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Review

# Molecular pathological insights into tumorigenesis and progression of giant cell tumor of bone

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## HIGHLIGHTS

- This review presents macroscopic to microscopic pathological phenotypes of GCTB.
- H3.3-G34W alters chromatin landscape and tumor microenvironment to drive GCTB.
- H3.3-G34W may be loss with malignant transformation of GCTB.

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## ABSTRACT

Giant cell tumor of bone (GCTB) is a primary bone tumor that typically exhibits benign histological appearance and clinical behavior in most cases, with local aggressiveness and rare metastasis. It predominantly affects individuals in the young adult age group. It is characterized by the presence of multinucleated osteoclastic giant cells and a stromal population of neoplastic cells. A key hallmark for GCTB pathogenesis is the G34W genetic mutation in the histone H3.3 gene, which is restricted to the population of cancerous stromal cells and is absent in osteoclasts and their progenitor cells. This review presents a comprehensive overview of the pathology of GCTB, including its histopathological characteristics, cytological features, histopathological variants, and their clinical relevance. We also discuss recent insights into genetic alterations in relation to the molecular pathways implicated in GCTB. A summary of the current understanding of GCTB pathology will update the knowledge base to guide the diagnosis and management of this unique bone tumor.

## 1. Introduction

The medical condition known currently as the giant cell tumor of bone (GCTB), was initially documented in 1818, and then named as osteoclastoma [1]. According to the classification scheme of the World Health Organization for tumors of soft tissue and bone, GCTB is categorized as a type of locally aggressive and rarely metastatic bone tumor of intermediate malignancy level [2]. GCTB accounts for approximately 5 % of the total number of primary bone tumors worldwide [2]. The condition is more common among individuals who have reached skeletal maturity, mostly between 20 to 40 years of age. Some GCTB studies report a modest prevalence of the disease among female patients [3,4].

GCTB primarily impacts the *meta*-epiphysis of long bones, with more cases in distal femur and proximal tibia, even though the disease can potentially affect any part of the skeletal system [5,6] (Fig. 1A). Clinically, patients typically experience pain and swelling at the afflicted site, with other specific symptoms depending on the location and size of the tumor, which include limited joint movement, neurological impairment, and pathological fracture [7,8]. Whereas surgery may be the major treatment for primary and recurrent GCTB, a range of systemic and localized treatments for this disease are also administered, such as drug treatment (with denosumab, bisphosphonates and interferon), radiotherapy and the embolization of serial arteries [9–11].

GCTB has unexpectedly perplexing clinical characteristics. Indeed,

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despite its histologically benign appearance, the aggressive nature and recurrence potential of GCTB necessitate a thorough understanding of its pathology for effective management. This review explores recent discoveries of GCTB pathology that encompass its histopathological characteristics, cytological features, histopathological variants and their clinical relevance, genetic alterations and associated molecular pathways.

## 2. Pathological features of GCTB

### 2.1. Histopathological characteristics

The histological hallmark of GCTB is the presence of numerous multinucleated giant cells scattered throughout a background of mononuclear stromal cells [2,5] (Fig. 1C). Understanding the interplay between these components is crucial to unravel GCTB pathogenesis. The local grade of GCTB is commonly assessed using the Campanacci radiological grading system, which classifies tumors into three grades: Grade I (clearly defined cortex), Grade II (relatively well-defined radiographic borders, expanded and thinned cortex), and Grade III (unclear or poorly defined borders with radiographic evidence of cortical bone destruction and soft tissue expansion) [12]. The grades of the disease is determined in conjunction with core needle biopsy or intraoperative frozen sectioning of samples to yield a more in-depth and definitive diagnosis to inform surgical decisions, with regards to the aggressive nature of the tumor and the occurrences of malignant transformation [13].

Gross examination of resected specimens often revealed GCTB as a voluminous, clearly or unclear defined, and irregularly enlarged neoplasm, with possible cortical thinning to complete destruction of the surrounding cortex (Fig. 1B). The cut surface exhibits heterogeneity, characterized by a soft, lobulated and dark crimson colored crumbly texture, in addition to yellowish-white regions that correlate to the fibrous tissue and areas of hemorrhage demonstrating blood-filled cystic cavities [2,14]. Macroscopically, GCTB consists of a well-developed vascular network amidst extensive bands of fibrous tissue constituted by cells or collagen fibers. Within such fibrous mass, areas of hemorrhage with an accumulation of hemosiderin, and presence of foamy macrophages can be discerned [14,15]. Nevertheless, GCTB manifests diverse appearance under imaging and histology attributable to the presence of necrosis and hemorrhage, particularly in large-sized tumors [15]. GCTB that shows co-occurrence of lung metastatic nodules often exhibits extensive hemorrhage and thrombus formation that are distinct from primary GCTB cases without local or distant recurrence [15].

### 2.2. Cytological features of GCTB

Conventional GCTB mainly exhibits four different microscopic morphological components [14,16–19] (Fig. 1D):

(1) Osteoclastic giant cells: These cells are most characteristic of GCTB as they morphologically resemble osteoclasts, albeit often larger and involve in bone resorption. These giant cells represent the reactive cell population characterized by an eosinophilic cytoplasm, multinucleated (up to 20–50, sometimes even more than 50), with prominent nucleoli.

(2) Neoplastic cells: These cells are round to oval-polygonal or elongated mononuclear stromal cells with a poorly defined cytoplasm and spindle-shaped nuclei. These stromal cells have a wide range of mitotic activity (from 2 to 20 per 10 high-power fields), expressed receptor activator of nuclear factor-kappa B (RANKL) ligand, and implicated in the recruitment and activation of the osteoclast-like giant cells.

(3) Mononuclear histiocytic cells: These are mainly tumor-associated mononuclear macrophages that are uncommitted to osteoclastogenesis.

(4) Various types of extracellular matrix proteins, interspersed fibroblastic cells, and a dense network of permeable blood vessels.

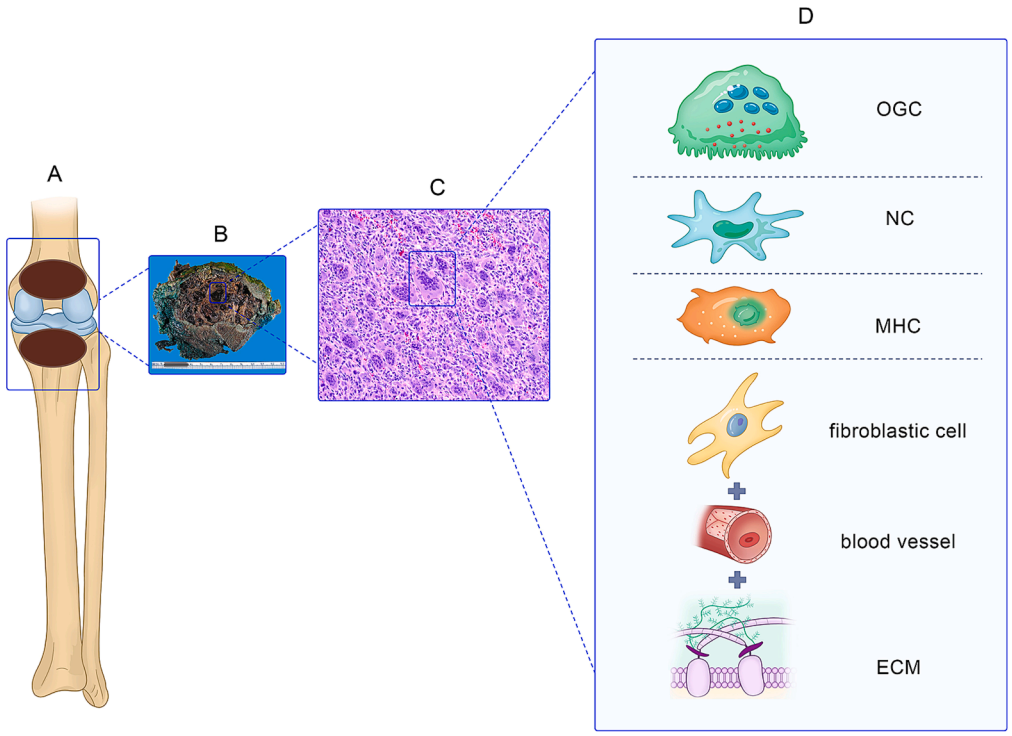


**Fig. 1. Schematic representation of the histopathological features of GCTB from macroscopic to microscopic level.** A, GCTB primarily impacts the *meta*-epiphysis of long bones, typically that of the distal femur and proximal tibia; B, Gross examination of a resected GCTB specimen (Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore); C, Typical histological morphology of GCTB tissue sections under microscope (100×,HE stain; Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore); D, Schematic diagram of the four different micromorphological components of GCTB. OGC, Osteoclastic giant cell; NC, Neoplastic cell; MHC, Mononuclear histiocytic cell; ECM, Extracellular matrix.

### 2.3. Histopathological variants and their clinical relevance

GCTB malignant transformation is rare, but may have one or several histological malignancies. GCTB malignancy typically presents excessive and rapid proliferation of neoplastic mononuclear cells of variable appearance, accompanied by abnormal mitotic events. Malignant GCTB shares common histological features with several high-grade sarcomas, comprising undifferentiated pleomorphic sarcoma, fibrosarcoma, and osteosarcoma [20]. The World Health Organization (WHO) classifies malignancy in GCTB into two categories: Primary malignant GCTB (PMGCTB) and secondary malignant GCTB (SMGCTB). PMGCTB is characterized by the presence of abnormal varied-shaped cancerous cells that occur alongside conventional GCTB. SMGCTB, on the other hand, refers to a lesion that is developed at the location where GCTB was previously treated [2]. Most MGCTBs are secondary in nature and arise following radiotherapy or repeated local recurrence after surgery, or late local recurrence. The prevalence rates of MGCTB, PMGCTB, and SMGCTB among GCTB patients are reported to be 1.1-11.3 %, 0.5-9.7 %, and 1.3-5%, respectively [21].

In addition to surgery and radiotherapy, patients may also be prescribed denosumab, a human monoclonal antibody, particularly those with unresectable GCTB or when surgery is expected to cause significant morbidity. Denosumab is a monoclonal antibody that inhibits the interaction between RANKL and its receptor to suppress differentiation, activation, and function of the osteoclasts. Denosumab thus effectively prevents osteoclast-mediated bone destruction and osteolysis [22]. CT imaging is employed to monitor potential malignancy development associated with denosumab treatment via observing the absence of fibro-osseous matrix and neocortex formation, and soft tissue component enlargement [23]. Favorable primary histological features upon denosumab treatment include reduction in osteoclastic giant cells and neoplastic stromal cells, increased spindling of cells, decreased cell division rate, and emergence of new bone regions with diverse configurations [22,24,25]. It is noted that these phenotypic manifestations are dependent on the duration of treatment with the antibody. While enhanced cellularity and disorganization of bone deposition can be observed during the early phase following denosumab treatment, extended exposure is associated with reduced cellularity and new bone is formed as wide and rounded cords or long and curved arrays [26]. These post-treatment morphologies resemble those detected in osteosarcoma, albeit there is an absence of noticeable nuclear abnormalities, mitotic activity, and existing bone infiltration [26]. In fact, an extensive clinical study conducted on 532 participants found approximately 1 % of denosumab-treated GCTB patients experienced verified sarcomatous transformation [27].

## 3. Pathogenesis and molecular Genetics

### 3.1. Cellular origin and pathogenesis

The neoplastic GCTB cells are postulated to originate from osteoprogenitor osseous stromal derived from mesenchymal tissue, based on expression of genes involved in the initial stage of normal osteoblastic differentiation (such as collagen I, bone sialoprotein, and osteonectin) and the genes that regulate this process (Cbfa-1, osterix, osteocalcin) [28,29]. Neoplastic GCTB cells also express markers associated with early osteoblastic development, such as Thy 1.1 and Stro1 [30,31]. These neoplastic cells are situated at a relatively primordial phase of mesenchymal differentiation [32], as suggested by not only the expression of fibroblast growth factor receptor (*FGF-R*) *3* but also the capacity of these cells to undergo osteoblastic development when exposed to retinoic acid. This view is further supported by the cancerous cells exhibiting characteristics of mesenchymal stem cells that express CD105 (SH2) and CD73 (SH3, SH4) markers [30]. Genes such as stathmin-like 2 (*STMN2*), insulin-like growth factor (*IGF*)*2*, and leptin (*LEP*) are downregulated in neoplastic stromal cells expressing the oncohistone H3.3 with G34W mutation. These genes are involved in the differentiation of mesenchymal stem cells and the formation of osteoclasts in mice that promote bone differentiation [33–35]. Analysis of immortalized stromal cell lines established from primary GCTB tumors revealed downregulation of 27 genes in G34W-mutant cells. Some of these genes (*ADORA1, MYL1, PKP2, TNNT2*) were associated with muscle contraction, suggesting that the G34W mutation in neoplastic stromal cells is potentially linked to muscle differentiation, which is a known lineage for mesenchymal progenitor cells in the bone marrow [36].

The expression of CD68 antigen—commonly expressed in mononuclear histiocytic cells and osteoclastic giant cells—suggest the affiliation of osteoclastic giant cells with the monocytic-histiocytic system [30]; even though it is acknowledged that the mononuclear histiocytic cells and osteoclastic giant cells of the GCTB are recruited as a secondary response to the neoplastic transformation, rather than a part of the initial neoplastic cell population.

It is also reported that formation of the giant cells in GCTB is promoted by an upregulation of osteoclastogenic cytokines and chemokines, such as interleukin (IL)-6, IL-11, IL-17, IL-34, and transforming growth factor (TGF)-β [37]. Furthermore, the high levels of macrophage colony-stimulating factor (M−CSF) and RANKL in these neoplastic cells further stimulate the recruitment of monocyte/macrophage mononuclear cells that favor their differentiation into receptor activator of nuclear factor-kappa B (RANK)-positive osteoclastic giant cells [38]. In a recent study expounding interaction of multiple cell types in GCTB, Feng et al. [39] examined the RNA sequencing data of 8033 cells derived from a GCTB patient, and proposed osteoclasts can be classified into three differentiation stages—progenitor, mature, and dysfunctional osteoclasts—based on the expression of specific molecules. Another study retrieved 15 archival GCTB cases with tumor samples from patients pre- and post-denosumab treatment identified FOS as an immunohistochemical marker for progenitor osteoclast and JDP2 and NFATc1 for matured osteoclasts [40]. These findings enable the authors to histologically delineate several cell types in distinct phases of osteoclastogenesis. The study further implicate NFATc1 as a key marker of osteoclast formation and a potential direct target of denosumab therapy.

### 3.2. Genetic alterations and molecular pathways involved

#### 3.2.1. H3F3A mutations

Eukaryotic genome is packaged into chromatin by the wrapping of DNA around structural histone proteins to form the nucleosomal complexes each contains ~ 146 bp DNA and eight histone proteins (a pair each of histone H2A, H2B, H3 and H4) [41]. The chromatin architecture plays an essential role in determining the accessibility of the DNA to other enzymatic protein complexes to regulate genome-wide gene expression. Eukaryotic cells also comprise variant histones with sequences that are highly similar to canonical histones, but with specific differences at certain amino acid residues [42]. Various histone mutations have been identified as being associated with malignancies. Of particular note here is the prominent variant in histone H3.3 [42,43].

Histone H3.3 is the predominant histone H3 protein in quiescent mammalian cells, and it is the sole variant of histone H3 that is consistently present throughout every phase of the cell cycle [44]. Indeed, the replacement of histone H3 with H3.3 leads to an inherently less stable nucleosome preferentially enriched at transcriptionally active chromosomal loci [45]. Histone H3.3 also facilitates repair of damaged DNA via reducing nucleosome density, in turn leading to chromatin unraveling to promote accessibility of DNA damage repair factors [46]. Histone H3.3 can also function as a nucleosomal gap-filler [47], and deposition of histone H3.3-containing nucleosome can facilitate replication fork progression through UV-damaged DNA tracts [48].

Histone H3.3 is translated from messenger RNAs that originate from one of two genes, *H3F3A* and *H3F3B* [44]. *H3F3A* gene is nested at 1q42.12 position of chromosome 1. Multiple reports have uncovered

somatic driver mutations in the *H3F3A* gene as being associated with GCTB, particularly the p.Gly34Trp (G34W) mutation is documented in 90–96 % of GCTB cases [49–51]. In contrast, the relatively less common mutated histone H3.3 variants with G34L, G34M, and G34V mutations, are more frequently found within the small bones of the hands and feet, patella, and the axial skeleton. Excluding these rarer sites from the analysis, the prevalence of the G34W mutation is found in 97.8 % of GCTB cases [49]. The H3.3-G34W alteration is confined to the cancerous stromal cell population and is not found in osteoclasts or their progenitors [49,50]. The significant frequency of H3.3 changes in GCTB, along with their strong specificity, makes this mutation a valuable diagnostic marker, particularly for identifying patients with ambiguous morphology and for differentiating GCTB from among other conditions such as chondroblastoma, aneurysmal bone cysts, and giant cell rich osteosarcoma variants [50,52].

Many human cancers-associated somatic missense mutations in histone genes appeared to be dominant in nature, manifesting their carcinogenic effects even if mutations are heterozygous (with at least one wildtype allele) and are thus referred to as "oncohistone" mutations. Fellenberg et al. [53] observed knockdown of the H3.3-G34W reversed the neoplastic characteristics of GCTB stromal cells (GCTSC), resulting in suppression of migration, proliferation, and colony-forming ability in cultured cells as well as *in vivo* GCTSC xenotransplantation model, attributing these tumorigenic phenotypes to this onco-histone mutation and the expression of oncohistone H3.3-G34W is adequate to promote GCTB carcinogenesis. Khazaei et al. also highlighted the role of the H3.3-G34W mutation in promoting bone destruction and the growth of osteoblast-like mesenchymal progenitors within GCTB [36]. These findings and others identified the H3.3-G34W mutation as a key driver in the pathological processes in GCTB development and progression.

Although GCTB is genetically characterized by a high recurrence of G34W mutation in the *H3F3A* gene, the implication of this mutation to the transformed MGCTB has not been thoroughly analysed [54]. Although some studies posited that *H3F3A* mutations may also be present in MGCTB [50,55], a recent finding by Yoshida et al. [56] confirmed the absence of this oncohistone mutation in 5 of the 7 MGCTB cases, either entirely or sub-clonally, yet detecting the presence of this mutation in the genome of their matched non-malignant GCTB tissue components. Fluorescence in situ hybridization (FISH) revealed the absence of G34W-mutated *H3F3A* alongside allelic deletion of the *H3F3A* gene in 75 % cases of MGCTB. Although the sample size was modest, the data suggest the possibility of deletion in the mutant gene allele, and support the hypothesis that the *H3F3A* G34W mutation being eliminated in the process of linear clonal evolution. Similarly, in a recent study [57], 2 out of 9 clinicopathologically confirmed MGCTB cases exhibited absence of the onco-histone *H3F3A* mutation, with a loss of H3.3-G34W expression and heterozygous deletion of *H3F3A* in the malignant components, in comparison to matched GCTB components in all cases. Organoids established from MGCTB patients also recorded loss of heterozygosity (LOH) of the *H3F3A* G34W mutation [58], lending further correlation of the absence of the oncohistone mutation upon malignant transformation.

One study proposed that chromatin regulation and RNA processing associated with *H3F3A* G34W in GCTB may be counterbalanced or made irrelevant by the emergence of additional diver mutations in MGCTB that no longer possesses the *H3F3A* mutation [59]. These counterbalancing mutations may include that in *TP53*, p53 overexpression, mitogen–activated protein kinase (MAPK) signaling pathway gene alterations, gene amplification of cyclin D1 (*CCND1*) and the mesenchymal epithelial transition (MET) proto-oncogene [60–62]. A report found in addition to having *H3F3A* or *H3F3B* mutations, the majority of MGCTBs also displayed characteristics linked to telomerase reverse transcriptase (TERT) promoter mutation, specifically C228T, implicating the contribution of telomere dysfunction to the malignant development of GCTB [63]. Additionally, nonrecurring instances of biallelic losses were also identified in the histone lysine demethylase genes *KDM4B* and *KDM6A*. In the aforementioned study of 9 clinically confirmed MGCTB cases [57], a p.D185H mutation in the enhancer of zeste homolog (*EZH)2* gene was found to be pathogenic in one case using next-generation sequencing (NGS), while 3 cases showed complete loss of both H3K27me2 and H3K27me3, as determined by immunohistochemistry (IHC), pointing to polycomb repressive complex (PRC)2 dysfunction in this MGCTB subgroup. Coincidentally, these 3 MGCTBs that exhibited H3K27me3 loss were primarily composed of spindle-shaped cells, resembling fibrosarcoma or malignant peripheral nerve sheath tumor (MPNST). Within the same study, the authors also identified *TP53* mutation in 3 cases using NGS, whereas 5 patients exhibited aberrant nuclear accumulation of p53 in the malignant, but not in the matched GCTB components, as ascertained by IHC. These findings offer proof of the potential involvement of *TP53* modification in the development of GCTB into a malignant form. Histologically, most p53-positive MGCTBs exhibited either epithelioid or pleomorphic morphology that suggest presence of a potential phenotypic-genotypic link in transition to MGCTB. Nevertheless, the precise molecular mechanism(s) responsible for such morphogenesis remains to be elucidated. Given the aforementioned discoveries, we suggest a theoretical framework for the malignant advancement of GCTB as shown in Fig. 2.

Epigenetic regulation, including histone modifications, plays a significant role in maintaining bone homeostasis and governing the process of bone cell differentiation [64]. Typically, during cell differentiation and maturation, cells undergo a loss of pluripotency, which is marked by epigenetic modifications that deactivate genes associated with stem cell properties [65]. Histone modifications including H3K9me3, H3KM27me3, and H3K4me3, effectively guide the process of osteoblastic development in mesenchymal stem cells. In addition, there is an increase in H3K36me3 during osteogenic differentiation [66]. In simple terms, genes that are responsible for maintaining embryonic stem cells have high levels of H3K27me3 and H3K9me3, whereas H3K36me3 levels must rise during osteogenic differentiation. The histone methyltransferase SETD2 is accountable for mediating H3K36 trimethylation, and thus G34W substitutions in H3.3 is expected to hinder the activity of SETD2 [67]. Indeed, G34W mutations sterically affect the adjacent K36 residue, leading to a reduction in H3.3K36me3 and a reciprocal increase in H3.3K27me3 on the mutant histone tail. The initial occurrence subsequent to the deletion of H3.3K36me3 from G34W histones results in the placement of the H3K27me3 mark to the mutated tail within regions of active genes. The genomic areas lack the H3.3K36me3 mark leads to the transfer of H3K27me3 from areas with lesser affinity to promoter and genic regions, most likely facilitated by the PRC2 complex [36,68].

G34W-induced epigenetic remodeling impacts determination of the mesenchymal cell lineage. Khazaei et al. [36] analyzed the epigenome, transcriptome, and secretory proteome of patient samples and tumor-derived cells that underwent editing using CRISPR-Cas9 to have H3.3-G34W mutation. The authors demonstrated that the loss of H3.3K36me3 on mutant H3.3 relocates the repressive H3K27me3 mark from intergenic to genic regions beyond the areas of histone H3.3 deposition. This led to the redistribution of several chromatin marks and an abnormal transcription, which changes the destiny of mesenchymal progenitors and impedes differentiation. Single-cell transcriptomics showed that H3.3-G34W stromal cells follow a cancerous path from an SPP1(+) osteoblast-like precursor group to an ACTA2(+) myofibroblast-like group. These myofibroblast-like cells produce extracellular matrix molecules that are expected to attract and stimulate osteoclasts. The authors further suggested that the histone H3.3-G34W mutation leads to the formation of GCTB through a sustained transformed state within the osteoblast-like progenitors.

Another discovery by Cottone et al. [69] identified H3.3-G34W-induced epigenetic remodeling of enhancer element to repress the expression of Signal Peptide CUB Domain and EGF Like Domain Containing 3 (*SCUBE3*) gene, which encodes a soluble TGFβ-like factor that can counteract osteoclast recruitment to tumor site [70]. Attenuated SCUBE3 level increases osteoclast targeting to promote osteoprogenitor

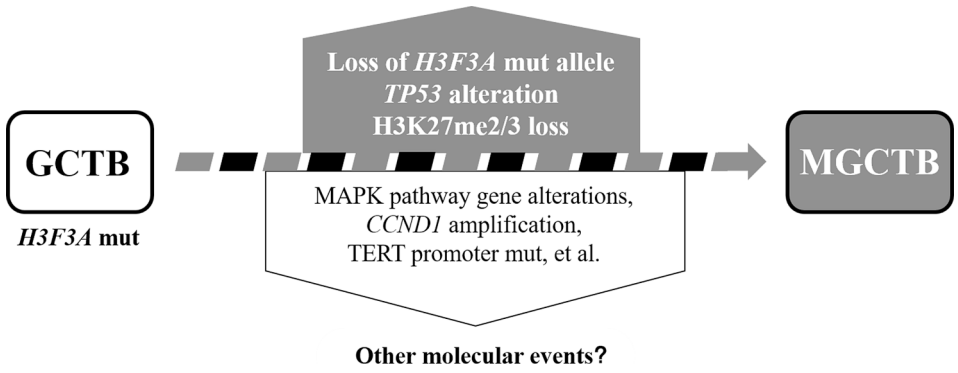


**Fig. 2. A hypothetical framework for malignant transformation of GCTB.** *H3F3A* mutant allele loss, *TP53* alteration (including *TP53* mutation, p53 overexpression), H3K27me2/3 loss, and other associated molecular events may play a role in the malignant transformation of GCTB, although the exact mechanisms underlying this process remain poorly understood. MGCTB, malignant GCTB.

proliferation to drive GCTB tumorigenesis via paracrine secretion [71]. Denosumab, which reduces osteoclast accumulation at tumor site reverses this carcinogenic cascade. The osteoclastic effect on osteoprogenitor maturation can however be decoupled by hTERT alterations often associated with MGCTB, hence predicts reduced responsiveness of MGCTB harboring the hTERT mutations to denosumab [69]. Taken together, these findings revealed an important molecular mechanism of H3.3-G34W in driving GCTB via the modulation of the tumor microenvironment.

Other research suggests that H3.3-G34W variant is physically integrated into chromatin: It is proposed that the alteration of a single amino acid in H3.3 leads to epigenomic changes that have significant consequences for the growth of neoplastic stromal cells and, more broadly, for the development of cancer [72]. The study conducted by Lutsik et al. [72] highlighted notable and reproducible differences in the epigenome of H3.3 MUT neoplastic stromal cells compared with H3.3 WT neoplastic stromal cells. The heterochromatic regions exhibited a significant decrease in methylation, whereas the bivalent domains displayed various changes, including an increase in DNA methylation, a decrease in chromatin accessibility, and a loss of several histone modifications (such as H3K27me3 and H3K4me3).

### 3.2.2. *Dysregulated RANK/RANKL pathway*

In H3.3-G34W mutant stromal cells, gene expression profiles indicate that the target genes of the transcription factor E2F are the most significantly downregulated, implying that the increased rate of cellular division in altered stromal cells is likely a result of the suppression of inhibitory factors that control the cell cycle, particularly the members of the E2F4-6 family. In consideration of the pertinent genes associated with GCTB that are affected by E2F transcription factors, it was shown that RANKL and osteoprotegrin (OPG) are targeted in a distinct manner. This process demonstrates a collaboration between histone H3.3 and the E2F transcription factors. G34W mutation causes a disruption in E2F regulation, leading to a release in the elevated amounts of RANKL and a decreased in the levels of its counteracting receptor OPG [59].

OPG and osteoprotegerin ligand (OPGL), act as components of a ligand receptor system that directly regulates the progression of osteoclast formation and bone resorption [73]. OPG is a soluble RANKL decoy receptor that is predominantly produced by osteoblasts and which prevents osteoclast formation and the resorption of bone by osteoclasts [73]. Evidence suggests that OPGL functions to stimulate the formation of osteoclasts and the cell surface receptor that interacts with OPGL is also the ligand for the TNF related protein receptor activator of nuclear factor-kappa B (RANK) [74].

The RANK-RANKL-OPG pathway is crucial in the process of osteoclastogenesis, as it stimulates the recruitment of osteoclastic cells from mononuclear osteoclast precursor cells in the bloodstream. These precursor cells then differentiate into multinucleated osteoclast-like giant cells, which are primarily responsible for the significant bone resorption caused by the tumor [75]. The neoplastic mononuclear stromal cells express a high level of RANKL, whereas the large, multinucleated osteoclast-like giant cells and their monocytic progenitors express the related receptor RANK. OPG functions as a soluble decoy receptor, effectively inhibiting the interaction between RANKL and its cellular receptor, RANK. The expression of RANKL and OPG is tightly regulated to modulate bone resorption and maintain bone density by governing the activation state of RANK on osteoclasts (Fig. 3). In 2013, the RANKL inhibitor Denosumab was approved for the treatment of GCTB [76]. However, Denosumab was ineffective in eradicating the neoplastic cells in the patients with GCTB due to the persistence of *H3F3A* mutations [77]. Gong et al. [78] utilized Sanger sequencing to verify such existence of *H3F3A* mutations in among 9 instances of GCTB following denosumab treatment. Udagawa et al. [79] further confirmed positive H3.3 G34W antibody staining after denosumab treatment among cases that were positive before treatment. In these cases, osteoblast-like cells surrounding the bone and osteocyte-like cells within the bone expressed the osteoblastic (SATB2) and osteocytic (sclerostin/SOST) differentiation markers, respectively, with the mononuclear stromal cells expressing both markers after denosumab administration. These findings suggest that, following denosumab treatment, GCTB cells that have differentiated into osteocytes lack the necessary biological signals through the RANK-RANKL signaling pathway to recruit and stimulate osteoclasts.

### 3.2.3. *Other genetic and epigenetic changes*

Numerous recent studies have assessed the involvement of non-coding RNAs in the development of GCTB. Oncogenic or tumor suppressive mechanisms of micro-RNA via targeting genes in the Akt signaling pathway: matrix metallopeptidase 9 (*MMP-9*), cytochrome *C* oxidase assembly factor 1 homolog (*COA1*), homeobox A1 (*HOXA1*), tartrate-resistant acid phosphatase (*TRAP*) and cathepsin K (*CK*) has been summarized elsewhere [80]. Jiang et al compared the expression of long non-coding RNA (lncRNA) of 20 cases each of primary, recurrent GCTB and non-GCTB bone trauma using microarray technology, and uncovered a pair of reciprocally expressing lncRNAs from the recurrent GCTB samples: upregulation of AK124776 and downregulation of RP11-160A10.2 [81]. This work points to plausible involvement of lncRNA in GCTB pathology and posits these lncRNAs as possible diagnostic markers for disease recurrence.

Malignant cells from GCTB patients were noted to exhibit telomere instability by several reports presumably due to *H3F3A* mutation-dependent H3.3 nucleosomes dysfunction giving rise to aberrant chromatin modifications that underlie telomere defects [18]. Telomere fusion was a frequent chromosomal abnormalities observed in 50 % to 70 % of GCTB cases, as determined by cytogenetic analysis [82,83]. This defect was proposed to arise from aberration in telomere maintenance resulting in the erosion of telomere that caps chromosomal ends [84].

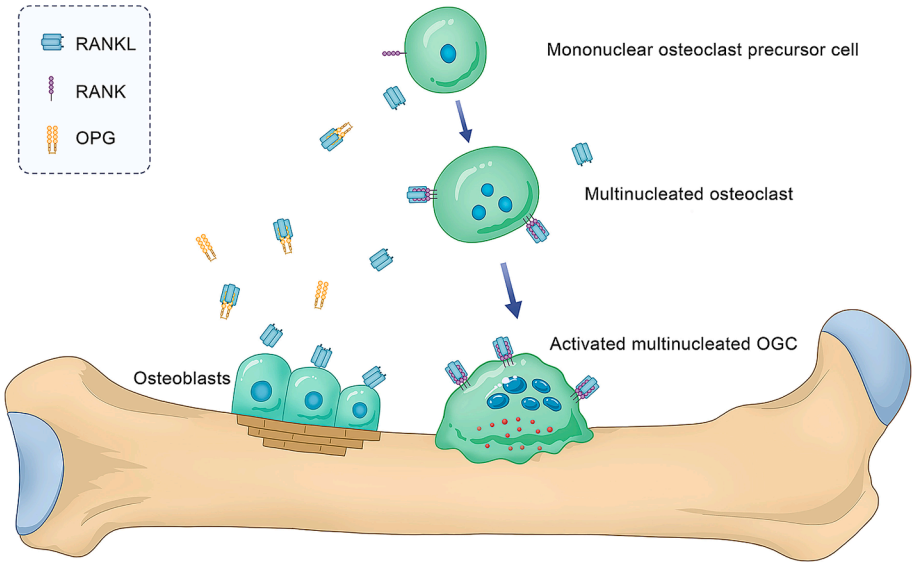


**Fig. 3. Coordination of osteoclastogenesis by RANK, RANKL and OPG.** Mononuclear osteoclast precursor cells express RANK (receptor activator of nuclear factor kappa-B). The high levels of RANKL (receptor activator of nuclear factor kappa-B ligand) expressed by osteoblasts stimulate the recruitment of mononuclear cells, which favor differentiation into RANK-positive multinucleated osteoclastic giant cells (OGCs). Osteoblasts also release OPG (osteoprotegerin), which binds to and inactivates RANKL. The expression of RANKL and OPG is therefore coordinated to regulate bone resorption and density by controlling the activation state of RANK on osteoclasts. OGC: Osteoclastic giant cell.

GCTB is positive for markers for telomerase maintenance, specifically hTERT and promyelocytic leukemia body-related antigens, in two types of cells: mononuclear osteoclast precursor cells and neoplastic stromal cells [84]. There is also a modest decrease in telomere length [85], albeit telomere dysfunction is not solely accountable for the underlying genetic instability [85,86]. One hypothesis suggests that centrosome anomalies may lead to chromosomal instability due to incorrect separation during the course of the cell cycle [87]. The occurrence of centrosome amplification and aneuploidy is more prevalent in recurrent and metastatic GCTB, indicating a potential association of such genomic instabilities with clinical behaviour [88,89].

## 4. Conclusion

GCTB presents unique challenges because of its benign histological appearance but locally aggressive behavior. Research has revealed relatively comprehensive understanding of the pathology of GCTB to achieve diagnostic aims. However, the unclarified carcinogenic mechanisms at the molecular level has prohibited significant advancement in the therapy of this disease, particularly when the disease become metastatic. Recent accounts however documented the loss of the pervasive *H3F3A* G34W from the genome of the malignant cells raising the differential implication of this pervasive mutant in disease initiation but its loss for metastatic event, pointing to potential applicability of employing the loss of this marker to monitor more advance malignancy. Revelation of novel epigenetic dysregulation in GCTB pathogenesis posit attractiveness of combining inhibitors of epigenetic enzymes to corroborate with conventional treatment agents such as denosumab for more effective disease management. Ongoing research in genetic and epigenetic regulation, genomic alterations, and the dynamic interplay between neoplastic stromal cells and the tumor microenvironment is expected to unlock more effective treatment of this intriguing bone tumor.

## CRediT authorship contribution statement

**Yibing Yao:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Victor Kwan Min Lee:** Writing – review & editing, Funding acquisition. **Ee Sin Chen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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