaling in diseases like GCT is considered as an astonishing prototype in case of indicated translational research. Giant cell bone tumor treatment includes the curettage irrespective of bone filler or adjuvants such as polymethylmethacrylate (PMMA) or phenol. In case of less possibility of surgical procedures, non-invasive methods for example; radiotherapy, radiofrequency thermal ablation, or chemoembolization, can be used. In some important cases in which surgery shows relatively minor functional impairment or some of the tumors expressing extensive local destruction, wide resection can be made available for such cases. However, resection may show a considerable morbidity [34].

Soft tissue recurrence in GCTB

In clinical practice, relapse in the soft tissue has been observed rarely in most of the cases as compared to local recurrence in bone. According to the latest reports, it has been shown that the soft tissue recurrence of GCTB oftenly takes place in the area that is adjacent to curettage site [55]. It may occur mostly with the help of contamination, when surgical removal of the tumor is performed. It has been reported that the reappearance chances of soft tissues in GCT are quite high post-surgery, in the range of 15–25% which goes up to 40–60%. Based on the category of the surgical methods being implemented, peripheral excision is related to an increased rate, while the spacious excision shows a decreased rate [42]. Tumor relapse has been commonly reported in the bone, at the site where the tumor was operated earlier and that this occurrence is depicted due to uninterrupted growth of the surplus tumor present in the bone. Soft tissue relapse irregularly manifests an outlying edge of ossification neighboring the mass, which is an idiosyncratic, related to the soft tissue recurrence of GCTB. Radiographic and histologic findings of ossified soft tissue recurrence were reported in previous studies. In case of the need of early detection of the recurrence, MR imaging tool was suggested by Balke [56], that can be used for the purpose of any suspicious finding. In order to detect a suitable indicator for soft tissue recurrence of GCTB, peripheral rim of ossification surrounding the mass was shown to be a potential source. Some previous studies observed a low detection rate in ossification based on the plain radiography. Also, the proper recognition of the radiographic characteristics of soft tissue recurrence of GCTB have not been shown in previous studies, an accurate diagnosis and appropriate treatment could be fecilitated with the help of in-depth studies [57].

The current advancement in radiological imaging has made it possible to detect small soft tissue abrasions with the help of sensitive modalities. The detection of soft tissue masses can be done with the help of MR imaging and it can be helpful to reveal the extent of the lesion, while there are chances that the outcome may be nonspecific. Ossification can be detected by plain radiography and CT scan because both the tools are sensitive enough in this case. A new classification was proposed regarding the soft tissue recurrence of GCTB and it is based on the integration of the plain radiograph and MR imaging [58]. A study was recently conducted in which 3 patients were identified and labelled accordingly as pure soft tissue recurrence without ossification (Type III), 2 as peripheral ossification (Type I) and the other one as central ossification (Type II). It was observed that ossification of soft tissue is occasionally identified in the central portion and usually at the periphery. Furthermore, it was also observed that there might be a recurrence lesion at high proportion irrespective of ossification in case of clinical practice. Plain radiography showed the suspicious soft tissue mass in the patients whereas, MR imaging was used to determine the recurrence of GCTB in the soft tissue. On the basis of requirement, some additional diagnostic studies, specifically a biopsy could be done with the help of computed tomography and it could be helpful to resolve the issue of soft tissue relapse [59].

Histopathological and radiological aspects in GCTB

According to the review literature, recently, a 16 year old male patient was observed in which a bony hard swelling involved the entire right side of face extending from malar region to lower border of mandible. Intra-oral examination showed that a well-defined inflammation expanded to a certain distance from the retro-molar region covering the soft tissue, the anterior ramus of mandible. The

palpable and non-tender positions of right submandibular lymph nodes were also observed [60]. According to the latest reports, the numbers of craniofacial osteosarcoma cases have been shown to be low, but the occurrence of jaw osteosarcoma is much higher than that of osteosarcoma in the total major parts of the skeleton in the body and jaws are shown to contribute as less as 0.86% of the total body volume. Giant cell rich osteosarcoma observed in long bones involving younger patients with mean age of 26 years has surfaced in recent studies [61].

In case of a recent case study performed, histopathological examination of the excised specimen taken from the patient was done in which pleomorphic tumor was shown to be organized in fascicular template as well as in sheets. The tumor cells were shown to be eminently pleomorphic and some of them were seen ellipsoidal to round or spindle shaped. The prevalence of abundant osteoclast like giant cells containing large pleomorphic nuclei with irregular nuclear membrane was also observed and clearly apparent nucleoli were also cited in few cells and also the vesicular nuclei and diverse mitotic shapes were crystal clear in tumor cells. Also the space covered with lace like osteoid deposits encircling the tumor cells and bone destruction was also clearly visible. There was no occurrence of blood filled cystic spaces or vascular spaces reported in this case. Thus, it was proclaimed that histopathological process supports easy requirements to perform the straight forward diagnosis of giant cell rich osteosarcoma [62]. The radiological features of both osteosarcoma rich in giant cells and the conventional osteosarcoma are entirely different. The distinctive radiological uncovering of reappearance has been reported to be the contemporary domain for the destruction of bone at the excision extremity alongside the intra-lesional bone graft matter resorption taking place as per the continuous imaging analysis reports. Assessment methods in case of tumor relapse could be effectively determined with the help of comparison of further observation of the images with the preliminary paradigm autopsy illustration and deliberate investigation of the images. These findings resemble to benign or malignant giant cell tumor in most of the cases. They have been reported as osteolytic contusion in both head and neck region as well as in long bones [63]. A 3D CT scan was shown to come up with a large tumor lump along with tumor osteoid that forms a composite network emanating from the body, angle of mandible and involving whole of ramus and condylar neck. According to histopathological study, it is difficult to diagnose giant cell rich osteosarcoma (GRCO) because it has impending analogy to benign or malignant giant cell tumor. GRCO being an allying sarcoma with sparse formation of osteoids and a large amount of osteoclast-like giant cells shows an impressive resemblance to giant cell tumor. The possibility of diagnosis prevails due to the subsistence of osteoid formation by the tumor cells. A few osteoclast-like giant cells present in about 13–25% of cases of osteosarcoma are mostly observed in haemorrhagic and perivascular areas.

Conclusion and Future Perspectives

The general mechanism of the giant cells was reviewed with respect to their important role in the emergence of bone tumor. *p63* expression contributes significantly towards the development of giant cell tumor. The *p63* nuclear expression was witnessed in 96.8% of bone related giant cell tumor, 75.0% of brown tumors (cases of tenosynovial giant cell tumors were precluded), 14.3% of osteosarcomas, 22.2% of aneurysmal bone cysts, 68.7% of chondroblastoma cases, 50% of fibromas with non-ossifiying state. Hence, *p63* expression helps to a greater extent in dealing with the giant cell tumor in bone in its diagnosis and effective treatment. The H3F3A alterations such as: p.Gly35Trp, p.Gly35Leu, p.Gly35Val, p.Gly35met, p.Gly35Glu were observed in giant cell tumor with the help of point mutations. Thus, gene mutation analysis of different genes responsible for GCTB contributes effectively towards understanding the giant cell tumor in bone. In case of histopathological analysis, plain radiography and CT scan can be a potential source to notify ossification process in GCTB. These tools are highly precise in this case because of high chances of accuracy. The correct medical diagnosis, in which the mutations in the H3F3A gene are revealed in all the possible sections, is an expeditive as well as a sensitive analysis method that particularly takes giant cell rich sarcomas into consideration. This advanced gene mutation analysis tool plays an exceptional role in case of demarcation of GCT from tumors with conformity at histological level. RANKL reactivation process also plays an important role in developing the giant cell bone tumor.

This overview will help the researchers with the basic idea about GCTB and it will help to understand the development of giant cell tumor of bone with respect to the different factors such as; *p63* gene expression, *H3.3* histone mutations, RANKL. etc. With the help of this review study, it could be possible for the researchers to shift the focus towards highly advanced techniques to detect detailed information regarding many more putative and procurable prognostic biomarkers, predictive biomarkers as well as diagnostic biomarkers for GCTB as the need of the hour is to develop highly specific gene inhibition strategy as well as effective therapeutic techniques in order to discover and develop highly accurate treatment methods to control these types of bone tumors at global level.

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