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Analysis of the giant cell tumor cell line of bone TIB 223 on the effect of quercetin

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Abstract

The uncontrolled and inappropriate growth of cells in the body is known as cancer. Giant Cell Tumor of Bone (GCTB) is a neoplasm with an aggressive behavior that, when it metastasizes, particularly manifests in the lungs. This study aimed to conduct a preliminary assessment of the activation of apoptosis as a cell death mechanism induced by quercetin TIB 223 cells. For this purpose, messenger RNA levels and the expression of proteins related to this pathway were analyzed. The TIB 223 cells were treated with two different concentrations of quercetin (91.1 µM and 220 µM). A flow cytometry analysis was performed to evaluate the expression of caspase-3 and Proliferating Cell Nuclear Antigen (PCNA) proteins. Quantitative Polymerase Chain Reaction (qPCR) analysis was performed to evaluate changes in the expression of genes regulating apoptosis (caspase-3) and proliferation (PCNA) after treatment. Flow cytometry analysis revealed a decrease in PCNA levels, indicating increased apoptosis and reduced proliferation, suggesting that quercetin effectively induces apoptotic pathways in GCTB cells. These results provide insight into the molecular mechanisms behind the anticancer activity of quercetin, highlighting its potential as a therapeutic agent for metastatic GCTB cells. We conclude that quercetin has the potential to be used in the future as a concomitant therapy alongside standard treatments to prevent the recurrence of GCTH tumors, either at the primary tumor site or in metastatic lesions.

Abbreviations:
CIPN = Chemotherapy-Induced Peripheral Neuropathy
GCTB = Giant Cell Tumor of Bone
PCNA = Proliferating Cell Nuclear Antigen
PSB = Phosphate Buffered Saline
qPCR = Quantitative Polymerase Chain Reaction

INTRODUCTION

The uncontrolled and inappropriate growth of cells in a tissue of the body is known as cancer. These cells threaten the normal function of organs and other cells. In more advanced cases, they travel through the bloodstream to reach different areas of the human body.[1]

A bone tumor is a proliferation of cells within the bones. This proliferation is abnormal and can be cancerous, either malignant or benign. Its cause is

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unknown; however, it is associated with various factors such as hereditary genetic abnormalities, radiation, and injuries.[2]

Giant Cell Tumors of Bone (GCTB) are aggressive neoplasms that arises from osteoclasts. They exhibit abundant vascularization with giant cells, are rare and develop when benign cells cluster together to form a large mass. These giant cells can spread to different parts of the body and become aggressively proliferating tumors.[3]

Beyond its biological aggressiveness, GCTB imposes a substantial disability burden that is increasingly recognized in clinical and epidemiological studies. Giant cell tumors frequently compromise structural bone integrity, leading to pain, functional limitations, and motor disability, largely due to cortical bone destruction and a high incidence of pathological fractures. Skeletal complications such as fractures, spinal cord compression and the need for orthopedic surgery are among the major sources of disability in bone tumors and bone metastases, often resulting in long-term mobility impairment and reduced independence in activities of daily living.[4,5]

Epidemiological analyses show that patients with bone tumors or skeletal metastatic disease experience significantly higher rates of mobility restriction, need for assistive devices, reduced quality of life, and greater dependence on healthcare resources compared with cancer patients without bone involvement.[6] In addition, conventional treatments used for GCTB such as repeated curettage, aggressive bone resections, radiotherapy, and systemic therapies can lead to persistent sequelae including chronic pain, neuropathy, fatigue, and limited joint function, all of which contribute to long-term disability.[7,8] Consequently, there is a growing interest in therapeutic strategies that may reduce both tumor burden and treatment-related morbidity, ultimately improving functional outcomes and reducing disability in affected patients.[8]

Metastasis originates when cells from the primary tumor travel through the body and begin to form a new tumor, either in the organs or tissues. Pulmonary metastasis is a neoplasm of various localizations that metastasize to the lungs. Generally, it does not cause symptoms, and when symptoms do occur, they are usually seen in advanced stages. There are several potential pathways through which neoplastic cells settle in the pulmonary parenchyma, including venous circulation, lymphatic circulation, direct extension, and bronchogenic dissemination.[9]

The most common oncological treatment for metastatic giant cell tumors is complete pulmonary resection with systematic lymph node dissection, which involves the anatomical resection of the lobes affected by the tumor. Other treatment modalities include chemotherapy, aimed at reducing or eliminating tumors through the use of pharmacological agents, and radiation therapy, which involves the administration of high-intensity radiation to eradicate tumors. Additionally, targeted therapy, which involves the use of specific medications to inhibit the growth of cancerous cells is employed. However, the side effects of these treatments may include peripheral neuropathy, arrhythmias, pulmonary inflammation or inflammation in other parts of the body, and hepatic damage.[3]

Currently, there are various alternative therapies, including combination therapies such as chemotherapy combined with hormone therapy. Hormone therapy involves the addition, blockade, or removal of hormones that are responsible for the initiation of certain types of cancer. Quercetin, a flavonoid found in various plant-based foods, is currently used in the treatment of certain allergies, asthma, and various types of cancer, including pancreatic, breast, ovarian, liver, glioblastoma, prostate, and lung cancers (primarily in experimental animal models). The described mechanism of action of quercetin in cancer involves inhibiting the growth of cancer cells both *in vitro* and in animal models, with its effects being implicated in apoptosis.[10,11]

To date, the cytotoxic activity of quercetin on isolated GCTB cells (TIB 223) derived from pulmonary metastases has not been described in depth. Therefore, the aim of this study was to conduct a preliminary assessment through messenger RNA analysis, as well as the expression of proteins related to cellular apoptosis, to determine if it is activated as a cell death mechanism in TIB 223 cells after being exposed to quercetin. Thus, the results generated will allow for further deepening the knowledge regarding the cell death model we are studying.

MATERIAL AND METHODS

Cell culture

The TIB 223 cell line of giant cell tumor was isolated from the lung of a male patient with fibrous histiocytoma, they were obtained from the *American Type Culture Collection* (Rockville, MD, USA). The cells

were cultured with culture medium (Corning McCoy's 5A [Iwakata & Grace modification]), the cells were cultured with 10% fetal bovine serum and 1% antibiotic-antimycotic in controlled humidity and temperature conditions. They were expanded in 150 cm² flasks, and once expanded, the cells were detached and aliquots of one million cells were frozen in cryovials in liquid nitrogen until further use. In a previous study conducted by our research group, it was established that quercetin-induced cell death was observed at 24 hours with an IC_{50} of 91.1 μM of quercetin.[12]

Flow Cytometry analysis

For this experiment, the previously established IC_{50} of 91.1 μM of quercetin was used. A slightly higher dose, more than twice the IC_{50}, corresponding to 220 μM, was also used.[12] The cells were cultured for approximately two months, the culture mean was changed every second day during those two months, after this period of time, quercetin was added and the cells were cultured again, once the time had passed to see the effect the culture mean was removed, all the plates were washed whit phosphate buffered saline (PBS). The phosphate buffered saline was removed and the cells, following aspiration of PBS, cells were mechanically detached using a cell scraper in fresh PBS. The cell suspension was transferred to a 1.5 mL microcentrifuge tube and centrifuged at 1,500 rpm for 20 minutes at 4 °C. Upon completion of centrifugation, the PBS supernatant was removed while preserving the cell pellet, which was subsequently stored in frozen conditions.

After 24 hours of exposure to TIB 223 cells, 2 mL of medium was extracted from each dish to initiate trypsinization. One milliliter of trypsin was added to each dish, and they were immediately placed in an orbital incubator to detach the cells. This process was carried out for five minutes at 150 rpm at 38 °C. Upon completion, the remaining medium from each dish was added, and the samples were centrifuged. Subsequently, 1 mL of the permeabilizing agent obtained from BD Biosciences (Cat: 554722, San José, CA, USA), was added to each dish, followed by 20 minutes of refrigeration. After the incubation period, the supernatant was decanted, and 600 μL of the washing solution was added. Following this step, 1 μL of each antibody, 1 μL of caspase-3 for the corresponding dishes, and 1 μL of Proliferating Cell Nuclear Antigen (PCNA) for the corresponding dishes were added, and the samples were refrigerated.

For flow cytometry analysis, 1 μL of washing with PBS were added to each sample before being placed in the cytometer for analysis.

Quantitative Polymerase Chain Reaction (qPCR) Analysis

Following treatment with two different concentrations of quercetin (91.1 and 220 μM), incubation was conducted for 24 hours. For relative qPCR, previously collected cells stored at -80 °C were thawed, and total RNA extraction was performed using Trizol reagent (Invitrogen, Carlsbad, California, USA). Subsequently, cDNA was synthesized using 1 μg of total RNA and kit reagents. For qPCR implementation, the reagents listed in *(Table 1)* were utilized.

The equipment was then programmed through four stages: the first stage consisted of one cycle at 42 °C for 15 minutes, followed by a second stage comprising another cycle at 95 °C for 3 minutes. The third stage included 40 cycles with the following temperatures: 15 seconds at 95 °C, 30 seconds at 57 and/or 60 °C, and the final stage consisted of the Melting analysis. All samples were analyzed in triplicate to ensure reproducibility of results. Finally, relative mRNA

Table 1: Genes used in quantitative polymerase chain reaction, considering the RPL27 gene as constitutive.

Gen	Forward	Reverse
HRPL27	CTGGGAAGGTGGTGCTTGTC	TAGCGGTCAATTCCAGCCAC
Caspase 8	ATTTGCCTGTATGCCCGAGC	CCTGAGTGAGTCTGATCCACAC
Caspase 3	AGAGGGATCGTTGTAGAAGTC	ACAGTCCAGTTCTGTACCACG
PCNA	CCTGCTGGGATATTTAGCTCCA	CAGCGGTAGGTGTCGAAAGC
RIP1K	TGGGGCTCATCATAGAGGAAG	TGGCCTTGGTGAGGTTTGATCC
VEGFA	ATGAACTTTCTGCTGTCTTGGGT	TGGCCTTGGTGAGGTTTGATCC
SNHG6	CTCTGCGAGGTGCAAGAAAG	AATACATGCCGCGTGATCCT

quantification was determined using the ΔΔCT (delta delta CT) method.

Statistical analysis

All experiments were performed in biological triplicates, and each analytical technique was carried out under identical experimental conditions to ensure reproducibility. Statistical analyses were performed using GraphPad Prism version 9.5.0 (GraphPad Software, San Diego, CA, USA).

For the flow cytometry assays, the percentage of positive cells for each marker (caspase-3 and PCNA) was quantified for the control, 91.1 μM quercetin, and 220 μM quercetin conditions. Because the three experimental groups consisted of independent samples, comparisons between groups were conducted using an unpaired two-tailed t-test. Each treatment concentration was compared independently against the control group to determine whether quercetin induced statistically significant changes in marker expression. In addition, a direct comparison was performed between the 91.1 and 220 μM groups to evaluate whether increasing the concentration produced additional significant effects.

For qPCR analysis, relative gene expression levels were calculated using the 2-ΔΔCT method, using RPL27 as the reference housekeeping gene. Each gene was analyzed in triplicate reactions, and the resulting fold-change values were compared across the three conditions. As with the flow cytometry data, unpaired two-tailed t-tests were used to identify significant differences in expression between the control and each quercetin concentration. Genes analyzed included caspase-3, caspase-8, PCNA, RIPK1, SNHG6, and VEGFA. Only genes with statistically significant differential expression in at least one of the treatment groups were considered biologically relevant. For all analyses, p-values < 0.05 were considered statistically significant.

RESULTS

The following are observations of morphological changes after 24 hours of treatment. These cells exhibit a morphology similar to that of fibroblasts *(Figure 1A-C)*.

Flow cytometry analysis at 24 hours of treatment

Flow cytometry analysis at 24 hours showed that the control had a mean expression of positive cells for the caspase 3 marker of 0.42 ± 0.11%, while for the 91.1 μM concentration, the mean expression for the marker was 93.77 ± 2.61%, and finally for the 220 μM concentration was 95.40 ± 2.75% *(Figure 1 D-E)*. Upon analysis, statistically significant differences were found between both conditions and the control ($p \leq 0.0001$ for 91.1 μM and $p \leq 0.0001$ for 220 μM); however, no significant difference was found between the two treatment concentrations ($p = 0.47$) *(Figure 1F)*.

On the other hand, for PCNA, flow cytometry analysis at 24 hours showed that the control had a mean expression of positive cells for the caspase 3 marker of 23.48 ± 4.14%, while for the 91.1 μM concentration, the mean expression for the marker was 1.56 ± 1.37%, and finally for the 220 μM concentration was 6.17 ± 0.06% *(Figure 1G-H)*. Upon analysis, statistically significant differences were found between both conditions and the control ($p \leq 0.0010$ for 91.1 μM and $p \leq 0.0006$ for 220 μM); however, no significant difference was found between the two treatment concentrations ($p = 0.15$) *(Figure 1I)*.

qPCR analysis

The control yielded an average RNA of 1.6, the 91.1 μM concentration yielded an average of 2.1, and the 220 μM concentration yielded an average of 12.92. The average RNA was adjusted from 1.4 to 10 μL. Similarly, we can observe the quantities used in the mix for performing qPCR. Notably, the same quantities of the mix were used throughout, while the RNA varied for each concentration and control. In the following figure, we can observe the different genes used in qPCR at 24 hours of treatment with 91.1 and 220 μM concentrations.

From these graphs, we can observe that PCNA, caspase 3, and the SNHG6 gene proved to be significant. However, we can observe that there was an increase for caspase 8, although it did not reach significance, and for the remaining genes, RIPK1 and VEGFA, we can observe that there was no significance whatsoever *(Figure 2)*.

DISCUSSION

This study demonstrated the effect of quercetin on cell death and proliferation in the giant cell tumor line TIB 223. Several in vitro studies have shown that quercetin can inhibit proliferation and induce apoptosis in different types of cancer cells, such as breast, lung, colon, prostate cancer, and leukemia.[13]

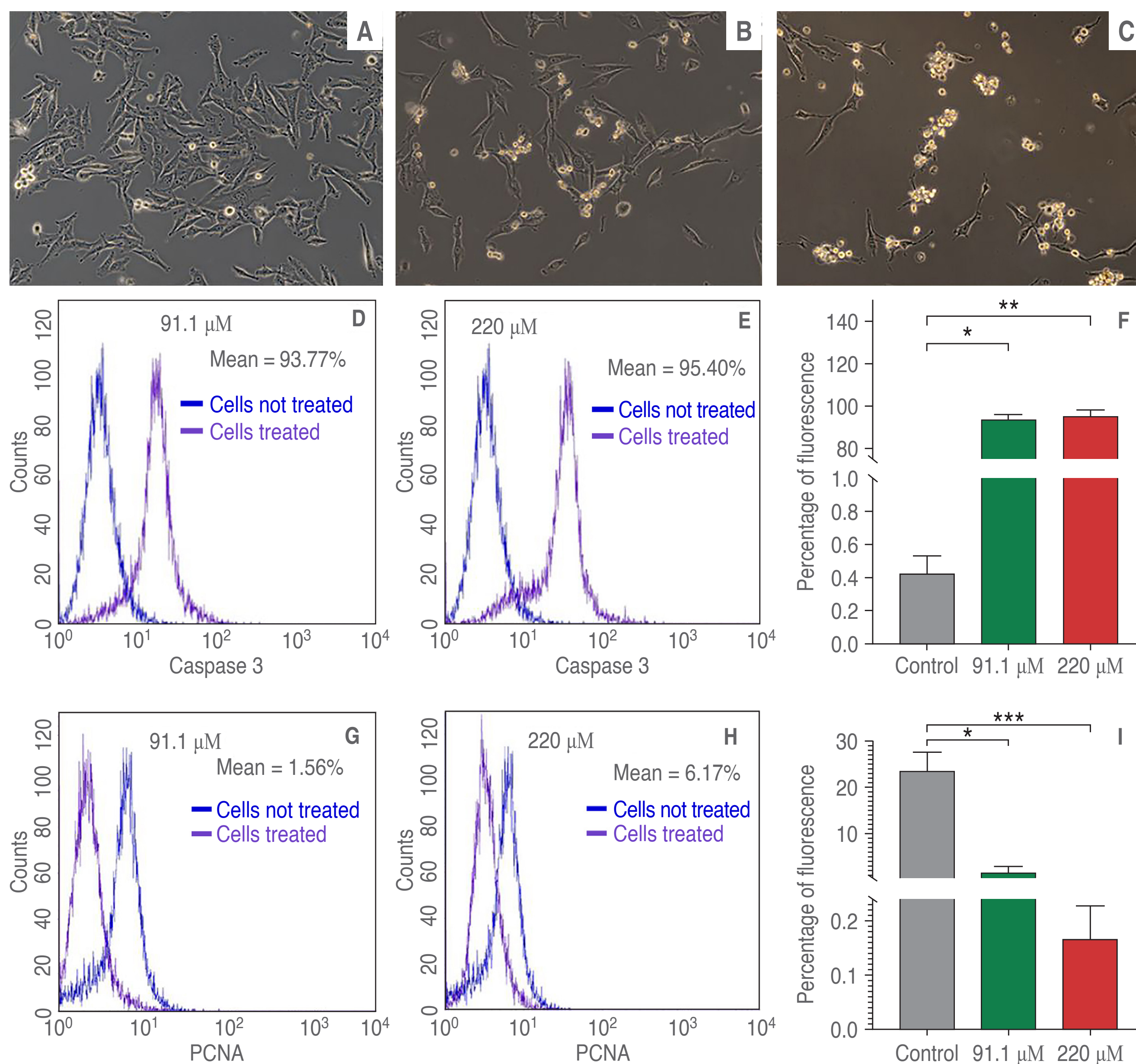

Figure 1: Morphological and flow cytometry analysis of TIB-223 cells after 24 hours of quercetin treatment. **A-C)** Bright-field micrographs showing morphological changes of TIB-223 cells after 24 hours. **(A)** Untreated control cells display an elongated, fibroblast-like morphology. **(B)** Cells treated with 91.1 μM quercetin show early signs of rounding and loss of adherence. **(C)** Cells treated with 220 μM quercetin exhibit marked cytoplasmic retraction, clustering, and increased detachment, consistent with apoptotic morphology. **(D-F)** Caspase-3 expression assessed by flow cytometry. In the histograms **(D-E)**, the lilac line represents untreated control cells, whereas the blue line represents quercetin-treated cells. A strong shift in fluorescence intensity indicates increased caspase-3 activation. Treatment with 91.1 μM resulted in 93.77% ± 2.61 caspase-3-positive cells, while 220 μM produced 95.40% ± 2.75, compared with only 0.42% ± 0.11 in the control. Statistical analysis **(F)** showed both concentrations were significantly different from control ($p < 0.0001$), with no significant difference between doses ($p = 0.47$). **(G-I)** PCNA expression assessed by flow cytometry. As in the previous panels, lilac = control and blue = treated cells. Treatment with quercetin markedly decreased PCNA-positive cells: 23.48% ± 4.14 in control vs 1.56% ± 1.37 at 91.1 μM and 6.17% ± 0.06 at 220 μM. Statistical comparison **(I)** confirmed significant reductions relative to control ($p = 0.0010$ and $p = 0.0006$, respectively) but no significant difference between the two quercetin concentrations ($p = 0.15$).

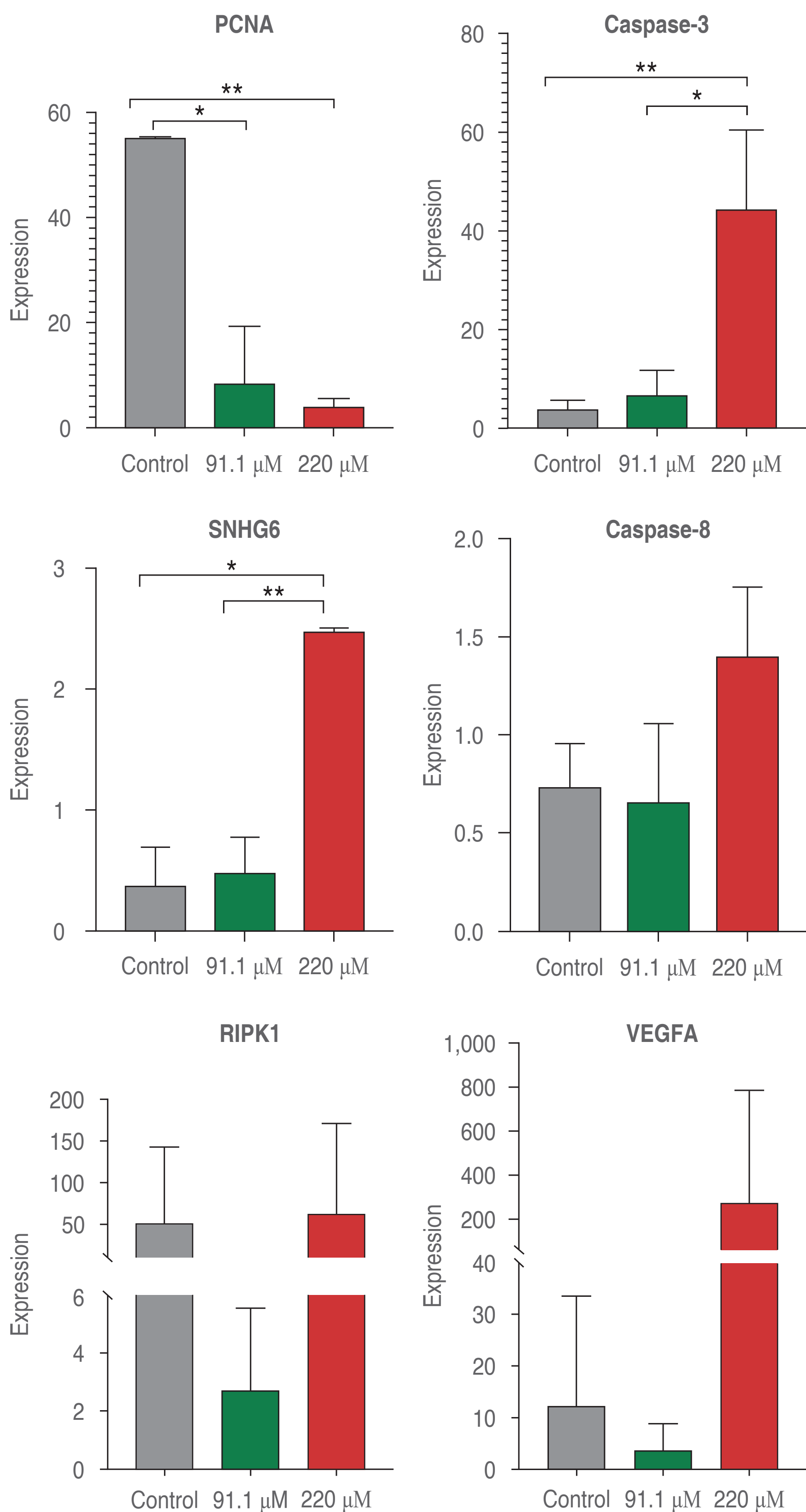

Figure 2:

The Quantitative Polymerase Chain Reaction (qPCR) results analyzing changes in gene expression within TIB 223 giant cell tumor of bone cells after a 24-hour exposure to quercetin. The data clearly illustrates the compound's potent and dose-dependent molecular effects. The figure is organized into distinct panels for key genes. In the panel showing Proliferating Cell Nuclear Antigen (PCNA), a critical marker for cell proliferation, a dramatic reduction in mRNA levels is evident. Expression decreases significantly at the 91.1 μM dose and falls even further at the higher 220 μM concentration. This demonstrates quercetin's powerful ability to halt the uncontrolled growth characteristic of these cancer cells. Conversely, the panel for Caspase 3, a central executioner enzyme in apoptosis, shows a striking opposite trend. Its mRNA expression is strongly upregulated upon treatment. The increase is moderate at 91.1 μM but becomes exceptionally pronounced at 220 μM, where expression rises to a level many times higher than the control. This confirms the direct activation of the cell's intrinsic suicide machinery. Furthermore, the panel for the long non-coding RNA SNHG6, a regulator often involved in cancer progression, also shows a statistically significant change in its expression profile following quercetin treatment, indicating a broader impact on the tumor's regulatory network. Collectively, these transcriptional results provide a coherent molecular narrative. They show that quercetin simultaneously downregulates a major proliferation driver (PCNA) and upregulates a key death promoter (caspase-3), effectively switching the cellular program from survival to apoptosis. This dual action, which intensifies with higher doses, offers a clear genetic explanation for quercetin's observed anticancer activity against these aggressive bone tumor cells.

The proposed mechanisms for these effects include the regulation of cell signaling pathways involved in cell proliferation and survival, the induction of oxidative stress, the modulation of gene expression related to apoptosis, and the inhibition of key enzymes in cellular metabolism.[14]

Scientific evidence indicates that quercetin, a flavonoid present in many fruits and vegetables, can exert anticancer effects through the negative regulation of the expression and function of PCNA, a key protein in cell proliferation.[15] The decrease in PCNA induced by quercetin contributes to inhibiting the uncontrolled proliferation of cancer cells, arresting the cell cycle, and sensitizing these cells to chemotherapy.[16]

In this study, PCNA was found to show significant inhibition after treatment. This protein, which is encoded by the PCNA gene, plays a crucial role in DNA replication and cell proliferation.[17] In cancer cells, quercetin can decrease PCNA levels, thus inhibiting the uncontrolled proliferation of these cells.[18] Previous work has shown that in many types of cancer, PCNA levels are significantly elevated compared to normal tissues.[19] Numerous *in vitro* studies have reported that quercetin treatment significantly reduces PCNA levels in different cancer cell lines, such as breast cancer, lung cancer, and colon cancer, among others.[20] Our results are consistent with the literature: untreated cells showed high PCNA expression, whereas treatment significantly reduced it.

On the other hand, caspases 8 and 3, which encode enzymes that participate in the process of apoptosis or programmed cell death,[21] were also evaluated. Quercetin has been reported as a major inducer of apoptosis in cancer cells by activating these caspases, leading to DNA fragmentation and cell death.[22] The activation of these caspases is described as a crucial mechanism by which quercetin exerts pro-apoptotic effects in various cancer cell lines, including TIB 223, suggesting its potential as an antitumor agent.[23]

Regarding RIPK1, it is important to note that this gene encodes a serine/threonine kinase that functions as a central regulator of cell survival, apoptosis, and necroptosis.[24] RIPK1 is subject to regulation by a variety of enzymes and modifications, including ubiquitination and phosphorylation. Under normal conditions, RIPK1 participates in the early cell-death checkpoint, where its ubiquitination within TNFR1 complex I promotes NF-κB activation and the transcription of pro-survival genes, thereby suppressing its cytotoxic activity.[25] When these regulatory ubiquitin modifications are lost, RIPK1 is redirected toward cytosolic complexes (complex IIa or IIb), where it can activate caspase-8 to induce apoptosis or interact with RIPK3 to promote necroptosis through MLKL activation.[26] As highlighted by DeRoo et al,[27] RIPK1 functions as a molecular switch determining whether cells activate apoptosis or necroptosis depending on caspase-8 availability and RIPK3 recruitment. Furthermore, pharmacological studies have demonstrated that the kinase activity of RIPK1 is essential for initiating necroptosis, and selective inhibition such as with Necrostatin-1 can block this pathway without impairing apoptosis, underscoring its clinical relevance in inflammatory and degenerative diseases.[28] In our study, RIPK1 expression did not show significant modulation following quercetin treatment, which may be explained by the fact that RIPK1 function is primarily regulated by post-translational modifications rather than changes in mRNA abundance. Thus, although quercetin may influence pathways associated with RIPK1, the interactions among these pathways are complex, and the activation of one can exert regulatory effects on the signaling of the others; therefore, a direct transcriptional effect on RIPK1 is not necessarily expected in the TIB 223 *in vitro* model.

Previous studies have also reported that VEGFA is a gene that encodes the Vascular Endothelial Growth Factor A protein, a key mediator of angiogenesis and tumor vascularization.[29] Quercetin has demonstrated anti-angiogenic and anticancer properties, and in several cancer models it can negatively regulate VEGFA expression, inhibiting angiogenesis and tumor growth.[30] In our study, however, VEGFA expression did not show significant differences after treatment, suggesting that quercetin's effects on angiogenesis may depend on cell type, tumor microenvironment, or longer exposure times.

Our *in vitro* observations indicate that quercetin reduces PCNA expression and increases caspase-3 activation. These results suggest that quercetin can limit tumor cell proliferation and promote apoptotic clearance. In the clinical setting of bone tumors and skeletal metastases, reduced tumor burden within bone is mechanistically linked to decreased osteolytic activity, lower risk of pathological fracture, and preservation of structural integrity-outcomes that directly influence mobility and motor function. Authors reviewing bone metastasis and skeletal complications highlight that tumor progression in bone promotes pain, cortical destruction and fractures, all major drivers of functional decline and disability in cancer patients. Therefore, therapies that reduce proliferation and

increase apoptosis in bone-infiltrating tumor cells may translate into decreased bone destruction and reduced motor disability.[4,31,32]

Regarding the use of less toxic adjuvant treatments (such as quercetin), it is important to note that the conventional systemic therapies for metastatic bone disease (chemotherapy, radiation) can cause persistent, disabling adverse effects notably Chemotherapy-Induced Peripheral Neuropathy (CIPN), chronic fatigue and mobility decline which independently worsen disability and quality of life among survivors. A less toxic adjunct such as quercetin (if shown effective and safe in vivo and clinically) could potentially reduce the cumulative exposure to more neurotoxic or myelosuppressive agents, thereby lowering the incidence or severity of treatment-related disability (for example CIPN-related falls, long-term gait impairment, or chronic fatigue limiting activities of daily living). These links between toxic treatment effects and long-term disability are well documented in survivorship literature.[7,33,34]

On the other hand, we can mention that the functional impairment and disability are frequent consequences of bone metastases and pulmonary metastatic disease. Large observational and registry studies report elevated rates of mobility limitation, increased short-term disability use, and greater health-care burden in patients with skeletal metastases compared with non-metastatic patients. Pulmonary metastases causing reduced respiratory reserve and systemic morbidity can further compound disability. When positioning the present *in vitro* results in a clinical context, it is therefore important to cite these epidemiologic data and to state explicitly that translation to reduced disability requires in vivo demonstration of tumor control, preservation of bone strength, and assessment of patient-centred functional outcomes.[6,35,36]

As a first approach to understanding the mechanisms by which quercetin induces cell death in the TIB 223 cell line, we can say that quercetin demonstrated potent pro-apoptotic and antiproliferative effects in TIB 223 cells *in vitro*, supporting its potential as an adjuvant therapeutic strategy for giant cell tumor of bone and metastatic lesions. Considering the above, we can hypothesize the following clinical applications: translation of these findings could include 1) evaluation of quercetin as an adjuvant to standard therapy to reduce tumor burden in bone metastases; 2) investigation of combination regimens that allow dose reduction of cytotoxic agents; and 3) assessment of functional outcomes (fracture incidence, mobility scores, activities of daily living) as clinical endpoints.

Likewise, the strengths we identified in the work are the following: the study reports both protein (flow cytometry) and gene expression (qPCR) evidence supporting apoptosis and reduced proliferation, and used a previously determined IC_{50} to select biologically relevant doses. The limitations of the study include the restriction to *in vitro* conditions quercetin bioavailability, metabolism, and pharmacokinetics in vivo may alter potency; the study does not assess effects on bone-resorbing osteoclast activity or biomechanical bone strength; sample size is modest (triplicates) and functional/behavioural outcomes were not measured.

Finally, continuing with this line of work, future studies should include: 1) *in vivo* studies using orthotopic or bone-metastasis models to assess tumor control, bone integrity and fracture risk; 2) pharmacokinetic and toxicology profiling to define tolerable systemic exposures; 3) combination studies to evaluate synergy with existing therapies and potential to reduce toxic agent doses; and 4) inclusion of functional/ disability-oriented endpoints (e.g., gait analysis, fracture incidence, validated patient-reported outcomes) in preclinical and clinical phases. These steps will be crucial to determine whether the molecular effects observed in vitro can meaningfully reduce bone destruction and disability in patients.

CONCLUSIONS

The results obtained in this study revealed that quercetin, a natural flavonoid, exhibits a potent pro-apoptotic effect in the TIB 233 giant cell tumor cell line. This observation is supported by the significant increase in caspase 3 activity and decrease in PCNA, which are key enzymes in the apoptotic pathway, following quercetin treatment.

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