RANKL, *OPG*, and RUNX2 expression and epigenetic modifications in giant cell tumour of bone in 32 patients

association with clinicopathological characteristics and recurrence

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Aims

The present study investigated receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), and Runt-related transcription factor 2 (RUNX2) gene expressions in giant cell tumour of bone (GCTB) patients in relationship with tumour recurrence. We also aimed to investigate the influence of CpG methylation on the transcriptional levels of RANKL and OPG.

Methods

A total of 32 GCTB tissue samples were analyzed, and the expression of RANKL, OPG, and RUNX2 was evaluated by quantitative polymerase chain reaction (qPCR). The methylation status of RANKL and OPG was also evaluated by quantitative methylation-specific polymerase chain reaction (qMSP).

Results

We found that RANKL and RUNX2 gene expression was upregulated more in recurrent than in non-recurrent GCTB tissues, while OPG gene expression was downregulated more in recurrent than in non-recurrent GCTB tissues. Additionally, we proved that changes in DNA methylation contribute to upregulating the expression of RANKL and downregulating the expression of OPG, which are critical for bone homeostasis and GCTB development.

Conclusion

Our results suggest that the overexpression of RANKL/RUNX2 and the lower expression of OPG are associated with recurrence in GCTB patients.

Article focus

- The overexpression of receptor activator of nuclear factor kappa-B ligand (RANKL)/Runt-related transcription factor 2 (RUNX2) and the lower expression of osteoprotegerin (OPG) are associated with tumour recurrence in giant cell tumour of bone (GCTB) patients.

Key messages

- RANKL, RUNX2, and OPG have a crucial role in the recurrence and aggressive behaviour of GCTB.

Strengths and limitations

- Our results indicate the upregulation of RANKL and RUNX2 and downregulation of OPG genes in recurrent GCTB cases

compared with non-recurrent cases. This finding underlines their involvement and crucial role in GCTB recurrence.
- We proved that changes in DNA methylation contribute to regulating the expression of OPG and RANKL genes, which are critical for bone homeostasis and GCTB development.
- A small sample size was used, although we plan to increase the number of patients in future research.

Introduction

Giant cell tumour of bone (GCTB) is a relatively uncommon intermediate tumour. It is painful, with a high tendency to recur locally, and in rare cases it can undergo transformation to a malignant form.[1] According to the most recent World Health Organization (WHO) classification,[2] these tumours fall into the category of lesions with intermediate behaviour and locoregional aggressiveness, and in exceptional cases develop distant metastases.[3] Tumour recurrence is a major problem: it is an enormous challenge encountered during the clinical treatment of GCTB,[4] with incidence rates of 0% to 65%.[5,6] Recurrence of GCTB is not fatal in most cases, but can lead to disability and poor quality of life as a result of repeated and radical operations, loss of bone stock, and secondary arthritis of the joints.[7]

The identification of risk factors influencing recurrence in GCTB is still under-developed. A previous study has reported that recurrence is influenced by clinical features, such as sex, age, location, tumour size, and surgery method.[8] Moreover, certain factors were proposed as predictors of recurrence and aggressiveness markers in GCTB. Indeed, the overexpression of interleukin-17A (IL-17A) and β-catenin is closely associated with GCTB progression and recurrence.[9]

Moreover, the receptor activator of nuclear factor kappa-B (*RANK*) pathway is often reported to be involved in the recurrence of GCTB. It is a key signalling system of bone remodelling that plays a critical role in the differentiation of precursors into multinucleated osteoclasts, which are responsible for bone resorption. The lack of RANK ligand (*RANKL*)-*RANK*-osteoprotegerin (*OPG*) signalling balance induces dysregulation of bone remodelling, which leads to changes in bone mass. This can be explained by an increase in bone destruction, bone metastasis, and the development of skeletal tumours.[10] Previous reviews have focused on the association of this pathway in the pathogenesis and progression of GCTB, as well as discussing the possible therapeutic strategies by targeting this pathway.[10,11] According to the Food and Drug Administration (FDA), some humanized monoclonal antibodies such as denosumab, which target the *RANKL* (RANKL inhibitor), have been employed in the treatment of GCTB when surgery is not possible, or is likely to result in severe morbidity.[12] Recent studies have shown an elevated incidence of recurrence and sarcomatous transformation in patients with GCTB following denosumab therapy.[13,14] Therefore, a new potential drug target is needed for GCTB.

Additionally, recent studies have identified that *RANKL* is bound to *RANK* on the surface of osteoblasts and transmits signals to increase the expression of downstream Runt-related transcription factor 2 (*RUNX2*), thereby promoting the differentiation and maturation of osteoblasts.[15] *RUNX2*, also known as core-binding factor α1 (*Cbfa1*), is a member of the Runt homology domain family of transcription factors, essential for osteoblast differentiation and bone formation. It is expressed in multipotent mesenchymal cells, osteoblast lineage cells, and chondrocytes.[16]

Several investigations have addressed the role of the trio *RANKL/OPG/RUNX2* in GCTB aggressiveness.[17,18] However, the identification of risk factors influencing recurrence, and the relationship between *RANKL/OPG/RUNX2* expression and recurrence, are still not well explored. Moreover, others have confirmed that DNA methylation influenced the transcriptional expression of *OPG* and *RANKL*, which probably take on a 'main switch' role in the pathogenesis of bone diseases.[19] Thus, for the first time, we aimed to explore the role of the 'RUNX2/RANKL/OPG' trio in GCTB recurrence. Our primary objectives were to identify and characterize the expression of the RANKL/OPG/RUNX2 trio in GCTB tissues using quantitative polymerase chain reaction (qPCR), and to examine their expression in relation to tumour recurrence. Additionally, we sought to investigate the impact of CpG-rich region methylation on the transcriptional messenger RNA (mRNA) levels of RANKL and OPG.

Methods

Patients and tissue samples

A total of 32 patients diagnosed with GCTB were enrolled in this study. Recruitment took place between 2018 and 2020 at our institution. All of the patients underwent their surgical procedures at our institution, and the tissue obtained during surgery underwent pathological examination reviewed by pathologists. All procedures performed in studies involving human participants were in accordance with the ethical standards of the National Committee of Medical Ethics, Tunisia.

Patients were scheduled for follow-up, which was defined as the time that elapsed from the confirmed diagnosis to the most recent follow-up date. These patients underwent their initial surgery for primary GCTB and were subsequently monitored for a maximum duration of 24 months. Based on their clinical status (recurrent or non-recurrent), we categorized the patients into two groups: recurrent GCTB and non-recurrent GCTB.

Ten control patients, comprising six females and four males, were included in the study. These controls were hospitalized due to fractures that occurred in healthy bones, which were non-tumoural, non-infectious, and not associated with any structural bone pathology. Samples were gathered from the cleaning products used during the fracture treatment process, without causing any harm or disruption to the patients' treatment, as these were typically discarded bone debris. We collected these debris fragments to use them as references, since they consisted of fragments of healthy bone (patients consented beforehand to the collection of their bone samples).

Molecular analysis

RNA extraction and cDNA synthesis: TRIzol reagent (Invitrogen; Thermo Fisher Scientific, USA) was used to extract RNA from specimens according to the manufacturer's protocol followed by quantification with NanoDropND-1000 (Thermo Fisher Scientific). A quantity of 300 ng of RNA served as a template for complementary DNA (cDNA) synthesis using hexamer primers (50 nmol) and a dNTP mix (10 nmol). This

Table I. Primer sequences for quantitative polymerase chain reaction.

Gene	F or R	Primer sequences (5′-3′)	Annealing T, °C
RANKL	Forward	5′-CAA CAT ATC GTT GGA TCA CAG CA-3′	60
	Reverse	5′-GAC AGA CTC ACT TTA TGG GAA CC-3′	
OPG	Forward	5′-CAC AAA TTG CAG TGT CTT TGG TC-3′	60
	Reverse	5′-TCT GCG TTT ACT TTG GTG CCA-3′	
GAPDH	Forward	5′-GCTCTCTGCTCCTCCTGTTC -3′	60
	Reverse	5′-CGCCCAATACGACCAAATC C-3′	
RUNX2	Forward	5′-CGGAATGCCTCTGCTGTTAT -3′	60
	Reverse	5′-AGCTTCTGTCTGTGCCTTCT -3′	

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor kappa-B ligand; RUNX2, Runt-related transcription factor 2; T, temperature.

step was followed by ten-minute incubation at 70°C. Next, buffer, DTT (0.2 μmol), and SuperScriptM II RTase (200 units) were added. The reaction was then incubated for 12 minutes at 25°C followed by 50 minutes at 42°C, and a final incubation at 70°C for 15 minutes.

Quantitative polymerase chain reaction: qPCR was performed using SYBR green master mix (Bio-Rad, USA). Primer sequences and annealing temperature (T°) are described in Table I. The reaction was performed in a volume of 10 μl using the following protocol: denaturation at 95°C for five seconds, annealing at 58°C for 15 seconds, and extension at 72°C for 20 seconds, for 40 cycles. The relative amount of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, which was expressed by the mean (standard error). Experiments were performed in triplicate. The mean Ct values were calculated from triplicate PCRs for *RANKL, OPG, RUNX2,* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), and the ΔCt value for each specimen was obtained by subtracting these two values. Then the relative amount of *RANKL, OPG,* and *RUNX2* expressions were calculated by the $2^{-\Delta\Delta Ct}$ method.

Methylation analysis by qMSP

Genomic DNA was isolated with phenol-chloroform: isoamyl alcohol (15593031; Invitrogen). A volume of 1 μg of genomic DNA was modified by bisulfite treatment for 12 hours using EZ DNA Methylation kit (D5005; Zymo Research) according to the manufacturer's instructions. After bisulfite conversion, the DNA was bound to a Zymo spin column and desulfonated. The bisulfite converted DNA was eluted from the column in 10 μl of elution buffer. This treatment deaminates unmethylated cytosines into uracil but does not affect 5-methylcytosines. DNA samples were subjected to quantitative methylation-specific polymerase chain reaction (qMSP) analysis.

qMSP is based on the amplification of bisulfite-converted DNA. Sodium bisulfite converts unmethylated cytosines to uracils, while methylated cytosines remain unaffected. Thus, it enables distinction between methylated and unmethylated DNA. Primers, which targeted CpG-rich regions within the *RANKL* and *OPG* promoter regions, were designed with Methyl Primer Express software (Applied Biosystems, USA). For each region, a pair of primers was selected: one specifically recognized the bisulfite-treated methylated DNA, and the other recognized only unmethylated bisulfite-treated DNA.[20]

Statistical analysis

All statistical analysis was performed with SPSS 20.0 (IBM, USA). Data are expressed as mean (standard deviation (SD)). The normality of distribution was checked using the Shapiro-Wilk test. Levene's test was performed to verify the homogeneity of variances. The independent-samples *t*-test was used to test the two groups of data. Analysis of variance (ANOVA) was used to test whether multiple sets of data were statistically significant. We used Mann-Whitney U test to determine if there was a significant difference between two groups (recurrent and non-recurrent patients). Pearson's chi-squared test was used to determine if there was a significant association between two categorical variables (e.g. age, recurrence, gender). Pearson correlation coefficient to correlate the two study groups.

After performing qPCR and qMSP, the relative expression levels for each gene were determined using the $2^{-\Delta\Delta Ct}$ method, normalized to the expression level of the housekeeping gene *GAPDH*. Statistical significance was set at $p < 0.05$.

Results

Clinicopathological characteristics of GCTB patients

The clinical and pathological features of 32 GCTB cases (11 male and 21 female) are summarized in Table II. Their ages ranged between 12 and 74 years, with a majority between 20 and 40 years (56.2%, n = 18). Tumours were mainly located in the radius (n = 10, 31%), tibia (n = 9, 28%), femur (n = 7, 22%), fibula (n = 3, 9.5%), and other rare locations (n = 3, 9.5%). The local recurrence rates were 47% overall, and 15/32 developed a recurrent tumour after surgery. Time of recurrence ranged from six to 24 months. The mean duration of follow-up from the time of diagnosis of GCTB was ten months (SD 5,154).

Curettage generally has a significantly higher recurrence rate than wide resection.[21] Indeed, 21 of the total patients underwent curettage as a treatment. In addition, the present study revealed that the type of surgery is significantly associated with recurrence (p = 0.031, chi-squared test). In fact, 13 of 21 patients treated with curettage had a recurrence, while this occured in only two of the seven patients treated with wide resection. Therefore, there was no significant correlation between the location of the tumour, age, sex, and recurrence (p = 0.091, p = 0.072, p = 0.061, chi-squared test) (Table II).

Gene expression in recurrent GCTB disease

The expression levels of *RANKL* and OPG were investigated in 32 GCTB cases, and in ten normal bone tissues. The level of *RANKL* was significantly higher in GCTB tissues than in normal bone tissues (p = 0.01, chi-squared test). By contrast, the

Table II. Clinicopathological characteristics of giant cell tumour of bone patients.

Categories	With recurrence	Without recurrence	Total	p-value*
Cases, n (%)	15 (47)	17 (53)	32	
Sex, n (%)				0.061
Male	3 (20)	8 (47.1)	11 (34.4)	
Female	12 (80)	9 (52.9)	21 (65.6)	
Age group, n (%)				0.072
< 20 yrs	1 (6.7)	3 (17.6)	4 (12.5)	
20 to 39 yrs	10 (66.7)	8 (47.1)	18 (56.2)	
≥ 40 yrs	4 (26.7)	6 (35.3)	10 (31.2)	
Location, n (%)				0.091
Femur	2 (13.3)	5 (29.4)	7 (21.9)	
Tibia	4 (26.7)	5 (29.4)	9 ((28.1)	
Radius	7 (46.7)	3 (17.6)	10 (31.2)	
Fibula	1 (6.7)	2 (11.8)	3 (9.4)	
Other locations	1 (6.7)	2 (11.8)	3 (9.4)	
Surgical method, n (%)				0.031
Biopsy	0 (0.0)	4 (23.5)	4 (12.5)	
Intralesional curettage	13 (86.7)	8 (47.1)	21 (65.6)	
Wide resection	2 (13.3)	5 (29.4)	7 (21.9)	

*Chi-squared test.

expression level of *OPG* was slightly, although still significantly, lower in GCTB tissues compared to normal bone tissues (p = 0.010, chi-squared test) (Figure 1).

Additionally, as demonstrated in Figure 2, *Runx2* was highly expressed in GCTB tissues compared to normal bone tissues (p = 0.010, chi-squared test).

Furthermore, as shown in Figure 3, the expression status of *RANKL* and *RUNX2* was significantly associated with sex (p = 0.011), age (p = 0.011), and the method of surgery (p = 0.011), while it was not correlated with the location of GCTB (p = 0.010, all chi-squared test). In fact, their expressions were associated specifically with female sex, higher age, and curettage. Moreover, the expression of *OPG* was only correlated with the method of surgery (p = 0.011, chi-squared test) (Figure 3).

Interestingly, *RANKL* and *RUNX2* were strongly expressed in recurrent GCTB (p = 0.030, Pearson correlation coefficient). Fold change (Fc) values of 1.26 and 1.37 for RANKL and RUNX2, respectively, suggest that the expression of RANKL and RUNX2 was 26% and 37% higher in the recurrent group compared to the non-recurrent group, respectively (Table III). A significant association between the overexpression of RANKL/RUNX2 and recurrence was observed in all GCTB cases (p = 0.020, chi-squared test). More importantly, we confirmed the involvement of RANKL and RUNX2 in GCTB recurrence.

However, minimal amounts of *OPG* mRNA were detected in recurrent GCTB patients compared to non-recurrent GCTB patients. Indeed, a significant association was found between the lower expression of *OPG* and recurrence in GCTB patients (p = 0.041, chi-squared test) (Figure 4).

Accordingly, the identification of suitable molecular markers of malignancy, such as *RANKL* and *RUNX2*, would facilitate further exploration of the biological behaviour of GCTB, and identification of effective angiogenesis inhibitors.

Influence of DNA methylation on the expression of *OPG/RANKL* in GCTB

Previous bioinformatics analysis revealed two CpG islands in the *RANKL* gene: the upstream one (18 CpG sites), located at -14,415 bp from the transcription start site of isoform I (TSS I), the major *RANKL* transcript; and the downstream one (59 CpG sites), which spans from -260 bp to +615 bp of the TSS I. In this study, chose the downstream *RANKL* CpG region because the upstream one does not play an important role in the regulation of gene expression.[20]

We explored the methylation degree of the CpG-rich regions of the *RANKL* gene in GCTB tissues and in normal bone tissues. qMSP analysis showed that GCTB tissues, which expressed higher amounts of *RANKL*, showed lower methylation levels in the CpG island compared to normal tissues. *RANKL*'s lower methylation was correlated with higher transcriptional levels (p = 0.023, chi-squared test).

In terms of OPG gene expression and methylation, one island was found in the *OPG* gene (56 CpG sites), spanning from -402 to +850 bp of the TSS I. We explored the methylation degree of the CpG-rich regions of the *OPG* gene via qMSP in GCTB tissues and in controls. Then, GCTB tissues, which expressed lower amounts of OPG, showed higher methylation in the CpG island compared to control tissues. *OPG* aberrant methylation was correlated with transcriptional silencing.

Relationship between RANKL and OPG gene expression and their methylation degree

The inverse relationship observed between methylation status and mRNA expression of *OPG* and *RANKL* genes suggests that DNA methylation plays an important role in the regulation of gene expression during development. Methyl moieties at CpG residues suppress transcription by disrupting DNA-protein interactions, thus altering the accessibility of genes to trans-acting factors in the cell. Indeed, in GCTB tissues, *RANKL* overexpression correlates with a lower degree of methylation in the CpG regions. Conversely, lower expression of *OPG* correlates with hypermethylation in its promoter region (p < 0.01, Mann-Whitney U test).

To summarize, we revealed that the hypermethylation of the CpG-rich regions of *OPG* induced a decrease in *OPG* gene expression in GCTB tissues than in control tissues. However, lower methylation of the CpG regions of *RANKL* induced an increase in *RANKL* gene expression in the GCTB tissues, but not in the controls.

Discussion

The local recurrence rate in GCTB varies from 0% to 65% (4/19). There is a risk of pulmonary metastasis development in

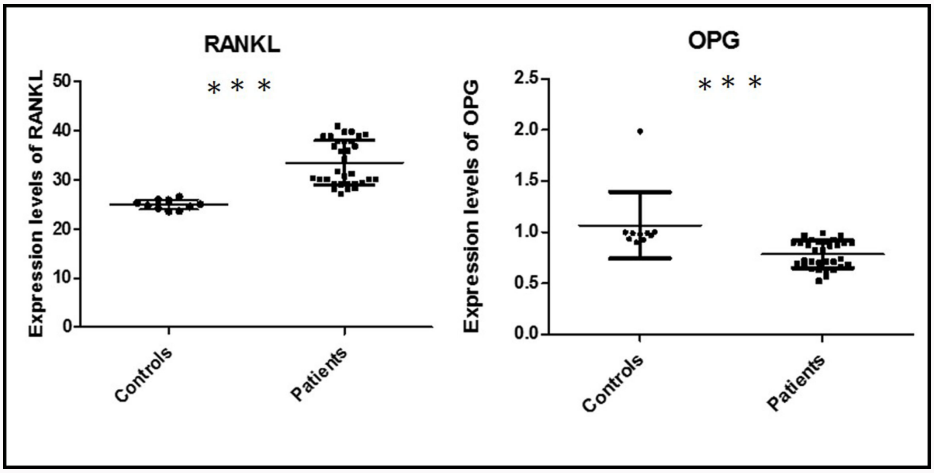

Fig. 1
Expression levels of receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) in giant cell tumour of bone (GCTB) tissues and in normal tissues. ***p < 0.05.

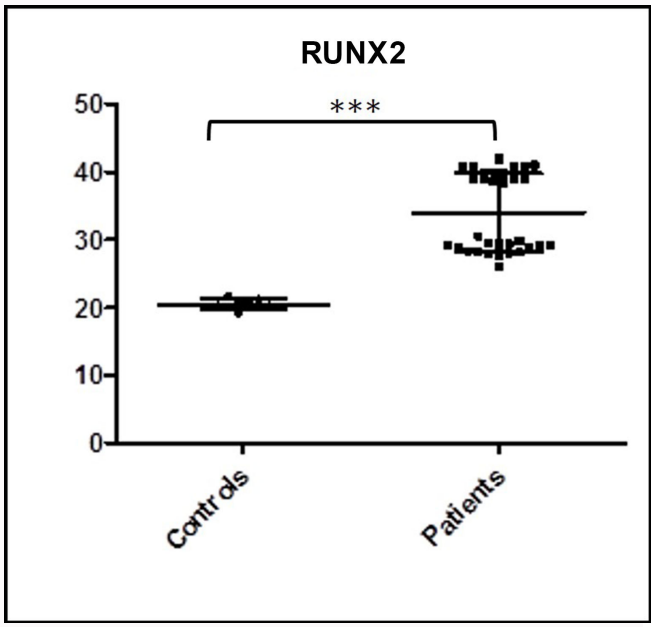

Fig. 2
Expression level of Runt-related transcription factor 2 (RUNX2) in giant cell tumour of bone (GCTB) tissues and in normal bone tissues. ***p < 0.05.

advanced or recurrent GCTB patients, with approximately 3% of cases metastasizing to the lungs.[22] In this study, we aimed to investigate *RANKL*, *OPG*, and *RUNX2* gene expressions in a cohort of GCTB patients in relation to tumour recurrence. We also aimed to investigate the influence of CpG methylation on the transcriptional levels of *RANKL* and *OPG*.

Within our cohort, no significant correlation was found between sex, age, location, and recurrence. However, some studies have reported a positive association between sex, age, and recurrence,[23,24] whereas others have reported that location in the radius and proximal tibia was more frequently associated with tumour recurrence.[25]

RUNX2 is a multifunctional transcription factor and is considered to be one of the most important markers in the process of osteoblast differentiation and maturation.[26] In this study, we found that the mRNA levels of *RANKL* and *RUNX2* in tumours were significantly overexpressed more often in GCTB tissues than in normal bone tissues. Furthermore, *OPG* was upregulated more often in GCTB tissues than in controls. Indeed, *RANK*, which is secreted from the maturing osteoclasts, binds osteoblastic *RANKL* and promotes bone formation by triggering *RANKL* reverse signalling, which activates *RUNX2*.[15] *RUNX2* is the most upstream transcription factor, essential for osteoblast differentiation. It regulates the expression of *Sp7*, the protein of which is a crucial transcription factor for osteoblast differentiation, as well as regulating the expression of bone matrix genes including *Spp1, Ibsp,* and *Bglap2*.[27] *RUNX2,* which is a vital factor in the *RANKL-RANK* pathway, is a key target for the regulation of bone destruction and is known to be associated with cell proliferation.[28] Here, we have demonstrated the positive correlation between *RUNX2* and *RANKL* expression and recurrence; this result suggests that, despite the multinuclear giant cells demonstrating bone absorption, the pivotal aspects of this process involve the recruitment and induction of osteoclast cells. These results would suggest that, during the development of GCTB, *RUNX2* promotes osteoblast maturation through regulating *RANKL* and *OPG*, and this trio has a crucial role in the development and differentiation of GCTB.

Since we found an epigenetic modulation of *RANKL* and *OPG* gene expression levels, we explored whether differential levels of DNA methylation could explain the differences in gene transcription between non-recurrent GCTB tissues, recurrent GCTB tissues, and controls. It has previously been reported that epigenetic mechanisms, particularly DNA methylation, are known to be a principal contributor to gene transcription in many tissues, as extensively studied in neoplastic disorders including bone tumours.[29,30] DNA methylation tends to block gene expression by mechanisms that are not well known, including the interference of the binding of transcription factors to the regulatory sites in DNA.[31] Although there are some indications for the role of DNA methylation of the *RANKL/OPG* genes in murine models and cancer cells,[32] it is still unknown whether DNA meth-

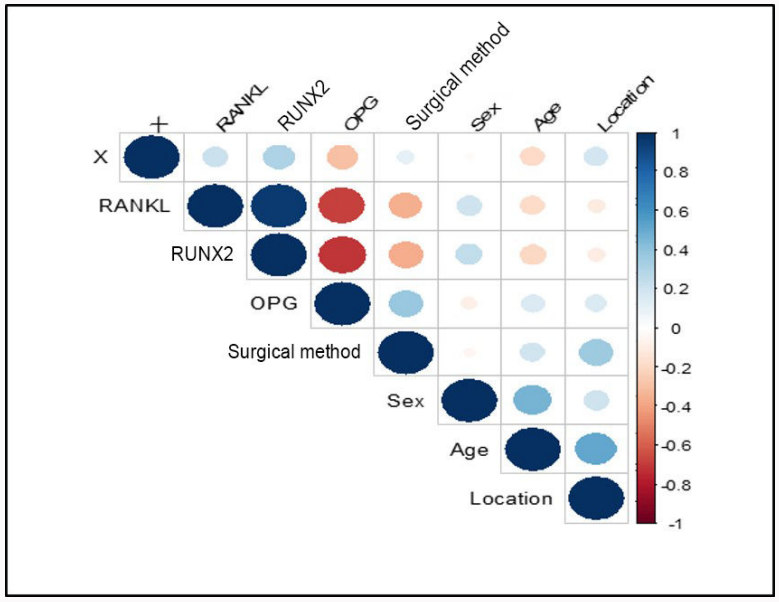

Fig. 3
Correlation of receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), and Runt-related transcription factor 2 (RUNX2) expressions with clinical data (Pearson correlation coefficient (r)).

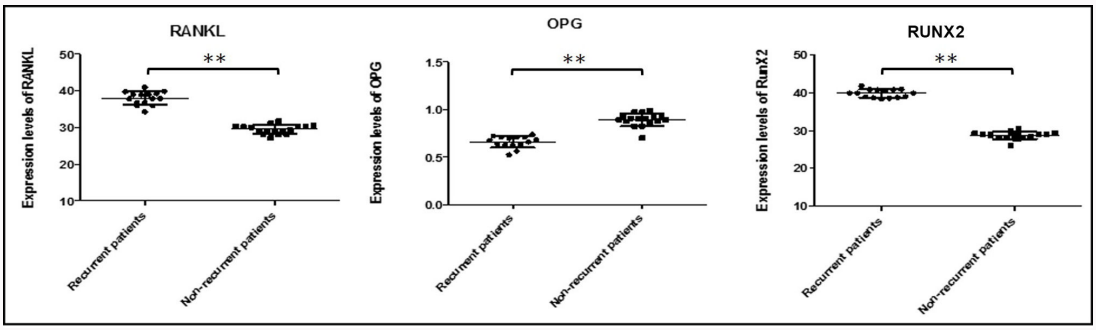

Fig. 4
Expression levels of receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), and Runt-related transcription factor 2 (RUNX2) in non-recurrent and recurrent giant cell tumour of bone (GCTB) tissues and in controls. Association of the expression levels of RANKL and OPG with recurrence in GCTB. Correlations were tested using the non-parametric Mann-Whitney U test. **p < 0.05.

Table III. Comparison of receptor activator of nuclear factor kappa-B ligand and Runt-related transcription factor 2 expression levels in non-recurrent giant cell tumour of bone (GCTB) patients versus recurrent GCTB patients.

Group	RANKL					RUNX2				
	Mean	Fc	SD	SE	p-value*	Mean	Fc	SD	SE	p-value*
Non-recurrent patients (n = 17)	30.059	1.26	0.67	0.16		29.147	1.37	0.988	0.239	
Recurrent patients (n = 15)	37.951	0.7	1.68	0.43	0.010	39.94	0.7	0.72	0.18	0.010

*Independent-samples *t*-test.
Fc, fold change; RANKL, receptor activator of nuclear factor kappa-B ligand; RUNX2, Runt-related transcription factor 2; SD, standard deviation; SE, standard error.

ylation regulates the expression of these genes in human bone. Therefore, we have studied the methylation profile of the *RANKL* downstream CpG-rich region, and the *OPG* CpG region in GCTB tissues and normal bone tissues. As a result, GCTB tissues that expressed higher amounts of *RANKL*, showed lower methylation in the CpG island than the control tissues. Meanwhile, for *OPG*, GCTB tissues that expressed lower amounts of OPG showed higher methylation in the CpG island than the control tissues. We can speculate that

DNA methylation represents an on/off regulator of *RANKL/OPG* gene expression.

In our study, we identified an inverse relationship between DNA methylation levels and gene expression, which is consistent with findings from a previous study that have also confirmed this association.[33] Thus, the CpG-rich regions of GCTB tissues, which highly expressed *RANKL* transcripts, were hypomethylated, whereas the CpG-rich regions of GCTB tissues, which hardly expressed *OPG*, were hypermethylated. A previously published study confirmed that changes in DNA methylation contribute to regulating the expression of *RANKL* and *OPG* genes, which are critical for bone homeostasis.[19] In addition, another study demonstrated that DNA methylation influenced the transcriptional expression of *OPG* and *RANKL*, which probably took on a 'main switch' role in the pathogenesis of primary osteoporosis.[20]

In conclusion, this study unveiled a more pronounced upregulation of RANKL and RUNX2 genes and a more significant downregulation of OPG genes in GCTB tissues compared to controls. Intriguingly, this pattern was even more prominent in recurrent cases than in non-recurrent cases. This finding underlines the involvement of *RANKL*, *RUNX2*, and *OPG* and their role in GCTB recurrence. Moreover, we demonstrated that changes in DNA methylation contribute to regulating the expression of *OPG* and *RANKL* genes, which are critical for bone homeostasis and GCTB development.

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Ethical review statement

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