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Review

Singe nucleotide polymorphisms in osteosarcoma: Pathogenic effect and prognostic significance

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ABSTRACT

Purpose: Osteosarcoma (OS) is a common malignant bone tumor in children and adolescents. Pathogenesis and prognosis of OS can be associated with several environmental and genetic factors. Single nucleotide polymorphisms (SNPs) are crucial genetic changes that can be involved in clinical and therapeutic outcomes of OS. The aim of this review is to present a synopsis of the role of SNPs in pathogenesis and prognosis of OS tumor cells as well as their potential as therapeutic targets to improve the outcomes of patients.

Method: The content used in this paper has been obtained by an electronic databases search of English language (1998–2018) articles using the terms "Single nucleotide polymorphisms", "Osteosarcoma", "Pathogenesis", "Prognosis", and "Clinical Outcomes".

Discussion: SNPs can affect a number of biological processes such as proliferation, apoptosis, adhesion, invasion, and drug resistance of OS tumor cells, playing a key role in pathogenesis, prognosis, and clinical outcomes after chemotherapy in this disease.

Conclusion: Considering the importance of SNPs in OS pathophysiology, these genetic changes may be used as potential pathogenic and prognostic biomarkers for OS. It is hoped that targeting these changes using new therapeutic approaches leads to the effective treatment of this debilitating tumor. However, better understanding of OS biology and further clinical trials are needed to achieve this goal.

1. Introduction

Osteosarcoma (OS), the most common malignant bone tumor among young children and adolescents, is derived from bone marrow (BM) mesenchymal cells. This malignancy is characterized by the rapid growth of bones, especially long bones (Geller and Gorlick, 2010). OS is divided to a number of subtypes based on histological criteria, including chondroblastic, telangiectatic, fibroblastic, small cell, giant cell rich, and sclerotic/osteoblastic (Hauben et al., 2003). Patients with OS show several specific clinical symptoms such as severe bone pain and swelling in the affected area and are at risk of fractures following blows and heavy exercises (Marina et al., 2004; Geller and Gorlick, 2010). Because of rapid progression and metastasis capacity to other bones and even soft tissues (e.g. lung), OS is associated with a short survival and poor prognosis (Marko et al., 2016). Studies show that 25% of OS patients enter the metastasis phase and that the lung is the most common site for metastasis (Marko et al., 2016). The precise pathogenesis of OS is unknown, although rapid BM growth has been recognized as the main cause of this malignancy. In this respect, recent studies have identified several factors such as age, gender, race, environment, and the genetic context of patients as risk factors of OS (Paioli et al., 2017; Mirabello et al., 2011a; Longhi et al., 2005), and the genetic context has recently been taken into account. Singe nucleotide polymorphisms (SNPs) are a common genetic variation (usually 1% or more) and can cause rare phonotypes when occurring in coding regions of genes in comparison to mutations, which are defined as any change in a DNA sequence due to errors during DNA replication and their frequency is lower than SNPs (Kim and Misra, 2007). SNPs can cause genetic diversity and predispose to OS by influencing the essential processes in cell growth such as proliferation, differentiation, and apoptosis in normal cells, which leads to the development of several diseases or changes in response to therapy or environmental toxins (Windsor et al., 2012; Tost and Gut, 2005). In addition, these genetic changes can affect the genes involved in immune and inflammatory responses of the body, especially in enzyme activity and secretion of biological mediators such as

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interleukins (ILs), growth factors, and tumor suppressors and thus play a role in OS pathogenesis and prognosis by deviating these processes (Xiao et al., 2014; Oliveira et al., 2007).

The standard OS treatment includes pre-surgery chemotherapy, primary tumor removal, and secondary chemotherapy following surgery (Chou et al., 2008). Methotrexate (MTX), cisplatin, cyclophosphamide, vincristine, and doxorubicin are the main chemotherapy drugs for this malignancy (Goorin et al., 2003). The results of various studies in recent years suggest that a combination of chemotherapy and surgery can result in an overall survival of five years in 60–70% of patients (Bielack et al., 2002). Despite the application of these potent therapeutic protocols, nearly 40% of patients do not respond well to treatment and enter into the relapse phase (Goorin et al., 2003; Khanna et al., 2014). Although the exact mechanism of response to therapy is complicated in this disease, investigations have identified various environmental and genetic causes (including SNPs) affecting carriers and enzymes of drugs metabolism pathway as factors limiting the response to treatment (Caronia et al., 2011; Chou and Gorlick, 2006; Kempf-Bielack et al., 2005).

Despite the fact that several environmental and genetic factors, including exposure to ionizing radiation and carcinogens, mutations, and SNPs, have been introduced as risk factors of susceptibility to OS, the exact pathogenesis of this disease is still unknown (Gianferante et al., 2017). Recent developments in the field of molecular biology as well as discovery of the relationship between SNPs in various genes with OS pathogenesis have highlighted the key role of this genetic change in the onset and progression of this malignancy. For this reason, with the selection of studies based on SNPs of the most common biological mediators in OS (Fig. 1), we discuss their possible mechanisms in the development, progression, and prognosis of OS.

2. Polymorphisms and clinicopathological characteristics of OS

2.1. Cytokines

Cytokines are biological agents secreted in response to a wide range of cellular changes such as stress, inflammation, and damage from malignant tumors (Goldszmid and Trinchieri, 2012). Several studies have investigated the association between G/A polymorphism in −308 and −238 loci of TNF-α gene, as an inflammatory cytokine, with OS risk (Oliveira et al., 2007; Jiang et al., 2017), the results of which indicate the association between TNF-α polymorphism with OS risk as a controversial issue. Zhoa et al. investigated the relationship between TNF-α polymorphisms with OS risk and found that there is no significant relationship between the presence of −238 G/A polymorphism and the occurrence of OS (Zhao et al., 2015). In contrast, results of this study have shown that TNF-α 308 polymorphism plays a key role in the development and progression of OS in Asian populations (Zhao et al., 2015). However, Oliveria et al. and Patio-Garcia et al. noted that there was no association between this type of TNF-α polymorphism and OS pathogenesis in Brazilian and Caucasian patients (Oliveira et al., 2007; Patino-Garcia et al., 2000). Interestingly, Bian et al. in their meta-analysis study reported no association between TNF-α 308 polymorphism and OS susceptibility in both Asian and Caucasian populations (Bian et al., 2015). These controversial results show that the influence of TNF-α 308 polymorphism on OS susceptibility is unclear and may reflect that SNPs have a difference impact in different populations, so that further studies are required to provide sufficient evidence on the impact of TNF-α 308 SNP in different worldwide populations. Similar to TNF-α 308 SNP, there are controversial results on the effect of +252 A > G polymorphism in TNF-β on OS susceptibility. While some studies reported that this polymorphism is associated with increased OS occurrence and reduced overall survival in Asian patients (Oliveira et al., 2007), inconsistent results have been reported on Asian and Caucasian populations (Jiang et al., 2017; Xie et al., 2008). TNF-β plays vital roles in bone tissues and stimulates the growth of mesenchymal cells (such as osteoblasts), and high levels of it is involved in OS metastasis (Tu et al., 2014). It seems that +252 A > G polymorphism can be a poor prognostic factor in OS; however, the precise effect of this polymorphism on OS susceptibility in Asian population is unclear. IL-16 is another inflammatory cytokine that plays a recognized role in inflammatory and

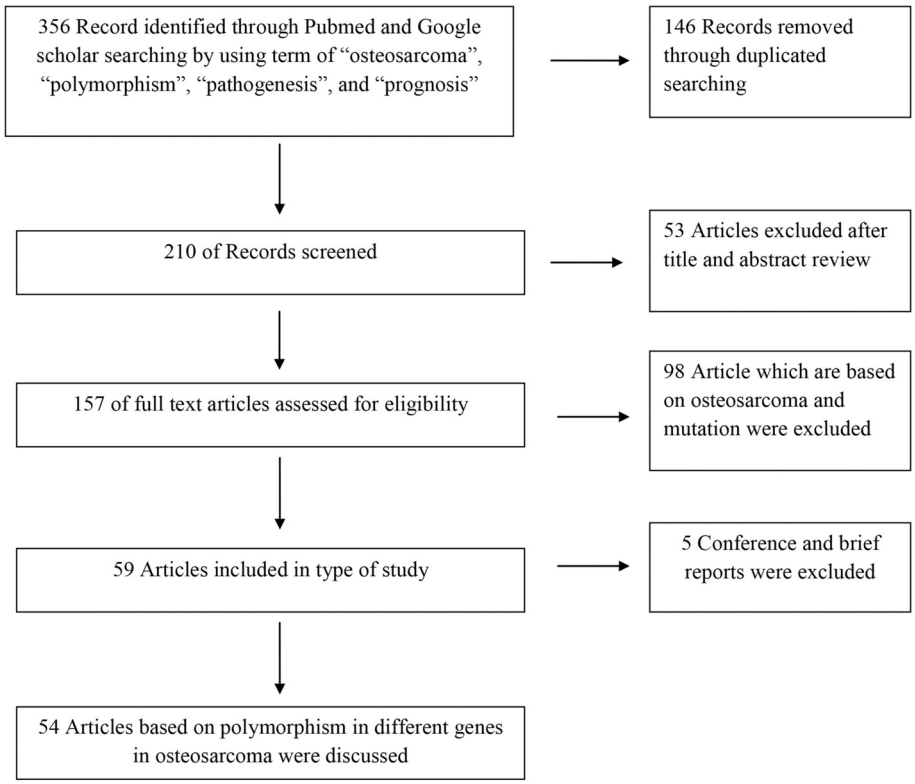

Fig. 1. Flow chart of study selection and inclusion process.

tumorigenesis processes. It has been shown that IL-16 TG genotype caused by an SNP in the gene of this cytokine has a direct correlation with increased IL-16 plasma levels as well as OS onset (Tang et al., 2016). On the other hand, increased secretion of IL-16 can increase the release of some other inflammatory cytokines such as TNF-α and IL-1β (Mathy et al., 2000). Since the increase in TNF-α is associated with OS metastasis (Jiang et al., 2017), the polymorphisms associated with elevated levels of IL-16 may indirectly exacerbate OS metastasis. Unlike IL-16 and TNF-α polymorphisms, the expression of a number of polymorphisms in IL-1β gene is reduced in OS patients, and it has been shown that the reduction of these polymorphisms is not associated with the occurrence of OS but is related with a good prognosis in patients (He et al., 2014b). Interleukin 1 receptor antagonist (IL-1Ra) is an inhibitor of IL-1 function, which competes with it through binding to IL-1 receptor. In addition to the inhibitory effect of IL-1Ra on IL-1, it has been shown that this antagonist can reduce the release of IL-1, IL-6, and IL-8 from human U-2 OS cell line (Hönicke et al., 2012). The main function of IL-8 is chemotaxis of neutrophils in immune and inflammatory conditions. Chen et al. in their recent study showed that −252 T/A and +781C/T polymorphisms could affect the expression of IL-8 gene. In addition, these researchers demonstrated that both the polymorphisms were associated with an increased incidence of OS, with the difference that the risk of metastasis and disease progression was higher in patients with −252 T/A polymorphism (Xie et al., 2008). Also, they showed that OS patients with a high level of IL-8 expression had larger tumors than those with low expression levels of this cytokine (Chen et al., 2016). Under such circumstances, tumor size may not indicate the progression of tumor mass growth, but increased IL-8 secretion from −252 T/A and +781 C/T polymorphisms is likely to cause chemotaxis of neutrophils and immunological mediators at tumor site and thereby increase the tumor size. Therefore, proving this hypothesis can lead to new perspectives on the role of these polymorphisms in the process of OS development. IL-6 is among the inflammatory cytokines produced in many inflammatory conditions and infections, which has anti-apoptotic properties (Naugler and Karin, 2008). Moreover, this cytokine plays a significant role in the progression of many solid tumors, including myeloma, prostate cancer, and ovarian cancer (Jourdan et al., 2009). Xiao et al. in their study stated that IL-6 levels were increased in OS patients, especially in the osteoblastic subtype (Xiao et al., 2014). In addition, patients with IL-6 -174 G/C SNP have a higher risk of progression towards metastatic OS (Qi et al., 2016). Moreover, it has been shown that −174 G/C genotype correlates with a poorer survival compared to homozygote GG genotype (Qi et al., 2016). Although −174 G/C genotype is associated with poor clinicopathological characteristics in OS, there is no report on the correlation between −572 G/C genotypes with pathological fracture, tumor location, and OS metastasis (Qi et al., 2016). These findings show that IL-6 -174 G/C promoter polymorphism can be associated with susceptibility to and progression of OS. On the other hand, it has been shown that this cytokine can indirectly contribute to the increase in mobilization, proliferation, and metastasis of tumor cells in OS by inducing the expression of signaling mediators, adhesion molecules, and vascular endothelial growth factor (VEGF) (Fong and Tang, 2013; Tu et al., 2012; Tzeng et al., 2013). VEGF is one of the most important angiogenesis regulators, which is a necessary process for the progression and metastasis of tumor cells (Ferrara et al., 2003). Similar to IL-8, the expression of this factor can be affected by several SNPs in many cancers (Lee et al., 2005b; Yapijakis et al., 2007). For example, +936C > T polymorphism is associated with an increase in the expression of this factor among OS patients and is closely related to the increased risk of OS development (Wang et al., 2014b). Since a high level of VEGF expression is associated with increased microvessel density in tumor tissues and advanced disease stages (Iordache et al., 2010) and given its importance for tumor cell metastasis, +936C > T polymorphism of this factor is likely to enhance metastasis and be associated with a worse prognosis in patients who carry this type of polymorphism. Also, Tie et al. in a study on the involvement of other VEGF SNPs in OS pathogenesis demonstrated that the AA-genotype of −2578C/A and GG genotype of −634 G/C polymorphisms are respectively associated with an increased risk of OS occurrence in men and women at an early age with a family history of cancer (Tie et al., 2014). Although previous studies reported that VEGF polymorphisms have an important role in OS progression, no clear relationship has been reported between VEGF SNPs and clinical situation or response therapy in OS patients.

IL-12 is a cytokine with a special role in the suppression of growth and regulation of processes such as angiogenesis and metastasis in tumor cells via activation of macrophages (Trinchieri et al., 2003). This cytokine can also increase anti-tumor activity by recruiting cytotoxic lymphocytes to the tumor site. Wang et al. in a case–control study showed that IL-12 rs3212227 polymorphism is associated with a reduction in the level of this cytokine as well as an increased risk of OS development (Wang et al., 2013). It seems that this polymorphism provides the conditions for tumor escape from defensive mechanisms activated by IL-12 via targeting the frequency and function of IL-12. However, no association has been reported between these SNPs and patient's gender, tumor location, metastasis, and response to therapy (Wang et al., 2013). Regarding the involvement of polymorphic cytokines mediating immune phenomena, inflammation, and angiogenesis, SNPs appear to be involved in OS pathogenesis and clinicopathological outcomes by affecting the release rate and function of cytokines. In fact, these findings suggest that cytokines can be diagnostic and therapeutic targets for OS. Although several case-control and meta-analysis studies have stressed on the role of polymorphic variants of cytokines in OS onset and progression, there is no decisive evidence about pathogenic importance of cytokine SNPs and interaction of these genetic alterations with each other or environment-based risk factors in response to therapy in patients.

2.2. Growth factors

Since OS is the most common bone malignancy in adolescents and young adults, evidence shows that OS is most likely to occur among adolescents during their growth age (Mirabello et al., 2009). Although OS is seen at a younger age in women than men due to earlier puberty, the incidence risk of this disease is higher in men, especially tall men (Savage and Mirabello, 2011). In addition, studies show that the risk of developing OS is higher in individuals with a higher birth weight (Mirabello et al., 2011a). It seems that rapid growth, both during fetal development and during puberty, is a risk factor for increasing incidence of OS. In other words, any environmental or genetic factor resulting in rapid bone growth can be associated with an increased risk of OS development. Meanwhile, the role of growth hormone (GH) and other growth factors is significant in this regard, which have an essential role in the growth and maturity of individuals. Although the important effects of these factors on the development and maturity of individuals have been confirmed, genetic variation in these genes seems to be a risk factor for OS susceptibility. Since bone growth and puberty are two related processes, genetic changes that increase the expression of GH and growth factors can be associated with an increased OS risk. GH is a main hormone for bone growth during puberty as well as being an important factor in increasing height in this age (Kronenberg, 2003). In this regard, Mirabello et al. in a case-control study showed that individuals harboring rs11079515 and rs7921 SNPs in downstream of the gene of this hormone had a higher risk of OS development (Mirabello et al., 2011c). In addition, in this study, a number of SNPs in growth factors and their receptors such as fibroblast growth factor-2 (FGF2), fibroblast growth factor-3 receptor (FGFR3), insulin-like growth factor-1 (IGF-1), and gonadotropin releasing hormone 2 (GNRH2) were introduced as a risk factor for increasing OS susceptibility (Table 1) (Mirabello et al., 2011c). IGF-1, which is secreted by liver due to GH stimulation, is a factor for increasing cell growth and metabolism as well as being a biomarker to measure GH in human body (Brick et al.,

Table 1
Summary of genetic polymorphisms involved in incidence and clinicopathological characteristics of OS.

Genes	Chro.	Function	Location and nucleotide change	Rs number	Method	Outcome	Ref.
Cytokines							
TNF-α	6p21.33	Involved in the regulation of a wide spectrum of biological processes	308 G > A	1,800,629	PCR-RFLP	Associated with susceptibility to metastatic OS	(Oliveira et al., 2007; Jiang et al., 2017; Zhao et al., 2015)
			−238 A/G	361,525	PCR-RFLP	No effect on OS susceptibility	(Zhao et al., 2015)
TNF-β	6p21.33	Involved in proinflammatory processes	+252 A > G	909,253	PCR	Tumor progression and a poor event-free survival	(Jiang et al., 2017)
IL-10	1q32.1	Has effects in immunoregulation and inflammation, enhancing B-cell survival and antibody production	−1082 A/G	1,800,896	PCR	Associated with advanced tumor stages, metastasis, and poor survival	(Jiang et al., 2017; Cui et al., 2016)
			−819C/T	1,800,871	PCR	Associated with susceptibility to OS	(Cui et al., 2016)
			−592 A/C	1,800,872			
IL-6	7p15.3	Functions in inflammation and the maturation of B-cells	174 G/C	1,800,795	PCR-RFLP	May be associated with increased mobility, proliferation, and metastasis of tumor cells by inducing ICAM-1, STAT3 and VEGF production	(Jiang et al., 2017; Qi et al., 2016)
VEGF	6p21.3	Induces proliferation and migration of vascular endothelial cells and is essential for angiogenesis	−2578C/A	699,947	PCR-RFLP	Increased risk of OS in males with shorter age and family history of cancer	(Tie et al., 2014; Zhang et al., 2015a)
			−634 G/C	2,010,963		Increased risk of OS in females with shorter age and family history of cancer	(Tie et al., 2014)
			+936C > T	3,025,039	PCR-RFLP	Increased risk of OS and may be associated with metastasis	(Wang et al., 2014b)
IL-8	4q12.13	Neutrophils chemotactic	−251 A/T	4073	PCR-RFLP	Associated with development and incidence of metastasis	(Chen et al., 2016)
IL-1β	2q14.1	Is an important mediator of the inflammatory response and a mediator for cellular processes	−511 TT	16,944	PCR-RFLP	Not associated with OS risk and incidence of metastasis	(He et al., 2014b)
			−31 T/C	1,143,627			
			+3954C/T	1,143,634			
IL-16	15q25.1	A modulator of T-cell activation	TG/GG	11,556,218	PCR-RFLP	Can be associated with increased metastasis by inducing TNF-α and IL-1β	(Tang et al., 2016)
IL-12	10q21	Stimulating the production of IFN-γ and TNF-α inflammatory responses	CC/AC	3,212,227	PCR-RFLP	Can be associated with OS risk via reduced IL-12 level	(Wang et al., 2013)
Growth factors							
GH	17q23.3	Plays an important role in growth control	G allele	11,079,515	PCR	Associated with OS after Bonferroni correction	(Mirabello et al., 2011c)
			A allele	7921			
IGF1	12q23.2	Involved in mediating growth and development	IVS2 + 10,605	7,956,547	PCR	Associated with a decreased risk of OS	(Mirabello et al., 2011c)
IGF2R	6q25.3	A receptor for IGF2 and has various functions, including the activation of TGF-β and the degradation of IGF2	Ex16 + 88G > A	998,075	PCR	Associated with increased of OS risk	(Savage et al., 2007b)
			IVS16 + 15C > T	998,074			
			Intronic	1,003,737			
FGF2	4q28.1	Involved in diverse biological processes, such as limb and nervous system development, wound healing, and tumor growth	C/T	11,737,764	RT-PCR	Can be associated with OS risk via rapid bone growth	(Wang et al., 2015)
FGFR3	4p16.3	This receptor binds acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance	A allele	6,599,400	PCR	Associated with increased OS susceptibility	(Mirabello et al., 2011c)
IGFALS	16p13.3	Binds insulin-like growth factors and increasing their half-life and their vascular localization	2,575,352	upstream	PCR	May be associated with OS risk in children via promoting bone growth	(Mirabello et al., 2011c; Musselman et al., 2012)
TGF-β1	19q13	It is involved in immune cells activation, regulation of inflammatory responses, and differentiation of HSCs	^{29}T/C	1,982,073	AS-PCR	Associated with reducing the expression TGF-β gene and increased risk of osteosarcoma	(Jiang et al., 2017; Bian et al., 2015)
			T/C	1,800,470	PCR-RFLP	Associated with an increased level of TGF-β and metastatic OS	(Xu et al., 2014; Kaneda et al., 2004)

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Table 1 (*continued*)

Genes	Chro.	Function	Location and nucleotide change	Rs number	Method	Outcome	Ref.
GNRH2	20p13	Regulates reproductive function by stimulating the production and release of the FSH and LH	C allele	3,761,243	PCR	May contribute to OS pathogenesis because of its function in puberty	(Mirabello et al., 2011b; Bierie and Moses, 2010)
Enzymes							
LOX	5q23.1	Functions in crosslinking of collagen and elastin	−22 G/C	2,956,540	PCR-RFLP	Can be associated with increased incidence of OS	(Jia et al., 2013; Liu et al., 2012)
			437 G/A	1,800,449		Associated with decreased activity of LOX and a higher incidence of metastasis	(Liu et al., 2012)
XRCC3	14q32.33	Protects the DNA from different DNA-damaging agents	TT CC	861,539	PCR-RFLP	Elevated risk of OS via decreased DNA repair damages induced by X-ray	(Yang et al., 2015; Guo et al., 2015)
APE1	14q11.2	Participates in cellular processes such as the response to oxidative stress, cell cycle control, and apoptosis	TG and GG allele	1,760,944	PCR	Lower risk of developing OS and metastasis	(Xiao et al., 2017)
PON1	7q21.22	Binds to HDL particles and hydrolyzes thiolactones and xenobiotics	192 Q allele	662	PCR–RFLP	Associated with the formation of low activity alloenzyme and increased risk of OS development	(Egeblad and Werb, 2002)
MMP-3	11q22.2	Degrades fibronectin, laminin, collagens III, IV, IX, X, and cartilage proteoglycans	E45K AG genotype	679,620	PCR-RFLP	May be associated with OS development via enhanced osteoblast differentiation	(Adiguzel et al., 2016)
MMP-9	20q13.12	Degrades type IV and V collagens	AA and CC	1,056,629	RT-PCR	Associated with increased interaction between MMP-9 and miR-491 that leads to decreased risk of metastasis of OS cells	(Tian and Zhang, 2016)
GSTM1	1p13.3	Detoxification of carcinogenic compounds by conjugation with glutathione	Null genotype	Not mentioned	PCR-RFLP	Associated with lung relapse and poor survival	(Salinas-Souza et al., 2010)
			Null genotype	Not mentioned	PCR-CTPP	Associated with increased risk of OS in young males than females	(Lu et al., 2011)
			A/unknown B/unknown	Not mentioned	PCR	An increased risk of acute OS	(Barnette et al., 2004)
GSTM3	1p13.3	Detoxification of carcinogenic compounds by conjugation with glutathione	AA	1,332,018	PCR	Associated with a low overall survival of metastatic patients	(Barnette et al., 2004)
			A/B B/B	1,799,735	PCR-RFLP	An increased risk of acute OS	(Barnette et al., 2004)
GSTT1	22q11	Catalyzes the conjugation of reduced glutathione to a variety of electrophilic and hydrophobic compounds	Null genotype	Not mentioned	PCR-RFLP	Related to a better survival	(Salinas-Souza et al., 2010)
			Wild genotypes	Not mentioned	PCR-RFLP	Associated with a worse survival in metastatic patients	(Salinas-Souza et al., 2010)
			Null genotype	Not mentioned	PCR-CTPP	Associated with increased risk of OS in young patients	(Lu et al., 2011)
GSTP1	11q13.2	Function in xenobiotic metabolism and play a role in susceptibility to cancers	Ile105Val	Not mentioned	PCR-CTPP	Associated with higher risk of death	(Zhang et al., 2012)
			A/A A/B B/B	Not mentioned	PCR	An increased risk of acute OS	(Barnette et al., 2004)
TERF	8q21.11	Functions as an inhibitor of telomerase, acting in cis to limit the elongation of individual chromosome ends	Upstream T allele	2,929,593	Q-PCR	These polymorphisms may be associated with short telomeres length and increased risk of osteosarcoma especially in females	(Mirabello et al., 2011b)
			Upstream T allele	9,298,211			
			IVS1–718 G allele	2,929,586			
			IVS2 + 640 G allele	2,929,585			
			IVS8–124 G allele	2,306,494			
			IVS9 + 448 A allele	2,306,492			
			Downstream A allele	7,001,277			
RECQL5	17q25.1	Prevents aberrant homologous recombination formation in the genome	CC	820,196	TaqMan method	Associated with increased risk for osteosarcoma	(Zhi et al., 2014)
			AA	4,789,223			
Tumor suppressors							
TP53	17p13.1	Responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, DNA repair, or changes in metabolism	Arg72 Pro	1,042,522	PCR	Associated with OS risk and have a prognostic value for overall survival	(Savage et al., 2007a; Toffoli et al., 2009)
			C > G	1,642,785		May be associated with OS risk via decreased tumor suppressor function of TP53	(Toffoli et al., 2009; Savage et al., 2007a)

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Table 1 (*continued*)

Genes	Chro.	Function	Location and nucleotide change	Rs number	Method	Outcome	Ref.
MDM2	12q15	Can promote tumor formation by targeting tumor suppressor proteins, such as p53, for proteasomal degradation	T309G	2,279,744	PCR	Increased risk of OS in females and can be associated with lung metastasis and death	(Toffoli et al., 2009; Wang et al., 2014a)
			AA	1,690,916		Associated with a decreased risk of OS	(Wang et al., 2014a)
CD markers							
CD152	2q33.2	Transmits an inhibitory signal to T cells	49 A/G	231,775	PCR-RFLP	May be associated with OS risk via suppressing T cell response against tumor cells	(Xiao et al., 2014; Bian et al., 2015; Liu J et al. 2013)
			75 G > C	Not mentioned	CRS-PCR	Related to OS risk	(He et al., 2014a)
			326 G > A	Not mentioned			
			−318C > T	5,742,909	–Not mentioned	Maybe positively associated with the development of OS	(Chang et al., 2014)
CD86	3q13.33	Transmits costimulatory signal for activation of the T-cell	+1057G/A	1,129,055	PCR-RFLP	Associated with increased susceptibility to osteosarcoma via decreased T lymphocytes stimulation	(Wang et al., 2011)
CD49C	17q21.33	Joins with a beta 1 subunit to form an integrin that interacts with extracellular matrix proteins	AA	2,230,392	TaqMan-PCR	Increased risk of OS metastasis and reduced survival rate	(Yang et al., 2014b)
CD95	10q23.31	Plays a central role in the physiological regulation of programmed cell death	18,272 A > G	2,229,521	PCR-RFLP	Maybe associated with increased susceptibility to OS by decreased tumor cells apoptosis	(Koshkina et al., 2007b)
Signaling pathways molecules							
PIK3A		Functions as a secondary messenger in cell growth	CC	7,646,409	TaqMan-PCR	Higher risk of suffering Enneking's stage II B of OS in males than females	(He et al., 2013)
AKT	14q32.33	Activate through PI3K and is a critical mediator of growth factor-induced neuronal survival	AA	6,973,569	TaqMan-PCR	Higher risk of chondroblastic and metastatic OS	(He et al., 2013)

Abbreviations: Chro: chromosome; TNF-α: tumor necrosis factor alpha; TNF-β: tumor necrosis factor beta; IL-10: interleukin 10; IL-6: interleukin 6; ICAM-1: intercellular adhesion molecule 1; STAT3: signal transducer and activator of transcription 3; VEGF: vascular endothelial growth factor; IL-8: interleukin 8; IL-1β: interleukin 1 beta; IL-16: interleukin 16; IL-12: interleukin 12; IFN-γ: interferon gamma; GH: growth hormone; IGF1: insulin like growth factor 1; IGF2R: insulin like growth factor 2 receptor; IGF2: insulin like growth factor 2; TGF-β: transforming growth factor beta; FGF2: fibroblast growth factor 2; FGFR3: fibroblast growth factor receptor 3; IGFALS: insulin like growth factor binding protein acid labile subunit; HSCs: hematopoietic stem cells; GNRH2: gonadotropin releasing hormone 2; FSH: follicle-stimulating hormone; LH: luteinizing hormone; LOX: lysyl oxidase; XRCC3: X-ray repair cross complementing 3; APE1: aporinic/apyrimidinic endonuclease 1; PON1: paraoxonase 1; HDL: high density lipoprotein; MMP-3: matrix metallopeptidase 3; MMP-9: matrix metallopeptidase 9; miR-491: microRNA-491; GSTM1: glutathione S-transferase mu 1; GSTM3: glutathione S-transferase mu 3; GSTT1: glutathione S-transferase theta 1; glutathione S-transferase pi 1; TERF: telomeric repeat binding factor 1; RECQL5: RecQ like helicase 5; TP53: tumor protein p53; MDM2: MDM2 proto-oncogene; PIK3A: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; OS: osteosarcoma; PCR: polymerase chain reaction; PCR-RFLP: polymerase chain reaction -restriction fragment length polymorphism; RT-PCR: real-time polymerase chain reaction; Q-PCR: quantitative polymerase chain reaction; CRS-PCR: created restriction site- polymerase chain reaction.

2010). GH/IGF signaling axis is an important pathway for the regulation of bone tissue growth and development. Due to the association between OS and rapid bone growth, it has been shown that the biological changes in this signaling pathway can be related to the incidence of dormancy and gross metastasis in adults and children with OS (PosthumaDeBoer et al., 2011). Dormancy is a pathologic condition with an extended survival period of single cells or small gross micrometastases, which has a critical role in drug resistance in metastatic OS (Hauben et al., 2006). Interestingly, rs7956547 IGF-1 polymorphism is associated with a reduced risk of OS (Mirabello et al., 2011c), while two SNPs (i.e. Ex16 + 88G > and IVS16 + 15C > T) in insulin-like growth factor 2 receptor (IGF2R) gene have been recognized as risk factors for increasing the likelihood of OS (Savage et al., 2007b). These polymorphisms appear to be able to affect the GH/IGF signaling pathway as a positive feedback, inducing OS by increasing the ectopic growth and development of bones. On the other hand, GH is a strong stimulator for IGF-1, which is an essential factor in the growth of tumor cells in OS (Naranjo et al., 2002). In validation of this finding, studies in animal models have shown that increasing IGF-1 level is associated with the progression of OS and that the reduced level of this factor is related with the inhibition of tumor cells growth and progression of disease (Rankin et al., 2012). Given that GH and IGF-1 can interact with each other, and more importantly, because the peak of GH production occurs during puberty, it seems that any SNP or genetic alteration increasing the expression of these factors can be associated with a higher risk of OS. However, confirmation of this hypothesis requires further studies.

Transforming growth factor-beta (TGF-β) is another growth factor that mediates the regulation of several genes and enzymes by binding to its receptor of TGF-βR (Bierie and Moses, 2010). However, the accurate function of TGF-β in tumors is unclear, and it can be both a suppressor and enhancer of tumor growth (Elliott and Blobe, 2005). It has been shown that some patients with OS, especially those with metastasis, have a type of polymorphism in their TGF-β gene, which increases TGF-β levels in their plasma and predisposes to OS development (Table 1) (Bian et al., 2015; Xu et al., 2014). Although various studies have suggested these polymorphisms as predisposing factors for the onset of OS, the precise mechanism of these polymorphisms on OS pathogenesis is not clear. Our assumption here is that these polymorphisms are likely to affect the function of TGF-β, thereby enhancing the tumor mass growth. In support of this hypothesis, the results of Ma et al. study can be mentioned, in which TT genotype caused by an SNP in exon 29 of TGF-β gene was shown to be associated with a decrease in the level of this factor and an increase in the risk of OS (Ma and Zhou, 2010). In fact, TGF-β function, not necessarily its level, seems to be the factor affecting these polymorphisms. It has also been shown that impaired TGF-β signaling leads to unresponsiveness of tumor cells to inhibitory function of TGF-β (Massagué et al., 2000). On the other hand, the expression of TGFβR1*6A, which is a variant of TGF-βR, is associated with an increased risk of OS and metastasis in patients (Hu et al., 2010). In these situations, TGFβR1*6A may be an unresponsive variant to inhibitory function of TGF-β, and tumor cells may have acquired this feature as a factor for their survival.

According to these statements, the vital role of growth factor SNPs is due to the relationship between OS and bone growth. In fact, it appears that the occurrence of growth factor SNPs in children and adolescents in puberty age is associated with a higher risk of OS than adults.

2.3. Enzymes

Bone microenvironment is composed of several types of cells and minerals, and the space between them is filled with bone matrix. Type I collagen is a main component of bone matrix maintaining its shape and stability (Muraglia et al., 2000). The strength of bone tissue is reinforced via creation of cross-links between collagen and elastin by lysyl oxidase (LOX). Moreover, LOX can act as a tumor suppressor (Lucero and Kagan, 2006). However, recent studies have shown that the expression of this enzyme is reduced in many cancers, including OS (Kaneda et al., 2004; Ren et al., 1998). Liu et al. evaluated the role of SNPs in reduction of this enzyme among patients with OS and showed that −22 G/C polymorphism in LOX gene was associated with an increased risk of OS development and progression (Liu et al., 2012). In addition to the crucial role of LOX in maintaining the integrity of bone tissue, this enzyme can also participate in the metabolism of estrogen hormone and downstream signaling pathways (Weitzel et al., 2014). Biphenol A (BPA), a peripheral estrogen type that mimics the function of normal estrogen, is known as a carcinogen in many cancers (Melzer et al., 2011; Fernandez and Russo, 2010). Interestingly, patients with OS having −22 G/C polymorphism in their LOX enzyme cannot metabolize BPA when exposed to it (Jia et al., 2013). This polymorphism seems to pave the way for increasing incidence of OS via reduction of LOX function in the elimination of undesirable effects of this carcinogen. Matrix metalloproteinases (MMPs) are another group of enzymes participating in the degradation and regeneration of bone tissue by binding to various components of bone microenvironment such as collagen and proteoglycans (Visse and Nagase, 2003). Evaluation of the function of these enzymes in animal models indicates that some members of this enzyme group, including MMP-3, play an important role in the differentiation of osteoblasts and the development of bone tissue (Sasaki et al., 2007). Interestingly, Adiguzel et al. recently showed that the level of this enzyme is increased in OS patients bearing E45K polymorphism in MMP-3 gene, which is associated with the progression of this disease (Adiguzel et al., 2016). Considering the crucial role of MMP-3 in maturation of osteoblasts (Sasaki et al., 2007), this type of polymorphism, which is associated with increased expression of MMP-3, may result in excessive growth and expansion of osteoblasts and thus lead to the formation of a neoplastic niche. MMP-9 is another member of MMPs family that can increase the migration of cancer cells and act as an oncogene (Egeblad and Werb, 2002). As this enzyme possesses collagenase activity, it can cause cancer cell migration and metastasis by degrading collagen in the basement membrane of different tissues (Hong et al., 2005). Therefore, increased expression and function of this enzyme in many cancers has been described as a poor prognostic factor (Liu et al., 2010). However, the function of this enzyme can be controlled by binding to miR-491. In fact, under such conditions, miR-491 acts as an antimetastatic gene (Zhou et al., 2013). Foukas et al. stated in their study that patients in an advanced phase of OS (stage IIB) show increased expression of MMP-9 and lung metastasis but have decreased survival (Foukas et al., 2002). Remarkably, SNP 1056629rs in 3′ untranslated region (3'UTR) of MMP9, which is the binding site of miR-491, can suppress postoperative metastasis among patients (Tian and Zhang, 2016). This finding suggests that this type of polymorphism is likely to enhance the binding of MMP-9 and miR-491 and lead to the induction of inhibitory activity of miR-491. However, other polymorphisms, including rs1056628 polymorphism in the same region, can be associated with impairment of MMP-9 and miR-491 interaction and uncontrolled MMP-9 function (Tian and Zhang, 2016). Thus, although the increased expression and function of MMP-9 can be associated with the progression of OS metastasis, the presence of 1056629rs SNP in 3'UTR of this gene can be related with a better prognosis due to the enhanced binding of this enzyme to its inhibitor (miR-491) and thereby reducing metastasis following surgery.

Exposure to ionizing radiation as well as BPA is another environmental risk factor for OS, which has recently been considered. X-ray repair cross complementing (XRCC3) enzyme plays a vital role in maintaining the integrity of the genome and preventing the incidence of DNA lesions from ionizing radiation (Griffin et al., 2000). Several SNPs have been identified in the XRCC3 gene, which are associated with many cancers like breast, lung, head and neck (Xuan et al., 2015; Namazi et al., 2015). In this regard, studies have shown that individuals bearing Met/Met genotype of XRCC3 Thr241Met polymorphism are not able to repair the damage caused by ionizing radiation and are thus susceptible to OS in codominant, dominant, and recessive models (Yang

et al., 2015; Guo et al., 2015). Although these studies have shown that the identification of XRCC3 Thr241Met polymorphism may help in the prediction of OS risk, there is no report in these studies on the correlation between these genetic changes with clinical findings among patients.

The instability of DNA, damage to this important biological molecule, and lack of its repair are other risk factors of OS, and the effect of SNPs in DNA repair system enzymes is of particular importance. It has been shown that people bearing rs1760944 polymorphism in the gene of apurinic/apyrimidinic endonuclease 1 (APE1), which is an enzyme involved in the DNA repair system, are more likely to develop OS than those without this polymorphism. In addition, GG genotype of this polymorphism is associated with less metastasis and longer survival in patients than TT genotypes (Xiao et al., 2017). These finding suggest that GG genotype is a good prognostic biomarker in the onset of OS diagnosis. However, no association has been reported between these polymorphic genotypes with clinicopathological characteristics, including age at diagnosis, gender, tumor size and location, tumor differentiation grade, metastasis at diagnosis, and response to chemotherapy (Xiao et al., 2017).

Additionally, SNPs in other DNA repairs enzymes such as telomeric repeat binding factor 1 (TERF1) and RecQ like 5 (RECQL5) helicase, which are respectively involved in maintaining chromosome telomere length and repairing replication errors such as mismatch, nucleotide excision, and direct repairs, can be associated with an increased risk of OS (Mirabello et al., 2011b; Zhi et al., 2014). Interestingly, Mirabello et al. showed that the risk of OS in females having chromosomes with shorter telomeres is higher than men, although the age of patients does not correlate with telomere length and OS risk (Mirabello et al., 2011b). Moreover, in this study, seven SNPs of TERF1 were found to have an inverse relationship with OS risk (Table 1). Considering the role of TERF1, these SNPs may result in the loss of TERF1 function in protecting the length of telomeres and lead to chromosomal abnormalities in OS patients. Although previous studies demonstrated that TERF1 polymorphisms could be associated with a higher risk of OS in females than males, the exact mechanism of this difference is not clear, and these associations probably refer to the impact of sexual hormones on chromosome stability. Rs820196 and rs4789223 are two common SNPs in RECQL gene among OS patients, which may indicate their association with OS pathogenesis (Zhi et al., 2014). However, different haplotypes resulting from the combination of these SNPs can be associated with different outcomes. For example, the TTA haplotype that results from a combination of these SNPs is associated with increased OS risk, while the TTG haplotype reduces the risk of OS (Zhi et al., 2014).

Oxidative stress is another risk factor for carcinogenesis in a variety of cancers, which can be involved in the development and progression of these disorders by exerting harmful effects on biological molecules such as DNA. Paraoxonase 1 (PON1) is an enzyme with a protective effect against the harmful effects of these toxic compounds (Wang et al., 2010). Nonetheless, several studies have shown that the activity of this enzyme is reduced in cancers, including solid tumors and hematological malignancies (Stevens et al., 2006; Lee et al., 2005a). By examining the function of this enzyme in OS patients, Ergen et al. showed that an SNP in codon 199 of this enzyme's gene leads to the formation its Q genotype, which has a weaker function than the wild genotype (Ergen et al., 2011). Although this polymorphism is associated with an increased risk of OS, there is no report on the relationship between this genetic change and clinical findings. Because Lee et al. in their study showed that PON1Q genotype increased the risk of lung cancer (Lee et al., 2005a), this genotype may be responsible for the incidence of lung metastasis in OS patients, as described in Ergen et al. study.

Glutathione S-transferase (GST) proteins are a group of enzymes that play a major role in detoxification of a wide range of peripheral and non-peripheral environmental carcinogens such as polyaromatic hydrocarbons as well as chemotherapy agents like anthracyclines and reactive oxygen species (Nebert and Vasiliou, 2004). The four subclasses of GST enzyme family include GSTM1, GSTM3, GSTP1, and GSTT1, which are responsible for detoxifying carcinogen agents by connecting these agents to their glutathione component (Nebert and Vasiliou, 2004). Generally, GST enzymes are highly polymorphic in human populations, and studies have shown that some alleles of these enzymes are associated with various types of solid tumors (particularly lung cancer) among smokers (Hosgood et al., 2007). Through evaluation of the functions of these enzymes in OS patients, Lu et al. showed that the risk of OS incidence is higher among people bearing the null genotype of GSTT1 and GSTM1 enzymes. Also, they showed that the risk of OS is higher in men bearing this genotype than women (Lu et al., 2011; Barnette et al., 2004). Although no physiological factor has been reported for this gender difference, environmental factors such as increased prevalence of cigarette smoking, more exposure to carcinogen agents in men, and the inability of GSTT1 and GSTM1 null genotype to eliminate the adverse effects of these agents may cause increased OS incidence in men relative to women. In addition to null genotype, SNPs that affect the sensitivity and specificity of GSTs can be associated with an increased risk of OS in children (Table 1) (Lu et al., 2011). Remarkably, GSMT1 null genotype is associated with poor clinical findings, including an increased risk of lung metastasis, while the presence of at least one allele of this enzyme can reduce metastasis and lead to a favorable response to treatment (Salinas-Souza et al., 2010). Evidence suggests that a combination of multiple alleles from these enzymes can be associated with different clinical findings. For instance, the simultaneous presence of GSMT1 null genotype and GSTT1 wild genotype is associated with a shorter survival and increased lung relapse in metastatic patients, while the presence of GSTT1 null genotypes alone is associated with a better survival of patients (Salinas-Souza et al., 2010). Therefore, these findings suggest that the null genotypes or a genotype caused by SNPs have a weaker function than wild genotype and can be associated with pathogenesis and a poor clinical outcome in OS. It seems that the presence of at least one of these altered alleles in an individual can act as a dominant allele to wild genotype and predispose to increased OS risk, a poor clinical outcome, and even weaker response to treatment by preventing the function of the wild allele. However, stronger evidence is needed to demonstrate the definitive effects of GST SNPs in OS pathogenesis and prognosis and to use these enzymes as therapeutic targets.

2.4. Tumor suppressor genes

Suppression of tumor growth is the main function of tumor suppressor genes; however, genetic changes such as mutations and SNPs can cause loss of function of tumor suppressors in a wide range of cancers (Jones and Thompson, 2009). Tumor protein p53 (TP53) is a tumor suppressor which can act as a transcription factor in processes such as cell growth, apoptosis, and DNA repair and have a particular importance in suppression of malignant tumors (Vogelstein and Kinzler, 2004). P53 induces apoptosis by promoting BCL-2 proteins in tumor cells. It has been shown that P53-null osteoblast progenitor cells can lead to the development of OS in mouse models. Also, several studies have been reported the reduced expression and function of TP53 as a result of some SNPs in OS patients (Savage et al., 2007a; Toffoli et al., 2009). The absence of these SNPs in normal people compared with OS patients can indicate the association of this genetic change with OS risk (Toffoli et al., 2009; Savage et al., 2007a). Considering the role of TP53 in apoptosis, these SNPs may reduce the apoptosis of tumor cells. Since the inhibition of P53 polymorphism seems a difficult task, upregulation of BCL-2 can be possible mechanism for overcome the adverse effects of these genetic changes. On the other hand, the increased expression of murine double minute 2 (MDM2), as a negative regulator of TP53, has been shown to be related with the progression of OS disease (Toffoli et al., 2009; Wang et al., 2014a). Similar to TP53, several common SNPs capable of affecting MDM2 expression and function are reported as risk factors of OS in several studies. Although the majority of these SNPs do

not show evidence of association with patient survival, Toffoli et al. in their study stated that 44% of patients carrying MDM2 polymorphism had clinical findings such as lung metastases, local recurrences, and increased risk of death (Toffoli et al., 2009). Moreover, there is a twofold risk of OS development in females carrying MDM2 309 GG genotype or at least one G allele compared to males carrying the G allele (TG + GG). In contrast, the risk of OS in these females (bearing the GG genotype) is four times higher than females harboring the TT genotype (Toffoli et al., 2009). Considering the fact that TP53 function can be controlled by MDM2, it appears that polymorphisms associated with increased expression and function of MDM2 along with polymorphisms reducing the expression and function of TP53 are potential risk factors for OS. Because these genetic changes affect disease pathogenesis as well as its prognosis, they could be considered as new biomarkers to diagnose patients and apply appropriate therapies.

2.5. CD markers

The cytotoxic T-lymphocyte antigen-4 gene (CTLA4) or CD152 is a surface marker with a key role in inhibiting the function of T lymphocytes (Yuan et al., 2011). Increasing expression of this marker in autoimmune diseases indicates a relationship between the changing expression of this CD marker with the pathogenesis and prognosis of these disorders (Scalapino and Daikh, 2008; Behzad et al., 2017; Behzad et al., 2018). Similarly, it have been shown that increased CD152 expression can be associated with the progression of tumor growth. Nevertheless, Contardi et al. showed that CD152 interactions with CD80 or CD86 recombinant ligands could induce apoptosis in tumor cell lines (Contardi et al., 2005). The association between rs231775 SNP with increasing CD152 expression in OS has been reported in several studies, which indicates the important role of this SNP in OS pathogenesis (Table 1) (Bian et al., 2015; Liu et al., 2013; Chang et al., 2014). Some studies have shown that increased expression of CD152 on human tumor cells interferes with antitumor therapies (Chang et al., 2014; He et al., 2014a). With these findings, in addition to reducing the function of T lymphocytes, it seems that increasing expression of CD152 following SNPs may result in lack of effective immune response defense against tumor cells. Since CD152 is a potent suppressor of antitumor responses, the changes in the expression of this immunoregulatory molecule via SNPs may lead to the development of OS tumor cells. Unlike CD152, CD86 (B7–2) is a costimulatory molecule on antigen presenting cells, which has a recognized role in the stimulation of T lymphocytes defense responses against pathogens as well as immunosuppression in vivo (Odobasic et al., 2005). However, the binding of this costimulatory molecule to CD28/CD152 complex can modulate and reduce the function of T lymphocytes (Salomon and Bluestone, 2001). Wang et al. showed that newly diagnosed OS patients with +1057G/A polymorphism in their CD86 gene are unable to sufficiently stimulate T lymphocytes and transduce activation signals for their function and that this genetic change was directly related to the progression of OS (Wang et al., 2011). Although the relationship between this polymorphism with CD86 and CD28/CD152 complex interactions hasn't been investigated, this genetic variation may suppress the function of T lymphocytes by increasing interaction between CD86 and CD28/CD152. In fact, it seems that +1057G/A polymorphism is involved in OS pathogenesis by affecting CD86 expression and function.

Integrins are membrane proteins composed of α and β chains acting as cell surface adhesion molecules. Integrin subunit alpha 3 (ITGA3), also called CD49C, is a surface integrin of cells that can interact with extracellular matrix proteins (Pozzi and Zent, 2003). This integrin can also be integrated into the structure of α3β1 integrin complex, which plays a role in regulating several functions of cancer cells such as cell adhesion (Kreidberg, 2000). Research has indicated that the overexpression of this surface molecule is associated with the progression of many cancers towards invasive and metastatic stages. In this regard, Yang et al. investigated the effect of SNPs on CD49C expression in OS patients and found that individuals who carry AA genotype due to CD49C rs2230392 polymorphism are susceptible to OS (Yang et al., 2014b). Furthermore, they showed that the risk of metastasis and reduced survival is twice higher in patients who carry AA genotype relative to those carrying GG genotype (Yang et al., 2014b). In addition, male patients harboring AA genotype had a higher risk for OS than females, but no difference was reported concerning the association of Enneking stage, tumor location, and histological type with rs2230392 genotype (Yang et al., 2014b). Increasing risk of OS and metastasis by this genetic change can be linked to the relationship between CD49C and focal adhesion kinase (FAK) signaling pathway. FAK is a signaling pathway mediating several vital processes such as differentiation, proliferation, and migration of various cells, including osteoblasts (Kim et al., 2007). Integrin-β1 is the main trigger of this pathway, which can lead to increased proliferation and migration of osteoblasts via phosphorylation and activation of FAK (Yang et al., 2014a). Because CD49C is a component of α3β1 integrin complex on cancer cells, SNPs in CD49C gene may enhance the expression of this surface complex and lead to metastasis of tumor cells in OS by activating FAK signaling. In fact, the expression of CD49C can be a marker of poor prognosis for increased OS risk and worse clinicopathological outcomes.

CD95 (FAS) is another surface marker of cells that induces apoptosis by binding to CD95L (FAS ligand). Studies have shown that different variants of this surface marker are associated with pathogenesis of a range of diseases, including autoimmune diseases, hematological malignancies, and solid tumors (Kanemitsu et al., 2002; Sibley et al., 2003). Induction of cancer cells' resistance to apoptosis by these variants is a hypothesis widely supported by many researchers to link the expression of CD95 variants with the pathogenesis of these abnormalities (Owen-Schaub et al., 2000). Evidence suggests that CD95 expression is closely related to OS pathogenesis. In the study of Koshkina et al., children with OS who carried 18272 A > G SNP in the promoter region of CD95 showed decreased expression of this marker and were prone to increased risk of OS (Table 1) (Koshkina et al., 2007b). It seems that this type of SNP leads to resistance of OS tumor cells to apoptosis by decreasing the expression and function of CD95 and subsequently helps them to escape from immune mechanism. Interestingly, decreased expression of CD95 has been introduced as a potential risk factor for lung metastasis in OS (Koshkina et al., 2007a). However, reduced expression of CD95 does not result in resistance to treatment, and it has been shown that increased expression of this marker is associated with decreased lung metastasis (Lafleur et al., 2004). Despite the important role of CD95 SNPs in OS pathogenesis, there is insufficient information about the possible association mechanism of this genetic change with lung metastasis. Therefore, further studies are required for better understanding of the exact mechanism of this connection. As lung metastasis is associated with a poor prognosis, timely diagnosis of the underlying cause of changing CD95 expression is likely to avoid this outcome. Given that previous studies have shown that lung metastasis due to reduced CD95 expression is not resistant to treatment, preventing disease progression by targeting variants of CD95 SNPs is a seemingly new therapeutic option.

2.6. Signaling pathway molecules

Signaling pathways include a set of related biological molecules involved in a wide range of cellular processes. Basic research on these pathways suggests that their function is essential for the development of cells and maintenance of balance in cell growth processes such as cell cycle, differentiation, and proliferation. Hence, the disruption of these pathways can lead to a variety of cancers. Phosphatidylinositol-3-kinase (PI3K)/AKT is a pathway that plays a vital role in various cellular processes, including growth, survival, apoptosis, and motility of cells (He et al., 2013). It has been determined that all these functions are mediated by AKT phosphorylation followed by a cascade of intermediate molecules such as glycogen synthase kinase 3 (GSK3), Bad and

Table 2
Prognostic value of genes polymorphism for clinical outcomes after chemotherapy in OS.

Enzymes genes	Chro.	Function	Genotype	Rs number	Method	Clinical outcome	Ref.
Drug metabolism enzymes							
MTHFR	1p36.22	Catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate	1298A > C	1,801,131	RT-PCR	MTX toxicity	(Patiño-García et al., 2009)
			677TT	1,801,133	PCR	Hematological toxicity including aplasia, neutropenia, anemia, leukopenia	(Patiño-García et al., 2009; Windsor et al., 2012)
MTR	1q43	Is a cobalamin-dependent methionine synthase and catalyzes the final step in methionine biosynthesis	A2756G	1,805,087	RT-PCR	Gastrointestinal toxicity including increased alkaline phosphatase, bilirubin, or transaminases	(Patiño-García et al., 2009)
MTHFD1	14q23.3	Possesses three distinct enzymatic activities for methionine, thymidylate, and de novo purine syntheses	1958G > A	2,236,225	PCR	The histological response, MTX toxicity, anemia	(Windsor et al., 2012)
RFC1	21q22.3	Transporter of folate and involved in the regulation of intracellular concentrations of folate	G80A	1,051,266	RT-PCR	Resistant to MTX via inhibition of drug transport into tumor cells	(Patiño-García et al., 2009)
ABCC2	10q24.2	Transports various molecules across extra- and intra-cellular membranes.	24C > T	717,620	PCR	Associated with MTX toxicity and poor histological response	(Windsor et al., 2012)
ABCC3	17q21.33	Transports various molecules across extra- and intra-cellular membranes.	G1013G	4,148,416	Golden Gate Genotyping Assay	Higher risk of death	(Caronia et al., 2011)
			TT	4,148,416	PCR	Poor response to chemotherapy	(Liu et al., 2014; Yang et al., 2013)
ABCB1		Is a member of ABC superfamily of transporters that transport various molecules across extra- and intra-cellular membranes	GG	4,148,737	Golden Gate Genotyping Assay	Poor overall survival	(Caronia et al., 2011)
			G allele	1,128,503		Associated with better overall survival	
			1236C > T	1,128,503	PCR	Poor response to chemotherapy and reduced overall survival	(Li et al., 2014b)
			TT	1,128,503	PCR	Good response to chemotherapy	(Liu et al., 2014; Yang et al., 2013)
			CC	10,276,036	Golden Gate Genotyping Assay	Associated with better overall survival	(Caronia et al., 2011)
			3435TT	1,045,642	TaqMan methodology	Poor response to chemotherapy and high risk of death	(Xiaohui et al., 2014)
CCND1	11q13.3	Function as regulator of CDK kinases	870A > G		PCR	Can be associated with metastatic disease	(Windsor et al., 2012)
DNA repair-related enzymes							
ERCC1	19q13.32	Function in the nucleotide excision repair pathway and required for the repair of DNA lesions such as those induced by UV ray	354 T > C	11,615	PCR	Metastasis at diagnosis	(Windsor et al., 2012)
			118 T/T	11,615	Sequenom MassARRAY platform	Increased event-free survival	(Hao et al., 2012)
			TT	11,615	PCR-RFLP	Poor response to chemotherapy and increases risk of death	(Sun et al., 2015)
			CC		PCR-RFLP	Good response to chemotherapy and decreased risk of death	(Ji and He, 2015; Zhang et al., 2017)
			CC AC	2,298,881	PCR-RFLP	Poor response to chemotherapy and unfavorable survival	(Sun et al., 2015)
ERCC2	19q13.32	Involved in transcription-coupled nucleotide excision repair	751A/A	13,181	PCR	Associated with shorter event-free survival	(Hao et al., 2012)
			312A/A	1,799,793	PCR	Associated with a better response to chemotherapy and longer overall survival of patients	(Caronia et al., 2009)
ERCC5	13q33.1	Is a DNA endonuclease that makes the 3′ incision in DNA excision repair following UV-induced damage	TT	1,047,768	PCR-RFLP	Good response to treatment and increases overall survival	(Sun et al., 2013; Li et al., 2014a)
XPC	3p25.1	Important for damage sensing and DNA binding	939C/C	2,228,001	PCR	Increased cisplatin response	(Caronia et al., 2009)
GSTP1	11q13.2	Plays an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione	313A > G	1695	PCR	Poor histological response	(Windsor et al., 2012)
						Poor response to chemotherapy and shorter overall survival	(Li et al., 2014b; Liu et al., 2014)

Abbreviations: Chro: chromosome; MTHFR: methylenetetrahydrofolate reductase; MTX: methotrexate; MTR: 5-methyltetrahydrofolate-homocysteine methyltransferase; MTHFD1: methylenetetrahydrofolate dehydrogenase 1; RFC1: reduced folate carrier1; ABCC2: ATP binding cassette subfamily C member 2; ABCC3: ATP binding cassette subfamily C member 3; ABCB1: ATP binding cassette subfamily B member 1; ABC: ATP-binding cassette; CCND1: cyclin D1; CDK: cyclin-dependent kinase; ERCC1: excision repair cross-complementation1; ERCC2: excision repair cross-complementation2; ERCC5: excision repair cross-complementation 5; GSTP1: glutathione S-transferases p1; PCR: polymerase chain reaction; PCR-RFLP: polymerase chain reaction -restriction fragment length polymorphism; RT-PCR: real-time polymerase chain reaction.

caspase-9, mammalian target of rapamycin (mTOR), and transcriptional factors such as forkhead (FOXO-1) and NF-kappa B (Zhang et al., 2015b). This pathway can play a role in OS pathogenesis by affecting various processes, including tumorigenesis, proliferation and invasion, cell cycle progression, apoptosis, angiogenesis, metastasis, and chemoresistance (Zhang et al., 2015b). Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3K3CA) and AKT are the main molecular components of this pathway. Mutations and SNPs in the genes of these molecules are associated with tumorigenesis in a variety of cancers, including OS (He et al., 2013; Xing et al., 2012). He et al. in a study on OS patients demonstrated that the AA genotype of AKT caused by rs6973569 SNP increased the risk of chondroblastic and metastatic OS in humans (He et al., 2013). On the other hand, PI3K/AKT /mTOR/p70s6k pathway plays a major role in migration and motility of cells (Qian et al., 2004). Moreover, Fendri et al. showed that the activity of this pathway could increase the expression of MMP-2, an enzyme that plays an important role in the degradation of extracellular matrix, leading to an increase in the metastasis of tumor cells (Fendri et al., 2009). According to these findings, AKT SNPs in OS tumor cells appear to increase the metastasis of these cells by increasing the expression of this molecule and induce the activity of PI3K/AKT/mTOR/p70s6k. Although the risk of OS development following AKT SNPs does not show a significant difference between men and women, the CC genotype of PI3K3CA rs7646409 polymorphism is associated with a fourfold increase in the risk of Enneking stage II B OS in men compared with women (He et al., 2013). Given that SNPs can change the normal function of PI3K3CA and AKT by converting them to oncogenes, these genetic variations appear to play a role in pathogenesis and prognosis (even response to the treatment) of OS and may thus be considered as potential therapeutic targets in this disease.

3. Polymorphism and clinical outcome after chemotherapy in osteosarcoma

The current standard treatments for OS include preoperative chemotherapy, surgical removal of primary tumor, and finally post-surgical chemotherapy. The drugs used for OS chemotherapy often include high doses of MTX, cisplatin, cyclophosphamide, vincristine, and doxorubicin (Marina et al., 2004). These therapeutic protocols can cause complications such as heart failure, infertility, and even secondary malignancies; moreover, environmental and genetic factors like age, sex, tumor site and size, metastatic disease, and SNPs can affect the sensitivity to these drugs (Gianferante et al., 2017). The resistance of cancer cells to various drugs is one of the main obstacles to achieve a successful treatment. Genetic studies in recent decades on carriers as well as function of drug-metabolizing enzymes show that SNPs can impair the function of these proteins, resulting in incomplete metabolism of drugs, increased toxicity due to higher drug concentrations, and finally drug resistance in patients (Windsor et al., 2012; Patiño-García et al., 2009). Given the diversity of these genetic variations in OS patients, a number of common SNPs that can affect the response to therapy and clinical findings after chemotherapy will be mentioned.

3.1. Polymorphism in drug metabolism enzymes

A high dose of MTX is the most commonly used treatment for patients with OS. This drug is a folate analog binding to dihydrofolate reductase (DHFR) and preventing the reduction of dihydrofolate to methylenetetrahydrofolate, which can inhibit the synthesis of thymidilate and subsequently DNA, RNA, and protein synthesis in cells (Robien et al., 2005). In addition to inhibiting DHFR, MTX is able to exert its effect by inhibiting several other enzymes of folate metabolism, including folate carrier1 (RFC1), 5,10-methylenetetradihydrofolate reductase (MTHFR), and thymidylate synthetase (TYMS) (Weisberg et al., 1998). SNPs in folate metabolism pathway may alter the therapeutic effects of MTX. For instance, MTHFR 677TT polymorphism generates a genotype of this enzyme that is associated with a reduction of its function compared to the normal genotype (Patiño-García et al., 2009; Weisberg et al., 1998). Children with OS carrying this genotype are faced with toxic effects of MTX (i.e. increased MTX concentration in serum) and grade G3/G4 hematological toxicity; however, their overall survival is not affected (Windsor et al., 2012; Patiño-García et al., 2009). MTHFR 677TT polymorphism seems to cause resistance of this enzyme to inhibitory effects of MTX in addition to reducing its function. In fact, it may be an obstacle to MTX in patients who require optimal dose of this drug to achieve a favorable response. Similarly, A2756G polymorphism in 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), which is an enzyme catalyzing the final stage of methionine biosynthesis, reduces MTR activity and can cause severe gastrointestinal toxicity (Table 2) (Patiño-García et al., 2009). These finding suggest that the reduced function of MTR due to SNPs is a possible mechanism by which OS tumor cells can become resistant to chemotherapeutic agents. Additionally, resistance to MTX can occur due to disruption in the transfer of this chemotherapeutic drug into cancer cells. RFC1 is an MTX carrier from cell membrane, and studies suggest that 80G > A polymorphism can reduce its function and expression at the beginning of OS diagnosis (Windsor et al., 2012; Laverdiere et al., 2002). Patino-García et al. showed that 80G > A polymorphism could lead to MTX resistance in children with OS (Patiño-García et al., 2009). In addition to MTX, the susceptibility to cisplatin, which is a chemotherapy drug for OS patients and other solid tumors, has been shown to be associated with RFC1 expression in tumor cell lines (Charasson et al., 2009). These findings suggest that SNPs can contribute to drug resistance in OS by inhibiting the transfer of chemotherapy drugs into tumor cells as well as increased or decreased affinity for target enzymes of these drugs.

Similar to RFC1 or ABC proteins, the ATP-binding cassette forms a group of important carriers for various types of chemotherapy drugs. Today, research is focused on eradicating drug resistance by these carriers in a variety of metastatic tumors. ATP binding cassette subfamily C member 3 (ABCC3) is a member of this family, which has been shown to play a key role in drug resistance in cancer cells (Zeng et al., 2000). Caronia et al. in their study on patients with OS showed that the G1013G genotype of this enzyme could be associated with eightfold increase in the risk of death and reduced survival of patients (Caronia et al., 2011). Similarly, the TT genotype of ABCC3 that is caused by an SNP in the same region of G1013G genotype is associated with reduced survival of patients and a poor response to treatment (Liu et al., 2014; Yang et al., 2013). The ATP binding cassette subfamily B member 1 (ABCB1) is another known carrier of vincristine, the function of which can be changed by several SNPs that can affect response to therapy and survival rates of patients (Table 2) (Caronia et al., 2011; Liu et al., 2014; Yang et al., 2013; Xiaohui et al., 2014). It seems that SNPs in these carriers contribute to resistance caused by reduced drug delivery into cancer cells. Overcoming this type of drug resistance is conceivable by identifying SNPs in patients and importing drugs through other carriers into cancer cells.

Therefore, it is inferred that the efficacy of chemotherapy drugs can be influenced by SNPs both in carriers and in their metabolizing enzymes. In fact, SNPs can be an appropriate biomarker to predict survival of patients by affecting response to treatment. Identification of these risk factors is likely to help design therapeutic protocols based on genetic background of the individual, thereby contributing to improvement of conditions and increasing overall survival by overcoming drug resistance in OS.

3.2. Polymorphisms of DNA repair pathway enzymes

DNA repair systems include a set of enzyme complexes that contribute to sustained replication, genome integrity, and cell life by repairing DNA damages. Excision repair cross-complementations (ERCC) are a group of nucleotide excision repair (NER) enzymes involved in the

detection and correction of DNA damages. If these enzymes do not perform their function correctly, DNA repair is impaired and cell death occurs (Costa et al., 2003). Cisplatin is one of the most commonly used chemotherapy drugs that is used together with doxorubicin and MTX to treat patients with OS. The mechanism of this drug is causing lesions in DNA, inhibiting DNA repair pathway enzymes (including ERCCs), stopping cell cycle, and ultimately inducing apoptosis in tumor cells (Rabik and Dolan, 2007). However, SNPs in DNA repair pathway enzymes are a mechanism that can lead to cisplatin resistance. The association of SNPs in DNA repair pathway enzymes (especially ERCC1) with response to cisplatin-based chemotherapy has been investigated in various clinical studies (Hao et al., 2012; Sun et al., 2015; Ji and He, 2015), which indicate that different genotypes of ERCC1 can have different clinical outcomes (Table 2) (Hao et al., 2012; Sun et al., 2015). For instance, the TT genotype of ERCC1 rs11615 polymorphism is associated with poor response to treatment and reduced survival relative to the wild-type, while CC genotype shows a favorable response and reduced mortality in patients (Sun et al., 2015; Hao et al., 2012; Ji and He, 2015; Zhang et al., 2017). Similar to TT genotype of ERCC1, the 751A/A genotype of ERCC2 can related with reduced survival and worse prognosis in OS patients (Caronia et al., 2009). XPC is another DNA repair pathway enzyme that plays a key role in the detection of DNA damage. XPC polymorphisms have been shown to be associated with an increased risk of solid tumors (Hu et al., 2005). Caronia et al. in their study on OS patients revealed that Lys939Gln SNP in the gene of this enzyme produced a genotype with a weak function that reduced its ability to detect DNA lesions, which ultimately led to apoptosis of cells (Caronia et al., 2009). In fact, this polymorphism can enhance the therapeutic effects of cisplatin and may be a good prognosis factor for OS patients. However, studies on XPC polymorphisms are limited, and further studies are needed to evaluate the role of these genetic changes in response to cisplatin-based chemotherapy to be able to predict XPC polymorphisms as a prognostic factor in OS.

Another research topic concerning the effects of polymorphisms on response to chemotherapy is devoted to the role of SNPs in GST enzymes, especially GSTP1. Although the main function of this enzyme is detoxification of carcinogens, research indicates that the 313A/G genotype of this enzyme is associated with a poor response to treatment, decreased susceptibility to chemotherapy drugs, and worse clinical outcome in OS relative to the wild-type (Zhang et al., 2012; Liu et al., 2014; Li et al., 2014b). It seems that genetic variation in this enzyme can be useful as a prognostic factor for OS patients undergoing chemotherapy. These findings suggest that despite advances in the discovery of new drugs for treatment of OS patients, SNPs can cause resistance against drug combinations. Therefore, recognizing these risk factors in patients and designing a drug strategy consistent with the genetic structure of patients may be helpful in overcoming drug resistance and reducing undesirable clinical complications to improve their clinical condition.

4. Discussion

The genetic basis is an important factor in OS that can affect several aspects such as the development, progression, and prognosis of this disease. In the past years, several meta-analyses and case-control studies, especially in European and Asian populations, have investigated the pathogenesis and biology of OS. A number of these studies have shown that polymorphic changes caused by SNPs in many biological agents such as cytokines, enzymes, tumor suppressors, growth factors, and CD markers are essential genetic changes that can be independently involved in OS pathogenesis by affecting various processes such as OS tumor cell proliferation, apoptosis, adhesion, invasion, and metastasis (Gianferante et al., 2017). Given that the peak of bone tissue growth takes place during adolescence, the occurrence of SNPs inducing excessive bone growth can be a reason for the onset and prevalence of OS in this period. Therefore, recognition of the basic mechanism of this disorder can lead to timely treatment of patients. In addition, the relationship between height and OS risk has been evaluated in several studies. Although some meta-analyses demonstrated the association between tall structure and OS risk, other studies have not observed any association between height and OS risk. These controversial results may be attributed to the impact of SNPs on genetic backgrounds in different populations. Therefore, better-designed studies are required to specify the association between SNPs and OS risk in different races. Drug resistance and relapse, usually in the form of pulmonary metastasis, are the most important challenges faced by OS patients. Although several factors such as metastatic disease, tumor size and location as well as response to chemotherapy before surgery are essential factor in complete OS treatment (Marina et al., 2004), they are not by themselves accountable for response to therapy in this disease; however, potential SNPs in drug-related metabolism enzymes and transporters can also be effective in response to therapy. SNPs in MTX metabolism pathways and drug carriers, including ATP binding cassette subfamilies and RFC1, are the most common causes of drug resistance in patients with OS (Windsor et al., 2012; Li et al., 2014b). It is inferred that impaired absorption and transport of drugs into tumor cells and their reduced sensitivity to drug compounds due to incomplete metabolism are the main mechanism of multidrug resistance caused by these SNPs. In particular, drug metabolism enzymes and transporter SNPs may be responsible for adverse clinical outcomes including drug resistance and progression of OS towards the high-grade metastatic form. A possible strategy to overcome multidrug resistance due to SNPs may be based on the timely diagnosis of these genetic changes and the use of appropriate treatments with the aim of increased tumor cell sensitivity and reversion of chemotherapy resistance. Therefore, accurate understanding of SNPs mechanism in inducing drug resistance as well as consequent design and application of therapies with targets other than tumor mass may be helpful in achieving a successful treatment for OS patients. However, overcoming drug resistance in OS is not a simple process and few studies have assessed the detailed mechanisms of this process. Further clinical trials are needed to assess whole-genome sequencing and improve response to therapy in OS.

5. Conclusion

SNPs appear to play an important role in the complex physiology of OS and response to therapy by creating genetic diversity. In spite of comprehensive analysis on the association between SNPs with OS pathogenesis and prognosis, few studies have compared the effects of these genetic changes on OS risk in different populations and analyzed the mechanisms of SNPs in long-term clinicopathological outcomes of OS. Therefore, the evaluation of these genetic changes as well as a better understanding of their role in tumorigenesis and clinical outcome after chemotherapy can be helpful in answering the question of why some patients respond favorably to treatment but others do not complete treatment. In fact, targeting SNPs with special treatments may have a significant impact in reducing relapses and improving the clinical conditions, especially in metastatic OS patients. However, clinical trials with large patient groups are needed to be able to use SNPs with higher certainty to design and implement appropriate treatment protocols, even based on genetic backgrounds of patients.

Authors' contributions

N.S. conceived the manuscript and revised it; A.A.A., M.M.B., M.Sh., M.Gh. and N.S. wrote the manuscript and prepared the Tables.

Conflict of interest

The authors declare no conflict of interest.

Research involving human participants and/or animals

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

Informed consent

Informed consent is not required for this type of study.

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