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Molecular genetics of osteosarcoma

Kirby Rickel^{a,b}, Fang Fang^{a,b}, and Jianning Tao^{a,c,*}

^aSanford Children's Health Research Center, Sanford Research, Sioux Falls, SD 57104 USA

^cDepartment of Pediatrics, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD 57105 USA

Abstract

Osteosarcoma is the predominant form of bone cancer, affecting mostly adolescents. Recent progress made in molecular genetic studies of osteosarcoma has changed our view on the cause of the disease and ongoing therapeutic approaches for patients. As we draw closer to gaining more complete catalogues of candidate cancer driver genes in common forms of cancer, the landscape of somatic mutations in osteosarcoma is emerging from its first phase. In this review, we summarize recent whole genome and/or whole exome genomic studies, and then put these findings in the context of genetic hallmarks of somatic mutations and mutational processes in human osteosarcoma. One of the lessons learned here is that the extent of somatic mutations and complexity of the osteosarcoma genome are similar to that of common forms of adult cancer. Thus, a much higher number of samples than those currently obtained are needed to complete the catalogue of driver mutations in human osteosarcoma. In parallel, genetic studies in other species have revealed candidate driver genes and their roles in the genesis of osteosarcoma. This review also summarizes newly identified drivers in genetically engineered mouse models (GEMMs) and discusses our understanding of the impact of nature and number of drivers on tumor latency, subtypes, and metastatic potentials of osteosarcoma. It is becoming apparent that a synergistic team composed of three drivers (one 'first driver' and two 'synergistic drivers') may be required to generate an animal model that recapitulates aggressive osteosarcoma with a short latency. Finally, new cancer therapies are urgently needed to improve survival rate and quality of life for osteosarcoma patients. Several vulnerabilities in osteosarcoma are illustrated in this review to exemplify the opportunities for next generation molecularly targeted therapies. However, much work remains in order to complete our understanding of the somatic mutation basis of osteosarcoma, to develop reliable animal models of human disease, and to apply this information to guide new therapeutic approaches for reducing morbidity and mortality of this rare disease.

*Corresponding author: Jianning Tao, Ph.D., Sanford Children's Health Research Center, 2301 East 60th Street North, Sioux Falls, SD 57104 USA, Fax: +1 605 312 6071, Jianning.Tao@SanfordHealth.Org (J. Tao).

^bThese authors contribute equally to this work

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Keywords

Bone cancer; Osteosarcoma; genomic analysis; driver mutations; animal modeling; targeted therapy

1. Introduction

Cancer of the bones and joints is a rare genetic disease. In the United States, about 3,300 new diagnoses and approximately 1,490 deaths as a result of the disease are projected for 2016 [1]. The three most common forms of primary bone cancer are osteosarcoma, Ewing tumors, and chondrosarcoma. Osteosarcoma (OS), also referred to as osteogenic sarcoma, is the most frequent, accounting for approximately 20% of all benign and malignant bone neoplasia and 2% of pediatric cancers [2]. Each year, about 800 new cases of OS are diagnosed, and half of these are reported in children and young adults. The majority of OS cases are sporadic, but they occur at increased rates in individuals with Paget's disease of bone (PDB), after therapeutic radiation, and in certain cancer predisposition syndromes. OS affects patients of all ages but shows a bimodal distribution, with the first peak at the age of 15-19 years (8 cases/million/year) and the second peak at 75-79 years (6 cases/million/year), with a middle lower plateau (1~2 cases/million/year) in persons aged 25-59 years [3]. OS epidemiology studies have provided additional etiological clues, such as associations with puberty, height, and disorders of bone growth and remodeling; however, strong environmental risk factors have not been identified [3, 4].

OS can arise in any bone, but it preferentially affects the metaphyses of long bones (distal femur > proximal tibia > proximal humerus). Its distribution in the elderly is more variable and often includes the axial skeleton and skull [2]. The clinical diagnosis of OS is mainly based on the observation of malignant osteoblasts and their products, i.e. immature bone or osteoid. OS can be histologically divided into conventional, telangiectatic, small cell, high-grade surface, secondary, low-grade central, periosteal, and parosteal variants. Conventional OS (intramedullary high-grade), the most common type in childhood and adolescence, includes about 85% of all OS cases and can be subdivided based on the presence of specific cell types (i.e., osteoblastic, fibroblastic, chondroblastic) [5]. Although some subtypes display characteristic genetic features and biological behaviors, the molecular basis for each subtype is not well understood [6]. In the clinic, OS patients are treated in an identical manner, irrespective of subtype [7]. Since 1970, the use of chemotherapy and surgery has led to a dramatic improvement in long-term survival rates, from less than 20 to 70%. However, continued progress in standard therapy to increase survival rates has slowed over the past three decades. Furthermore, OS patients with metastases, mostly in the lungs, show poor 5-year survival rates, on the order of 40% or less [8]; hence additional treatment approaches and agents are needed.

The etiological factors and pathogenetic mechanisms underlying OS development are complex, but significant progress has been made toward understanding its causes. The efforts made over the past few decades have focused on identifying so-called 'driver' mutations present in cases of inherited predisposition, as well as in sporadic OS [5, 6, 9, 10].

Cancer-causing genes (often called driver genes or drivers) contain driver mutations, which confer a proliferative advantage to cancer cells, leading to tumor clone outgrowth. This is in contrast to ‘passenger’ mutations which do not result in a growth advantage [11]. Drivers can be divided into at least two types: activated oncogene and inactivated tumor suppressor gene (TSG). At present, only TSG drivers have been identified in inherited familial syndromes with a predisposition to OS [5]. Specifically, TSGs including *p53*, *Rb*, *RECQL4*, *BLM*, and *WRN* play a critical role in the development of OS in patients with Li-Fraumeni, hereditary retinoblastoma, Rothmund-Thomson, Bloom or Werner syndromes, respectively (Table 1). A causal role for p53 and/or Rb has been revealed across species [10, 12-15]. Genetically engineered mouse models (GEMMs) equipped with p53 and/or Rb mutations have been used to identify new driver genes, to model human osteosarcomagenesis, and to study different OS subtypes as well as metastatic and non-metastatic features [16-24]. Understanding the functional roles of driver genes in GEMMs has broadened our knowledge of the molecular genetics of OS and will eventually advance preclinical investigations into new therapeutic strategies and drugs [5, 24-27]. However, in recent years the genomic analysis of human OS samples has provided new insights into driver genes and dependent signaling pathways implicated in several key events in OS pathogenesis including initiation, progression, chromosomal instability (CIN), chromothripsis, invasion, and metastasis. In this review, we discuss novel candidate driver genes that have been identified by next-generation sequencing as well as by *in vivo* forward and reverse genetic studies in GEMMs, the roles of these driver genes and their interactions in OS development, and driver mutation-dependent genetic pathways as targets for cancer therapy.

2. The genomic landscape of osteosarcoma

2.1 Osteosarcoma somatic mutations revealed by next-generation sequencing

To identify driver mutations conferring clonal advantage and the processes by which somatic mutations are generated, several groups have performed whole genome sequencing (WGS) of 47 OS samples with paired normal controls, whole exome sequencing (WES) of 111 samples with paired normal controls, and whole transcriptome sequencing of 36 samples [28-33]. These studies detected distinct classes of DNA mutations such as somatic point mutations, which include single base substitutions, and indels, which are insertions or deletions of small segments of DNA. Other DNA mutations are classified as structural variations (SV), which include rearrangements and somatic copy number alterations (SCNAs). Rearrangements occur when DNA strands are broken and then rejoined to a different DNA segment on either the same or another chromosome. SCNAs occur when copy number changes in the normal diploid genome. Copy number increase may activate an oncogene driver while copy number reduction or loss of a DNA fragment may inactivate a TSG driver.

In a WGS study of 34 pediatric OS samples, Chen et al. identified 50,426 validated somatic sequence mutations and 10,806 structural variations (SV), with considerable difference in the number of each type of mutation between individual samples. The average number of sequence mutations and SVs in OS was 1,483 and 317 per sample, respectively, which is significantly higher than in medulloblastoma and T-cell acute lymphoblastic leukemia

samples [28]. Chen et al. also identified 122 cancer genes with at least one SV breakpoint, implying that they may serve as candidate drivers in the development of OS. However, since protein-coding regions account for only ~1% of the genome, most of these sequence mutations and SVs likely occur outside coding regions. In other words, the majority of the alterations in noncoding regions and “gene deserts” are presumably passenger mutations. Although a driver gene by definition contains driver mutations, it may also contain passenger mutations. As for bone cancer, during the pre-malignant stage, bone cells that are continuously replacing old bone tissue accumulate somatic mutations. All of these pre-malignant mutations are likely passenger mutations that have no effect on tumor initiation. In general, the number of mutations in adult cancers is directly correlated with age [34]. Thus, the majority of mutations found by DNA sequencing are likely passengers rather than drivers.

In a second WES study of 59 samples and WGS of 13 samples, Perry et al. reported localized hypermutations and complex chains of rearrangements characteristic of OS in almost all cases. The median number of somatic rearrangements was 230 per tumor, and the mean nonsilent somatic mutation rate was 1.2 mutations per megabase [32]. This high frequency ranks OS as having the highest somatic mutation rate among childhood cancers. In contrast, neuroblastoma, which shows the second highest frequency, has 0.5 mutations per megabase, and Ewing sarcoma has a frequency of only 0.15; for comparison, the frequency for adult breast cancer is 1.2 [35, 36]. This implies that the catalog of driver genes and mutational processes in OS may be unique compared to other childhood tumors, such as Ewing sarcoma, which is an example of a fusion gene-driven cancer.

In the third study, comprised of 123 tumors, Kovac et al. observed that SCNA affected 0.2 to 87% of OS genomes. A typical genome they examined contained 69 SCNA events. Within protein-coding regions, individual tumors contained a median of 21 potentially functional single-nucleotide variants (SNVs) (range 4-174) and 7 small indels (range 2-210) [30]. This suggests that OS harbors far more point mutations than other pediatric solid tumors and leukemias, which have on average 9.6 point mutations per tumor [34]. Moreover, Kovac et al. detected validated mutations in 388 genes and identified 14 genes as the main drivers, which are evenly responsible for 87% of OS cases. These 14 genes include: *TP53*, *RBI*, *BRCA2*, *BAP1*, *RET*, *MUTYH*, *ATM*, *PTEN*, *WRN*, *RECQL4*, *ATRX*, *FANCA*, *NUMA1*, and *MDC1*, the majority of which are either well-known cancer drivers or have been reported in the context of cancer susceptibility. Additionally, most of them have been identified as somatically mutated genes by several groups [28, 29, 31-33]. However, the roles of several genes are unknown in the context of OS, such as *FANCA*, *NUMA1*, and *MDC1* [30]. Table 2 lists selected genes that are somatically mutated in at least two OS samples. These genes can be classified into cancer signaling pathways regulating three core cellular processes: cell fate, cell survival, and genome maintenance [34].

2.2 Complexity of genomic rearrangement and chromothripsis

Before the advent of DNA GWS studies, hallmarks of OS such as aneuploidy and genome instability, as well as tumoral heterogeneity had been observed with conventional approaches such as: karyotyping, comparative genomic hybridization (CGH), fluorescence in situ

hybridization (FISH), quantitative polymerase chain reaction (qPCR), and single-strand conformation polymorphism analysis [6]. Cancer genome studies have not only systematically assessed the mutational rate, driver gene mutations, rearrangements, and copy number alterations during OS development, but have also revealed a novel mechanism for these mutation events. “Chromothripsis” (from the Greek, “thripsis” = “shattering”) is the process by which massive genomic rearrangements and localized hypermutations (also termed kataegis) are acquired in a single catastrophic event [37]. Most cells suffering tens to hundreds of DNA breaks in this one-off cataclysmic event would be expected to die, but any cell that survives this crisis will likely have a significant selective growth advantage, promoting progression toward cancer. During chromothripsis, driver genes may arise simultaneously by several mechanisms: decreased copy number (deletion of TSGs), increased copy number (amplification of oncogenes), juxtaposition of coding sequences from two genes (production of a fusion onco-protein), or bringing together of an intact gene with the promoter of different gene resulting in dysregulation of its gene expression. This process is fundamentally different from the more gradual stepwise, or progressive acquisition of driver gene mutations by most tumor cells [11]. “Kataegis” (from the Greek, “kataegis” = “shower” or “thunderstorm”) is a phenomenon often accompanying chromothripsis, whereby regional hypermutation characterized by multiple base mutations occurs in nearby rearrangement breakpoints. This process likely involves the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC) protein families [38]. In human OS samples, approximately 50-85% showed kataegis patterns [28, 32]. Chromothripsis has been frequently detected in select OS cases (3 out of 9 primary OS samples in one study, 4 out of 34 in another study) [28, 37]. Interestingly, chromothripsis has been recently linked to both somatic and germline TP53 mutations in pediatric medulloblastoma and acute myeloid leukemia [39]. Therefore, further investigation on extent of association between TP53 mutation and chromothripsis in osteosarcoma will likely improve our understanding of the genetic basis of this aggressive disease. Cancer cells have also evolved to tolerate genome complexity in order to avoid apoptosis, for example, by acquiring mutations in genes such as TP53 that control cellular response to DNA damage. Hence, genomic complexity is in part, the result of cancer rather than the cause [34].

What induces chromothripsis in the OS genome? One recent study showed that telomere crisis can induce chromothripsis and kataegis during tumorigenesis [40]. Telomeres maintain genomic integrity in normal cells, and their progressive shortening after many cell divisions induces chromosomal instability. During telomere crisis, telomere loss promotes end-to-end chromosome fusions and dicentric chromosomes, which are broken during mitosis and undergo breakage-fusion-bridge (BFB) cycles, resulting in hundreds of DNA breaks through TREX1-mediated fragmentation [40]. The DNA repair machinery can rescue the genome by quickly stitching chromosomal fragments together in random order and orientation. In fact, BFB has been previously reported as a mechanism for generating OS cytogenetic abnormalities and genetic heterogeneity [41]. In cancer cells, replicative senescence is inhibited by maintaining telomere length either through activation of telomerase (the enzyme normally responsible for telomere replication) or the alternative lengthening of telomeres (ALT) pathway, a telomerase-independent telomere maintenance mechanism. Longer telomeres and high ALT activity are common in OS, suggesting a contribution of ALT to

genomic instability [42]. ALT in tumors is associated with alteration of ATRX, which is recurrently mutated in OS patients (Table 2) [28, 30, 32]. The important contribution of ALT and ATRX to OS development and implications for therapies are discussed further in Section 4.

Another factor in the induction of chromothripsis is physical chromosomal damage, such as that caused by ionizing radiation [43]. A pulse of ionizing radiation induces DNA double-strand breaks, leading to the unfettered circulation of hundreds of shards of genomic DNA in the nucleus. These pieces, in turn, are randomly pasted together by the DNA repair machinery. This phenomenon explains why radiation-induced OS in either human or animals is dose-dependent (a higher dose induces more driver mutations at once) [44]. Most cases of secondary OS arise due to ionizing radiation, with or without chemotherapy [9]. Radiation is estimated to cause up to 3% of OS cases, some of which can appear up to 30 years after exposure. This is possible because if only one cell acquires a driver mutation from such an event, this clone will then present a considerable latency period.

2.3 Cataloging cancer driver genes in human osteosarcoma

The identification of cancer driver genes has been a central goal of cancer research over the past few decades [11]. Currently, there are at least 595 driver mutation genes (2.7% of 22,000 genes that encode proteins in the human genome) reported in an assortment of cancers with strong evidence that these contribute to tumorigenesis [45] (<http://cancer.sanger.ac.uk/census>). To explore the feasibility of creating a comprehensive catalog of cancer genes, Lawrence et al. analyzed 4,742 human cancers with paired normal controls across 21 cancer types [36]. Using WES genomic analysis, nearly all known cancer genes in these tumor types could be identified. They used rigorous statistical methods to estimate that near-saturation could be achieved with 600-5,000 samples per tumor type, depending on background mutation frequency. For human OS with an average of 1.2 mutations per megabase, approximately 1,000 samples are needed to obtain a complete catalog of cancer driver genes (at least those of intermediate frequency). This is a tremendous challenge due to the rarity of OS. The International Cancer Genome Consortium (ICGC, see <http://www.icgc.org/home>) has proposed to sequence 250 OS samples, and the TARGET OS project, through a multi-center collaboration, has been characterizing 100 OS samples using a variety of methods, including WGS and WES (<https://ocg.cancer.gov/programs/target/projects/osteosarcoma>). So, there is still much work that needs to be done to generate a comprehensive catalog of candidate driver genes in human OS.

Comprehensive sequencing efforts over the past decade have revealed the genomic landscapes of common forms of human cancer [34, 36, 46]. A given cancer type consists of a small number of driver genes that are mutated at high frequency (>20%). These highly mutated genes have been termed “mountains” [34]. However, there are many more driver genes that are found mutated at intermediate frequencies of 2-20% and lower frequencies of <2% (so called “hills” and “tails”, respectively) [38]. A mountain in one type of cancer can be either a hill or a tail in another type, in line with evidence showing that the same driver gene can be repeatedly “re-discovered” with different frequencies in distinct tumor types. Most of the mountains and a portion of hills detected through GWS have been previously

identified through low-resolution genome-wide screens using biological assays for transforming activity of whole cancer cell DNA, or through targeted mutational screens guided by biologically well-informed guesswork to find gene mutations in the germ line or in somatic cells [11]. How many more genes containing germline or somatic driver mutations have yet to be discovered? Studies of common forms of cancer suggest that a plateau is being reached [34]. For example, Nik-Zainal et al. performed WGS for 560 breast cancers and detected a total of 93 cancer driver genes, and 90% of them overlapped with the 595 known driver genes {Nik-Zainal, 2016 #585}. Among the 90%, only five cancer genes had prior absent or equivocal evidence. Nik-Zainal et al. also found that dominantly active fusion genes and non-coding driver mutations seem to be rare, although additional infrequently mutated cancer genes probably exist. They therefore concluded that the substantial majority of driver mutations include these genes; the mountains and hills in breast cancer are now known. OS has a somatic mutation rate similar to that of breast cancer (1.2 per million megabases). Thus, it is conceivable that a near complete catalog of OS contains approximately 100 driver genes, with most of them likely overlapping with the 595 known drivers.

OS mountain driver genes including *p53*, *Rb*, *CDKN2A* and *PTEN* have been identified by prior targeted investigations, as well as through current unbiased GWS [28-33, 47-50]. The genes listed in Table 2 are good candidates for mountain or hill drivers. They completely overlap with the 595 known drivers, except for *DLG2*. OS mountains are nearing saturation, but hills and tails will certainly be revealed as GWS continues. Ultimately, *DLG2* or other candidate hill and tail driver genes will need to be validated by biological assays. A recent study implied that multiple oncogenic pathways drive chromosomal instability during OS progression and result in the acquisition of *BRCA1/2*-deficient traits [30]. Kovac et al. found a pattern of base substitution signatures in a set of OS cases similar to base substitution signatures 3 and 5 of breast cancer. Their presence is strongly associated with rearrangement signatures 1, 3, and 5 within breast cancer [46]. Cancers exhibiting rearrangement signature 1 frequently show *TP53* mutations, enrichment for base substitution signature 3, and a high homologous recombination deficiency (HRD) index. Those with rearrangement signatures 3 and 5 were strongly associated with the presence of *BRCA1/2* mutations or *BRCA1* promoter hyper-methylation. The significance of *BRCA1/2*-deficient traits in therapeutic applications is discussed further in Section 4. The status of mutation signatures and *BRCA1* promoter hyper-methylation need to be investigated in future OS genomic studies.

The majority of OS samples subjected to GWS so far are of pediatric origin, and the subtypes are largely osteoblastic and chondroblastic OS, with a few cases of fibroblastic and telangiectatic OS [28, 30-32]. Candidate driver mutations responsible for adult OS and other subtypes have been reported, but their specific roles as drivers are not well understood. On one hand, germline and somatic mutations in sequestosome 1 (*SQSTM1*) are not found in GWS samples of OS. However, mutations in *SQSTM1* frequently exist in patients with PDB, which is characterized by extensive bone remodeling with enlarged and weakened bone tissue, affecting mostly individuals >50 years of age [51]. PDB is the second most common metabolic bone disease after osteoporosis, and about 1% of affected individuals will subsequently develop OS [52, 53]. One remaining question is whether *SQSTM1* can serve as a driver for PDB-induced OS. Other novel fusion drivers have not been reported in

those sequencing studies, but some have been found in small cell OS, a subtype of human OS, including EWSR1-CREB3L1, LRP1-SNRNP25, and KCNMB4-CCND3 [54, 55]. On the other hand, GNAS, MDM2, and CDK4 (Table 2) have been frequently reported as driver genes in parosteal osteosarcoma, which is characterized by ring chromosomes [56, 57]. In the future, sufficient WGS data accumulated from each individual subtype will help define whether subtype-specific cancer driver genes exist, and their frequencies. This information will increase our understanding of the genomic landscape for each subtype of OS and eventually guide clinicians to differentially treat individual patients.

The cancer genetic landscape may not be wholly complete until we fully understand the significance of yet another type of driver gene, called Epi-driver genes. These genes are not frequently mutated, but instead have epigenetic modifications which confer selective growth advantage to the tumor [34]. Epi-driver genes are expressed aberrantly in tumors due to changes in DNA methylation or chromatin modification and persist as the tumor cells divide. It is not known how many Epi-drivers exist or what their role is as drivers of tumorigenesis. In the context of OS, Wnt inhibitory factor 1 (WIF1) may be considered as an Epi-driver gene because its aberrant expression is regulated by epigenetic modification [58, 59]. Detailed studies on OS-specific epigenetic mechanisms, miRNA regulation, proteomics, non-coding RNAs, and expression profiling have been recently reviewed elsewhere [60-64]. In contrast to point mutations in coding regions, our ability to discover and understand other types of drivers such as Epi-drivers is still limited. Many other important cancer drivers may be lurking in places that we cannot easily interrogate [38]. These include copy number alterations, chromosomal rearrangements, and drivers found in noncoding regions. Further research on Epi-drivers and other unknown drivers will be essential [34]. In summary, while the field of OS genomics has made significant progress, further exploration and WGS analysis of OS patient samples will be required to complete our understanding of the molecular basis of the disease.

3. Understanding the role of driver gene mutations in the development of osteosarcoma

3.1 New driver genes identified in forward and reverse genetics studies

Driver mutations in the *p53* gene have been detected in 65-90% of pediatric patients with OS [28, 30, 32]. The role of *p53* as a triggering factor in the initiation of OS has been confirmed by studies in GEMMs through a full spectrum of mutations. These include germline deletion of one or both alleles (*p53*^{+/-} or *p53*^{-/-}), osteoblast-specific deletion of one or both alleles using the Cre-LoxP system (*Cre*⁺*p53* flox/⁺ or *Cre*⁺*p53* flox/flox), and introduction of known point mutations (*p53* R172H or R270H) which are prevalent in human OS patients with Li-Fraumeni syndrome [13, 14, 18, 19, 22, 65, 66]. To identify new driver genes that contribute to OS development, Moriarity et al. performed a Sleeping Beauty transposon-based forward genetic screen in mice with or without the *p53* R270H mutation [19]. In total, they identified 191 candidate genes that may depend on *p53* mutations for OS development. Notably, they also found 65 candidate genes that can drive tumor formation without *p53* somatic mutations. Among them, *Sema4d* and *Sema6d* were functionally validated as oncogenes in human OS [19]. In addition, 33 newly identified cancer driver genes overlap

with the 595 human consensus driver genes, and many are components of the ErbB, PI3K-AKT-mTOR and MAPK signaling pathways. After functional validation, they will be valuable future candidates with which to generate new mouse models, and for development of new therapeutic targets.

Over the past several decades, a handful of new driver genes have been identified through reverse genetic studies in mice, including *Prkar1a*, *Wif1*, *Brca2*, *Apc*, *Twist1*, *Nf2*, *Notch1*, *FOS*, *Pten*, and *Ptch1* [16, 19, 58, 67-72]. Before these GEMMs were generated, somatic driver mutations for these genes in human OS had not been reported, however, most of them are consensus genes in other common forms of human cancer. For example, somatic mutations in components of the Notch signaling pathway had not been discovered in any mesenchyme-derived rare cancers such as OS, until recent GWS and forward genetic screens in mice identified mutations in *FBXW7*, *MAML2*, *Notch1*, *Notch 2*, *Notch3* and *Notch4* [19, 28-33]. Tao et al. generated a mouse model of OS (cNICD mice), and found that expression of an activating truncated form of the Notch1 receptor in osteoblasts was sufficient to drive OS development [73]. Notably, cNICD mice developed severe osteosclerotic lesions and showed increased bone remodeling before OS presented [74]. On one hand, this type of pre-cancerous bone lesion has so far only been reported in GEMMs based on mutations in genes such as *Notch1*, *Apc*, *Prkar1a*, and *Ptch1*, but not in mice with *p53* mutations [16, 67, 68, 72]. This implies that a distinct mutational process may be characterized by driver mutations typically arising from genes involved in evolutionarily conserved signaling pathways. It is conceivable that tumors derived from this group of GEMMs mimic human OS in patients with a bone-lesion condition such as PDB. On the other hand, through whole-transcriptome sequence and pathway analysis, Tao et al. also found that two types of tumors (Notch-driven and *p53*-driven) share several canonical cancer genetic pathways, including Wnt, PI3K/mTOR, and *p53*, as well as BRCA1 in DNA damage response [16]. It is noteworthy to point out that driver mutations in the PI3K/mTOR and BRCA1 pathways have recently been detected in GWS studies of human OS [30, 32].

3.2 Number of driver genes required to develop osteosarcoma

Decades of functional studies, as well as recent genome sequencing efforts have revealed there is a limited number, perhaps between two and eight, of large-effect driver genes in most tumors [34, 75]. In any given cell type, these drivers form a synergistic team promoting tumorigenesis and clone generation. There may be a larger number of small-effect drivers that help expand the clone, but by definition they do not contribute to tumor initiation and to most of the tumorigenic phenotype. The contribution and functional roles of large-effect driver genes in OS development have been investigated in GEMMs. A driver that can trigger the bone tumorigenic process is classified as a first driver. The group of first drivers includes *p53*, *Notch1*, *Myc*, *Fos*, *Nf2*, *Wif1*, *Brca2*, *Apc*, *Ptch1*, and *Prkar1a*. The strength and nature of each driver is distinct. For example, *p53* and *Notch* can drive OS formation with complete penetrance, whereas *Wif1* and *Brca2* can induce OS in just a small percentage of mice [16, 17, 58, 70]. Furthermore, *Apc*, *Ptch1*, and *Prkar1a* can only induce benign or low-grade malignant bone tumors, but not advanced OS [67, 68, 72]. A driver that cannot trigger the bone tumorigenic process is classified as a synergistic driver. A synergistic driver must team up with a first driver in order to accelerate tumor initiation and growth, but it may exist as a

germline mutation before a first driver arises from a somatic mutation. The group of synergistic drivers includes *Rb1*, *Twist*, *Pten*, and *Jun*. For example, 56% of OS patients have both TP53 and RB1 inactivation mutations [32]. Consistent with this, although inactivation of Rb1 alone in mice could not induce formation of bone tumors, the synergistic pairing of p53/Rb1 mutations shortened the average latency of malignant OS from 292 to 128 days (*Osx-Cre⁺p53^{fl/fl}* vs. *Osx-Cre⁺p53^{fl/fl}pRb^{fl/fl}* mice) [17]. Notably, two or more first drivers can also form a team to accelerate tumorigenesis. This is exemplified by the synergistic p53/Notch pair, which shortened the average latency of OS from 346 to 154 days in mice (*Col1a1 2.3kb Cre⁺p53^{fl/fl}* vs. *Col1a1 2.3kb Cre⁺p53^{fl/fl}Notch ICD⁺* mice) [16]. In this case, Notch may take on the role of a synergistic driver, and vice versa. Recently, a study of the synergistic combination of three drivers showed that mice with mutations in p53, Rb1, and Prkar1a (*Col1a1 2.3kb Cre⁺p53^{fl/fl}pRb1^{fl/fl}Prkar1a^{fl/+}* or *Rb^{fl/fl}p53^{fl/fl}Prkar1a^{+/OB}*) developed tumors within 44 days and lived no longer than 77 days (an average of 63 days). This was a significantly shorter latency compared to that seen in mice with mutations in just two of these drivers, p53 and Rb1 (*Col1a1 2.3kb Cre⁺p53^{fl/fl}pRb1^{fl/fl}* or *Rb^{fl/fl}p53^{fl/fl}*) (140 to 267 days, with an average of 203 days) [24]. Thus, the number of drivers significantly affects the latency of tumor development; as exemplified here, from one driver (346 days in *p53^{fl/fl}*), to two drivers (203 days in *Rb^{fl/fl}p53^{fl/fl}*), to three drivers (63 days in *Rb^{fl/fl}p53^{fl/fl}Prkar1a^{+/OB}*). Here we need to point out that the genetic mouse model described by Chen et al. was not originally designed to prove that three mutations of p53, Rb and Prkar1a are actually seen in the pediatric OS patients. Lower expression of PRKAR1A has been associated with the response to chemotherapy in a study of 54 human OS patients, but somatic mutations of Prkar1a or Prkar1b have only recently been found in a limited number of OS pediatric patients [30, 32, 72]. Therefore, it remains to be determined whether these three mutations co-exist in the same tumor of human OS pediatric patients. However, it is conceivable that the “three drivers” model we propose in this review more closely mimics the development of aggressive OS since the majority of the GEMMs generated so far have a much longer latency. Furthermore, a recent study demonstrated that three sequential mutations in *p53*, *Kras*, and *Myc* can convert wild-type porcine mesenchymal stem cells (MSCs) into sarcoma cancer cells, mimicking key molecular aspects of human sarcomagenesis [12]. Our proposed model correlating the number of drivers and tumor latency is also in line with a mathematic model generated by epidemiology studies of a large colon cancer cohort, which found that only three driver gene mutations are required for the development of lung and colorectal cancers [76].

Undoubtedly, besides affecting onset, latency, and survival time, both the number and nature of drivers can significantly shape other features of OS development. Histologically, human conventional OS can be sub-classified as: osteoblastic (50%), which has a variable degree of mineralization and is characterized by abundant production of osteoid matrix; chondroblastic (25%), which presents a variable degree of chondroid areas admixed with malignant spindle cells and is characterized by production of cartilaginous matrix; or fibroblastic (25%), which comprises high-grade spindle cells or undifferentiated high-grade pleomorphic cells [77]. The specific driver mutations for each subtype of human OS are not well understood, but genetic studies of GEMMs on driver mutations may provide additional clues. In mouse

models (Figure 1), osteoblastic OS can be efficiently initiated by one driver, such as *p53* (loss of two alleles) [17], or by two drivers, such as the *Ptch1* (loss of one allele) and *p53* (loss of one allele) with about 70% penetrance [67]. Fibroblastic OS can be efficiently initiated by one driver, such as *Notch* [16], or by two drivers, such as *p53* (loss of two alleles) and *Rb1* (loss of two alleles) [17, 21, 23]. In the latter case, the *Rb1* driver not only accelerates tumor development, but also switches tumor subtype from osteoblastic to fibroblastic. Notably, *Rb1* regulates fate choice and lineage commitment in MSCs and MSC lineage cells, which explains the observation in mice, that loss of *p53* and *Rb1* (*Osx-Cre⁺p53^{fl/fl}pRb^{fl/fl}* and *Prx1-Cre⁺p53^{fl/fl}pRb^{fl/fl}*) also causes a high percentage of other tumor types such as hibernomas and rhabdomyosarcomas [17, 21, 23, 78]. To examine whether the cell of origin contributes to the final differentiation state in transformed OS cells, Quist et al. removed *p53* and *Rb1* using *Prx1-Cre*, *Col1a1 2.3kb Cre*, and *OC-Cre* constructs and found that the differentiation state of tumors did not correlate with the differentiation state of the cells' lineage of origin [23]. They suggested that the final differentiation state of an OS cell (e.g., extent of mineralization and osteoid matrix production) may instead be influenced by the silencing of epigenetic regulators such as DNA methyl transferases. They also found similar latency periods in osteosarcomagenesis in the three groups of mice, which reinforces the concept that the number of drivers plays a critical role in OS initiation and progression. Furthermore, chondroblastic OS can be induced by either one driver such as *Fos*, two drivers (*Apc* and *Twist*), or three drivers (*p53*, *Rb1*, and *Prkar1a*) (Figure 1) [24, 68, 69]. However, tumors in mice induced from the same set of initiating drivers sometimes manifest as fibroblastic, chondroblastic, or osteoblastic OS, as well as mixtures of these subtypes. This raises the question of whether an additional driver is needed to promote a specific subtype such as chondroblastic OS.

The conclusion that virtually all of the mutations in metastatic lesions are already present in a large number of primary tumor cells helps our understanding of the role initiating drivers play in metastasis, which is responsible for most cancer patient deaths [34]. Irrespective of the starting number of drivers, OS displays similar incidences of metastases (32% in *Osx-Cre⁺p53^{fl/fl}* vs. 37% in *Osx-Cre⁺p53^{fl/fl}pRb^{fl/fl}* mice) [21]. How the nature of a driver affects metastatic formation is a different story. Zhao et al. observed that 31% of *Cre⁺F/+* mice (*Col2.3-Cre⁺p53^{fl/+}*) exhibited metastatic lesions compared with 55% for *Cre⁺R/+* mice (*Col2.3-Cre⁺p53^{R172H/+}*) [18]. In addition, the *p53* point mutation was associated with earlier onset and a higher metastatic rate in human OS than the null mutation [66]. They further observed downregulation of gene expression of the naked cuticle homolog 2 (*NKD2*), a negative regulator of Wnt signaling, in metastatic OS. This implies that the *R172H* mutation (corresponding to a hotspot for the human *R175H* mutation) may promote increased Wnt pathway signaling and increase the metastatic potential of the cells. This indicates that a driver-dependent signaling pathway may be a useful vulnerability in OS that might be exploited for a targeted therapy. In another study, Mutasaers et al. observed that 70% of shRNA mice (*Osx-Cre⁺p53.1224⁺pRb^{fl/fl}*) had metastatic disease compared to 29% of *Cre:lox* animals (*Osx-Cre⁺p53^{fl/fl}pRb^{fl/fl}*) [20]. The strength of the *p53* driver (or *p53* dosage) was controlled through shRNA-based knockdown of *p53*, which significantly impacted metastatic potential in the shRNA mice. In addition, the nature of a driver also influences the mutational process of chromosomal rearrangements. For example, *Notch*-

induced OS seldom presents small chromosomes or fragments, which are frequently detected in p53-induced OS [16]. Taken together, this suggests that the nature, but not the number, of driver mutations, strongly influences the state of OS metastasis.

GEMMs also help our understanding of mutations co-existing in both normal and tumor cells. We found that deletion of Recombination Signal Binding Protein for Immunoglobulin Kappa J Region (Rbpj), a transcription factor in the Notch pathway, completely blocked tumor formation in the cNICD mice, but rarely affected tumor formation in the p53 mutant mice [16]. This confirms the concept that mutations in various components of a single pathway are mutually exclusive and do not occur in the same tumor [34]. In the context of p53 mutant tumor, the Rbpj deletion is a passenger mutation, which also suggests that the Rbpj-dependent Notch canonical pathway is not indispensable for the tumor initiation process [16]. However, this does not rule out the possibility that p53-induced OS uses the Notch signaling pathway at later stages of tumor progression, such as invasion and metastasis, or angiogenesis induction, two hallmarks of cancer [79, 80]. In breast cancer, the Notch signaling pathway is critical for spreading tumor cells to the bone [80]. Likewise, mutations in the *RECQL4* gene have long been recognized as responsible for OS in patients with autosomal recessive Rothmund-Thomson Syndrome (RTS), a third familial cancer syndrome in addition to Li-Fraumeni and hereditary retinoblastoma syndrome (Table 1) [81]. Complete loss of function of Recql4 in mice leads to embryonic death, which implies that the mutated *RECQL4* gene in RTS patients may have enough residual activity to maintain cell survival [82]. In skeletal cells, complete loss of function of Recql4 leads to developmental bone abnormalities and adult osteoporosis, but does not induce the development of OS [83, 84]. Lu et al. suggested that *RECQL4* is critical for skeletal development by modulating p53 activity *in vivo* [83]. Ng et al. further suggested that mutant, not null, alleles of *RECQL4* may account for tumor suppression and susceptibility for OS [84]. These mouse data are indeed consistent with a previous proposal based on clinical studies, that there are two types of RTS: Type II, which presents OS features and Type I, which does not [81]. Further investigation is needed to determine how Recql4 genetically interacts with p53, and which type of *RECQL4* mutations can cause OS. Overall, our understanding of the molecular basis of OS development has dramatically improved by using a cross-species approach, especially through GEMMs. Other publications on modeling OS using mouse, rat, dog, pig, and zebrafish exist, but will not be described in detail in this review [12, 26, 27, 85-88].

4. Driver-dependent genetic vulnerabilities as targets of osteosarcoma therapy

Like a double-edged sword, a driver gene not only confers a growth advantage, but also creates vulnerabilities in cancer cells. These may derive from the driver itself, from altered pathways that the driver participates in, or from unique cancer cell traits that normal cells lack. A leading paradigm for driver-dependent targeted cancer therapy is the use of imatinib (a small molecule tyrosine kinase inhibitor) to turn off the mutated KIT driver gene that produces a constitutively activated form of tyrosine kinase in patients with gastrointestinal stromal tumor (GIST). In an open-label phase II clinical trial, imatinib induced a complete

or partial response in 80 to 90% of patients with unresectable or disseminated disease [89]. Similar to OS, GIST is a rare type of mesenchyme-derived cancer, but it is the most common sarcoma of the intestinal tract known to be refractory to chemotherapy. Most of the clinically approved drugs that target genetically altered genes are directed against kinases. Kinases have been extensively studied at the biochemical, structural, and physiologic levels; therefore, they are relatively easy to be targeted with small molecule inhibitors [34]. Recently, the PI3K/mTOR pathway, a kinase-enriched pathway, has been identified as a common vulnerability for therapeutic exploitation in the context of OS [32]. Perry et al. found that 24% of OS patients had genetically altered genes in this pathway. Interestingly, multiple genes were also identified by a forward genetic mutation screen [19]. Some of the mutated genes in this pathway are listed in Table 2 and highlighted in Figure 2. Coincidentally, dysregulation of most genes in the PI3K/mTOR pathway has also been observed in whole-transcriptome sequencing of p53-driven and Notch-driven OS (Figure 2) [16]. This further suggests that PI3K/mTOR pathway kinases possessing mutations and/or high expression levels will be good targets for small molecule inhibitors. Indeed, a phase II clinical trial has yielded encouraging data; in 54 patients with metastatic or unresectable bone sarcomas, treatment with ridaforolimus (a small molecule inhibitor of mTOR kinase) led to a confirmed partial response for three patients and clinical benefit response (i.e. complete or partial response, or stable disease for >16 weeks) for 17 patients [90]. An ongoing phase II clinical trial with sirolimus (also known as rapamycin, inhibitor of mTORC1) in combination with chemotherapy will provide additional information (ClinicalTrials.gov Identifier: NCT02574728). However, since only a fraction of OS patients have mutations in the PI3K/mTOR pathway, introducing treatments with mTOR inhibitors in patients who lack these mutations may not prove beneficial. Besides mTOR inhibitors, Campbell et al. applied parallel small interfering RNA (siRNA) screens to identify the kinase dependencies of 117 cancer cell lines derived from ten cancer types, including OS, and demonstrated an increased sensitivity of OS cell lines to fibroblast growth factor receptor (FGFR) inhibitors [91]. Recent clinical trials for OS have tested multiple small molecule inhibitors and monoclonal antibodies targeting different genes and pathways, including ERBB2, IGF1R, EGFR, PDGFR, VEGFR, KIT, FGFR, SRC, RANKL, AURKA, HDACs, WNT/beta-catenin, Notch, and Hedgehog. The functions of most of these molecules and pathways in bone development and disease have been studied in detail [73, 92-100]. Results from these completed trials, as well as from studies evaluating other types of treatments such as immunotherapy, have been reviewed recently and will not be described here [9, 101-105]. In general, the findings from these targeted therapy trials have been unsatisfactory. One possible reason could lie in the approach of using those targeting agents without knowing whether the target cells have activating mutations. Thus, in the clinic, the status of driver mutations in OS should be considered before administering such drugs to patients.

The biggest vulnerability in OS is the *p53* driver. With more than half of all human tumors carrying mutations in this gene, therapeutic targeting of p53-mutant tumors is one of the major goals of cancer research. Intense efforts have been made towards the development of drugs that can activate or restore the p53 pathway. Several are now undergoing clinical evaluation, with promising results for two types of small molecule drugs: nutlin-like and

APR-246 [106]. Nutlin-like restores wild-type p53 protein function by blocking the interaction of p53 with MDM2, which negatively regulates p53 by inducing p53 protein degradation. Nutlin may thus be an effective drug to treat OS with an intact p53 gene and high levels of MDM2 expression such as small cell OS. APR-246 promotes the formation of covalent adducts on mutant p53 R175H and p53 R273H proteins, frequently found in human OS, and restores p53 activity [106]. GEMMs with p53 R172H or p53 R270H mutations (corresponding to the human R175H or R273H mutation) will be suitable animal models for preclinical trials of APR-246. However, nutlin-like and APR-246 are not suitable for other types of p53 mutations such as truncated p53 proteins, delta133p53 and delta160p53. These two mutations are caused by TP53 intron 1 hotspot rearrangements, which are specific to sporadic OS and can cause Li-Fraumeni syndrome [28, 107].

The BRCA-like trait or 'BRCAness' is a unique cancer trait that seems to be linked to genetic vulnerability in OS. Kovac et al. found that more than 80% of cases of OS exhibit a mutation signature similar to that of breast cancer, with BRCA1/2 inactivation in 91% (112/123) and 78% (96/123) of OS tumors, respectively [30]. BRCAness is a phenocopy of the BRCA1 or BRCA2 mutation; it describes the situation in which a homologous recombination repair (HRR) defect exists in a tumor in the absence of a germline BRCA1 or BRCA2 mutation [108]. In OS, mutations in different 'BRCA' genes (67 binding partners in total) such as PTEN and ATM can be functionally equivalent analogues of BRCA1/2 mutations, causing HRR deficiencies that result in chromosomal instability and the polyclonal nature of OS [30]. Tumors with BRCAness may also respond to similar therapeutic approaches as BRCA-mutant tumors [108]. This suggests that a high percentage of OS tumors may be HRR-deficient and therefore be vulnerable to additional DNA damage caused by double-strand breaks (DSBs). ADP ribose polymerase (PARP1) is an important enzyme that repairs DNA single strand breaks (SSBs). Since cancer cells divide more frequently and have more SSBs than normal cells, inhibiting PARP1 causes SSBs to accumulate and become DSBs. Therefore, inhibition of PARP1 by PARP inhibitors leads to more DSBs, resulting in the death of cells with BRCAness. Olaparib, the first PARP inhibitor licensed in 2015, is effective against ovarian and breast tumors with known BRCA mutations. Multiple clinical trials of olaparib are now recruiting patients with refractory or advanced solid tumors, including bone tumors (ClinicalTrials.gov Identifier: NCT02511795; NCT02398058; NCT02338622; NCT01894243).

In contrast to cancer HRR-defective cells that are susceptible to PARP inhibition, cancer cells with a highly active ALT pathway are HRR-proficient [109]. Recently, several studies found that ALT activity is present in 85% of OS samples (12 of 14 tumors) and that ATRX is mutated in 10-50% of tumors [28, 30, 32]. ATRX is part of a multiprotein complex that regulates telomere maintenance; loss of the chromatin-remodeling protein ATRX is associated with ALT in cancer [109]. ALT is used in approximately 5% of all human cancers, but it is prevalent in specific cancer types, including OS. Although there are no therapies that specifically target ALT, its reliance on recombination raises the possibility that recombination might be a vulnerability of ALT-positive cancers that could be exploited for targeted therapy. Using OS cells including SJSA1, U2OS, MG63, and SAOS2, Flynn et al. showed that loss of ATRX leads to the formation of a recombinogenic nucleoprotein complex consisting of ATR, replication protein A, and TERRA. Inhibition of ATR, a

specific protein kinase that is essential for ALT, disrupts ALT and triggers chromosome fragmentation and apoptosis in ALT-positive cells. Cell death induced by ATR inhibitors is highly selective for cancer cells that rely on ALT, suggesting that such inhibitors may be useful for treatment of ALT-positive cancers [109]. Currently, two highly selective and potent ATR inhibitors, AZD6738 and VX-970, are in phase I clinical trials. They are being used either as a monotherapy or paired with a variety of genotoxic chemotherapies for the treatment of solid tumors and refractory cancer [110]. These trials will provide important insights into the potential role of inhibiting ATR in the treatment of human cancers, including OS.

A genetic pathway that is required by cancer cells but spares normal cells is undoubtedly a target worth considering for OS therapy. Recently, Walia et al. demonstrated that normal osteoblasts survive depletion of both parathyroid hormone-related protein (PTHrP) and CREB1, whereas osteoblasts lacking p53 in OS depend on the continuous activation of the PTHrP-cAMP-CREB1 pathway. In the absence of PTHrP or CREB1, OS cells undergo proliferation arrest and apoptosis [111]. PTHrP and PTH are crucial regulators of bone formation and remodeling, and administration of high-dose human PTH increased incidence of OS in rats [112, 113]. GEMM studies, genomic analysis, and genome-wide association studies (GWAS) of OS have identified several candidate driver genes involved in this pathway, including *GRM4*, *RANK*, *GNAS*, and *Prkar1a* [28, 72, 114-116]. Is there a way to block this pathway to halt p53-induced OS growth? Most recently, Chen et al. established receptor activator of nuclear factor kappa-B ligand (RANKL) as a therapeutic target for the suppression and prevention of OS [24]. They first showed that GEMMs containing three drivers (Rb^{-/-} OB p53^{-/-} OB pPrkar1a^{+/-} OB) developed aggressive OS tumors characterized by protein kinase A, RANKL, and osteoclast hyperactivity. The three drivers “therapy cohort” (i.e. mice bearing tumors) injected with RANK-Fc (a recombinant protein containing the murine extracellular domain of RANK fused to the Fc portion of murine immunoglobulin G1), which binds to RANKL and blocks its function, had a 50% extension in lifespan (average survival increased from 8.9 weeks to 13.3 weeks). In the “prevention cohort” (i.e. mice with no tumors) injected with RANK-Fc, the three drivers mice showed no evidence of OS during the 20-week treatment period. In less aggressive GEMMs harboring a genetic deletion of RANKL or undergoing treatment with RANK-Fc, they observed a marked suppression of OS development for more than 40 weeks, suggesting that RANKL is required for OS development. Denosumab, an antibody against RANKL, is currently being used in a phase II trial study for the treatment of patients with recurrent or refractory OS (ClinicalTrials.gov Identifier: NCT02470091). Together, these data provide a strong rationale for blocking RANKL in the treatment and prevention of aggressive RANKL-overexpressing OS in humans, including heritable cancers in patients with Li-Fraumeni syndrome. One of the questions remaining is why tumor cells cannot continue to grow in osteopetrotic bone caused by RANKL blocking? This implies that the bone-remodeling microenvironment maintained by RANKL-stimulated osteoclasts is required for tumor expansion. A recent study of an osteogenic niche that promotes early-stage bone colonization of cancer cells residing in bone may provide an important clue [117]. Another question is how long can cancer cells be suppressed before they evade RANKL inhibition? These questions certainly warrant further investigation.

5. Conclusions and perspectives

Molecular genetic studies of OS have contributed to the enormous progress in the field over the past few decades. This review highlights recent findings from OS genomic analysis and mouse genetic studies that have not only shaped our new understanding of the role of driver gene mutations in OS development, but have also set the foundation for the next generation of molecularly targeted therapies. OS genomics is emerging from its first phase, but further analysis of whole-genome sequences from patients with different OS subtypes will be required to complete the catalog of driver genes and mutation signatures and eventually fulfill our understanding of the genetic basis of the disease. Even now, genome-based medicine in OS should be sufficient to guide clinical trials for treating tumors that contain targetable driver genes, as well as to improve standard treatment for relieving the side effects of chemotherapy. The nature and number of driver genes profoundly impact the onset, latency, survival time, aggressiveness, and metastatic potential in the development of OS. However, our understanding of the roles driver genes play in the genesis of the disease is in its infancy. Additionally, the role of driver genes in maintenance of OS stem cells, microRNA regulation, epigenetic mechanisms, heterotypic interactions between cancer cells and normal cells, tumor-promoting inflammation, reprogramming of metabolism, and tumor evasion from immune destruction is still largely unknown. The continuous development of new GEMMs and large animal models will help identify new driver genes and elucidate their roles, but will also establish a platform for preclinical tests using new therapeutic strategies and drugs. We may yet win the war on cancer by attacking the genetic vulnerabilities that driver genes confer to cells. We are currently far from victory, but precision medicine and drug combination therapy are promising. Going forward, additional efforts should be directed at “defense”, through cancer prevention (e.g. seeking genetic counseling prior to starting a family for patients with familial cancer predisposition disorders such as Li-Fraumeni syndrome) and early detection (e.g. in patients with Paget disease of the bone). Increasing our knowledge obtained from cancer genome and molecular genetic studies will help reduce morbidity and mortality of all bone cancers, including OS.

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References

1. American Cancer Society. Cancer Facts & Figures. 2016.
2. Unni, KK., Inwards, CY. Dahlin's Bone Tumors: General Aspects and Data on 10,165 Cases. 6. Philadelphia: Lippincott Williams & Wilkins; 2009.
3. Savage SA, Mirabello L. Using epidemiology and genomics to understand osteosarcoma etiology. *Sarcoma*. 2011; 2011:548151. [PubMed: 21437228]
4. Ottaviani G, Jaffe N. The etiology of osteosarcoma. *Cancer Treat Res*. 2009; 152:15–32. [PubMed: 20213384]
5. Tao, J., Bae, Y., Wang, LL., Lee, B. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. John Wiley & Sons, Inc; 2013. Osteogenic Osteosarcoma; p. 702-10.

6. Martin JW, Squire JA, Zielenska M. The genetics of osteosarcoma. *Sarcoma*. 2012; 2012:627254. [PubMed: 22685381]
7. Gorlick R, Khanna C. Osteosarcoma. *J Bone Miner Res*. 2010; 25:683–91. [PubMed: 20205169]
8. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: Current Treatment and a Collaborative Pathway to Success. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015; 33:3029–35. [PubMed: 26304877]
9. Kansara M, Teng MW, Smyth MJ, Thomas DM. Translational biology of osteosarcoma. *Nature reviews Cancer*. 2014; 14:722–35. [PubMed: 25319867]
10. Morrow JJ, Khanna C. Osteosarcoma Genetics and Epigenetics: Emerging Biology and Candidate Therapies. *Crit Rev Oncog*. 2015; 20:173–97. [PubMed: 26349415]
11. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. *Nature*. 2009; 458:719–24. [PubMed: 19360079]
12. Saalfrank A, Janssen KP, Ravon M, Flisikowski K, Eser S, Steiger K, et al. A porcine model of osteosarcoma. *Oncogenesis*. 2016; 5:e210. [PubMed: 26974205]
13. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Butel JS, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*. 1992; 356:215–21. [PubMed: 1552940]
14. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, et al. Tumor spectrum analysis in p53-mutant mice. *Curr Biol*. 1994; 4:1–7. [PubMed: 7922305]
15. Lin PP, Pandey MK, Jin F, Raymond AK, Akiyama H, Lozano G. Targeted mutation of p53 and Rb in mesenchymal cells of the limb bud produces sarcomas in mice. *Carcinogenesis*. 2009; 30:1789–95. [PubMed: 19635748]
16. Tao J, Jiang MM, Jiang L, Salvo JS, Zeng HC, Dawson B, et al. Notch activation as a driver of osteogenic sarcoma. *Cancer Cell*. 2014; 26:390–401. [PubMed: 25203324]
17. Walkley CR, Qudsi R, Sankaran VG, Perry JA, Gostissa M, Roth SI, et al. Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease. *Genes and Development*. 2008; 22:1662–76. [PubMed: 18559481]
18. Zhao S, Kurenbekova L, Gao Y, Roos A, Creighton CJ, Rao P, et al. NKD2, a negative regulator of Wnt signaling, suppresses tumor growth and metastasis in osteosarcoma. *Oncogene*. 2015; 34:5069–79. [PubMed: 25579177]
19. Moriarity BS, Otto GM, Rahrmann EP, Rathe SK, Wolf NK, Weg MT, et al. A Sleeping Beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and metastasis. *Nat Genet*. 2015; 47:615–24. [PubMed: 25961939]
20. Mutsaers AJ, Ng AJ, Baker EK, Russell MR, Chalk AM, Wall M, et al. Modeling distinct osteosarcoma subtypes in vivo using Cre:lox and lineage-restricted transgenic shRNA. *Bone*. 2013; 55:166–78. [PubMed: 23486187]
21. Berman SD, Calo E, Landman AS, Danielian PS, Miller ES, West JC, et al. Metastatic osteosarcoma induced by inactivation of Rb and p53 in the osteoblast lineage. *Proc Natl Acad Sci U S A*. 2008; 105:11851–6. [PubMed: 18697945]
22. Lengner CJ, Steinman HA, Gagnon J, Smith TW, Henderson JE, Kream BE, et al. Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling. *The Journal of Cell Biology*. 2006; 172:909–21. [PubMed: 16533949]
23. Quist T, Jin H, Zhu JF, Smith-Fry K, Capecchi MR, Jones KB. The impact of osteoblastic differentiation on osteosarcomagenesis in the mouse. *Oncogene*. 2015; 34:4278–84. [PubMed: 25347737]
24. Chen Y, Di Grappa MA, Molyneux SD, McKee TD, Waterhouse P, Penninger JM, et al. RANKL blockade prevents and treats aggressive osteosarcomas. *Science translational medicine*. 2015; 7:317ra197.
25. Janeway KA, Walkley CR. Modeling human osteosarcoma in the mouse: From bedside to bench. *Bone*. 2010; 47:859–65. [PubMed: 20696288]
26. Jones KB. Osteosarcomagenesis: modeling cancer initiation in the mouse. *Sarcoma*. 2011; 2011:694136. [PubMed: 21403899]
27. Guijarro MV, Ghivizzani SC, Gibbs CP. Animal models in osteosarcoma. *Front Oncol*. 2014; 4:189. [PubMed: 25101245]

28. Chen X, Bahrami A, Pappo A, Easton J, Dalton J, Hedlund E, et al. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. *Cell Rep.* 2014; 7:104–12. [PubMed: 24703847]
29. Bousquet M, Noirot C, Accadbled F, Sales de Gauzy J, Castex MP, Brousset P, et al. Whole-exome sequencing in osteosarcoma reveals important heterogeneity of genetic alterations. *Ann Oncol.* 2016; 27:738–44. [PubMed: 26787232]
30. Kovac M, Blattmann C, Ribi S, Smida J, Mueller NS, Engert F, et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat Commun.* 2015; 6:8940. [PubMed: 26632267]
31. Joseph CG, Hwang H, Jiao Y, Wood LD, Kinde I, Wu J, et al. Exomic analysis of myxoid liposarcomas, synovial sarcomas, and osteosarcomas. *Genes Chromosomes Cancer.* 2014; 53:15–24. [PubMed: 24190505]
32. Perry JA, Kiezun A, Tonzi P, Van Allen EM, Carter SL, Baca SC, et al. Complementary genomic approaches highlight the PI3K/mTOR pathway as a common vulnerability in osteosarcoma. *Proc Natl Acad Sci U S A.* 2014; 111:E5564–73. [PubMed: 25512523]
33. Reimann E, Koks S, Ho XD, Maasalu K, Martson A. Whole exome sequencing of a single osteosarcoma case--integrative analysis with whole transcriptome RNA-seq data. *Hum Genomics.* 2014; 8:20. [PubMed: 25496518]
34. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science.* 2013; 339:1546–58. [PubMed: 23539594]
35. Brohl AS, Solomon DA, Chang W, Wang J, Song Y, Sindiri S, et al. The genomic landscape of the Ewing Sarcoma family of tumors reveals recurrent STAG2 mutation. *PLoS Genet.* 2014; 10:e1004475. [PubMed: 25010205]
36. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature.* 2014; 505:495–501. [PubMed: 24390350]
37. Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell.* 2011; 144:27–40. [PubMed: 21215367]
38. Garraway LA, Lander ES. Lessons from the cancer genome. *Cell.* 2013; 153:17–37. [PubMed: 23540688]
39. Rausch T, Jones David TW, Zapatka M, Stütz Adrian M, Zichner T, Weischenfeldt J, et al. Genome Sequencing of Pediatric Medulloblastoma Links Catastrophic DNA Rearrangements with TP53 Mutations. *Cell.* 2012; 148:59–71. [PubMed: 22265402]
40. Maciejowski J, Li Y, Bosco N, Campbell Peter J, de Lange T. Chromothripsis and Kataegis Induced by Telomere Crisis. *Cell.* 2015; 163:1641–54. [PubMed: 26687355]
41. Selvarajah S, Yoshimoto M, Park PC, Maire G, Paderova J, Bayani J, et al. The breakage–fusion–bridge (BFB) cycle as a mechanism for generating genetic heterogeneity in osteosarcoma. *Chromosoma.* 2006; 115:459–67. [PubMed: 16897100]
42. Scheel C, Schaefer KL, Jauch A, Keller M, Wai D, Brinkschmidt C, et al. Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcomas. *Oncogene.* 2001; 20:3835–44. [PubMed: 11439347]
43. Morishita M, Muramatsu T, Suto Y, Hirai M, Konishi T, Hayashi S, et al. Chromothripsis-like chromosomal rearrangements induced by ionizing radiation using proton microbeam irradiation system. *Oncotarget.* 2016; 7:10182–92. [PubMed: 26862731]
44. Kansara M, Leong HS, Lin DM, Popkiss S, Pang P, Garsed DW, et al. Immune response to RB1-regulated senescence limits radiation-induced osteosarcoma formation. *The Journal of Clinical Investigation.* 2013; 123:5351–60. [PubMed: 24231354]
45. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, et al. A census of human cancer genes. *Nature reviews Cancer.* 2004; 4:177–83. [PubMed: 14993899]
46. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature.* 2016; 534:47–54. [PubMed: 27135926]

47. Varley JM, McGown G, Thorncroft M, Santibanez-Koref MF, Kelsey AM, Tricker KJ, et al. Germ-line mutations of TP53 in Li-Fraumeni families: an extended study of 39 families. *Cancer Res.* 1997; 57:3245–52. [PubMed: 9242456]
48. Toguchida J, Ishizaki K, Sasaki MS, Nakamura Y, Ikenaga M, Kato M, et al. Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. *Nature.* 1989; 338:156–8. [PubMed: 2918936]
49. Mohseny AB, Tieken C, van der Velden PA, Szuhai K, de Andrea C, Hogendoorn PC, et al. Small deletions but not methylation underlie CDKN2A/p16 loss of expression in conventional osteosarcoma. *Genes Chromosomes Cancer.* 2010; 49:1095–103. [PubMed: 20737480]
50. Freeman SS, Allen SW, Ganti R, Wu J, Ma J, Su X, et al. Copy number gains in EGFR and copy number losses in PTEN are common events in osteosarcoma tumors. *Cancer.* 2008; 113:1453–61. [PubMed: 18704985]
51. Merchant A, Smielewska M, Patel N, Akunowicz JD, Saria EA, Delaney JD, et al. Somatic Mutations in SQSTM1 Detected in Affected Tissues From Patients With Sporadic Paget's Disease of Bone. *Journal of Bone and Mineral Research.* 2009; 24:484–94. [PubMed: 19016598]
52. Hiruma Y, Kurihara N, Subler MA, Zhou H, Boykin CS, Zhang H, et al. A SQSTM1/p62 mutation linked to Paget's disease increases the osteoclastogenic potential of the bone microenvironment. *Human Molecular Genetics.* 2008; 17:3708–19. [PubMed: 18765443]
53. Shaker JL. Paget's Disease of Bone: A Review of Epidemiology, Pathophysiology and Management. *Therapeutic advances in musculoskeletal disease.* 2009; 1:107–25. [PubMed: 22870432]
54. Debelenko LV, McGregor LM, Shivakumar BR, Dorfman HD, Raimondi SC. A novel EWSR1-CREB3L1 fusion transcript in a case of small cell osteosarcoma. *Genes Chromosomes Cancer.* 2011; 50:1054–62. [PubMed: 21987447]
55. Yang J, Annala M, Ji P, Wang G, Zheng H, Codgell D, et al. Recurrent LRP1-SNRNP25 and KCNMB4-CCND3 fusion genes promote tumor cell motility in human osteosarcoma. *Journal of Hematology & Oncology.* 2014; 7:76. [PubMed: 25300797]
56. Carter JM, Inwards CY, Jin L, Evers B, Wenger DE, Oliveira AM, et al. Activating GNAS mutations in parosteal osteosarcoma. *Am J Surg Pathol.* 2014; 38:402–9. [PubMed: 24525511]
57. Hang JF, Chen PC. Parosteal osteosarcoma. *Archives of pathology & laboratory medicine.* 2014; 138:694–9. [PubMed: 24786129]
58. Kansara M, Tsang M, Kodjabachian L, Sims NA, Trivett MK, Ehrich M, et al. Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. *J Clin Invest.* 2009; 119:837–51. [PubMed: 19307728]
59. Baker EK, Taylor S, Gupte A, Chalk AM, Bhattacharya S, Green AC, et al. Wnt inhibitory factor 1 (WIF1) is a marker of osteoblastic differentiation stage and is not silenced by DNA methylation in osteosarcoma. *Bone.* 2015; 73:223–32. [PubMed: 25571841]
60. Chang L, Shrestha S, LaChaud G, Scott MA, James AW. Review of microRNA in osteosarcoma and chondrosarcoma. *Med Oncol.* 2015; 32:613. [PubMed: 25920607]
61. Li B, Ye Z. Epigenetic alterations in osteosarcoma: promising targets. *Mol Biol Rep.* 2014; 41:3303–15. [PubMed: 24500341]
62. Bernardini G, Laschi M, Geminiani M, Santucci A. Proteomics of osteosarcoma. *Expert review of proteomics.* 2014; 11:331–43. [PubMed: 24654989]
63. Kuijjer ML, van den Akker BE, Hilhorst R, Mommersteeg M, Buddingh EP, Serra M, et al. Kinome and mRNA expression profiling of high-grade osteosarcoma cell lines implies Akt signaling as possible target for therapy. *BMC medical genomics.* 2014; 7:4. [PubMed: 24447333]
64. Li Z, Yu X, Shen J. Long non-coding RNAs: emerging players in osteosarcoma. *Tumour Biol.* 2016; 37:2811–6. [PubMed: 26718212]
65. Harvey M, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A, Donehower LA. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. *Nat Genet.* 1993; 5:225–9. [PubMed: 8275085]
66. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, et al. Mutant p53 Gain of Function in Two Mouse Models of Li-Fraumeni Syndrome. *Cell.* 2004; 119:847–60. [PubMed: 15607980]

67. Chan LH, Wang W, Yeung W, Deng Y, Yuan P, Mak KK. Hedgehog signaling induces osteosarcoma development through Yap1 and H19 overexpression. *Oncogene*. 2014; 33:4857–66. [PubMed: 24141783]
68. Entz-Werle N, Choquet P, Neuville A, Kuchler-Bopp S, Clauss F, Danse JM, et al. Targeted apc;twist double-mutant mice: a new model of spontaneous osteosarcoma that mimics the human disease. *Transl Oncol*. 2010; 3:344–53. [PubMed: 21151473]
69. Wang ZQ, Liang J, Schellander K, Wagner EF, Grigoriadis AE. c-fos-induced osteosarcoma formation in transgenic mice: cooperativity with c-jun and the role of endogenous c-fos. *Cancer Res*. 1995; 55:6244–51. [PubMed: 8521421]
70. McAllister KA, Houle CD, Malphurs J, Ward T, Collins NK, Gersch W, et al. Spontaneous and irradiation-induced tumor susceptibility in BRCA2 germline mutant mice and cooperative effects with a p53 germline mutation. *Toxicologic pathology*. 2006; 34:187–98. [PubMed: 16546942]
71. McClatchey AI, Saotome I, Mercer K, Crowley D, Gusella JF, Bronson RT, et al. Mice heterozygous for a mutation at the Nf2 tumor suppressor locus develop a range of highly metastatic tumors. *Genes Dev*. 1998; 12:1121–33. [PubMed: 9553042]
72. Molyneux SD, Di Grappa MA, Beristain AG, McKee TD, Wai DH, Paderova J, et al. Prkar1a is an osteosarcoma tumor suppressor that defines a molecular subclass in mice. *J Clin Invest*. 2010; 120:3310–25. [PubMed: 20697156]
73. Tao J, Chen S, Lee B. Alteration of Notch signaling in skeletal development and disease. *Ann N Y Acad Sci*. 2010; 1192:257–68. [PubMed: 20392245]
74. Tao J, Chen S, Yang T, Dawson B, Munivez E, Bertin T, et al. Osteosclerosis owing to Notch gain of function is solely Rbpj-dependent. *J Bone Miner Res*. 2010; 25:2175–83. [PubMed: 20499347]
75. Sidow A, Spies N. Concepts in solid tumor evolution. *Trends in genetics : TIG*. 2015; 31:208–14. [PubMed: 25733351]
76. Tomasetti C, Marchionni L, Nowak MA, Parmigiani G, Vogelstein B. Only three driver gene mutations are required for the development of lung and colorectal cancers. *Proc Natl Acad Sci U S A*. 2015; 112:118–23. [PubMed: 25535351]
77. Papagelopoulos PJ, Galanis EC, Vlastou C, Nikiforidis PA, Vlamis JA, Boscainos PJ, et al. Current concepts in the evaluation and treatment of osteosarcoma. *Orthopedics*. 2000; 23:858–67. quiz 68-9. [PubMed: 10952054]
78. Calo E, Quintero-Estades JA, Danielian PS, Nedelcu S, Berman SD, Lees JA. Rb regulates fate choice and lineage commitment in vivo. *Nature*. 2010; 466:1110–4. [PubMed: 20686481]
79. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–74. [PubMed: 21376230]
80. Tao J, Erez A, Lee B. One NOTCH Further: Jagged 1 in Bone Metastasis. *Cancer Cell*. 2011; 19:159–61. [PubMed: 21316596]
81. Wang LL, Gannavarapu A, Kozinetz CA, Levy ML, Lewis RA, Chintagumpala MM, et al. Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. *Journal of the National Cancer Institute*. 2003; 95:669–74. [PubMed: 12734318]
82. Ichikawa K, Noda T, Furuichi Y. Preparation of the gene targeted knockout mice for human premature aging diseases, Werner syndrome, and Rothmund-Thomson syndrome caused by the mutation of DNA helicases. *Nihon yakurigaku zasshi Folia pharmacologica Japonica*. 2002; 119:219–26. [PubMed: 11979727]
83. Lu L, Harutyunyan K, Jin W, Wu J, Yang T, Chen Y, et al. RECQL4 Regulates p53 Function In Vivo During Skeletogenesis. *J Bone Miner Res*. 2015; 30:1077–89. [PubMed: 25556649]
84. Ng AJ, Walia MK, Smeets MF, Mutsaers AJ, Sims NA, Purton LE, et al. The DNA helicase recql4 is required for normal osteoblast expansion and osteosarcoma formation. *PLoS Genet*. 2015; 11:e1005160. [PubMed: 25859855]
85. Mohseny AB, Hogendoorn PC. Zebrafish as a model for human osteosarcoma. *Adv Exp Med Biol*. 2014; 804:221–36. [PubMed: 24924177]
86. Fenger JM, London CA, Kisseberth WC. Canine osteosarcoma: a naturally occurring disease to inform pediatric oncology. *ILAR journal / National Research Council, Institute of Laboratory Animal Resources*. 2014; 55:69–85.

87. Janeway KA, Walkley CR. Modeling human osteosarcoma in the mouse: From bedside to bench. *Bone*. 2010; 47:859–65. [PubMed: 20696288]
88. Mutsaers AJ, Walkley CR. Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells? *Bone*. 2014; 62:56–63. [PubMed: 24530473]
89. Schlemmer M, Bauer S, Schutte R, Hartmann JT, Bokemeyer C, Hosius C, et al. Activity and side effects of imatinib in patients with gastrointestinal stromal tumors: data from a German multicenter trial. *European journal of medical research*. 2011; 16:206–12. [PubMed: 21719393]
90. Chawla SP, Staddon AP, Baker LH, Schuetze SM, Tolcher AW, D'Amato GZ, et al. Phase II study of the mammalian target of rapamycin inhibitor ridaforolimus in patients with advanced bone and soft tissue sarcomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012; 30:78–84.
91. Campbell J, Ryan CJ, Brough R, Bajrami I, Pemberton HN, Chong IY, et al. Large-Scale Profiling of Kinase Dependencies in Cancer Cell Lines. *Cell Rep*. 2016; 14:2490–501. [PubMed: 26947069]
92. Zanolini S, Canalis E. Notch Signaling and the Skeleton. *Endocrine reviews*. 2016; 37:223–53. [PubMed: 27074349]
93. Cai Y, Cai T, Chen Y. Wnt pathway in osteosarcoma, from oncogenic to therapeutic. *J Cell Biochem*. 2014; 115:625–31. [PubMed: 24190862]
94. Engin F, Yao Z, Yang T, Zhou G, Bertin T, Jiang MM, et al. Dimorphic effects of Notch signaling in bone homeostasis. *NatMed*. 2008; 14:299–305.
95. Engin F, Bertin T, Ma O, Jiang MM, Wang L, Sutton RE, et al. Notch signaling contributes to the pathogenesis of human osteosarcomas. *Human Molecular Genetics*. 2009; 18:1464–70. [PubMed: 19228774]
96. Hilton MJ, Tu X, Wu X, Bai S, Zhao H, Kobayashi T, et al. Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation. *Nature medicine*. 2008; 14:306–14.
97. Gordon JA, Stein JL, Westendorf JJ, van Wijnen AJ. Chromatin modifiers and histone modifications in bone formation, regeneration, and therapeutic intervention for bone-related disease. *Bone*. 2015; 81:739–45. [PubMed: 25836763]
98. Bradley EW, Carpio LR, van Wijnen AJ, McGee-Lawrence ME, Westendorf JJ. Histone Deacetylases in Bone Development and Skeletal Disorders. *Physiological reviews*. 2015; 95:1359–81. [PubMed: 26378079]
99. O'Brien CA, Nakashima T, Takayanagi H. Osteocyte control of osteoclastogenesis. *Bone*. 2013; 54:258–63. [PubMed: 22939943]
100. Glass DA 2nd, Karsenty G. In vivo analysis of Wnt signaling in bone. *Endocrinology*. 2007; 148:2630–4. [PubMed: 17395705]
101. Zhou W, Hao M, Du X, Chen K, Wang G, Yang J. Advances in targeted therapy for osteosarcoma. *Discov Med*. 2014; 17:301–7. [PubMed: 24979249]
102. Abarrategi A, Tornin J, Martinez-Cruzado L, Hamilton A, Martinez-Campos E, Rodrigo JP, et al. Osteosarcoma: Cells-of-Origin, Cancer Stem Cells, and Targeted Therapies. *Stem cells international*. 2016; 2016:3631764. [PubMed: 27366153]
103. Yang J, Zhang W. New molecular insights into osteosarcoma targeted therapy. *Curr Opin Oncol*. 2013; 25:398–406. [PubMed: 23666471]
104. Yan GN, Lv YF, Guo QN. Advances in osteosarcoma stem cell research and opportunities for novel therapeutic targets. *Cancer Lett*. 2016; 370:268–74. [PubMed: 26571463]
105. Adamopoulos C, Gargalionis AN, Basdra EK, Papavassiliou AG. Deciphering signaling networks in osteosarcoma pathobiology. *Exp Biol Med (Maywood)*. 2016
106. Khoo KH, Verma CS, Lane DP. Drugging the p53 pathway: understanding the route to clinical efficacy. *Nature reviews Drug discovery*. 2014; 13:217–36. [PubMed: 24577402]
107. Ribi S, Baumhoer D, Lee K, Edison, Teo AS, Madan B, et al. TP53 intron 1 hotspot rearrangements are specific to sporadic osteosarcoma and can cause Li-Fraumeni syndrome. *Oncotarget*. 2015; 6:7727–40. [PubMed: 25762628]
108. Lord CJ, Ashworth A. BRCAness revisited. *Nature reviews Cancer*. 2016; 16:110–20. [PubMed: 26775620]

109. Flynn RL, Cox KE, Jeitany M, Wakimoto H, Bryll AR, Ganem NJ, et al. Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. *Science*. 2015; 347:273–7. [PubMed: 25593184]
110. Karnitz LM, Zou L. Molecular Pathways: Targeting ATR in Cancer Therapy. *American Association for Cancer Research*. 2015; 21:4780–5.
111. Walia MK, Ho PM, Taylor S, Ng AJ, Gupte A, Chalk AM, et al. Activation of PTHrP-cAMP-CREB1 signaling following p53 loss is essential for osteosarcoma initiation and maintenance. *eLife*. 2016; 5
112. Martin TJ. Parathyroid Hormone-Related Protein, Its Regulation of Cartilage and Bone Development, and Role in Treating Bone Diseases. *Physiological reviews*. 2016; 96:831–71. [PubMed: 27142453]
113. Vahle JL, Sato M, Long GG, Young JK, Francis PC, Engelhardt JA, et al. Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1-34) for 2 years and relevance to human safety. *Toxicologic pathology*. 2002; 30:312–21. [PubMed: 12051548]
114. Savage SA, Mirabello L, Wang Z, Gastier-Foster JM, Gorlick R, Khanna C, et al. Genome-wide association study identifies two susceptibility loci for osteosarcoma. *Nat Genet*. 2013; 45:799–803. [PubMed: 23727862]
115. Karlsson EK, Sigurdsson S, Ivansson E, Thomas R, Elvers I, Wright J, et al. Genome-wide analyses implicate 33 loci in heritable dog osteosarcoma, including regulatory variants near CDKN2A/B. *Genome Biol*. 2013; 14:R132. [PubMed: 24330828]
116. Hsiao EC, Boudignon BM, Chang WC, Bencsik M, Peng J, Nguyen TD, et al. Osteoblast expression of an engineered Gs-coupled receptor dramatically increases bone mass. *Proc Natl Acad Sci U S A*. 2008; 105:1209–14. [PubMed: 18212126]
117. Wang H, Yu C, Gao X, Welte T, Muscarella AM, Tian L, et al. The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. *Cancer Cell*. 2015; 27:193–210. [PubMed: 25600338]

Highlights

- We review recent advances in the area of human osteosarcoma genomic analysis and mouse forward and reverse genetic studies.
- Somatic mutations, genetic hallmarks, and catalogs of driver genes in human osteosarcoma are described.
- Impact of the nature and number of driver genes on tumor latency, subtypes, and metastatic potentials of osteosarcoma are discussed.
- A synergistic three driver model may better recapitulate aggressive osteosarcoma.
- Genetic vulnerabilities in osteosarcoma are illustrated to exemplify the opportunities for next generation molecularly targeted therapies.

Figure 1. Models for development of osteosarcoma subtypes

(a) The “single driver” hypothesis states that each subtype is induced by a subtype-specific first driver (A, B, or C) in proliferative cells, which then determines three osteosarcoma (OS) subtypes: osteoblastic OS, fibroblastic OS, and chondroblastic OS. (b) The “multiple drivers” hypothesis states that each subtype is induced by two or more drivers, illustrated here by three first drivers (D, F, H) in addition to synergistic drivers (E, G, I), which then determines the different OS subtypes. The red arrow indicates a proliferating mesenchymal stem cell-derived osteoblast.

Figure 2. PI3K-mTORC1 signaling components altered in osteosarcoma

Simplified summary of mutated and/or dysregulated genes in human and mouse osteosarcoma (OS) that participate in the mTORC1 signaling pathway. In the presence of growth factors (e.g. Insulin) or other stimuli, receptor tyrosine kinase (RTK) intracellular domains are trans-phosphorylated, leading to the recruitment of the regulatory subunit of class IA PI3K, p85 (encoded by gene PIK3R1) and release of the catalytic subunit p100 (encoded by gene PIK3CA). One of the signaling components activating PI3K is Ras. Binding of a ligand to RTKs promotes dimerization of the receptor and subsequent autophosphorylation of tyrosine residues. This allows the RTK to interact with SH2 domain-containing proteins, such as Growth Factor Receptor Bound Protein 2 (GRB2), that can bind and activate Son of Sevenless (SOS), which in turn, activates RAS. Neurofibromin 1 (NF1) negatively regulates this process. Thus, p110 is activated by RAS independently of p85. Upon activation, PI3K catalyzes the formation of the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the plasma membrane, which can be down-regulated by the tumor suppressor, phosphatase and tensin homolog (PTEN). Increased PIP3 levels lead to the recruitment of PIP3-binding proteins, such as Protein Kinase B Alpha (Akt) and PDK1. At the membrane, PDK1 (encoded by PDK1) phosphorylates Akt at Thr308 leading to partial activation of Akt. Akt then phosphorylates varied substrates, including tuberlin (encoded by TSC2), an inhibitor of mTORC1. This results in the activation of Ras Homolog Enriched in Brain (Rheb). Activated Rheb binds to and activates mTORC1. Active mTORC1 promotes protein synthesis, lipogenesis, and energy metabolism, but inhibits autophagy and lysosome biogenesis. Other regulators of mTORC1 in OS, including Neurofibromin 2 (NF2) and Glycogen Synthase Kinase 3 Beta (GSK3b), which mediates Wnt signaling to phosphorylate and promote TSC2 activity and p53 in response to DNA damage. Rapamycin selectively inhibits mTORC1. Round shape: oncogene. Rectangular shape: tumor suppressor gene (TSG). Red text: mutations identified in human OS samples [28-32]. Asterisks (*) denote mutations identified by Sleeping Beauty

forward genetic screen [19]. Green background: change of expression identified by transcriptome analysis of p53 and Notch OS [16]. Gray text: targeted agents being tested in clinical trials. Orange bar-headed lines or arrows: drug mechanism (inhibition or activation, respectively).

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Table 1
Genetics of inherited osteosarcoma syndromes

Gene Mutations	Syndrome	Phenotype
<i>p53</i>	Li-Fraumeni Syndrome	Higher incidence of cancer including breast, brain, adrenocortical, leukemia, and sarcomas. Incidence of OS is 3%.
<i>RB1</i>	Hereditary Retinoblastoma	Tumor in the retina occurring in childhood. Incidence of OS is 12.1%.
<i>RECQL4</i>	Rothmund-Thomson Syndrome	Poikiloderma, short stature, sparse scalp hair, eyelashes and eyebrows, skeletal abnormalities, radial ray defects, and features of premature aging. Also, higher incidence of cancers. Incidence of osteosarcoma is 32% from a cohort study. The actual incidence is not known.
<i>BLM (RECQL2)</i>	Bloom's Syndrome	Dermatologic features including photosensitivity, and telangiectatic erythema and malignancies of the gastrointestinal tract, genitalia, and urinary tract. Patients have high pitched voices, short stature, and reduced subcutaneous fat along with a higher incidence of cancer including OS.
<i>WRN(RECQL3)</i>	Werner Syndrome	Accelerated aging beginning in adolescence. Incidence of OS is 7.7%.

Table 2

Validated somatic mutations in candidate cancer genes identified in genomic studies.

Genes	signaling pathway	Frequency
Oncogenes		
CDK4	Cell cycle/ apoptosis	1/20 (Perry et al), * (Kovac et al)
MDM2	Cell cycle/ apoptosis	2/52 (Chen et al), 3/59 (Perry et al)
MYC	Cell cycle/ apoptosis	3/20 (Perry et al)
CARD11	Cell cycle/ apoptosis	1/34 (Chen et al), 3/20 (Perry et al)
EGFR	PI3K-mTOR; RAS	2/34 (Chen et al)
GNAQ	PI3K-mTOR; RAS; MAPK	2/34 (Chen et al)
GNAS	APC; PI3K-mTOR; TGF-beta; RAS	2/34 (Chen et al)
JAK1	IFN	2/34 (Chen et al), 2/20 (Perry et al)
MAML2	Notch	1/34 (Chen et al)
FBXW7	Notch	1/34 (Chen et al), * (Kovac et al)
ALK	PI3K-mTOR; RAS	1/34 (Chen et al), 2/20 (Perry et al)
PDGFRA	PI3K-mTOR; RAS	2/34 (Chen et al), 3/20 (Perry et al)
PDGFRB	PI3K-mTOR; RAS	1/34 (Chen et al), 1/20 (Perry et al)
PIK3CA	PI3K-mTOR	1/34 (Chen et al), 5/20 (Perry et al)
APC	APC	* (Kovac)
CTNND1	Cell cycle/ apoptosis	* (Kovac)
BLM	Cell cycle/ apoptosis	1/34 (Chen)
CCNE1	Cell cycle/ apoptosis	2/20 (Perry et al) * (Kovac)
COPS3	Cell cycle/ apoptosis	4/20 (Perry et al)
PDPK1	PI3K-mTOR	1/20 (Perry et al)
AKT1	PI3K-mTOR	1/20 (Perry et al)
EIF4B	PI3K-mTOR	2/20 (Perry et al)
WRN	DNA damage control	2/123 (Kovac et al)
Notch1-3	Notch	* (Kovac et al)
Notch4	Notch	1/20 (Perry et al) * (Kovac)
PRKCA	Cell cycle/ apoptosis	* (Kovac et al)
Tumor suppressor genes		
EP300	Chromatin modification; APC; TGF-beta; NOTCH	1/34 (Chen et al), 3/123 (Kovac et al)
SMAD4	TGF-beta	2/34 (Chen et al), 1/20 (Perry et al)
Runx1	Transcriptional regulation	1/34 (Chen)
ARID1A	Chromatin modification	3/20 (Perry et al) * (Kovac)
ATM	DNA damage control	2/20 (Perry et al), 3/123 (Kovac et al)
RB1	Cell cycle/ apoptosis	61% (Perry et al), 10/34 (Chen et al), 47% (Kovac et al)
CDKN2A	Cell cycle/ apoptosis, DNA damage control	6/20 (Perry et al), 15% SNCA (Kovac et al)

Genes	signaling pathway	Frequency
TP53	Cell cycle/ apoptosis, DNA damage control	75% (Perry et al.), 90% (Chen et al), 47% (Kovac et al)
ATRX	Chromatin modification	10/20 (Chen et al), 7/20 (Perry et al), 11/123 (Kovac et al)
FANCA	Chromatin modification	1/34(Chen et al), 2/20 (Perry et al), 3/123 (Kovac et al)
RECQL4	Chromatin modification	1/20 (Perry et al), 3/123 (Kovac et al)
BRCA1	DNA damage control	2/34 (Chen et al), 91% SCNA (Kovac et al)
BRCA2	DNA damage control	78% SCNA (Kovac et al)
MLH1	DNA damage control	1/34 (Chen et al), 1/20 (Perry et al)
CBL	PI3K-mTOR; RAS	1/34 (Chen et al), 1/20 (Perry et al)
PTCH1	Hedgehog	1/34 (Chen et al), *(Kovac et al)
NF1	RAS	2/34 (Chen et al), 3/20 (Perry et al)
MAP2K4	MAPK	1/34 (Chen et al), 2/20 (Perry et al)
AKT2	PI3K-mTOR	1/34 (Chen et al), 1/20 (Perry et al)
PIK3R1	PI3K-mTOR	1/34 (Chen et al), 1/20 (Perry et al)
PTEN	PI3K-mTOR	2/34 (Chen et al), 7/20 (Perry et al), 50% SNCA (Kovac et al)
TSC2	PI3K-mTOR	3/20 (Perry et al), 1/7 (Bousquet et al)
GAS7	Transcriptional regulation	5/34 (Chen et al), 1/20 (Perry et al)
MLLT3	Transcriptional regulation	1/34 (Chen et al), 2/20 (Perry et al)
DLG2	Wnt	18/34 (Chen et al), 5/20 (Perry et al), 24% SCNA (Kovac et al)
VHL	PI3K-mTOR; RAS; STAT	*(Kovac et al)
BAP1	DNA damage control	38% SCNA (Kovac et al)

Note:

*
Number not stated in paper