RESEARCH ARTICLE

Editorial Process: Submission:06/13/2023 Acceptance:09/16/2023

Possible Role of *IL-6R/STAT3/MiRNA-34a* Feedback Loop in Osteosarcoma

Marwa Fathy Amer[1], Abbas Mohamed[1], Abeer Ismail[2], Lamiaa Ali Bayoumi[1], Passant Essam Eldin Shibel[3], Ghada Nabil Elnaggar[2]*

Abstract

Objective: Osteosarcoma is considered the most common primary malignant tumor that develops from the primary osteoblasts. MiRNAs are small non-coding RNAs that play a key role in tumorigenesis. The aim of this study was to detect the possible relationship between expression levels of *miRNA-34a* and levels of Signal transducer and activator of transcription 3 (*STAT3*) and interleukin-6 receptor (IL-6R) in osteosarcoma and the possible role of this relationship in development of metastases in these patients. **Methods:** A total of thirty-six (36) bone samples were included in the study. They were divided into 3 groups: Group (I): Twelve normal bone samples as control group. Group (II): Twelve patients with non-metastatic osteosarcoma. Group (III): Twelve patients with metastatic osteosarcoma. *MiRNA-34a* expression levels were estimated using qRT-PCR. *STAT3* and *IL-6R* levels were measured by ELISA. **Results:** Expression level of *miRNA-34a* was downregulated in osteosarcoma groups compared to control group. *STAT3* and IL-6R levels were upregulated in osteosarcoma groups compared to control group. This difference in expression levels was found to be more significant in the metastatic group than the non-metastatic one ($P<0.001$ each). There was a significant positive correlation between *STAT3* and IL-6R ($r=0.868$, $P<0.001$), and a significant inverse correlation between IL6 and *miRNA-34a* ($r=-0.993$, $P<0.001$). **Conclusion:** *miRNA-34a,* STAT3 and IL-6R feedback loop could be a potential target for treatment of osteosarcoma and can be used as prognostic indicator for this disease.

Keywords: Osteosarcoma- *miRNA-34a*- *STAT3*- IL-6R- metastasis

Asian Pac J Cancer Prev, **24 (9)**, 3269-3274

Introduction

Osteosarcoma is a mesenchymal-derived bone tumor that typically develops in the epiphyseal growth plates of the femur or the tibia during the rapid growth period of long bones (de Azevedo et al., 2020). MicroRNAs are considered a large subgroup of non-coding RNAs of 18-25 nucleotides. MicroRNAs interaction with target genes characterizes their role in growth, planed death, cellular differentiation and proliferation, and confirms the direct function of microRNAs in cancer (Nazarian et al., 2019).

It has been found via extensive research that members of the miRNA-34 family act as tumor suppressors in a range of human malignancies. Three miRNAs make up the miRNA-34 family: *MiRNA-34a*, *miRNA-34b,* and miRNA-34c. When compared to nearby normal tissues, human primary OS tumor samples have lower *MiRNA-34a* expression, which raises the possibility that *MiRNA-34a* dysregulation plays a role in the pathophysiology of the disease (Lopez et al., 2018). Signal transducers and activators of transcription (STATs) are a significant class of cytoplasmic factors that are activated to take part in gene regulation. The STAT family consists of seven different *STATs: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b*, and *STAT6*. There are four *STAT3* isoforms known as (α, β, γ and δ) (Liu et al., 2021).

STAT3 activation is a key component in the malignant transformation of cells caused by different protein tyrosine kinases, oncogenes, and viruses. It has been demonstrated that activation of *STAT3* may promote cell transformation by inhibiting apoptosis (Kamran et al., 2013, Tolomeo and Cascio 2021). A group of 212 amino acids make up human IL-6, including a signal peptide with 28 amino acids (Tanaka et al., 2014, Sellner et al., 2021). A ligand-binding glycoprotein (IL-6R), also known as CD126, and a signaling component (gp130), also known as CD130, make up the cell-surface type I receptor complex known as the *IL-6* receptor (Kaur et al., 2020). In order to start *IL-6* signaling, the *IL-6/IL-6R* complex subsequently binds to the gp130 membrane protein, causing it to dimerize. A number of signaling pathways, such as phosphatidylinositol-3-kinase, extracellular signal-regulated kinase, mitogen-activated protein kinase,

*[1]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt. [2]Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt. [3]Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt. *For Correspondence: ghada.elnaggar@nci.cu.edu.eg*

and *STAT3*, are activated after dimerization, including the Janus kinas (JAK) family. *STAT3* activation triggers a large number of effector genes that are important in cell survival, differentiation, and proliferation (Kitamura et al., 2017).

The aim of this study was to detect the possible relationship between expression levels of *MiRNA-34a* and levels of IL-6 receptor and *STAT3* in osteosarcoma. Also, the possible role of this relationship in development of metastasis in these patients.

Materials and Methods

Patients

A total of twenty-four (24) osteosarcoma bone samples and twelve (12) normal bone samples were included in the study. They were retrospectively collected in the period between 2021 and 2022 from the Sugical Pathology Department at the National Cancer Institute. Samples were anonymous and were given numbers for confidentiality issues. The subjects were divided into 3 groups:

- Group (I): Twelve normal bone samples as control group.
- Group (II): Twelve patients with non-metastatic osteosarcoma.
- Group (III): Twelve patients with metastatic osteosarcoma.

Inclusion criteria

Patients diagnosed as osteosarcoma as follows:

- Clinically, by age (bimodal) and by site of the tumor (mostly in the proximal part of tibia or the distal part of femur).
- Imaging: by X-ray or MRI.
- Biopsy, which is the gold standard.
- Metastases are detected using bone scan, PET scan and CT chest.

Both males and females were included in the study.

Patients aged between five and sixty-five years.

Exclusion criteria

1. Primary bone cancers other than osteosarcoma.
2. Patients presenting with bone metastasis for a primary tumor not in the bone.

Sample Collection and Preparation

Samples were formalin fixed and paraffin embedded tissue blocks (FFPE). Each selected paraffin block was sectioned at a thickness of 10 µm by the microtome and the retrieved section was put into a 1.5 ml collection tube. Deparaffination was done using a paraffin solvent (xylene) that was exposed to heat at 50°C for an overnight. Sample homogenization was performed through the addition of phosphate buffer saline, then disruption using a grinder.

Methods

Estimation of IL-6R by ELISA technique

The procedure was done using the Human Soluble Interleukin 6 Receptor (SIL-6R) ELISA kit, BT LAB, Cat. No. E0226Hu according to the manufacturer's instructions.

Estimation of STAT3 by ELISA technique

STAT3 was measured using Human Signal Transducer and Activator of Transcription 3 (*STAT3*) ELISA kit, BT LAB, Cat. No. E0650Hu according to the manufacturer's instructions.

Estimation of expression levels of miRNA 34a by qRT-PCR

Total RNA with preserved small-RNAs was extracted using the miRNeasy Mini kit (50) (Qiagen, Germany), HB-1277-006 © 2020 QIAGEN, Cat. No. 217004 according to the manufacturer's instructions. Then, Using a NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), RNA samples were submitted to quantification and purity evaluation.

Reverse transcription was done. In this step, complementary DNA (cDNA) was synthesized from total micro RNA samples using (TransScript® miRNA First-Strand cDNA Synthesis SuperMix, Beijing, China) Cat. No. AT351 according to the manufacturer's instructions. cDNA was then stored at -20 °C till usage. qRT-PCR was performed using a SYBR Green PCR Kit (PerfectStartTM Green qPCR SuperMix, Beijing, China) Cat. No. AQ601. Amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA).

After running RT-PCR, data were expressed in cycle threshold (Ct). PCR data sheet included Ct values of assessed *MiRNA-34a* and the house keeping (reference) gene; the gene that is normally and continuously expressed in the cell (snU6 RNA). The relative miR-34a gene expression level was calculated using the equation $2^{-\Delta\Delta CT}$ where $\Delta\Delta CT$= (CT miR-34a - CT U6) patient sample - (CT miR-34a - CT U6) control sample.

Statistical Analysis

Data were coded and entered using the statistical package SPSS version 22, Chi square test was used when comparing nonparametric data. Numerical data were summarized using mean and standard deviation and frequency for categorical data. Comparisons between groups were done using unpaired t test when comparing the two groups and analysis of variance (ANOVA) when comparing more than 2 groups. Quantitative variables were correlated using Pearson's correlation coefficient.

Results

Down-regulation of miR-34a in osteosarcoma patients

MiR-34a was significantly down-regulated in both osteosarcoma groups (group II and group III) compared to the control group (group I) ($P< 0.001$). A more significant decrease in its expression level was found in the metastatic group (group III) compared to non-metastatic (group II) ($P< 0.001$) as shown in Figure 1A and Table 1.

Up-regulation of STAT3 and IL-6R in osteosarcoma patients

There was a significant increase in *STAT3* and IL-6R levels in both osteosarcoma groups compared to normal control ($P<0.001$ each), with more significant increase in their levels in the metastatic group compared

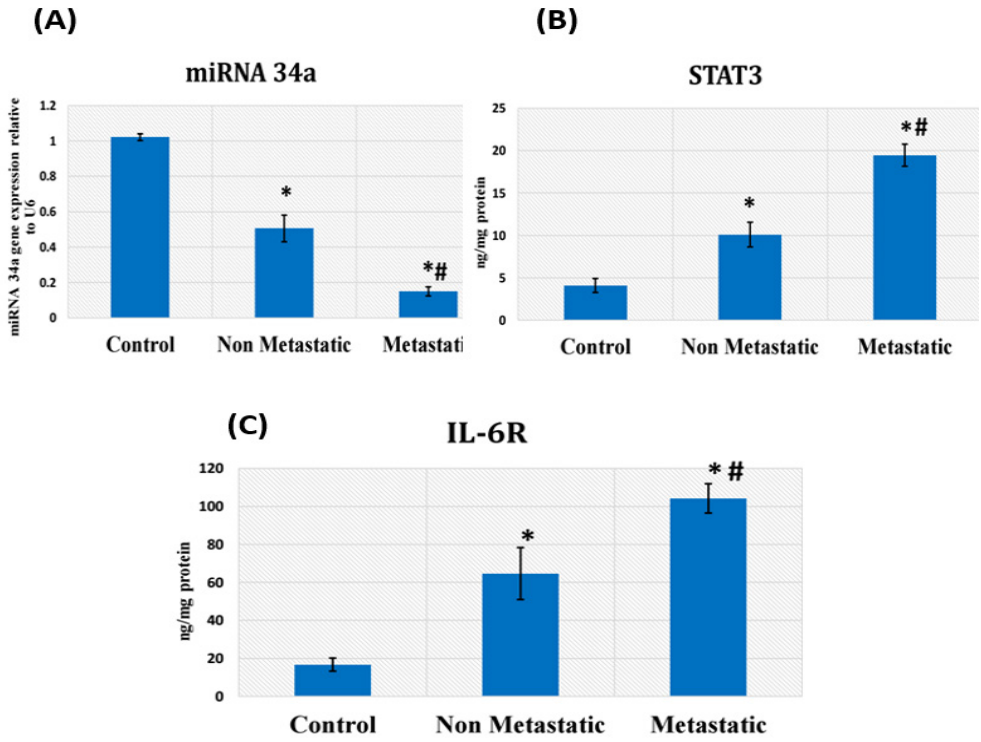

Figure 1. Box Plots for miR-34a, STAT3 and IL-6R Levels among Studied Groups. *, Significant difference versus control group; #, Significant difference versus non metastatic group; P value <0.05 is considered significant.

Table 1. Level of miRNA-34a, STAT3 and IL-6R in the Studied Groups

Variable	Control group	Non-metastatic group	Metastatic group	P1	P2	P3
MiRNA-34a (relative expression)	1.02 ± 0.02	0.51 ± 0.08	0.15 ± 0.03	<0.001*	<0.001*	<0.001*
STAT3 (ng/mg protein)	4.1 ± 0.82	10.1 ± 1.46	19.47 ± 1.31	<0.001*	<0.001*	<0.001*
IL-6R (ng/mg protein)	16.71 ± 3.45	64.64 ± 13.68	104.2 ± 7.73	<0.001*	<0.001*	<0.001*

*P value <0.05 is significant; P1 comparison between non metastatic and control; P2 comparison between metastatic and control; P3 comparison between non metastatic and metastatic group

to non-metastatic (P<0.001 each) as shown in Figure (1B and C) and Table (1).

Correlations of miR-34a, STAT3 and IL-6R in the studied groups

Using Spearman's correlation coefficient showed significant positive correlation between *STAT3* and IL-6R (r=0.868, P<0.001) as shown in Figure 2A. Our data also revealed a significant inverse correlation between IL6 and *MiRNA-34a* (r = -0.993, P<0.001) as shown in Figure 2B.

Table 2. Correlation between Studied Parameters in the Non-Metastatic Group

		MiRNA-34a	STAT3	IL-6R
Age	r	-0.088	-0.192	0.269
	p value	0.785	0.549	0.397
Tumor size	r	-0.735**	-0.578*	-0.535
	p value	0.006**	0.049*	0.073

**, Correlation is significant at the 0.01 level (2-tailed); *, Correlation is significant at the 0.05 level (2-tailed).

Relation between miR-34a, STAT3 and IL-6R Levels and Clinicopathological Characteristics of Osteosarcoma Group

As shown in Tables 2 and 3, our results showed that expression level of *MiRNA-34a* bears a significant correlation to tumor size in non-metastatic group (P=0.006), and bears a significant correlation to age (P<0.001) and tumor size (P=0.002) in metastatic group.

We also found that STAT3 level bears a significant correlation with tumor size in non-metastatic group (P=0.049), and bears a significant correlation with age only (P=0.002) in the metastatic group. Regarding IL-6R, its level bears a significant correlation with age and tumor size in the metastatic group only (P<0.001 each). As for the tumor grade, all parameters showed a significant difference

Table 3. Correlation between Studied Parameters in the Metastatic Group

		MiRNA-34a	STAT3	IL-6R
Age	r	-0.945**	-0.790**	0.901**
	p value	0.000**	0.002**	0.000**
Tumor size	r	-0.789**	-0.551	0.714**
	p value	0.002**	0.064	0.009**

**, Correlation is significant at the 0.01 level (2-tailed).

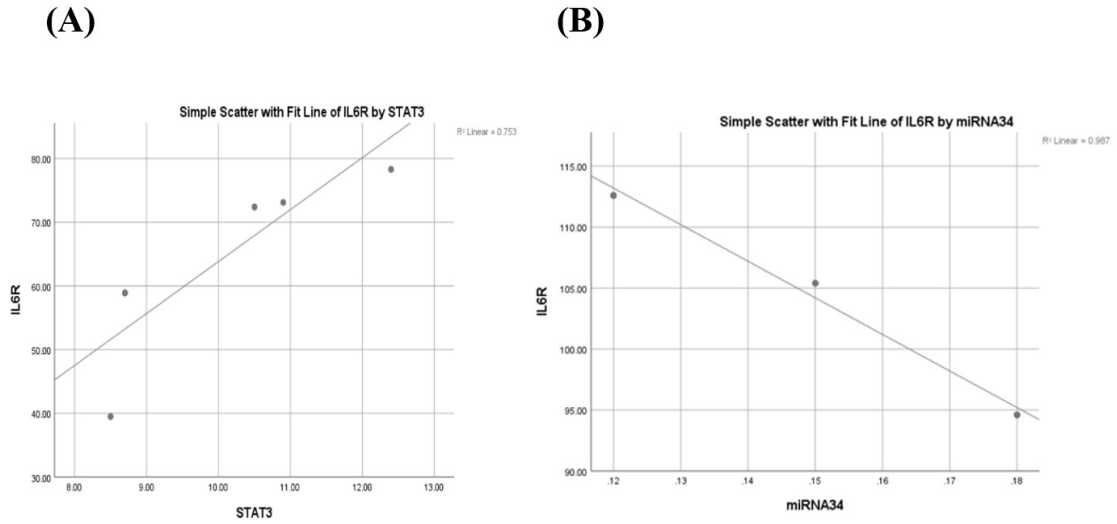

Figure 2. (A) Significant positive correlation between STAT3 and IL6-R (r=0.868, p <0.001). (B) Significant inverse correlation between IL6 and miRNA-34a (r=-0.993, p <0.001).

in high grade tumors compared to intermediate grade in the non-metastatic group only with p value <0.05 each.

Discussion

The most prevalent primary bone tumor that develops from primary osteoblasts is osteosarcoma. It is the most prevalent type of bone cancer in adolescents and children between the ages of 10 and 20 (Li et al., 2021). MicroRNAs are short non-coding RNAs with 20–24 nucleotides that play a key function in controlling the expression of genes and several biological activities. It's interesting that they can function as onco-miRNAs or tumor suppressors. (Llobat and Gourbault 2021). *MiRNA-34a* expression was discovered to be downregulated in several malignancies, including osteosarcoma, and it has a variety of target genes to operate upon, including P53, Wnt, and Notch. (Yang et al., 2020).

In this study, we aimed to assess the possible relationship between expression levels of *MiRNA-34a*, *STAT3* and IL-6R in osteosarcoma tissue samples compared to normal ones. Also, to detect the possible role of this signaling pathway in development of metastasis in this disease. Regarding *MiRNA-34a*, our data showed a statistically significant decrease in its expression levels in the two osteosarcoma groups (group II and group III) compared to the control group (group I) (P value < 0.001) and a more significant decrease in its level in the metastatic group (group III) compared to the non-metastatic group (group II) (P value < 0.001). We also found that expression level of *MiRNA-34a* bears a significant correlation to tumor grade and tumor size but not the age or tumor location in group (II), and bears a significant correlation to age, tumor size but not tumor location or tumor grade in group (III). This was in agreement with the studies of Wang et al., (2017) and Yang et al., (2020) who reported a significant decrease in serum and tissue level of *MiRNA-34a* in osteosarcoma group compared to healthy controls.

They also demonstrated that its level was lower in patients with distant metastasis, low grade, and poor response to chemotherapy. This implies that *MiRNA-34a* is a prognostic factor and an indicator for chemosensitivity. This result was also supported by several studies on osteoblastic cell lines. Wu et al., (2013) and Shi et al., (2019) stated that *MiRNA-34a* expression levels were lower in osteosarcoma tissues than in normal ones and osteoblastic cell lines. They referred this to the abnormal methylation of the *MiRNA-34a* promotor CpGs found in osteosarcoma cells. Increased *MiRNA-34a* expression downregulated DNA (cytosine-5)- methyltransferase1 DNMT1, an inhibitor of tumor suppressor genes, which, in turn, negatively regulates methylation of promotor CpGs. This inhibits cell proliferation and tumorigenesis and implies that *MiRNA-34a* acts as tumor suppressor.

A crucial cytoplasmic transcription factors family called STATs, including *STAT3*, is activated by cytokines like IL-6. Once activated, *STAT3* binds to specific promotor sequences to regulate the transcription of its target genes, including cyclin D1, c-myc, Bcl-2, and many other molecules involved in cellular proliferation and apoptosis (Sajjadi-Dokht et al., 2022). The IL-6/JAK/*STAT3* signaling pathway has been shown to promote osteosarcoma cancer progression, including cell transformation, invasion, and metastasis both in vivo and in vitro (Liu et al., 2021).

STAT3 therefore, may be considered as an oncogene. A study done by Zuo et al., 2018 proved this by inducing inhibition of *STAT3* signaling using Napabucasin, a *STAT3* inhibitor, through targeting its target genes; c-myc, survivin and vascular endothelial growth factor A (VEGF-A). This inhibition suppressed osteosarcoma cell growth and metastasis in vivo and in vitro. As for *STAT3*, our study showed a statistically significant increase in its level in groups (II) and (III) compared to group (I) (p value <0.001) with more significant increase in its level in group (III) compared to group (II) (p value <0.001). We found that level of *STAT3* bears a significant correlation with tumor grade and tumor size but not the age or tumor location in group (II) and bears a significant correlation with age but not tumor location or tumor size in group

(III).

These results were consistent with the study of Wang et al., (2011). They found a significant overexpression of *STAT3* level in osteosarcoma tissues compared to chondroma tissues and normal tissues. They also stated that *STAT3* protein expression was positively correlated with poor differentiation and presence of metastasis but not with age, gender, tumor size or tumor location. They demonstrated that downregulation of *STAT3* induces apoptosis and inhibits proliferation which may be caused by downregulation of anti-apoptotic genes; Bcl-xL, survivin and Mcl-1. Therefore, they concluded that *STAT3* may be a prognostic factor and a therapeutic target in osteosarcoma.

Similarly, studies done by Ryu et al., (2010); Ryu et al., (2010); and Liu et al., (2011) also reported overexpression of *STAT3* and its phosphorylated state in osteosarcoma tissues and cell lines using western blot and immunohistochemistry. In contrast with the previous studies, some papers implicated that *STAT3* has tumor suppressor functions. In thyroid cancer, activation of *STAT3* correlates with reduced metastasis and its inhibition leads to enhanced tumor growth (Sellier et al., 2013). In addition, in a mouse model of intestinal cancer, inhibition of *STAT3* promoted late tumor progression (Musteanu et al., 2010). Inflammation, oncogenesis, and immunological regulation are all impacted by the significant cytokine IL-6, especially in advanced stages of tumor development (Weng et al., 2019). After IL-6 binds to its receptor, the IL-6/IL-6R complex attaches to the gp130 signal-transducing membrane protein, causing dimerization and the activation of the JAK2/*STAT3* signaling pathway (Baran et al., 2018).

It has been evident that many malignancies, including osteosarcoma, have high IL-6 levels (Wu et al., 2017). Regarding IL-6R, it was discovered to be elevated in the sera of patients with specific malignancies, such as colorectal cancer (CRC), and that it associated with the size of the tumor, which raises the possibility that IL-6R plays a role in the development of this type of cancers. (Turano et al., 2021). However, there was little attention paid to the clinicopathological significance of IL-6R in the setting of osteosarcoma in the literature.

This study has shown a statistically significant increase in the level of IL-6R in both group (II) and group (III) compared to group (I) (p value <0.001) with more significant increase in its level in group (III) compared to group (II) (p value <0.001). Its level bears a significant correlation with tumor grade but not the age, tumor size or tumor location in group (II) and bears a significant correlation with age and tumor size but not with tumor location or tumor grade. Similarly, the overexpression of IL-6R was reported in various types of cancers including gastric carcinoma (Simondurairaj et al., 2019), CRC (Chung et al., 2006), cervical cancer (Luan et al., 2018) and breast cancer (Labovsky et al., 2016). Our results showed a significant correlation between IL-6R and *STAT3*. IL-6/IL-6R complex induces dimerization and activation of *STAT3*. High IL-6 levels regulate expression of IL-6R through activation of JAK/STAT (Johnson et al., 2018).

This study also shows a significant inverse correlation between *MiRNA-34a* and IL-6R. IL-6R is suggested to be a direct *MiRNA-34a* target as *MiRNA-34a* binds to the 3′ UTR of IL-6R mRNA (Zhang et al., 2020). *MiRNA-34a* is believed to be directly repressed by IL-6 mediated activation of *STAT3* which contributes to invasion and metastasis (Shi et al., 2019). In agreement with our results, a study done by (Rokavec et al., 2014) proved the presence of an IL-6R/*STAT3*/*MiRNA-34a* feedback loop in CRC, breast cancer and prostatic cancer cell lines. They showed that cell lines with mesenchymal phenotype presented higher expression of IL-6R and lower expression of *MiRNA-34a* than those with epithelial phenotype which indicates that this loop mediates EMT. They also proved that ectopic expression of *MiRNA-34a* in cancer cell lines repressed IL-6R, which suggests that IL6R is a direct *MiRNA-34a* target. Also, IL-6 mediated activation of IL-6R and *STAT3* induces direct repression of *MiRNA-34a*, a signaling pathway required for invasion and metastasis.

Same results were reported by Li et al., (2015). They performed a study on CRC cell lines. IL-6R level was measured using ELISA, and IL-6R mRNA and *MiRNA-34a* expressions were detected by qRT-PCR. Their study demonstrated that mesenchymal like CRC cell lines expressed higher IL-6R levels and lower *MiRNA-34a* levels. They proved that *MiRNA-34a* suppresses IL-6R mRNA and protein. Therefore, downregulation of *MiRNA-34a*, which is common in cancers, and enhanced production of IL-6R produces activation of the JAK/STAT pathway in tumor microenvironment.

Taken together, our findings point to a possible role of the IL-6R/*STAT3*/*MiRNA-34a* feedback loop in the molecular etiology of osteosarcoma and state that *MiRNA-34a*, *STAT3* and IL-6R are potential targets in osteosarcoma treatment.

Author Contribution Statement

All authors shared in planning and designing the research, interpretation of laboratory data and practical work, writing and editing the final version of the manuscript. Passant Essam Eldin Shibel participated in collecting patients' specimens.

Acknowledgements

The authors would like to thank Prof. Dr. Laila Ahmed Rashed, Professor of Medical Biochemistry and Molecular Biology, Cairo University for her great support and helpful supervision in the practical part of the study.

Funding Statement

This work was funded by Faculty of Medicine, Cairo University and was part of Lamiaa Ali Bayoumi approved Master thesis.

Ethical Approval and Consent

The study was approved by the Research Ethics Committee (REC), Faculty of Medicine, Cairo University.

Conflict of Interest

The authors declare no conflict of interest.

References

Baran P, Hansen S, Waetzig GH, et al. (2018). The balance of interleukin (IL)-6, IL-6·soluble IL-6 receptor (sIL-6R), and IL-6·sIL-6R·sgp130 complexes allows simultaneous classic and trans-signaling. *J Biol Chem*, **293**, 6762-75.

Chung YC, Chaen YL, Hsu CP (2006). Clinical significance of tissue expression of interleukin-6 in colorectal carcinoma. *Anticancer Res*, **26**, 3905-11.

de Azevedo JWV, de Medeiros Fernandes TAA, Fernandes JV, et al (2020). Biology and pathogenesis of human osteosarcoma. *Oncol Lett*, **19**, 1099-116.

Johnson DE, O'Keefe RA, Grandis JR (2018). Targeting the IL-6/JAK/*STAT3* signalling axis in cancer. *Nat Rev Clin Oncol*, **15**, 234-48.

Kamran MZ, Patil P, Gude RP (2013). Role of *STAT3* in cancer metastasis and translational advances. *Biomed Res Int*, **2013**, 421821

Kaur S, Bansal Y, Kumar R, Bansal G (2020). A panoramic review of IL-6: Structure, pathophysiological roles and inhibitors. *Bioorg Med Chem*, **28**, 11532.

Kitamura H, Ohno Y, Toyoshima Y, et al (2017). Interleukin-6/STAT 3 signaling as a promising target to improve the efficacy of cancer immunotherapy. *Cancer Sci*, **108**, 1947-52.

Labovsky V, Martinez LM, Calcagno ML, et al (2016). Interleukin-6 receptor in spindle-shaped stromal cells, a prognostic determinant of early breast cancer. *Tumour Biol*, **37**, 13377-84.

Li H, Rokavec M, Hermeking H (2015). Soluble IL6R represents a miR-34a target: potential implications for the recently identified IL-6R/*STAT3*/miR-34a feed-back loop. *Oncotarget*, **6**, 14026.

Li J, Qin B, Huang M, et al (2021). Tumor-Associated Antigens (TAAs) for the Serological Diagnosis of Osteosarcoma. *Front Immunol*, **12**, 665106.

Liu LH, Li H, Li JP, et al (2011). miR-125b suppresses the proliferation and migration of osteosarcoma cells through downregulation of *STAT3*. *Biochem Biophys Res Commun*, **416**, 31-8.

Liu Y, Liao S, Bennett S, et al (2021). *STAT3* and its targeting inhibitors in osteosarcoma. *Cell Prolif*, **54**, e12974.

Llobat L, Gourbault O (2021). Role of MicroRNAs in human osteosarcoma: Future Perspectives. *Biomedicines*, **9**, 463.

Lopez CM, Yu PY, Zhang X, et al (2018). MiR-34a regulates the invasive capacity of canine osteosarcoma cell lines. *PLoS One*, **13**, e0190086.

Luan S, An Z, Bi S, Chen L, Fan J (2018). Interleukin 6 receptor (IL-6R) was an independent prognostic factor in cervical cancer. *Histol Histopathol*, **33**, 269-76.

Musteanu M, Blaas L, Mair M, et al (2010). Stat3 is a negative regulator of intestinal tumor progression in Apc (Min) mice. *Gastroenterology*, **138**, 1003-11. e1-5.

Nazarian A, Mohamadnia A, Danaee E, Bahrami N (2019). Examining the Expression of miR-205 and CEA mRNA in Peripheral Blood of Patients with OSCC (Oral Squamous Cell Carcinomas) and comparing them with Healthy People. *Asian Pac J Cancer Biol*, **4**, 65-68.

Rokavec M, Öner MG, Li H, et al (2014). IL-6R/*STAT3*/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J Clin Invest*, **124**, 1853-67.

Ryu K, Choy E, Yang C, et al (2010). Activation of signal transducer and activator of transcription 3 (*Stat3*) pathway in osteosarcoma cells and overexpression of phosphorylated-*Stat3* correlates with poor prognosis. *J Orthop Res*, **28**, 971-8.

Ryu K, Susa M, Choy E, et al (2010). Oleanane triterpenoid CDDO-Me induces apoptosis in multidrug resistant osteosarcoma cells through inhibition of *Stat3* pathway. *BMC Cancer*, **10**, 187.

Sajjadi-Dokht M, Merza Mohamad TA, Sulaiman Rahman H, et al. (2022). MicroRNAs and JAK/*STAT3* signaling: A new promising therapeutic axis in blood cancers. *Genes Dis*, **9**, 849-67.

Sellier H, Rébillard A, Guette C, Barré B, Coqueret O (2013). How should we define *STAT3* as an oncogene and as a potential target for therapy?. *JAK-STAT*, **2**, e24716.

Sellner J, Sitte HH, Rommer PS (2021). Targeting interleukin-6 to treat neuromyelitis optica spectrum disorders: Implications from immunology, the FcRn pathway and clinical experience. *Drug Discovery Today*, **26**, 1591–601.

Shi J, Zhao C, Liu X, et al (2019). The regulatory role of aberrant methylation of microRNA-34a promoter CpGs in osteosarcoma. *Transl Cancer Res*, **8**, 2328-38.

Simondurairaj C, Krishnakumar R, Sundaram S, Venkatraman G (2019). Interleukin-6 Receptor (IL-6R) Expression in Human Gastric Carcinoma and its Clinical Significance. *Cancer Invest*, **37**, 293-8.

Tanaka T, Narazaki M, Kishimoto T (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*, **6**, a016295.

Tolomeo M, Cascio A (2021). The Multifaced Role of *STAT3* in Cancer and Its Implication for Anticancer Therapy. *Int J Mol Sci*, **22**, 603.

Turano M, Cammarota F, Duraturo F, Izzo P, De Rosa M (2021). A Potential Role of IL-6/IL-6R in the Development and Management of Colon Cancer. *Membranes (Basel)*, **11**, 312.

Wang YC, Zheng LH, Ma BA, et al (2011). Clinical value of signal transducers and activators of transcription 3 (*STAT3*) gene expression in human osteosarcoma. *Acta Histochem*, **113**, 402-8.

Wang T, Wu J, Liu X, Li S (2017). Serum miR-34a is a potential diagnostic and prognostic marker for osteosarcoma. *Int J Clin Exp Pathol*, **10**, 9683-9.

Weng YS, Tseng HY, Chen YA, et al (2019). MCT-1/miR-34a/IL-6/IL-6R signaling axis promotes EMT progression, cancer stemness and M2 macrophage polarization in triple-negative breast cancer. *Mol Cancer*, **18**, 42.

Wu X, Zhong D, Gao Q, et al (2013). MicroRNA-34a inhibits human osteosarcoma proliferation by downregulating ether à go-go 1 expression. *Int J Med Sci*, **10**, 676-82.

Wu Z, Yang W, Liu J, Zhang F (2017). Interleukin-6 upregulates SOX18 expression in osteosarcoma. *Onco Targets Ther*, **10**, 5329-36.

Yang W, Qi YB, Si M, Hou Y, Nie L (2020). A comprehensive analysis for associations between multiple microRNAs and prognosis of osteosarcoma patients. *Peer J*, **8**, e8389.

Zhang Z, Sun C, Li C, et al (2020). Upregulated MELK leads to doxorubicin chemoresistance and M2 macrophage polarization via the miR-34a/JAK2/*STAT3* pathway in uterine leiomyosarcoma. *Front Oncol*, **10**, 453.

Zuo D, Shogren KL, Zang J, et al (2018). Inhibition of *STAT3* blocks protein synthesis and tumor metastasis in osteosarcoma cells. *J Exp Clin Cancer Res*, **37**, 244.