

Alteration in the Expression of Circular Rnas and its association with the Development and Progression of Osteosarcoma, an Integrative Review with High Sensitivity Research

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Abstract

Background: Osteosarcoma is the most common primary malignant bone tumor, mainly affecting children, young adults, and the elderly. It is an aggressive cancer with a poor prognosis, exhibiting low survival rates even with standard treatment. Recently, circular RNA molecules capable of influencing gene expression through various functions, with their main role being acting as microRNA sponges and reducing their intracellular expression, have been identified. Recent studies have linked circular RNAs to osteosarcoma development and progression. Therefore, the present study aimed to investigate the alteration in circular RNA expression during osteosarcoma development and progression. **Methods:** An integrative literature review was conducted from September 10th to November 12th, 2021, using the following databases: PubMed/MEDLINE, SCOPUS, Web of Science, OVID, and EMBASE. 129 full articles were included in the review. The obtained data were organized using a standardized data collection instrument, which included the following information: altered expression profile of circular RNAs, associated cancer hallmarks, clinical-pathological relationships of circular RNAs, and perspectives on the studied circular RNAs. **Results:** A total of 94 distinct circular RNAs were identified, predominantly showing an increased expression pattern. Approximately 91% of the studies that aimed to identify the mechanisms of action of circular RNAs highlighted the function of circular RNAs as microRNA sponges. The most associated cancer hallmarks with the identified circular RNAs were proliferative signaling induction, invasion and metastasis, and resistance to cell death. The altered expression of these circular RNAs generally correlated with a worse prognosis for patients, as evidenced by clinical features such as shorter survival, advanced Enneking and/or TNM stage, higher incidence of metastasis, larger tumor size, and increased chemoresistance. **Conclusion:** These findings indicate the significance of circular RNA molecules in osteosarcoma carcinogenesis, suggesting their potential as new prognostic and/or diagnostic biomarkers, as well as alternative therapeutic targets in the fight against osteosarcoma.

Keywords: Circular RNA- Osteosarcoma- Oncogenesis

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Introduction

Osteosarcoma (OS) is a malignant primary tumor of the bone. It has a mesenchymal origin and is characterized by the production of immature osteoid matrix. Primary malignant bone neoplasms are rare, representing less than 5% of all malignant tumors in children and adolescents [1, 2]. The progression of this tumor is mainly related to cellular growth/proliferation processes, DNA damage, and alterations in cell cycle checkpoint control [3]. Studies

indicate an association between osteosarcoma and genetic syndromes such as hereditary retinoblastoma, Li-Fraumeni syndrome, Werner syndrome, Rothmund-Thomson syndrome, and Bloom syndrome. Additionally, there are a few reports indicating that isolated genetic alterations also play a role in the progression of osteosarcoma and that modifications in genes such as FOS and MYC are related to the development of OS [3, 4].

In this context, circular RNA (circRNA) has recently been identified as a class of molecules that potentially plays

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an important role in the development of OS. CircRNA is a type of endogenous non-coding single-stranded RNA that presents a closed, stable circular structure with tissue-specific expression that has been associated with the initiation and progression of different types of cancers [5].

The most well-established function of circRNAs is to act as “microRNA sponges” (Figure 1). In this mechanism, circRNA binds to and sequesters microRNA molecules in the cytoplasm, reducing their availability to the cytoplasmic environment. As a result, circRNA is capable of modulating gene expression, impacting in the modulation of different signaling pathways in a cell [5, 6]. In the osteosarcoma scenario, studies have shown that these molecules exhibit altered expression, related to characteristics of the carcinogenic process such as chemotherapy resistance, cellular proliferation, cellular metastasis, glycolysis, among others [7].

An important tool for characterizing the oncogenic process is the cancer hallmarks. They constitute an organizing principle to rationalize the complexities of neoplastic disease, and currently, there are 14 hallmarks proposed [8-10]. In this sense, several studies have shown that alterations in circRNAs expression have been associated with these hallmarks in the pathogenesis of osteosarcoma, being important for a better characterization of the carcinogenic process of this neoplasia [11-13].

In this context, more studies are needed to obtain a solid knowledge base about the role of circRNAs in OS carcinogenesis and progression. In the perspective of helping in the process of filling this gap, the present study presents an integrative review on the correlation between the expression of circular RNAs and the development and progression of osteosarcoma. For this purpose, we analyzed the influence of alterations in circular RNAs expression on the development and progression of osteosarcoma through an integrative literature review on the topic. Here, we summarize the past empirical or theoretical literature to provide a greater comprehensive understanding of the roles that circular RNAs play in osteosarcoma carcinogenesis and how they are associated with cancer hallmarks. Furthermore, we evaluated the relationship between circular RNA expression and clinical characteristics and patient prognosis.

Materials and Methods

Search strategy

This article is a literature review with a high-sensitivity search conducted between September 10th and November 12th, 2021. For the search process, we used the keywords “circular RNA” and “osteosarcoma” combined with their associated terms. The databases used, along with their respective search tools, were PubMed/MEDLINE, SCOPUS, OVID, EMBASE, and Web of Science (Supplementary materials and methods 1).

Selection eligibility criteria

Primary studies that focused on circular RNA in the context of osteosarcoma were included, with no minimum time restriction for publication. Reviews, abstracts, case reports, editorials, and works in languages other than

English, Spanish, or Portuguese were excluded (Figure 2).

For reference management, the digital platform Rayyan was used. The first step for article selection was the removal of duplicates. Subsequently, two independent reviewers, following the eligibility criteria, excluded studies based on titles and abstracts that did not fit the scope of this review. All conflicts were resolved without the need for a third reviewer. Finally, with the full-text reading of the articles, both reviewers performed standardized data extraction and made a final judgment regarding eligibility.

Circular RNAs classification system

For better understanding, the circRNAs were identified according to a specific code C and their respective number (Supplementary Table 1, 2). This classification was needed based on the fact that it was found a high number of circRNAs, as the authors had made comparison between the amount of circular RNA in healthy cells and/or tissues and cells and/or tissues affected by osteosarcoma.

In addition, a classification of the circRNAs was established according to the mechanism by which altered circRNAs expression impact in the different Cancer Hallmarks in osteosarcoma. Regarding the clinicopathological relationship, we examined the association between altered circular RNA and clinical data of patients, such as metastasis, overall survival, and degree of differentiation. Finally, the perspective analysis summarized the authors' expectations regarding the respective work's contribution in the context of osteosarcoma.

Results

Expression profile of circular RNAs

Ninety-four different circular RNAs with altered expression levels correlated to oncogenic processes in osteosarcoma were identified. From this total, it was observed that the majority of circRNAs, 84 (89.36%) exhibited increased expression in osteosarcoma. Among these, 7 (8.33%) circRNAs were specifically increased in osteosarcoma cell lines, while 77 (91.66%) were increased in both tissues and cells. No circular RNAs were found to be increased only in tissues affected by osteosarcoma.

A smaller number of molecules, 14 (14.89%) out of all identified circRNAs, showed decreased expression in osteosarcoma. Among these, 1 (7.14%) circRNA was decreased only in osteosarcoma cell lines, and no circRNA was specifically decreased in tissues affected by osteosarcoma. From this group, the remaining 13 (92.85%) circRNAs exhibited decreased expression in both cells and tissues affected by osteosarcoma.

It is important to note that there was a disagreement concerning the expression of 4 circular RNAs in osteosarcoma. For C32, two articles reported increased expression [14, 15], while one article reported decreased expression in OS [16]. For C34, one study reported increased expression [17], and another study reported decreased expression [18]. For C31, Li et al. 2020 [12] reported an increased expression, and one study reported its decreased expression in OS [19]. Lastly, for C84, one

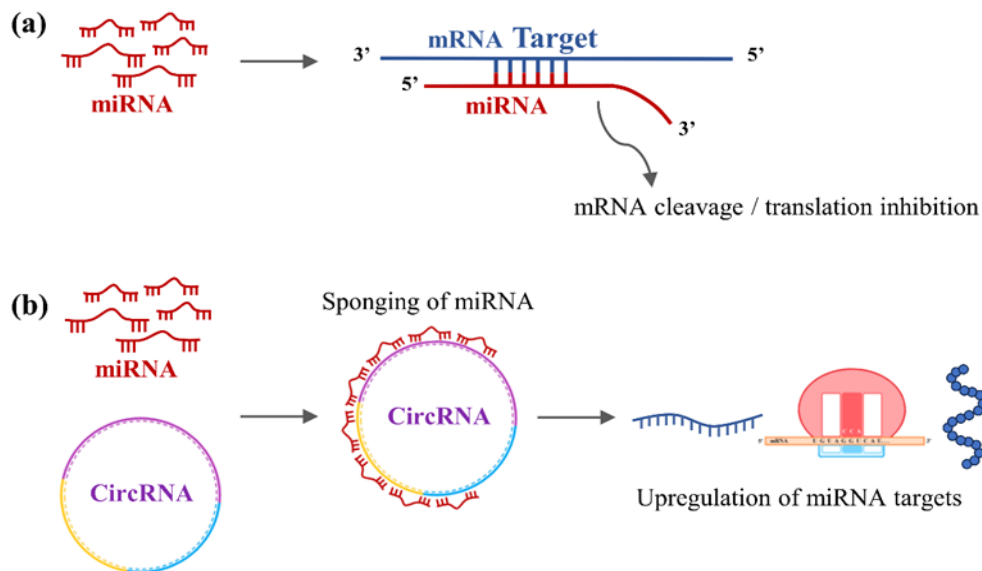


Figure 1. Role of CircRNA as a Sponge for MicroRNA. (a) Specific microRNAs interact in a sequence-specific manner with messenger RNA, inducing their cleavage and/or translation inhibition. (b) In the presence of circRNA, microRNAs are captured (sponging function) through base complementarity interactions, allowing for efficient translation of messenger RNAs.

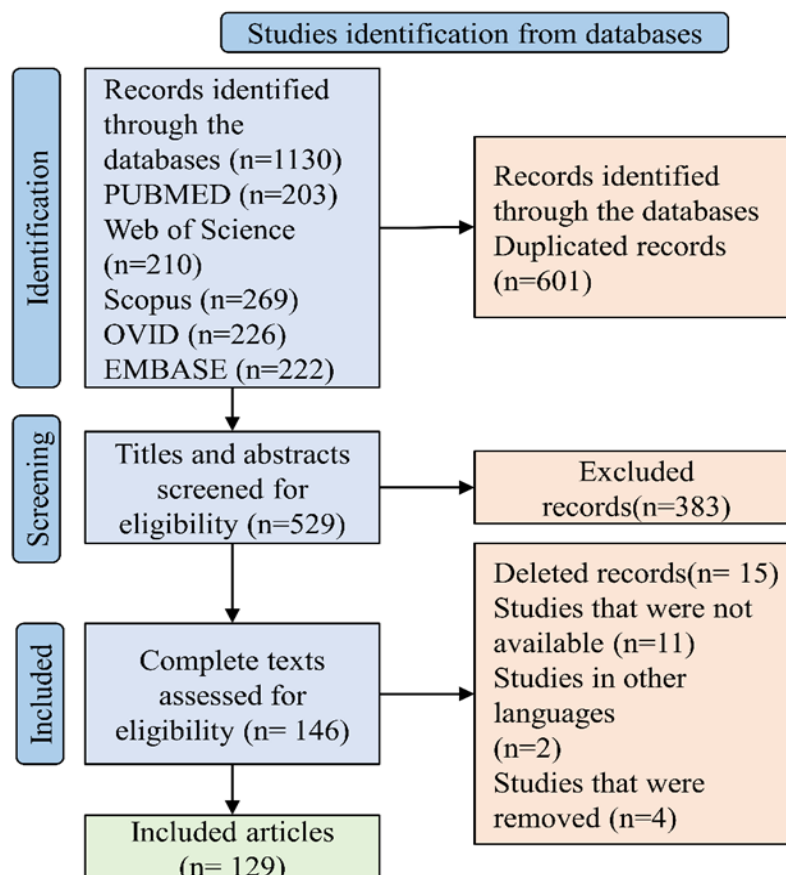


Figure 2. Flowchart for Obtaining the Selected Studies.

study reported increased expression [20], and one study reported decreased expression [21].

Mechanism of action

Among the 129 analyzed studies, 124 investigated the mechanisms by which circular RNAs influence the

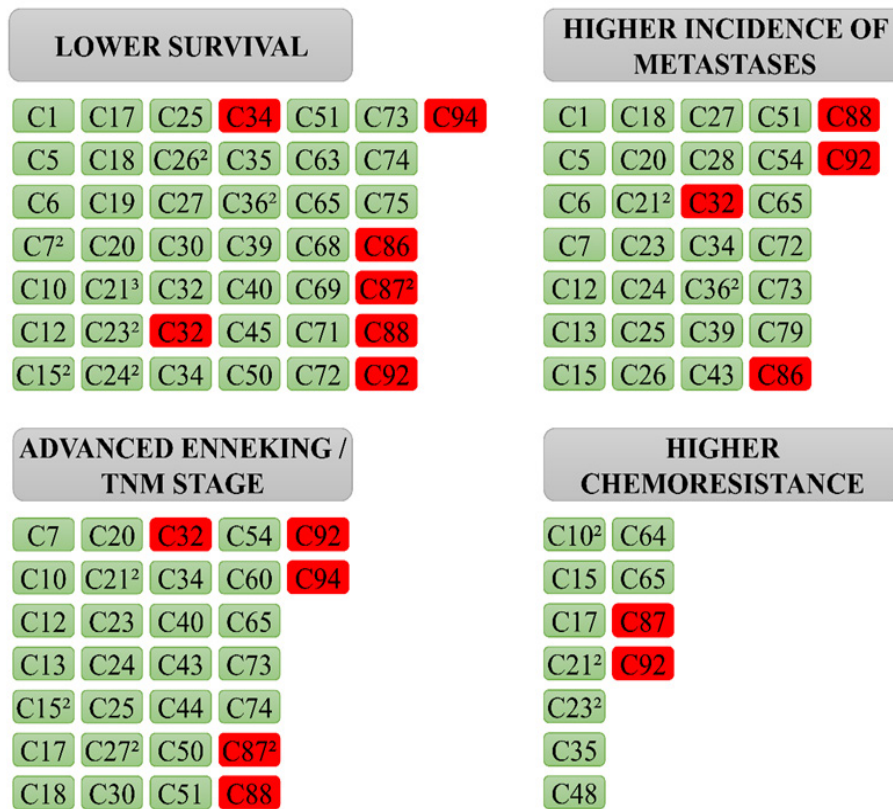


Figure 3. Correlation between the Expression of Circular RNAs and Key Clinical Characteristics of Patients. In red: Circular RNAs with decreased expression; in green: Circular RNAs with increased expression. For the C codes, please refer to their corresponding numbers in Supplementary table 1 and 2. Superscribed numbers indicate the number of papers referring to the same circRNA.

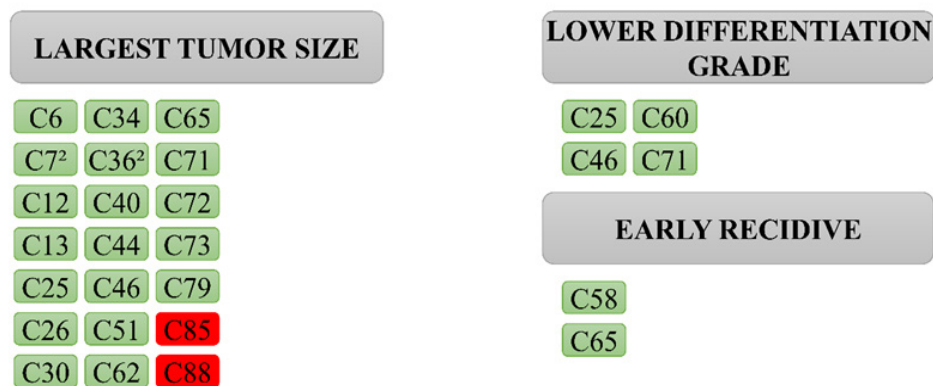


Figure 4. Continued Correlation between the Expression of Circular RNAs and Key Clinical Characteristics of Patients. In red: Circular RNAs with decreased expression; in green: Circular RNAs with increased expression. For the C codes, please refer to their corresponding numbers in Supplementary Table 1 and 2. Superscribed numbers indicate the number of papers referring to the same circRNA.

carcinogenic process in OS. The most predominantly observed mechanism of action was the capacity of circRNAs to act as microRNA sponges, acting as competitive endogenous RNAs that regulate the interaction between a microRNA and its target gene, which was seen in 113 (91.12%) of the studies that explored their mechanisms of action.

A less common mechanism mentioned was the capacity of circRNAs to act in an opposite direction, stabilizing microRNA molecules, which was reported in

4 (3.22%) studies. Also, 7 (5.64%) studies only mentioned the target genes and/or pathways without specifying the mechanism by which these processes were influenced.

Association of circular RNAs with Cancer Hallmarks

After identification, circRNAs were grouped according to the hallmarks with which they were associated. The most observed hallmark was the induction of proliferative signaling, present in approximately 87 (92.5%) of the 94 identified circular RNAs. The second most predominantly

Table 1. Correlation between the Expression Profile of Circular RNAs from All Studies and the associated Hallmarks. Identification of articles and circular RNAs with the C code and their respective numbers are available in Supplementary Table 1 and 2.

Associated Hallmark	Circular RNA	
	Increased expression	Decreased expression
Proliferative signaling	C1, C3, C4, C6-C22, C24-C64, C66-C68, C70-C84	C31, C32, C34, C84-C93
Invasion and metastasis	C1, C3, C4, C6-C18, C20-C28, C31-C53, C55, C56, C58, C59, C61-C64, C67-C70, C72-C76, C79-C81, C83, C84	C31, C32, C34, C84-C88, C90, C92, C93
Resistance to cell death	C2-C8, C10-C12, C14, C15, C17, C18, C20-C26, C35-C40, C45-C48, C51, C52, C54-C58, C60, C62-C64, C66, C70-C78, C80-C84	C31, C34, C87-C91, C93
Cellular energy dysregulation	C5, C7, C14, C21, C28, C36, C44	N/A
Induction of angiogenesis	C8, C9, C19	N/A
Inflammation promotion	C16	N/A
Evasion of suppressive mechanisms	C63	N/A
Genome instability and mutation	C67	C92

associated hallmark was invasion and metastasis, found in approximately 72 (76.6%) of the identified circRNAs. Lastly, resistance to cell death was the third most predominantly reported hallmark, present in 56 (59.5%) of the identified circRNAs (Table 1, 2).

Circular-RNAs expression and clinical pathological characteristics

A relationship between the expression profile of circRNAs and the clinical characteristics of patients was observed in 71 studies. Among the main characteristics identified, lower survival was reported in 52 (73.2%) studies, involving 43 distinct circular RNAs, with 36 (83.7%) circRNAs showing increased expression and 7 (16.2%) others showing decreased expression. Furthermore, the higher incidence of metastasis was found to be higher in 32 (45%) studies, involving 30 distinct circular RNAs, with 26 (86.6%) circRNAs showing increased expression and 4 (13.3%) circRNAs showing decreased expression (Figure 3).

The altered expression of circRNAs was also analyzed in relation to TNM (tumor, nodes, metastasis) staging and/or advanced Enneking stage, the latter being a surgical staging system used to guide the treatment of musculoskeletal tumors, including osteosarcoma. From this analysis, 34 (47.8%) studies reported that altered expression correlated with advanced Enneking and/or TNM stages, involving 30 distinct circular RNAs, with 26 (86.6%) showing increased expression and other 4 (13.3%) showing decreased expression (Figure 3).

Other clinical characteristics related to altered expression of circular RNAs included largest tumor size, reported in 23 (32.3%) studies, and higher chemoresistance, reported in 14 (19.7%) studies, among others (Figures 3, 4).

The altered expression of circRNAs was primarily associated with decreased overall survival, advanced Enneking and TNM stages, and increased incidence of metastasis, to name a few (Figures 3, 4). For example, the increased expression of C65 was related to 6 out of the 7 analyzed characteristics, except for lower differentiation grade, making it the circRNA most associated with pathological clinical features [22]. It was observed

that 54 (76%) studies reported that the analyzed RNAs were related to more than one clinical characteristic (Figures 3, 4).

Discussion

Circular RNAs were discovered approximately three decades ago, but only more recently they have received greater attention. It is now known that they can regulate many biological processes and play an important role in the development of various types of cancer, including osteosarcoma [23].

In this context, an altered pattern of circular RNA expression was observed in the context of OS, with a predominance of increased expression in both OS cell lines and tissues affected by the disease, and only a few circular RNAs showing decreased expression in OS. Thus, it can be observed that the expression profile of circRNAs is closely related to the carcinogenic process of OS.

When certain circular RNAs showed increased expression in OS, higher rates of proliferation, migration, cell viability, and lower apoptotic rates were observed. Examples of this include C82 [24], C80 [25], and C78 [26]. These characteristics directly affect cellular progression and are important for the carcinogenesis process, ultimately contributing to cancer progression or evolution. By decreasing the expression of these RNAs through experiments, a reversal of the previously established effects was generally observed, with decreased rates of proliferation, invasion, and metastasis, and increased rates of apoptosis. These findings reinforce the idea that altered expression of circRNAs contributes to the development of osteosarcoma.

On the other hand, low expression of certain circular RNAs promoted similar effects as mentioned above in carcinogenic development. However, when their expression was increased through experiments, a decrease in these effects was observed. Thus, the hypothesis arises that circular RNA may also play a protective role in cancer development, albeit to a lesser extent since the majority of the circRNAs studied had increased expression in OS context.

Furthermore, some studies showed conflicting

expression profiles for the same circular RNA, such as C32 [16, 14, 15], C34 [18, 27], C31 [19, 12], C84 [20, 21]. This raises the hypothesis that the expression pattern of circular RNA may change in OS due to the development of the carcinogenic process and may be influenced by other factors such as the primary site of the disease or the stage of cancer evolution. A large part of the studies, however, does not specify the bone site from which the affected tissue was obtained or the stage of disease progression in the patients, when the samples were collected.

Among the mechanisms described for the action of circRNA, its role as a microRNA sponge was the most prevalent, which is a well-known function of circular RNAs. In this mechanism, circular RNA negatively regulates the expression of a microRNA, thereby influencing the expression of its target gene as well as the activation or inhibition of other cellular pathways [28]. It was noted that, most of the time circRNAs have more than one microRNA target and can simultaneously regulate the expression of multiple microRNAs. For example, C21 targets microRNAs miR-423-5p, miR-137, miR-205-5p, and miR-256b, which were identified in independent studies [29, 30, 7, 31, 32]. Another example is C63 [33], which regulates the expression of miR-23a-3p, miR-23b-3p, miR-23c, and miR-130a-5p, and C17 [34], which regulates the expression of miR-145-5p and miR-151-3p, simultaneously.

In our investigation, we noticed that miR-145-5p plays a role in regulating the expression of genes related to the progression and metastasis of osteosarcoma [35]. Furthermore, the action of miR-145-5p is controlled by different circRNAs: C1 (hsa_circ_0001900) [36], C6 (circ_0008932) [37], C17 (hsa_circ_0000073) [38], and C37 (hsa_circ_0008934) [39].

Hsa_circ_0001900, a type of circRNA derived from the CAMSAP1 gene, acts as a “sponge” for miR-145-5p, restoring the expression of FLI1. This results in a complex regulatory axis, where hsa_circ_0001900, miR-145-5p, and FLI1 are interconnected in the biology of osteosarcoma. In this context, miR-145-5p acts as a tumor progression suppressor, and the reduction of FLI1, partially mediated by hsa_circ_0001900 and miR-145-5p, can impact on inhibiting metastasis and proliferation of osteosarcoma [36].

CircRNA circ_0008932 exerts a significant influence on the cellular functions of osteosarcoma by negatively regulating miR-145-5p. Circ_0008932 acts as a molecular competitor, establishing a direct physical binding with miR-145-5p, inhibiting its physiological regulatory action. This interaction leads to the suppression of miR-145-5p activity, which normally plays crucial roles in regulating cell proliferation, migration, invasion, and apoptosis. This suggests that the interaction between circ_0008932 and miR-145-5p plays a critical role in the pathogenesis of osteosarcoma [37].

It has been reported that the levels of miR-145-5p and miR-151-3p were significantly reduced in osteosarcoma tissues and cell lines, and these levels were inversely correlated with the levels of circRNA hsa_circ_0000073. Functional interaction demonstrated that hsa_circ_0000073 directly affects the levels of

miR-145-5p and miR-151-3p in osteosarcoma cells, and this regulation plays an important role in the expression of the NRAS gene. Therefore, it is suggested that hsa_circ_0000073 may increase the expression of NRAS by inhibiting the expression of miR-145-5p and miR-151-3p in osteosarcoma cells, with potential implications for osteosarcoma progression [40].

The interaction between hsa_circ_0008934 and miR-145-5p was investigated in the context of osteosarcoma, and the results revealed that hsa_circ_0008934 acts as a “sponge” for miR-145-5p, inhibiting its action. This inhibition results in an increase in the expression of the E2F3 gene, which plays an important role in the proliferation and migration of osteosarcoma cells. This cascade regulation, in which hsa_circ_0008934 acts as a modulator of gene expression by sequestering miR-145-5p, has a significant impact on the progression of osteosarcoma [40].

In addition to this usual function, it was identified that circRNA can also act through adsorption and/or stabilization of microRNAs in OS, leading to increased expression of the target microRNAs: C22 [41], C83 [42], C26 Zhang P, Li J (2021) [30] and C31 [38], to name some. C22 acts as a sponge for miR-217 and a stabilizer for miR-19b, which represents different functions in different studies [41, 19]. This fact raises the hypothesis that the same RNA can act as a sponge and stabilizer of microRNAs simultaneously, influencing different genes and pathways. It also raises the question of whether circular RNA alters its mechanism of action depending on the conditions under which the carcinogenic process occurs or the clinical status of the analyzed patient.

Among the hallmarks of osteosarcoma in which the identified circular RNAs were associated with its development, there was a predominance of proliferative signaling, invasion and metastasis, and resistance to cell death. As osteosarcoma is an aggressive type of cancer, it was expected that these characteristics would be prominently present. On the other hand, the lack of expression of other hallmarks can be explained by the absence of appropriate functional experiments to evaluate them correctly or directly. Therefore, this fact does not mean that the other hallmarks are less important or less common in the carcinogenic process of osteosarcoma.

It was also observed that the vast majority of circular RNAs were associated with different types of hallmarks, not just one. For example, C7 [43], C8 [44], C63 [33], to name a few. Therefore, it can be stated that circular RNAs play a very important role in the pathogenesis of osteosarcoma.

The evolution of osteosarcoma is rapid and aggressive, making it important to recognize factors that can predict a worse clinical and/or prognostic outcome. In this regard, altered expression of circular RNAs has been associated with various clinical characteristics of OS patients. In general, they have been associated with worse prognosis, including lower overall survival, disease-free survival, and 5-year survival. This is the case for C15 [45], C19 [46], and C34 [47] circRNAs.

Circular RNAs have also been linked to a higher incidence of metastases, particularly in the lungs, as

reported in the literature. C39 [48], C43 [49], and C36 [50] are some examples. Moreover, circRNAs have been associated with advanced Enneking/TNM stages of osteosarcoma, as it was seen with C51 [51], C17 [52], and C60 [40]. Additionally, an increased tumor size has been correlated with C71 [53], C26 [54], and C79 [55].

Of note, the altered expression of C65, which was associated with lower survival rates, higher incidence of metastases, advanced disease stage, larger tumor size, early recurrence, and increased chemoresistance. Thus, it is understood that this molecule has an important influence on the progression and development of osteosarcoma and may be the subject of future studies for alternative therapies in the treatment of this cancer [22].

When examining the correlation between the expression profile of circular RNAs and patient prognosis, some authors demonstrated that these molecules could be used as potential biomarkers. In this context, C23 was mentioned as a possible diagnostic and prognostic biomarker that is more sensitive and specific than alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), the current biomarkers used in osteosarcoma [56]. C65 was indicated as a possible diagnostic biomarker, referred as even more sensitive and specific than ALP and LDH, and it could also be used as a prognostic biomarker [22]. Furthermore, C21 was mentioned as a diagnostic biomarker that is more sensitive and specific than ALP in the context of osteosarcoma [29].

In addition to increased expression in tissues and cells affected by osteosarcoma, the aforementioned three circular RNAs also showed increased expression in serum. It was observed that the serum expression of these molecules decreased as chemotherapy and/or surgery treatments were performed. This raises the hypothesis that circular RNA could be used as a biomarker for monitoring osteosarcoma treatment.

In conclusion, a close relationship was observed between altered expression of circular RNAs, impacting on different hallmarks and the progression and development of osteosarcoma. In this aspect, C65 (hsa_circ_0003074) could be highlighted as their expression profile were closely associated to the clinical outcomes of OS patients. In this context, circular RNAs may provide a future complementary therapeutic alternative in the treatment of osteosarcoma, as well as serve as relevant prognostic and diagnostic biomarkers. Furthermore, these molecules can contribute to a better understanding of the pathogenesis of osteosarcoma, which remain unclear. Finally, studies focused on the relationship between circular RNAs and osteosarcoma are of paramount importance and should be conducted and encouraged to improve the prognosis and outcome of this condition, leading to better survival rates for patients in the future.

Author Contribution Statement

FAL was involved in the conception of the study, literature review and drafting the manuscript. FLF was involved in the conception of the study, literature review and drafting the manuscript. DRQA was involved in drafting the manuscript. AEC was involved in revising

the manuscript critically for important intellectual content. VDA was involved in literature review. GAC was involved in literature review. NMM was involved in literature review. TDR was involved in literature review. EGCN was involved in revising the manuscript critically for important intellectual content. ITO was involved in revising the manuscript critically for important intellectual content. CMB was involved in literature review. JVF was involved in revising the manuscript critically for important intellectual content. TAAMF was involved in the conception of the study, literature review and drafting the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

All authors have read and approved the content of the manuscript. The authors declare that they have no conflicts of interests.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Available of Data and Materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

References

- Ritter J, Bielack SS. Osteosarcoma. *Ann Oncol.* 2010;21 Suppl 7:vii320-5. <https://doi.org/10.1093/annonc/mdq276>.
- Moore DD, Haydon RC. Ewing's sarcoma of bone. *Cancer Treat Res.* 2014;162:93-115. https://doi.org/10.1007/978-3-319-07323-1_5.
- Gorlick R, Khanna C. Osteosarcoma. *J Bone Miner Res.* 2010;25(4):683-91. <https://doi.org/10.1002/jbmr.77>.
- Prater S, McKeon B. Osteosarcoma. Statpearls. Treasure Island (FL) ineligible companies: StatPearls Publishing LLC; 2023.
- Zhou WY, Cai ZR, Liu J, Wang DS, Ju HQ, Xu RH. Circular rna: Metabolism, functions and interactions with proteins. *Mol Cancer.* 2020;19(1):172. <https://doi.org/10.1186/s12943-020-01286-3>.
- Ng WL, Mohd Mohidin TB, Shukla K. Functional role of circular rnas in cancer development and progression. *RNA Biol.* 2018;15(8):995-1005. <https://doi.org/10.1080/15476286.2018.1486659>.
- Liu J, Feng G, Li Z, Li R, Xia P. Knockdown of circrim1 inhibits hdac4 to impede osteosarcoma proliferation, migration, and invasion and facilitate autophagy by targeting mir-432-5p. *Cancer Manag Res.* 2020;12:10199-210. <https://doi.org/10.2147/emar.S253130>.
- Hanahan D, Weinberg RA. The hallmarks of cancer.

- Cell. 2000;100(1):57-70. [https://doi.org/10.1016/s0092-8674\(00\)81683-9](https://doi.org/10.1016/s0092-8674(00)81683-9).
9. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646-74. <https://doi.org/10.1016/j.cell.2011.02.013>.
 10. Hanahan D. Hallmarks of cancer: New dimensions. *Cancer Discov*. 2022;12(1):31-46. <https://doi.org/10.1158/2159-8290.Cd-21-1059>.
 11. Yang J, Han Q, Li C, Yang H, Chen X, Wang X. Circular rna circ_0001105 inhibits progression and metastasis of osteosarcoma by sponging mir-766 and activating ythdf2 expression. *Oncotargets Ther*. 2020;13:1723-36. <https://doi.org/10.2147/ott.S234668>.
 12. Li X, Liu Y, Zhang X, Shen J, Xu R, Liu Y, et al. Circular rna hsa_circ_0000073 contributes to osteosarcoma cell proliferation, migration, invasion and methotrexate resistance by sponging mir-145-5p and mir-151-3p and upregulating nras. *Aging (Albany NY)*. 2020;12(14):14157-73. <https://doi.org/10.18632/aging.103423>.
 13. Ji X, Shan L, Shen P, He M. Circular rna circ_001621 promotes osteosarcoma cells proliferation and migration by sponging mir-578 and regulating vegf expression. *Cell Death Dis*. 2020;11(1):18. <https://doi.org/10.1038/s41419-019-2204-y>.
 14. Huang Z, Yuan C, Gu H, Cheng X, Zhou K, Xu J, et al. Circular rna circhipk3 promotes cell metastasis through mir-637/stat3 axis in osteosarcoma. *Biomed Res Int*. 2020;2020:2727060. <https://doi.org/10.1155/2020/2727060>.
 15. Wen Y, Li B, He M, Teng S, Sun Y, Wang G. Circhipk3 promotes proliferation and migration and invasion via regulation of mir-637/hdac4 signaling in osteosarcoma cells. *Oncol Rep*. 2021;45(1):169-79. <https://doi.org/10.3892/or.2020.7833>.
 16. Xiao-Long M, Kun-Peng Z, Chun-Lin Z. Circular rna circ_hipk3 is down-regulated and suppresses cell proliferation, migration and invasion in osteosarcoma. *J Cancer*. 2018;9(10):1856-62. <https://doi.org/10.7150/jca.24619>.
 17. Zhang C, Na N, Liu L, Qiu Y. Circrna hsa_circ_0005909 promotes cell proliferation of osteosarcoma cells by targeting mir-338-3p/hmgal1 axis. *Cancer Manag Res*. 2021;13:795-803. <https://doi.org/10.2147/cmar.S285118>.
 18. Wu Z, Shi W, Jiang C. Overexpressing circular rna hsa_circ_0002052 impairs osteosarcoma progression via inhibiting wnt/ β -catenin pathway by regulating mir-1205/apc2 axis. *Biochem Biophys Res Commun*. 2018;502(4):465-71. <https://doi.org/10.1016/j.bbrc.2018.05.184>.
 19. Zhou W, Liu Y, Wu X. Down-regulation of circitch promotes osteosarcoma development and resistance to doxorubicin via the mir-524/rassf6 axis. *J Gene Med*. 2021;23(10):e3373. <https://doi.org/10.1002/jgm.3373>.
 20. Amuti A, Liu D, Maimaiti A, Yu Y, Yasen Y, Ma H, et al. Doxorubicin inhibits osteosarcoma progression by regulating circ_0000006/mir-646/ bdnf axis. *J Orthop Surg Res*. 2021;16(1):645. <https://doi.org/10.1186/s13018-021-02782-y>.
 21. Gao P, Zhao X, Yu K, Zhu Z. Circ_0084582 facilitates cell growth, migration, invasion, and angiopoiesis in osteosarcoma via mediating the mir-485-3p/jag1 axis. *Front Genet*. 2021;12:690956. <https://doi.org/10.3389/fgene.2021.690956>.
 22. Lei S, Xiang L. Up-regulation of circrna hsa_circ_0003074 expression is a reliable diagnostic and prognostic biomarker in patients with osteosarcoma. *Cancer Manag Res*. 2020;12:9315-25. <https://doi.org/10.2147/cmar.S262093>.
 23. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular rnas are a large class of animal rnas with regulatory potency. *Nature*. 2013;495(7441):333-8. <https://doi.org/10.1038/nature11928>.
 24. Zhang H, Yu Z, Wu B, Sun F. Circular rna circfoxp1 promotes angiogenesis by regulating microRNA -127-5p/cdkn2aip signaling pathway in osteosarcoma. *Bioengineered*. 2021;12(2):9991-9. <https://doi.org/10.1080/21655979.2021.1989258>.
 25. Li JF, Song YZ. Circular rna gli2 promotes osteosarcoma cell proliferation, migration, and invasion by targeting mir-125b-5p. *Tumour Biol*. 2017;39(7):1010428317709991. <https://doi.org/10.1177/1010428317709991>.
 26. Song YZ, Li JF. Circular rna hsa_circ_0001564 regulates osteosarcoma proliferation and apoptosis by acting mirna sponge. *Biochem Biophys Res Commun*. 2018;495(3):2369-75. <https://doi.org/10.1016/j.bbrc.2017.12.050>.
 27. Zhang C, Zhou H, Yuan K, Xie R, Chen C. Overexpression of hsa_circ_0136666 predicts poor prognosis and initiates osteosarcoma tumorigenesis through mir-593-3p/zeb2 pathway. *Aging (Albany NY)*. 2020;12(11):10488-96. <https://doi.org/10.18632/aging.103273>.
 28. Panda AC. Circular rnas act as mirna sponges. *Adv Exp Med Biol*. 2018;1087:67-79. https://doi.org/10.1007/978-981-13-1426-1_6.
 29. Kun-Peng Z, Chun-Lin Z, Jian-Ping H, Lei Z. A novel circulating hsa_circ_0081001 act as a potential biomarker for diagnosis and prognosis of osteosarcoma. *Int J Biol Sci*. 2018;14(11):1513-20. <https://doi.org/10.7150/ijbs.27523>.
 30. Li Z, Fu Y, Ouyang W, He M, Wang Y, Wang X, et al. Circ_0016347 promotes osteosarcoma progression by regulating mir-1225-3p/kcnh1 axis. *Cancer Biother Radiopharm*. 2021. <https://doi.org/10.1089/cbr.2019.3349>.
 31. Wan J, Liu Y, Long F, Tian J, Zhang C. Circpvt1 promotes osteosarcoma glycolysis and metastasis by sponging mir-423-5p to activate wnt5a/ror2 signaling. *Cancer Sci*. 2021;112(5):1707-22. <https://doi.org/10.1111/cas.14787>.
 32. Yan M, Gao H, Lv Z, Liu Y, Zhao S, Gong W, et al. Circular rna pvt1 promotes metastasis via regulating of mir-526b/ foxc2 signals in os cells. *J Cell Mol Med*. 2020;24(10):5593-604. <https://doi.org/10.1111/jcmm.15215>.
 33. Jiang Y, Hou J, Zhang X, Xu G, Wang Y, Shen L, et al. Circ_xpo1 upregulates xpo1 expression by sponging multiple mirnas to facilitate osteosarcoma cell progression. *Exp Mol Pathol*. 2020;117:104553. <https://doi.org/10.1016/j.yexmp.2020.104553>.
 34. Li J, Zhang F, Li H, Peng F, Wang Z, Peng H, et al. Circ_0010220-mediated mir-503-5p/cdca4 axis contributes to osteosarcoma progression tumorigenesis. *Gene*. 2020;763:145068. <https://doi.org/10.1016/j.gene.2020.145068>.
 35. Li H, He L, Tuo Y, Huang Y, Qian B. Circular rna hsa_circ_0000282 contributes to osteosarcoma cell proliferation by regulating mir-192/xiap axis. *BMC Cancer*. 2020;20(1):1026. <https://doi.org/10.1186/s12885-020-07515-8>.
 36. Chen Z, Xu W, Zhang D, Chu J, Shen S, Ma Y, et al. Circcamsap1 promotes osteosarcoma progression and metastasis by sponging mir-145-5p and regulating flil1 expression. *Mol Ther Nucleic Acids*. 2021;23:1120-35. <https://doi.org/10.1016/j.omtn.2020.12.013>.
 37. Cao C, Shu X. Suppression of circ_0008932 inhibits tumor growth and metastasis in osteosarcoma by targeting mir-145-5p. *Exp Ther Med*. 2021;22(4):1106. <https://doi.org/10.3892/etm.2021.10540>.
 38. Li L, Kong XA, Zang M, Dong J, Feng Y, Gui B, et al. Hsa_circ_0003732 promotes osteosarcoma cells proliferation via mir-545/ccna2 axis. *Biosci Rep*. 2020;40(6). <https://doi.org/10.1042/bsr20191552>.
 39. Li S, Zeng M, Yang L, Tan J, Yang J, Guan H, et al. Hsa_

- circ_0008934 promotes the proliferation and migration of osteosarcoma cells by targeting mir-145-5p to enhance e2f3 expression. *Int J Biochem Cell Biol.* 2020;127:105826. <https://doi.org/10.1016/j.biocel.2020.105826>.
40. Li H, Lan M, Liao X, Tang Z, Yang C. Circular rna cir-itch promotes osteosarcoma migration and invasion through cir-itch/mir-7/egfr pathway. *Technol Cancer Res Treat.* 2020;19:1533033819898728. <https://doi.org/10.1177/1533033819898728>.
 41. Du YX, Guo LX, Pan HS, Liang YM, Li X. Circ_ankib1 stabilizes the regulation of mir-19b on socs3/stat3 pathway to promote osteosarcoma cell growth and invasion. *Hum Cell.* 2020;33(1):252-60. <https://doi.org/10.1007/s13577-019-00298-6>.
 42. Li L, Guo L, Yin G, Yu G, Zhao Y, Pan Y. Upregulation of circular rna circ_0001721 predicts unfavorable prognosis in osteosarcoma and facilitates cell progression via sponging mir-569 and mir-599. *Biomed Pharmacother.* 2019;109:226-32. <https://doi.org/10.1016/j.biopha.2018.10.072>.
 43. Hu R, Chen S, Yan J. Blocking circ-cnst suppresses malignant behaviors of osteosarcoma cells and inhibits glycolysis through circ-cnst-mir-578-ldha/pdk1 cerna networks. *J Orthop Surg Res.* 2021;16(1):300. <https://doi.org/10.1186/s13018-021-02427-0>.
 44. Feng ZH, Zheng L, Yao T, Tao SY, Wei XA, Zheng ZY, et al. Eif4a3-induced circular rna prkar1b promotes osteosarcoma progression by mir-361-3p-mediated induction of fzd4 expression. *Cell Death Dis.* 2021;12(11):1025. <https://doi.org/10.1038/s41419-021-04339-7>.
 45. Zhang H, Wang G, Ding C, Liu P, Wang R, Ding W, et al. Increased circular rna ubap2 acts as a sponge of mir-143 to promote osteosarcoma progression. *Oncotarget.* 2017;8(37):61687-97. <https://doi.org/10.18632/oncotarget.18671>.
 46. Zhang Q, Wang L, Cao L, Wei T. Novel circular rna circatrn1 accelerates the osteosarcoma aerobic glycolysis through targeting mir-409-3p/ldha. *Bioengineered.* 2021;12(2):9965-75. <https://doi.org/10.1080/21655979.2021.1985343>.
 47. Zhang Z, Pu F, Wang B, Wu Q, Liu J, Shao Z. Hsa_circ_0000285 functions as a competitive endogenous rna to promote osteosarcoma progression by sponging hsa-mirna-599. *Gene Ther.* 2020;27(5):186-95. <https://doi.org/10.1038/s41434-019-0112-5>.
 48. Wang J, Ni J, Song D, Ding M, Huang J, Li W, et al. The regulatory effect of has-circ-0001146/mir-26a-5p/mnat1 network on the proliferation and invasion of osteosarcoma. *Biosci Rep.* 2020;40(6). <https://doi.org/10.1042/bsr20201232>.
 49. Li JJ, Xiong MY, Sun TY, Ji CB, Guo RT, Li YW, et al. Circfam120b knockdown inhibits osteosarcoma tumorigenesis via the mir-1205/ptbp1 axis. *Aging (Albany NY).* 2021;13(20):23831-41. <https://doi.org/10.18632/aging.203657>.
 50. Gao AM, Yuan C, Hu AX, Liu XS. Circ_arf3 regulates the pathogenesis of osteosarcoma by sponging mir-1299 to maintain cdk6 expression. *Cell Signal.* 2020;72:109622. <https://doi.org/10.1016/j.cellsig.2020.109622>.
 51. Liu P, Liu W, Gao H, Zhang Y, Yan M, Wang X. Circ0085539 promotes osteosarcoma progression by suppressing mir-526b-5p and phlda1 axis. *Front Oncol.* 2020;10:1250. <https://doi.org/10.3389/fonc.2020.01250>.
 52. Ren Z, Yang Q, Guo J, Huang H, Li B, Yang Z, et al. Circular rna hsa_circ_0000073 enhances osteosarcoma cells malignant behavior by sponging mir-1252-5p and modulating ccne2 and mdm2. *Front Cell Dev Biol.* 2021;9:714601. <https://doi.org/10.3389/fcell.2021.714601>.
 53. Jin J, Chen A, Qiu W, Chen Y, Li Q, Zhou X, et al. Dysregulated circrna_100876 suppresses proliferation of osteosarcoma cancer cells by targeting microrna-136. *J Cell Biochem.* 2019;120(9):15678-87. <https://doi.org/10.1002/jcb.28837>.
 54. Jin Y, Li L, Zhu T, Liu G. Circular rna circ_0102049 promotes cell progression as cerna to target mdm2 via sponging mir-1304-5p in osteosarcoma. *Pathol Res Pract.* 2019;215(12):152688. <https://doi.org/10.1016/j.prp.2019.152688>.
 55. Huang L, Chen M, Pan J, Yu W. Circular rna circnasp modulates the malignant behaviors in osteosarcoma via mir-1253/foxf1 pathway. *Biochem Biophys Res Commun.* 2018;500(2):511-7. <https://doi.org/10.1016/j.bbrc.2018.04.131>.
 56. Kun-Peng Z, Xiao-Long M, Chun-Lin Z. Overexpressed circpvt1, a potential new circular rna biomarker, contributes to doxorubicin and cisplatin resistance of osteosarcoma cells by regulating abcb1. *Int J Biol Sci.* 2018;14(3):321-30. <https://doi.org/10.7150/ijbs.24360>.



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