ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 3/2023
671–678
https://doi.org/10.18388/abp.2020_6869

Regular paper

Osteosarcoma's genetic landscape painted by genes' mutations

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Purpose: Osteosarcoma (OS) is one of the most common primary bone tumors. Direct pathogenesis remains unknown, however, genes' mutations are proven to participate in the process. This study aimed to examine the most frequently mutated genes in OS to appoint candidates for the cancer markers. Methods: Using the COSMIC Catalogue twenty the most frequently mutated genes were selected leading to an up-to-date genetic OS landscape summary. The genes can be classified into four categories: suppressor genes (*TP53, RB1, NCOR1, SMAD2, NF1, TSC2, KMT2C*), proto-oncogenes (*GNAS, BRAF, MLLT3*), epigenetic and post-translational modification-related genes (*SMARCA4, ARID1A, ATRX, BCOR, H3F3A*) and cell growth and survival regulating genes (*EGFR, CAMTA1, LRP1B, PDE4DIP, MED12*). Results and conclusions: Their role in cancerogenesis was confirmed by the analysis of available articles published previously. The results of the study indicate that examination of selected genes' mutations might help to identify patients' predisposition to OS development, as well as monitor the disease progression, and establish prognosis. However, to fully understand the pathogenesis of OS further studies are required.

Keywords: osteosarcoma, genes'mutations, COSMIC catalogue, biomarkers

Received: 02 June, 2023; revised: 14 August, 2023; accepted: 14 August, 2023; available on-line: 17 September, 2023

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Abbreviations: ALP, alkaline phosphatase; ANPEP, alanyl aminopeptidase; AP-1, activator protein 1; ARID1A, AT-rich interactive domain-containing protein 1A; ATRX, alpha thalassemia/mental retardation syndrome X-linked; BCOR, BCL6 Corepressor; BRAF, serine/threonine kinase; c-Myc, cellular myelocytomatosis oncogene; CAMTA1, calmodulin binding transcription activator 1; CD133, CD133 antigen; COSMIC, Catalogue Of Somatic Mutations In Cancer; CRP, C-reactive protein; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FLNA, filamin A; FUCA1, alpha-L-fucosidase 1; GNAS, guanine nucleotide binding protein alpha stimulating; H3F3A, H3 histone family 3A; HER-4, human epidermal growth factor receptor 4; ICB, immune checkpoint blockade; ICIs, immune checkpoint inhibitors; KTM2C, lysine methyltransferase 2C; LAMA3, laminin subunit alpha 3; LGALS1, galectin 1; LRP1B gene, low density lipoprotein receptor-related protein 1B; MATN3, matrilin 3; MED12, mediator complex subunit 12; miRNA , microRNA; MLLT3, MLLT3 super elongation complex subunit 3; MMGL, myomegalin-like; MSCs, mesenchymal stem cells; NANOG, homeobox protein NANOG; NCOR1, nuclear receptor corepressor 1; NF1, neurofibromin 1; OS, Osteosarcoma; PDE4DIP, phospodiestrase 4D-interacting protein; Rb, retinoblastoma protein; RB1, RB transcriptional corepressor 1; SEC, super elongation complex; SGCG, sarcoglycan gamma; SMAD2, SMAD family member 2; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependant regulator of chromatin, subfamily a, member 4; SOX4, SRY-box transcription factor 4; TCGA, The Cancer Genome Atlas; TICs, tumor-initiating cells; TP53, tumor protein p53; TSC, tuberous sclerosis complex; TSC2, TSC complex subunit 2; TWIST, Twist Family BHLH Transcription Factor; VEGFA,Vascular endothelial growth factor A; WNT5A, Wnt Family Member 5A

INTRODUCTION

Osteosarcoma (OS) is one of the most common primary bone tumors. It occurs intraosseous, mainly in the metaphyseal region of the long bones. OS is inflicting an accelerated osteoid matrix production, which is connected to being developed at sites where bone grows expeditiously. Current data prove that OS has two main peaks of incidence: in childhood and adolescence. OS is the third most common type of tumor affecting young people, with the highest incidents in the early twenties (Wu & Livingston, 2020; Sun *et al.*, 2020; Misaghi *et al.*, 2018; Czarnecka *et al.*, 2020).

Osteosarcoma is divided into several subtypes (Fig. 1). It can be categorized depending on the region of bone it affects (the surface and central part of the bone, and within the medulla). The staging scheme divides OS into two classes depending on the grade of the tumor (low or high), as well as the localization of the compartment (extra- or intra-compartmental). Additionally, it is classified considering the metastatic level of the tumor. The most common classification groups OS as osteoblastic, chondroblastic, fibroblastic, and small cell subtypes. Among mention above types of ostesarcoma, the most often diagnosed one is a high-grade tumor, occurring extra-cortically (Misaghi *et al.*, 2018).

Even though the exact mechanisms responsible for OS pathogenesis are not known, it is confirmed that genetics play a role in the tumor origin. The most common characteristic of OS includes genome disorganization, together with alterations of tumor suppressor and DNA repair, as well as changes in cell cycle aneuploids with chromosomal alterations (de Azevedo *et al.*, 2020).

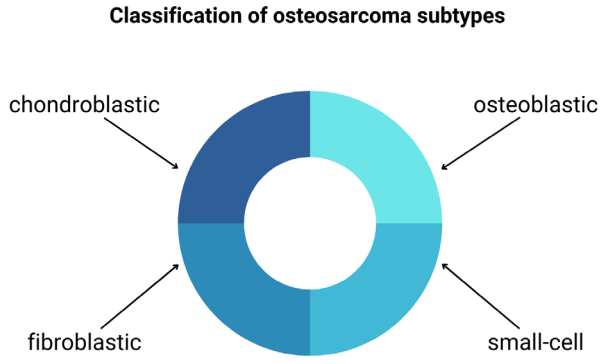

Figure 1. The most common classification of osteosarcoma subtypes, depending on the predominant type of cells affected by OS.

It was proved that OS can be developed as a consequence of alteration in tumor suppressor genes, including *TP53* and *Rb1,* during hereditary disorders such as Li-Fraumeni cancer family syndrome and retinoblastoma (Porter *et al.*, 1992; Fletcher & Unni, 2002; Misaghi *et al.*, 2018; Sun *et al.*, 2020). However, also other genes, such as transcription factors, and tumor suppressor genes, including *CAMTA1*, or *KMT2C* might participate in osteosarcoma development (He *et al.*, 2021; Chen *et al.*, 2016; Chiappetta *et al.*, 2019).

Currently, there are no laboratory tests or specific biomarkers to diagnose osteosarcoma (Misaghi *et al.*, 2018). Even though there are some molecules expressed in OS that are proposed as their potential markers. The most promising one appears to be alkaline phosphatase (ALP), which increased serum level and seems to correlate positively with tumor volume (Limmahakhun *et al.*, 2011). Additionally, C-reactive protein (CRP), Cathepsin D, osteocalcin, SATB2, and aspartic endoprotease can help distinguish OS (Misaghi *et al.*, 2018; Czarnecka *et al.*, 2020; Agustina *et al.*, 2018; Machado *et al.*, 2016; Tallegas *et al.*, 2022).

In addition to proteins, microRNA expression also seems to be valuable in the diagnosis of osteosarcomas. Overexpression of miR-421 and miR-191 proves to be linked with proliferation, migration, and malignant character of the tumors. Their levels in the serum of OS patients were higher compared to samples from healthy volunteers (Czarnecka *et al.*, 2020).

Moreover, the presence of tumor-initiating cells (TICs) may help in OS diagnostics. TICs expressing markers of stem cell phenotype, such as NANOG or SOX4 were observed in both primary and metastatic tumor tissue (Yan *et al.*, 2016). These proteins, together with CD133+, which is a distinctive marker of TICs observed in osteosarcoma cell lines (Czarnecka *et al.*, 2020). It has been proved that the expression of CD133 in OS patients is related to distant metastasis and poor prognosis. making this a tumor marker (Xie *et al.*, 2018).

Finally, the prevalence of mesenchymal stem cells (MSCs) connected with OS progression is proposed to be the tumor marker. Changes in the expression of c-Myc, Rb, AP-1 or TWIST were shown to contribute to the transformation of MSCs into osteosarcoma tumor cells (Yang *et al.*, 2020). Most sarcomas characterize a permanent mesenchymal state. However, their phenotype can change by factors regulating epithelial-mesenchymal transition – EMT. These factors have been shown to be highly correlated with the invasiveness and higher risk of metastasis in malignant OS (Wu *et al.*, 2019; Yu, Yustein & Xu, 2021). An EMT-related genes' panel (including *LAMA3, LGALS1, SGCG, VEGFA, WNT5A, MATN3, ANPEP, FUCA1*, and *FLNA*) was used as a predictive marker in a multi-cohort study of OS. The panel proved to be a reliable tool for estimating the overall survival of OS patients. Moreover, it was proposed as a selection method for patients with metastases for personalized treatment (Yiqi *et al.*, 2020).

Even though numerous studies describe molecules, whose expression may characterize osteosarcomas, all pointed proteins and/or miRNA are not specific to OS. Thus, further studies are needed to establish biomarkers helping to diagnose and treat OS.

The present study aims to provide a comprehensive, up-to-date study of genes the most frequently mutated in osteosarcoma using the COSMIC Catalogue. Such genes would become candidates for OS markers.

COSMIC, the Catalogue Of Somatic Mutations In Cancer (https://cancer.sanger.ac.uk) is a database providing tools to explore somatic mutations and their correlation with human cancers. The catalogue is the collection of somatic mutations. The data used to build this catalogue derives strictly from the scientific literature. COSMIC additionally provides information about patients' genetic predispositions and environmental factors participating in the process of cancerogenesis (Tate *et al.*, 2019).

MATERIALS AND METHODS

To identify the most common somatic mutations occurring in osteosarcoma patients, the COSMIC Cancer Browser tool (https://cancer.sanger.ac.uk/cosmic/browse/tissue) was used. The catalogue provides information about 282 osteosarcoma samples (classified into 9 histological subtypes). The biological relevance of the 20 most frequently mutated genes pointed out by the COSMIC Cancer Browser was investigated by the analysis of publications available on PubMed (https://pubmed.ncbi.nlm.nih.gov/). To search for the publications, specific keywords, such as: selected genes' name, osteosarcoma, cancer, and mutation were used. For each gene research was divided into two keyword panels – first: selected genes' name, cancer, mutation; second: selected genes' name, osteosarcoma, mutation.

RESULTS AND DISCUSSION

Genes the most frequently mutated in OS

The analysis of the COSMIC Catalogue provided a list of genes in which somatic mutation may participate in osteosarcoma development. Out of 18,309 entries of genes, the top 20 genes, based on the frequency of their

Table 1. The frequency of gene mutation in OS samples in the COSMIC Catalogue

Gene	Number of OS samples available in COSMIC catalogue	Number of mutated samples and % of frequency
TP53	371	91 (25%)
RB1	285	22 (8%)
ATRX	212	16 (8%)
KMT2C	215	13 (6%)
LRP1B	136	8 (6%)
CAMTA1	134	7 (5%)
NCOR1	208	9 (4%)
MLLT3	134	6 (4%)
PDE4DIP	134	6 (4%)
GNAS	352	9 (3%)
SMARCA4	211	6 (3%)
ARID1A	210	6 (3%)
H3F3A	453	11 (2%)
EGFR	280	5 (2%)
BRAF	257	6 (2%)
SMAD2	243	5 (2%)
NF1	216	5 (2%)
MED12	210	5 (2%)
TSC2	210	5 (2%)
BCOR	209	5 (2%)

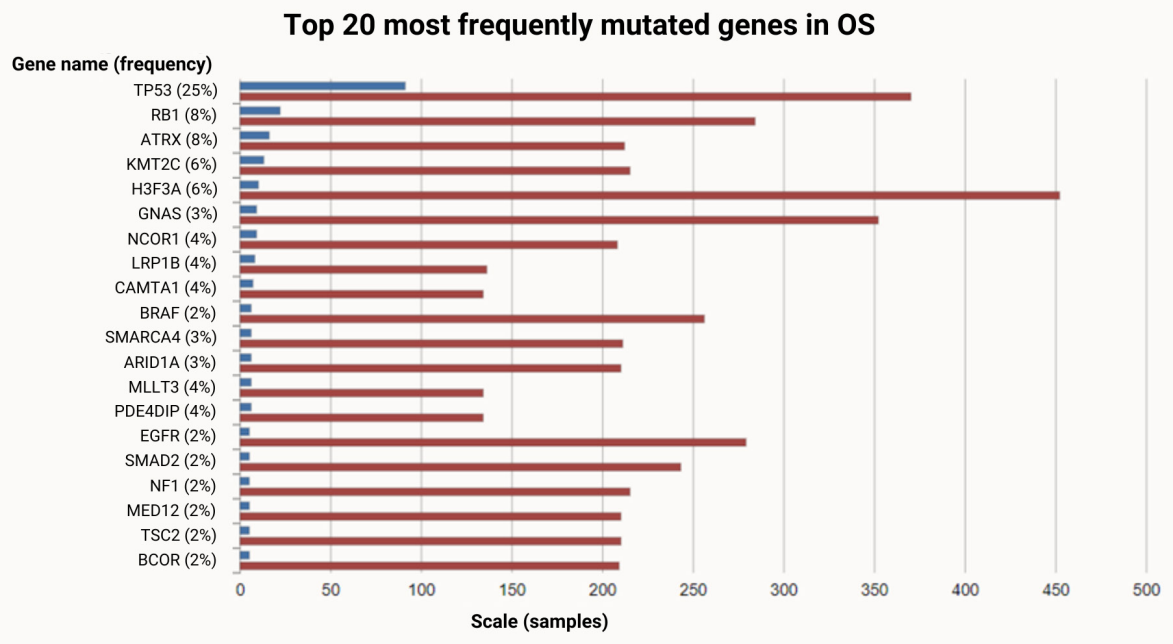

Figure 2. The top 20 most frequently mutated genes in OS according to COSMIC Catalogue

mutation were selected. They are shown in Table 1 and shown in Fig. 2.

All these genes are directly or indirectly involved in oncogenesis (Table 2). Seven out of these 20 genes – *TP53, RB1, NCOR1, SMAD2, NF1, TSC2, KMT2C* are known to be tumor suppressor genes. Thus, their mutations lead to the expression of malfunctioning protein products and strictly correlate with tumourigenesis. The following three genes: *GNAS, BRAF,* and *MLLT3* are protooncogenes. Other genes – *SMARCA4*, *ARID1A,*

Table 2. Genes the most frequently mutated in osteosarcomas and their function

Gene	Function	References
TP53	tumor suppression	(Synoradzki *et al*., 2021)
RB1	tumor suppression	(Li *et al*., 2022)
ATRX	epigenetics and post-translational modifications	(He *et al*., 2015)
KMT2C	tumor suppression	(Gala *et al*., 2018; Lian *et al*., 2022; Liu *et al*., 2021)
LRP1B	cell growth, metabolism and survival	(Wang & Xiong, 2021)
CAMTA1	cell growth, metabolism and survival	(Lu *et al*., 2018)
NCOR1	tumor suppression	(Tang *et al*., 2020)
MLLT3	protooncogene	(Sun *et al*., 2017)
PDE4DIP	cell growth, metabolism and survival	(Soejima *et al*., 2001; Lehnart *et al*., 2005)
GNAS	protooncogene	(Nomura *et al*., 2014; Zauber, Marotta & Sabbath-Solitare, 2016; Patra *et al*., 2018; Afolabi *et al*., 2022)
SMARCA4	epigenetics and post-translational modifications	(Xu *et al*., 2021; Jelinic *et al*., 2014)
ARID1A	epigenetics and post-translational modifications	(Xu *et al*., 2019)
H3F3A	post-translational modifications of histone H3.3	(Sturm *et al*., 2012; Park *et al*., 2016)
EGFR	cell growth, metabolism and survival	(Wang *et al*., 2004)
BRAF	protooncogene	(Śmiech *et al*., 2020)
SMAD2	tumor suppression	(Piek, Heldin & Ten Dijke, 1999; Pasche, 2001)
NF1	tumor suppression	(Trovó-Marqui & Tajara, 2006)
MED12	cell growth, metabolism and survival	(Ding *et al*., 2008)
TSC2	tumor suppression	(Inoki *et al*., 2002)
BCOR	epigenetics and post-translational modifications	(Huynh *et al*., 2000)

ATRX, BCOR, and *H3F3A* are related to the epigenetic status of gene expression or posttranslational histone modifications. Finally, *EGFR, CAMTA1, LRP1B, PDE4DIP,* and *MED12* are associated with cell growth, metabolism, and survival.

Molecular basis of OS

Genome destabilization, aneuploids with chromosomal changes, dysregulation of cell cycle and tumor suppressor genes, as well as a lack of DNA repair are some of the most prevalent characteristics of OS (de Azevedo *et al.*, 2020). Epigenetic changes are also recognized as risk factors for OS (Sharma, Kelly & Jones, 2009). Still, the pathogenesis of OS is complex and poorly understood. Thus, the identification of additional genes involved in oncogenesis may lead to advances in understanding OS biology, as well as shortening the time needed to establish diagnosis and introduce proper treatment.

The results of the COSMIC Catalogue analysis allowed distinguishing 20 genes the most frequently mutated in cancer. All recognized genes (Table 2) are known to be key regulators of vital cellular processes such as DNA repair, cell proliferation and differentiation. Thus, their mutations might lead to cancer development.

The most frequently mutated genes and their possible role in OS biology

Among the genes examined during the analysis of the COSMIC Catalogue, the most frequently mutated gene in OS was *TP53*. Its mutation characterized 25% of samples available in the database. *TP53* encodes the P53 protein, which is an essential transcription factor with a tumor suppressor function. It stimulates several processes that result in cell cycle arrest, DNA repair, alterations in metabolism in response to cellular stress, apoptosis, and cell senescence. Moreover, it plays an important role in ontogenesis, myogenesis, and angiogenesis (Synoradzki *et al.*, 2021). Thus, mutated *TP53* appears to be essential for cancer development and its progression. Mutations in *TP53* are known to increase protein instability, which affects clinical characteristics of osteosarcoma, such as metastatic potential, tumor type, and grade as well as its aggressiveness. According to Chen *et al. TP53* mutations are a reliable predictive indicator for osteosarcoma patient survival (Chen *et al.*, 2016).

Another gene commonly mutated in OS is Retinoblastoma transcriptional corepressor 1 gene – *RB1*. Its mutations were observed in 8% of samples available in the COSMIC catalogue. Between 50% and 70% of osteosarcoma cases exhibit mutations in *RB1* (Wu & Livingston, 2020). *RB1* encodes retinoblastoma protein controlling the transition from the G1 to S phase. This makes it a crucial regulator of cell cycle progression. It is suggested that during OS oncogenesis, alterations of *RB1* may correlate with *TP53* inactivation (Wu & Livingston, 2020; Li *et al.*, 2022). Indeed, using mice models it was proved that deletion of *RB1* leads to osteosarcomas development. Osteoblast development and mineralization as well as an increase in the expression of osteogenic markers were positively impacted by the gene deletion (Li *et al.*, 2022).

Yet another gene mutated in 8% of OS is *ATRX* – alpha thalassemia/mental retardation syndrome X-linked. The gene encodes the protein that plays a role in chromatin remodeling and DNA repair. Loss of *ATRX* function has been linked to the accumulation of repetitive DNA sequences and alterations in the epigenetic regulation of the gene expression, which may contribute to the development and progression of tumor (He *et al.*, 2015). Mutations in *ATRX* have been observed in a variety of cancer types (Haase *et al.*, 2018). In osteosarcoma, *ATRX* dysfunction was linked with chromosomal instability, which contributes the tumor development. The gene's mutations were also associated with poor prognosis and resistance to chemotherapy (Masliah-Planchon *et al.*, 2018). Thus, *ATRX* is pointed out as a promising biomarker of osteosarcoma.

6% of OS samples in the COSMIC catalogue manifested mutation of the *KTM2C* gene. KTM2C has been shown to act as a tumor suppressor. Its expression in OS has been shown to be downregulated, like in a variety of other human cancers, including breast and prostate cancer (Gala *et al.*, 2018; Lian *et al.*, 2022; Liu *et al.*, 2021). Loss of KTM2C expression is associated with poor prognosis and resistance to chemotherapy (Lian *et al.*, 2022; Liu *et al.*, 2021). What is more, KTM2C has been shown to regulate the other tumor suppressor genes, such as *TP53* and *p16INK4a*. By removing methyl groups from histones, KTM2C also activates the expression of oncogenes, such as *MYC* (Liu *et al.*, 2021; Lian *et al.*, 2022; Gala *et al.*, 2018). All these data demonstrate that the *KTM2C* gene can play an important role in OS development.

6% of available OS samples of the COSMIC catalogue were mutated in the *LRP1B* gene – Low Density Lipoprotein Receptor-Related Protein 1B that encodes cell surface receptor for LDL. *LRP1B* has been previously shown to play a role in tumor growth and progression. Additionally, it regulates angiogenesis in some of the cancer types, such as non-small cell lung cancer, renal cell cancer, and neuroglioma (Wang & Xiong, 2021). The gene overexpression observed in various cancers is linked to poor prognosis and decreased patient survival (Brown *et al.*, 2021; Príncipe *et al.*, 2021; Wang & Xiong, 2021). *LRP1B* is associated with the occurrence of osteosarcoma and its overexpression correlates with poor prognosis and decreased patient survival. Therefore the gene might be one relevant factor helping in OS diagnosis (Xu *et al.*, 2021).

A significant role in tumorigenesis and tumor progression is also played by the *CAMTA1* gene encoding a transcription factor – Calmodulin Binding Transcription Activator 1 (He *et al.*, 2021). The gene down-expression correlates with chemoresistance (Pan *et al.*, 2022). It has been shown that the expression of long noncoding RNA CAMTA1 (lncCAMTA1) in breast cancer affects cells viability and promotes their migratory state. Knock-down of lncCAMTA1 leads to cancer cell apoptosis (Lu *et al.*, 2018). *CAMTA1* mutations were observed in 5% of OS COSMIC samples. Even though there is a lack of information about the gene significance for osteosarcoma, still in Epithelioid hemangioendothelioma, that can arise from soft tissues and bones, CAMTA1 was proposed to be an immunohistochemical marker (Anderson & Jo, 2021).

Yet another gene shown to play a role in tumor development and its progression is *NCOR1* (Nuclear Receptor Corepressor 1) gene. COSMIC catalogue analysis showed that as many as 4% of OS samples showed *NCOR1* mutation. The gene encodes a corepressor involved in the regulation of gene expression (Tang *et al.*, 2020). *NCOR1* was observed to be frequently upregulated in breast cancer, and its high expression is associated with good prognosis and better patient survival (Noblejas-López *et al.*, 2018). Interestingly, in bladder cancer, *NCOR1* strongly correlated with immunogenicity, and its mutations were shown to cause activation of anti-tumor

immunity, as well as overexpression of immune-related genes (Lin *et al*., 2021). According to Luo *et al. NCOR* is widely amplificated in osteosarcoma, as it might participate in tumor growth as a regulator of other gene transcription (Luo *et al*., 2019).

Four percentage of OS samples of the COSMIC catalogue showed mutations of the *MLLT3* gene. MLLT3 protein is part of a super elongation complex (SEC), which is needed to increase the catalytic function of RNA polymerase II (Calvanese *et al*., 2019). The knockout of the *MLLT3* gene can lead to decreased proliferation of osteosarcoma cell lines, associated with the JNK signaling pathway. Thus, *MLLT3* can be an oncogene candidate involved in osteosarcomas development (Sun *et al*., 2017).

As much as 4% of samples deposited in the COSMIC catalogue were characterized by *PDE4DIP* gene mutations. Even though there is no information about the gene significance in osteosarcomas, still, PDE4DIP protein, also known as MMGL (myomegalin-like) protein is involved in several intracellular metabolic pathways, which may control OS biology (Soejima *et al*., 2001; Lehnart *et al*., 2005).

Three percentage of OS samples carried a mutation of *GNAS* (Guanine Nucleotide Binding Protein, Alpha Stimulating). The gene encodes a signaling molecule involved in many cellular processes involving cAMP signaling. It has been shown to play a role in tumorigenesis (Nomura *et al*., 2014; Zauber, Marotta & Sabbath-Solitare, 2016; Patra *et al*., 2018; Afolabi *et al*., 2022). *GNAS* mutations were observed in various cancers, such as colon, pancreas, and gastrin adenocarcinoma as well as in fibrous dysplasia, which is known to have the potential to form malignancies such as osteosarcoma (Sugiura *et al*., 2018).

Three percentage of samples deposited in the COSMIC catalogue manifested mutations of the *SMARCA4* gene. *SMARCA4* encodes the catalytic subunit of SWI/SNF complexes, which is associated with chromatin remodeling and making genes accessible for transcriptional factors. Unfortunately, there is a lack of information about the significance of gene alterations in osteosarcomas.

Also, the *ARID1A* gene product (AT-rich interactive domain-containing protein 1A) is a part of the SWI/SFI complex, associated with chromatin remodeling (Xu *et al*., 2019). *ARID1A* mutations were observed in many cancers before (Jones *et al*., 2010). Recent studies show that in osteosarcomas the expression of *ARID1A* is down-regulated compared to non-tumor tissues (Xu *et al*., 2019). This can be correlated with the gene mutations measured in 3% of OS samples of the COSMIC catalogue.

Yet another mutated gene affecting the structure of chromatin and contributing to tumorigenesis is *H3F3A* (Sturm *et al*., 2012; Park *et al*., 2016). *H3F3A* overexpression has been shown to correlate with aggressive phenotype and chemoresistance leading to lower survival rates and relapse in lung cancer patients (Park *et al*., 2016). COSMIC catalogue analysis showed that 2% of OS samples are mutated in *H3F3A*. This data finds approval in Koelsche *et al.* study showing hotspot mutations in *H3F3A* in six osteosarcoma cases analysed by the authors. Observed alternations were correlated with age and increased the incidence of osteosarcoma in patients over the age of 30 (Koelsche *et al*., 2017).

Two percentage of the samples of the COSMIC catalogue were carrying mutations in the *BCOR* gene. BCOR protein is involved in the process of deacetylation of histones and when over-expressed inhibits BCL-6 tumor suppressor (Huynh *et al*., 2000). Mutations in *BCOR* are associated with many types of cancer, such as clear cell sarcoma of the kidney, endometrial stromal sarcoma, or small round blue cell sarcomas (Astolfi *et al*., 2019; Ueno-Yokohata *et al*., 2015; Marinõ-Enriquez *et al*., 2018; Sbaraglia *et al*., 2020).

Among samples deposited in the COSMIC catalogue, approximately 2% carry mutations in the *MED12* gene. MED12 is part of a larger, multiprotein complex that mediates polymerase II RNA during the process of transcription (Ding *et al*., 2008). *MED12* is associated with 70% of uterine leiomyomas and fibroepithelial tumors of the breast (Croce & Chibon, 2015; Lerwill *et al*., 2022). As for now, only two clinical case reports described *MED12* mutations in osteosarcomas. Le and others (Le *et al*., 2021) showed that the patient with *MED12*-mutated high-grade uterine sarcoma developed osteosarcoma with the same L36R missense mutation. Another team described *MED12* mutation in osteosarcoma which further led to the development of breast malignant phyllodes tumor (Tokoyoda *et al*., 2018). More study is needed to assess the link between *MED12* mutations and the occurrence of osteosarcoma, especially since according to The Cancer Genome Atlas (TCGA) data, mutations in *MED12* can be biomarkers helping to select patients for the therapy with immune checkpoint inhibitors (ICIs) (Zhou *et al*., 2022).

Two percentage of OS samples from the COSMIC catalogue displayed changes in the *EGFR* gene encoding receptor for the epidermal growth factor (EGFR). The receptor is known for its crucial role in the signaling pathway responsible for cell proliferation, differentiation, and/or survival (Wang *et al*., 2004). Mutations of *EGFR* in a variety of malignancies are well documented. Most frequent is the gene's amplification leading to EGFR overexpression increasing cellular signaling (Sigismund, Avanzato & Lanzetti, 2018; Cheng *et al*., 2021). The gene importance for OS has been already documented. Sheng-Lin and others (Sheng-Lin *et. al.,* ????) reported that high expression of EGFR together with HER-4 (human epidermal growth factor receptor 4) was associated with distant metastasis and the level of *EGFR* expression was proposed to be a potential prognostic biomarker of OS (Wang *et al*., 2018).

Among OS samples deposited in the COSMIC catalogue, 2% manifested mutations of the *SMAD2* gene. This low number of samples can indicate that the gene mutations are not osteosarcoma specific. However, is worth mentioning that SMAD2, as a part of the TGF-beta superfamily, plays an important role in cell signaling, proliferation, and differentiation (Piek *et al*., 1999) and may act as an oncosuppressor (Piek *et al*., 1999; Pasche, 2001). Moreover, it has been shown that there are statistically significant changes in *SMAD2* expression in OS compared to healthy tissue. The correlation between alterations of Smad signaling, the high proliferation rate, and the invasive phenotype of osteosarcoma cells was observed in OS with Serine/threonine kinase 39 (STK39) knockdown (Won *et al*., 2010; Yang *et al*., 2013; Huang *et al*., 2017).

Another gene selected during our study was the *NF1* gene. According to the data from the COSMIC catalogue 2% of OS samples were carrying the gene's mutations. The product of *NF1* is neurofibromin 1 – a cytoplasmic protein expressed in neurons, Schwann cells, and leukocytes. The protein is a part of the RAS/MAPK signaling pathway controlling cell proliferation (Huang *et al*., 2017). It is suggested that NF1 can act also as an oncos-

uppressor. Although the *NF1* gene is associated mostly with neural malignancies, its mutations are observed in many cancers, e.g. lung cancers or melanomas (Trovó-Marqui & Tajara, 2006). Further studies are needed to prove *NF1* role in OS development.

An additional gene involved in the inhibition of cell proliferation is the tumor-suppressor gene *TSC2* (Inoki *et al.*, 2002). The gene's mutations were described in tuberous sclerosis complex (TSC) and non-small cell lung cancer. It was proved that patients with TSC1/TSC2 loss can benefit from target therapy including treatment with immune checkpoint blockade (ICB) (Huang *et al.*, 2022). Kuroda *et al.* reported a clinical case of a young patient with tuberous sclerosis complex (TSC) who developed osteosarcoma. Analysis of the tumor sample showed additional mutations in *TP53* as well as *TSC2* genes (Kuroda *et al.*, 2021). This observation is in agreement with COSMIC catalogue data showing that approximately 2% of OS samples were characterized by mutated *TSC2*.

Two percentage of OS samples were also characterized by mutated proto-oncogene *BRAF*. BRAF activates signaling pathways involved in the cell cycle, including proliferation, differentiation, and cell death. The gene mutations may result in malignancies development. In fact, dysregulation of gene expression was observed in several cancers, including melanoma, colorectal cancer, and non-small cell lung cancer (Śmiech *et al.*, 2020). Pignochino *et al.* reported that in 4 of 30 osteosarcoma patients analysed in their study, *BRAF* gene mutation was confirmed (Pignochino *et al.*, 2009).

CONCLUSIONS

COSMIC catalogue analysis allows to identify genes that are the most frequently mutated in osteosarcoma. Since 20 of the selected genes have previously been shown to be involved in cancerogenesis, thus their mutation might also play a role in OS development. However, to prove their clinical significance and the possibility of using them as OS biomarkers further studies are needed. The results of such a study would help to identify patient predisposition to OS development, follow cancer progression, and establish patients' treatment and prognosis.

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