Chapter 2

Characterizing Osteosarcoma Through PTEN and PI3K: What p53 and Rb1 Can't Tell Us

Matthew G. Cable and R. Lor Randall

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67071

Abstract

Attention has been given to the fact that overall survival of osteosarcoma has plateaued over the last 30 years despite the addition of chemotherapy regimens. Elucidating the involvement of p53 and Rb1 in osteosarcoma has not yielded many novel treatments, but recent studies have started to characterize how the PTEN and the PI3K pathway can contribute to osteosarcoma. PTEN is a tumor suppressor that regulates a variety of signal transduction pathways and cellular processes, mainly by antagonizing PI3K activity and shutting down the PI3K/Akt pathway. Loss of PTEN function with concurrent PI3K activation has been detected frequently in a multitude of cancers, including osteosarcoma. This chapter aims to characterize PTEN and the PI3K/Akt pathway in osteosarcoma, their effects on primary bone tumor behavior, and potential therapeutic targets.

Keywords: osteosarcoma, phosphatase and tensin homolog, phosphatidylinositol 3-kinase, therapeutic applications

1. Introduction

Osteosarcoma (OS) is a malicious cancer that affects predominantly children and adolescents, and is the most common primary sarcoma of bone. After the advents of adjuvant multi-agent chemotherapy, in combination with surgery, the 5-year survival rate for OS has increased from 40 to 76% in children under 15 and from 56 to 66% in adolescents 15–19 years old [1]. Despite these advances, the prognosis for the 20% of patients that present with stage IV disease remains poor, and survival rates have plateaued [2]. In addition, with roughly 60% of cases occurring in just the second decade of life, the societal cost of OS exceeds that of many other cancers.

OS also represents a unique entity among pediatric cancers, with cases arising de novo and already exhibiting high-grade pathology, heterogeneous karyotypes, and frequent genomic mutations. This genomic instability is further characterized by unusually high numbers of chromosomal structural variants and not single nucleotide mutations [3]. An OS genome can often contain over 200 of these structural variants, making it the most disordered among childhood cancers [4] (**Figure 1**).

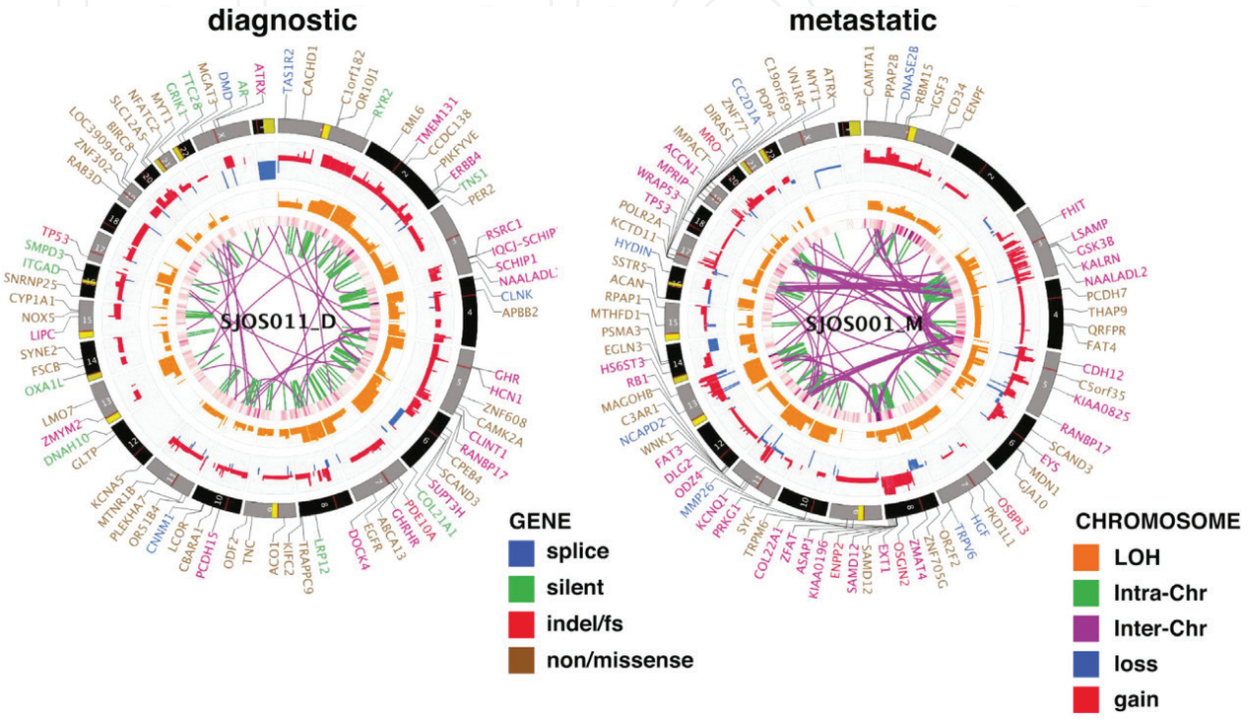

Figure 1. CIRCOS plots of osteosarcoma tumors. Representative CIRCOS plots of validated mutations and chromosomal lesions in diagnostic and metastatic osteosarcoma tumors from different patients. Loss of heterozygosity (orange), gain (red), and loss (blue) is shown. Intra-chromosomal (green lines) and inter-chromosomal (purple lines) translocations are indicated. Sequence mutations in RefSeq genes included silent single nucleotide variations (green), nonsense and missense single nucleotide variations (brown), splice-site mutations (dark blue), and insertion/deletion mutations (red). An additional track was added to the innermost ring of the plot showing the density of single nucleotide variations to highlight regions adjacent to structural variations characteristic of kataegis. Adapted from **Figure 1A**, Chen et al. [3].

To overcome the stagnation in survival rates, the cellular etiology and biology of OS need to be more completely understood. Toward this end, molecular targets that actively modulate essential cell processes such as cell cycle regulation, migration, mitosis, metabolism, and apoptosis have been studied to develop potential therapies. The most well-known and frustrating examples are the frequent inactivation mutations of cell cycle regulator gene tumor protein 53 (TP53) or tumor suppressor gene retinoblastoma protein 1 (Rb1), and attempts in translating these targets into applicable therapies have been met with much difficulty. Recent advances in cell signaling have broadly identified tyrosine kinase receptors (TKRs) as prominent targets for cancer therapies, with many receptors confluencing on the second messenger phosphatidylinositol (3,4,5)-triphosphate (PIP3), and it is activation by phosphatidylinositol-4,5-bisphosphonate 3-kinase (PI3K). More importantly, PI3K and its inhibitor, phosphatase and tensin homolog (PTEN), may play significant roles in

http://dx.doi.org/10.5772/67071

OS and represent therapeutic targets [4]. Investigations have also been centered on the effects of PTEN on osteoclastogenesis, and how the bone microenvironment may facilitate tumor expansion with PTEN loss. Therapeutic targets are expanding as strategies focus on restoring normal PTEN function and inhibiting PI3K pathway activation.

2. The PI3K pathway and PTEN

Class I PI3K is a family of heterodimeric signal transduction enzymes that phosphorylates the 3′ hydroxyl group of the inositol ring on phosphatidylinositol-4,5-bisphosphate (PIP2) to PIP3. The implications of that biochemical mouthful are that PI3K activates one of the most influential pathways in cell cycle regulation and cell proliferation, the PI3K/Akt pathway (**Figure 2**).

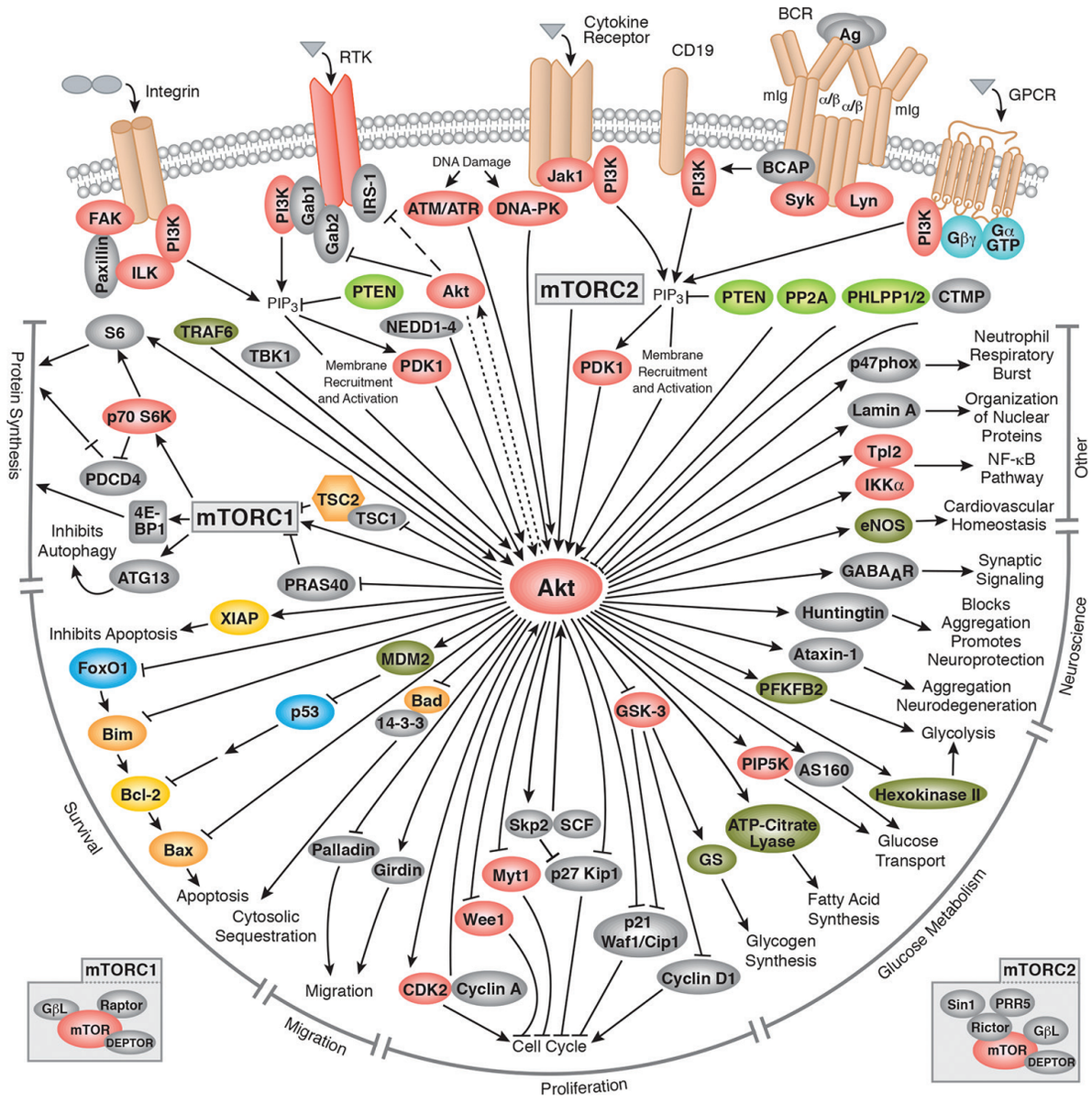

Figure 2. PI3K/Akt signaling pathway. Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).

The PI3K/Akt pathway was first identified when attempting to characterize insulin signaling and discovering the tyrosine kinase receptor type-1 insulin-like growth factor receptor (IGF1R) [5]. IGFR1 is one of the many tyrosine kinase receptors (TKRs) in the cell membrane, in addition to G protein-coupled receptors, that can instigate signaling through the canonical PI3K pathway. Although there are three classes of PI3Ks, class I is the most involved in oncogenesis and divided into class IA (PI3K alpha, PI3K beta, and PI3K delta) and class IB (PI3K gamma) [6]. All class I PI3Ks form a heterodimer consisting of a catalytic and regulatory subunit. The catalytic subunits forming class IA PI3Ks are p110 alpha, beta, and delta, and encodedby the genes PIK3CA, PIK3CB, and PIK3CD, respectively. The regulatory subunits for class IA PI3Ks are p85 alpha, p85 beta, and p55 gamma encoded by PIK3R1, PIK3R2, and PIK3R3, respectively [7]. Class IB PI3K is formed exclusively from the catalytic subunit p110 gamma (encoded by PIK3CG) and and the regulatory subunit p101 (encoded by PIK3R5). Class IA PI3Ks can be activated by TKRs including IGFR1, platelet-derived growth factor (PDGF), and epidermal growth factor receptor (EGFR). Class IB PI3Ks are activated only by G protein-coupled receptors.

Once activated, PI3K converts PIP2 to PIP3 [8]. PIP3 acts as a second messenger by recruiting and activating proteins containing a pleckstrin homology domain to the plasma membrane, notably the phosphoinositide-dependent kinase 1 (PDK1) and serine-threonine kinase Akt (also known as protein kinase B) [9]. Akt is activated via two phosphorylation sites, and the first occurs at threonine 308 by none other than PDK1. The second phosphorylation required for Akt activation occurs at serine 473 by a number of kinases including PDK1 or even Akt itself [7]. Once activated, Akt translocates to the cell cytoplasm or nucleus to set in motion a number of downstream effects to inhibit apoptosis and induce protein synthesis.

One of the most important downstream targets of Akt is mammalian target of rapamycin (mTOR), a 289 kDa serine/threonine kinase that drives one of the two complexes in mammalian cells, mTORC1 or mTORC2. mTORC1 has been implicated as a driver of many cancers, and mTORC2 can create a positive feedback loop by phosphorylating and activating Akt at serine 473 [10]. mTOR is activated when Akt phosphorylates and inactivates a regulatory protein in the mTORC1 complex called proline-rich Akt substrate of 40 kDa (PRAS40). Akt also inhibits the tumor suppressor protein tuberous sclerosis protein 2 (TSC2) which can result in activation of mTOR [11]. The activation of mTORC1 promotes protein synthesis and cellular proliferation by phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and ribosomal protein S6 kinase polypeptide 1 (S6K1) which in turn phosphorylates ribosomal protein S6 (RPS6). The majority of mTOR inhibitors (including rapamycin derivatives) function by inhibiting mTORC1.

In addition to mTOR, important downstream targets of the PI3K/Akt pathway include promotion of cyclin-dependent kinase 4 (CDK4) to support cell cycle progression [12] and activation of nuclear factor-kappa B (NF-κB) which initiates transcription of many target genes including those seen in drug-resistant malignancies [13]. Akt also inhibits Bcl-2-associated death promoter (BAD), caspase-9, and forkhead box O3 (FOXO3), while activating cyclic AMP response element-binding protein (CREB), all of which serve to prevent apoptosis [14–16].

http://dx.doi.org/10.5772/67071

The grandiosity and arborous nature of the of the PI3K/Akt pathway emphasize the current attention being given to phosphatase and tensin homolog (PTEN) (**Figure 3**), a lipid phosphatase that directly antagonizes PI3K during the initiation steps of the PI3K/Akt pathway [17]. PTEN is a 200 kb gene located on chromosome 10q23.3 [18], which encodes a 60-kDa dual-specificity phosphatase that cleaves phosphate groups from phospholipids (including phosphatidylinositols) as well as proteins (serine, threonine, and tyrosine residues including those on TKRs) [19]. PTEN functions as a tumor suppressor by dephosphorylating and inactivating the second messenger PIP3 to PIP2, cogently shutting down the PI3K/Akt pathway and promoting cell cycle regulation and apoptosis [20]. The function of PTEN is further supported by phosphorylation at tyrosine 336 by RAK, which prevents ubiquitin-mediated proteosomal degradation [21]. PTEN also has tumor suppressor functions outside of the canonical PI3K pathway and regulates a variety of cellular processes and signal transduction pathways, such as controlling cell proliferation through cyclin D1 levels [22, 23]. A 576 amino acid translational variant of PTEN has been discovered, termed PTEN long, that can be secreted and enter other cells, enabling tumor suppressor effects in a paracrine-like manner [24]. The C-terminus of PTEN is thought to be essential in maintaining heterochromatin structure and genomic stability [25].

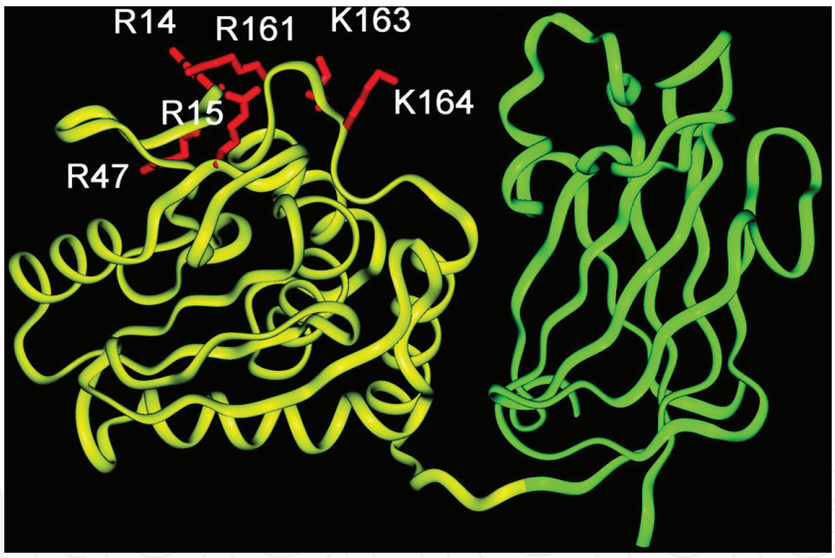

Figure 3. A ribbon diagram of the PTEN tumor suppressor. The phosphatase domain and the C2 domains are shown in yellow and green ribbons, respectively. Reproduced from Das et al. [98].

3. Relevance of PTEN and PI3K to cancer processes

OS is already prone for structural genomic variations and chromothripsis, and whether this is due to selective loss of the TP53 gene or an intrinsic feature of OS itself is beyond the scope of this paper. What this intrinsic genomic instability does allow for is the selection of cancer cells that consistently develop predictable mutations that are beneficial for tumor survival [3]. This

phenomenon of localized hypermutation in a cancer genome is called kataegis. Two of these predictable mutations in OS are the frequent loss of PTEN and activation of the PI3K/Akt pathway [26, 27]. The PI3K/Akt pathway is activated in a multitude of cancers, as its overall effects are to promote cellular proliferation and survival while reducing apoptosis. Akt upregulates expression of murine double minute 2 (MDM2) which further inhibits release of tumor protein 53 (p53) [28] and can result in very aggressive phenotypes since p53 is already stunted in many OS cancers. p53 itself has been shown to be a potent dual inhibitor of mTORC1 and mTORC2 in OS cells, further supporting a highly active PI3K/Akt pathway in OS tumors [29].

Loss of PTEN function has been detected frequently in many different forms of cancer including breast, prostate, lung, gastric, colon, and skin cancer, as well as endometrial carcinoma. The frequency of mono-allelic mutations of the PTEN locus is estimated to be 50–80% in sporadic tumors, with complete loss generally associated with advanced malignancies and metastases [30]. In fact, the PTEN protein was initially referred to as MMAC1 for "mutated in multiple advanced cancers" [18]. PTEN deletion mutations were first identified in canine OS cell lines [31], and retrospectively, it is interesting to note that chromosomal loss of 10q has been a frequent occurrence in over 50% of human OS tumor samples analyzed in some studies [32]. Specific mutations in PTEN have been identified in 44% of pediatric OS [3].

The genetic disruption of PTEN often leads to unchecked PI3K/Akt signaling [33]. In breast cancer cell lines, a functional PTEN can directly inhibit cell growth [21]. More recently, the PI3K/Akt pathway and PTEN have risen to the forefront of OS research since the surprise finding by Perry et al. that identified biologically and clinically relevant alterations in the PI3K/Akt pathway in 24% of OS samples [4]. Furthermore, when comparing the same OS tumors to a murine model of OS (with conditional deletions of TP53 and Rb1 in the preosteoblast), both contained somatic mutations in PTEN and PIK3R1.

4. How is PTEN lost in cancer?

There are numerous methods by which the PI3K/Akt pathway is activated in malignancies, often the consequence of upregulation via a multitude of TKRs and G-protein-coupled receptors. With PTEN having a pronounced effect on the PI3K/Akt pathway, PTEN function is accordingly inhibited in many ways. Recurrent PTEN germline mutations often involve exon 5, which codes for its phosphatase domain, and missense and nonsense somatic mutations also can occur in exons 5–8 [34]. Cowden's disease (also known as multiple hamartoma syndrome) is an autosomal dominant condition with increased risk of thyroid, breast, uterine, and kidney cancers that results from similar somatic inactivating mutations of PTEN [35]. The end result is a functional loss of heterozygosity, which is sufficient to produce oncogenesis, as PTEN is essential for embryonic development, and homozygous loss results in embryonic lethal phenotype in mice [36].

At the epigenetic level, PTEN is inhibited by aberrant promoter hypermethylation of CpG islands, which is a poor prognostic indicator in numerous cancer types including breast,

http://dx.doi.org/10.5772/67071

colorectal, uterine, and malignant melanoma [37–40]. While hypermethylation has been identified in several soft tissue sarcomas and in murine models of OS, it has only been studied in human OS tumors in vitro [41]. PTEN protein translation can be negatively regulated by many microRNAs including miR-92a, miR-17, miR-128, and miR-130/131 [42–44]. Theoretically, structural features of PTEN itself could prove to be potential sites for compromising protein stability. Post-translational modification such as phosphorylation at tyrosine 336 could be abrogated, thus promoting protein degradation. Protein localization to the cell membrane or nucleus could be affected if the C2 or PDZ-binding domains of PTEN were impaired. Although the majority of inactivating mutations affect the phosphatase domain of PTEN, preventing it from cleaving PIP3 to PIP2, there are several ways that PTEN expression and dysregulation can be imparted in malignancies.

5. PTEN in bone and osteosarcoma

PTEN is frequently deactivated through deletions in human OS tumor samples [26]. A sleeping beauty forward genetic screen by Moriarity et al. supports the role of PTEN loss as a key driver in osteosarcomagenesis in mice, with a concordant enrichment of genes involved in the PI3K/Akt pathway [45]. The PI3K/Akt pathway is already recognized as a common effector for RTK-activating mutations in cancer [46]. The loss of PTEN only further promotes this, but what factors in the bone microenvironment that facilitate tumor expansion in the absence of PTEN is unknown. It would be very un-Darwinian for OS, multiple myeloma, and bone metastases to all have PTEN derangements for unrelated reasons. The effects of PTEN on osteoclastogenesis and receptor activator of nuclear factor-kappa-B ligand (RANKL) may be the commonality among these cancers.

There is a vicious cycle that begins when a malignancy metastasizes or originates in bone that allows it to propagate and induce osteolysis. Metastatic tumor cells can secrete interleukin-1 (IL-1) and parathyroid hormone-related protein (PTHrP), which stimulate RANKL production and secretion from osteoblasts. IL-1 also stimulates osteoblasts to secrete IL-6 via the PI3K/Akt pathway, and IL-6 is a potent stimulator of osteoclastogenesis [47]. OS cells also directly produce RANKL. RANKL binding to the RANK receptor on osteoclast precursors activates osteoclast differentiation and osteolysis. The increased bone resorption results in the release of bone morphogenetic proteins (BMPs) and insulin-like growth factor 1 (IGF-1) that further attract and stimulate the growth of cancer cells in bone, thus initiating a vicious cycle of tumor expansion and osteolysis [48, 49].

PI3K/Akt acts as a cog in the wheel of this vicious cycle, being activated by RANKL and resulting in downstream expression of NFATc1, a key transcription factor of osteoclastogenesis [50]. This gives PTEN the opportunity to prevent bone resorption by inhibiting the PI3K/Akt pathway, thus suppressing RANKL-induced osteoclast differentiation and stopping the vicious cycle [51]. This would support the loss of PTEN in aggressive bony malignancies, preventing stimulation of osteoblasts by IL-1 and osteoclasts by RANKL. Murine models with PTEN deletions have shown increased osteoblast proliferation and bone mass [52], in addi-

tion to increased osteoclast differentiation [53]. Fibroblast growth factor 18 (FGF18) is another stimulator of bone growth, and with all FGF receptors being RTK's, its effects are mediated via the PI3K/Akt pathway and can be inhibited by PTEN [54]. Vascular endothelial growth factor (VEGF) and EGFR are both upregulated in OS and are key activators of the PI3K/Akt pathway [26, 55].

The finding that WNT5A may phosphorylate Akt is further support for the bone microenvironment being conducive to tumor growth [56]. WNT5A is expressed on osteoblast-precursor cells and activates RTK-like orphan receptors (Ror) on osteoclast precursors, culminating in increased expression of RANK on osteoclasts and enhancing RANKL osteoclastogenesis [57]. The result of PTEN loss in OS would be twofold in promoting the effects of WNT5A: (1) further increasing RANKL production by osteoblasts and (2) enabling WNT5A-related Akt activation, both increasing osteoclastogenesis and osteolysis.

6. PTEN in osteosarcoma and metastasis

WNT5A also provides segue into the realm of OS metastases. Metastatic melanoma cell motility and invasiveness are increased through WNT5A and Akt. Akt is increased in metastatic OS specimens, and inhibiting Akt in mice decreases pulmonary metastases [7]. WNT5A is even implicated in helping to initiate the epithelial to mesenchymal transition (EMT), a key event in malignant cancers developing metastatic potential [58, 59]. The effect of PTEN loss in promoting EMT has been shown in prostate, colorectal, and OS cancer cells [60–62]; however, the role of EMT in mesenchymal tumors including OS is still a topic of debate and a focus of current research.

IL-6 is also increased in OS tissues and can promote ICAM-1 expression and cell motility in OS in vitro, possibly correlating to metastatic potential. These effects of IL-6 in OS can be negated by Akt inhibition [63], suggesting that PTEN could also negate this effect in bone by preventing IL-1 from stimulating IL-6 through PI3K/Akt. Another promoter of metastasis is the chemokine CXCL12 and its receptor CXCR4, both induced by IL-1 and strongly linked to bone metastasis [64]. PTEN is involved in the negative regulation of CXCR4, and in prostate cancer, PTEN loss induces CXCL12/CXCR4 expression [65].

Focal adhesion kinase (FAK) deserves special recognition as a direct target of PTEN outside of the canonical PI3K pathway, as it is a target of the protein phosphatase (not lipophosphatase) domain of PTEN [66]. Activation of FAK by phosphorylation (pFAK) promotes proliferation and invasion of tumor cells and increases matrix metalloproteinases that can degrade the extracellular matrix. FAK and pFAK are overexpressed in human OS samples and independently predict overall and metastasis-free survival [67]. This effect may be reversed in OS cells with PTEN transfections, which exhibit decreased migration and adhesion capabilities and concomitant downregulation of pFAK and MMP-9, further supporting the loss of PTEN in OS [68]. Just as FAK can be prognostic in human OS, the presence of PTEN in tumor resections is significantly associated with improved survival prognosis [69]. Unfortunately neither can be correlated with response to current chemotherapy regimens.

7. Therapeutic applications targeting PI3K/Akt pathway and PTEN in osteosarcoma

There is an abundance of convincing evidence supporting a key role for PTEN in OS, but from a therapeutic standpoint, this assumes that there is causality and not just correlation between PTEN loss and poor patient prognosis. Two major therapeutic strategies are restoring normal PTEN function and inhibiting the PI3K pathway. Comparing these two strategies could also aid in distinguishing how PTEN serves as a tumor suppressor beyond the canonical PI3K pathway.

Many chemotherapy agents already target various levels of the PI3K/Akt pathway, and several are in various phases of clinical trials involving OS. These include small molecule inhibitors of PI3K, Akt, and numerous mTOR inhibitors (**Table 1**). Countless PI3K small molecule inhibitors have been developed, but several have specifically been effective in OS including GSK458, LY294002, BYL719, and BKM120 (Buparlisib) [4, 70–72]. Pictilisib (GDC-0941) is a pan PI3K inhibitor that has entered phase I clinical trials for advanced solid tumors [73]. Aminopeptidase N (also known as CD13) is a surface receptor activated by IL-6 that can stimulate PI3K and is involved in tumor invasion. An aminopeptidase N inhibitor, ubenimex, is currently used for acute myeloid leukemia treatments and may help prevent OS metastases [74]. The Akt inhibitors perifosine and MK-2206 both exert anti-OS activity in vitro [75, 76]. Inhibitors of the mTOR complex are the most numerous of this pathway, being extensively studied and developed since the discovery of the mTORC1 inhibitor rapamycin in 1975. Many new inhibitors of the mTORC1/2 complex are currently being developed, but several in particular have been tested on OS either in vivo or are in various phases of clinical trials: temsirolimus [77], ridaforolimus [78], everolimus [79], XL388 [80], and NVP-BEZ235 [81]. Apitolisib (GDC-0980) has entered phase I clinical trials for patients with advanced solid tumors and could precede future studies involving OS patients [82].

With current chemotherapy regimens for OS having reached seemingly maximum efficacy, attention has been generous in identifying new agents to increase PTEN function in OS. MicroRNA-21 (miR-21) is one of the first mammalian microRNA's discovered that happens to be highly expressed in bone marrow and post-transcriptionally regulates a number of tumor suppressors including PTEN. miR-21 is overexpressed, suppresses PTEN in OS, and has been identified as a potential therapeutic target [83]. MicroRNAs are small non-coding pieces of RNA, similar to small interfering RNA (siRNAs) that bind to complimentary pieces of messenger RNA, effectively preventing translation of the mRNA into proteins. In multiple myeloma, miR-21 inhibitors have been used in vivo using murine models to cause tumor suppression, with tumors exhibiting increased PTEN and decreased p-Akt levels [84]. Targeting microRNAs could show promise, as numerous microRNAs are specifically upregulated in OS cell lines [44]. In addition to miR-21, miR-17 and miR-221 are also increased and can inhibit PTEN in OS cells and tissues, potentially being therapeutic targets [42, 85]. Further incentive for therapeutic inhibition

of miR-221 is its association with cisplatin resistance, an agent frequently included in chemotherapy regimens for OS.

Agent	Target	Therapies
GSK458	PI3K	Phase I trial for advanced solid tumors
LY294002	PI3K	Efficacy against OS cells in vitro
BYL719 (Alpelisib)	PI3K alpha	Phase I for advanced solid tumors
BKM120 (Buparlisib)	PI3K	Phase Ib for advanced solid tumors
GDC0941 (Pictilisib)	PI3K	Phase I for advanced solid tumors
Bestatin (Ubenimex)	Aminopeptidase N	Efficacy against OS cells in vitro
KRX0401 (Perifosine)	PI3K, Akt	Efficacy against OS cells in vitro
MK-2206	Akt	Phase I for advanced solid tumors and metastatic breast cancer
Rapamycin (Sirolimus)	mTORC1	Phase II for soft tissue sarcoma and osteosarcoma
CCI779 (Temsirolimus)	mTORC1	Phase II for soft tissue sarcoma and recurrent/ refractory sarcoma
MK8669 (Ridaforolimus)	mTORC1	Phase II and III for advanced soft tissue sarcoma and osteosarcoma
RAD001 (Everolimus)	mTORC1	Phase II for advanced osteosarcoma
XL388	mTORC1/2	Efficacy against OS cells in vitro
NVP-BEZ235 (Dactolisib)	PI3K, mTORC1/2	Phase I for advanced solid tumors Efficacy in vivo against OS in mice
GDC0980 (Apitolisib)	PI3K, mTORC1/2	Phase I for advanced solid tumors
Sorafenib	RTK	Phase II for advanced osteosarcoma

"Advanced" denotes tumors that have metastasized, recurred, failed prior chemotherapies, or are not surgically resectable.

Table 1. Current agents targeting PI3K/Akt pathway with potential against osteosarcoma.

Targeted molecular therapy using RTK inhibitors has been fruitful in many cancers. EGFR causes activation of Akt in OS, and resistance to EGFR inhibitors can be seen in tumors with PTEN deletions leading to unchecked Akt activity. This is encouraging for possible combination therapies that restore PTEN function and inhibit either the PI3K pathway or RTKs. One RTK inhibitor in particular, sorafenib, may have interactions with PTEN. Sorafenib is a small molecule inhibitor of many RTKs including platelet-derived growth factor receptors (PDGFR), VEGF, EGFR, and Raf family kinases and is currently used in advanced renal cell carcinoma, thyroid cancer, and hepatocellular carcinoma. In thyroid cancers in vivo, sorafenib reversed tumor growth that had been attributed to PTEN loss [86]. Interactions between sorafenib and PTEN can also explain why acquired resistance to sorafenib in hepatocellular

carcinomas is partly due to miR-21-mediated inhibition of PTEN [87]. Notably, sorafenib is the first targeted chemotherapy agent used for treatment of OS. Sorafenib did demonstrate some activity as a single agent in patients with unresectable OS, with median progression free and overall survival of 4 and 7 months, respectively [88]. Additional effect was seen with combination of sorafenib and the mTOR inhibitor everolimus [78], and further improvement could be achieved with combination therapy specifically targeting PTEN or miR-21.

Demethylating agents may be able to restore PTEN function by removing hypermethylated promoter regions in cancer cells. In many cancerous processes, methyl groups are added throughout the genome preferentially in the promoter regions of tumor suppressor genes, to the five position of cytosine of a CpG dinucleotide (i.e., where a guanine is preceded by a cytosine). These 5-methylcytosines act as roadblocks on a cell's DNA, preventing transcription of tumor suppressor genes. Although hypermethylation occurs in many cancers, it is difficult to show that this occurs in OS in vivo. 5-Azacytidine is a commonly used demethylating agent approved for treatment of myelodysplastic syndrome. It has been shown in human OS in vitro that the PTEN gene promoter is hypermethylated and that 5-azacytidine treatments activate PTEN expression [89]. Initial uses of 5-azacytidine as an isolated agent in OS were disappointing [90]; however, recent investigations show a role for combination therapies targeting PTEN through epigenetic regulation [91].

Many other specific activators of PTEN have been recently identified, but at this time, they remain tested only in vitro. Tepoxalin, a 5-lipogenase inhibitor, appears to increase PTEN activity by preventing its alkylation or oxidation in canine OS cell lines [92]. Evodiamine, derived from the fruit of the *Evodia rutaecarpa* plant, inhibits human OS cell proliferation by increasing both protein and gene levels of PTEN [93]. Celecoxib, a cyclooxygenase-2 inhibitor, inhibits hepatocarcinoma tumor growth and angiogenesis in mice by concurrently increasing PTEN and decreasing PI3K levels [94]. Similarly, using a VEGF inhibitor in combination with celecoxib may prove beneficial in human OS [95]. Even caffeine has been shown to induce PTEN activation and many other tumor suppressor genes, while decreasing expression of IL-6 and matrix metallopeptidase 2 [96, 97]. Overall there is much promise for improving OS treatments by honing our understanding of PTEN and the PI3K pathway (**Table 2**).

Agent	Target	Effect on PTEN
None yet available	miR17, miR21, miR221	Reduced inhibition of PTEN by miR
5-Azacytidine	Demethylating agent	Increases PTEN in human OS cells
Tepoxalin	5-Lipogenase inhibitor	Increases PTEN in canine OS cells
Evodiamine	Unknown	Increases PTEN in human OS cells
Celecoxib	Cyclooxygenase-2 inhibitor	Increases PTEN and decreases PI3K in vitro
Caffeine	Unknown	Activates PTEN, decreases expression of IL-6 and MMP2

Table 2. Potential future agents for targeting PTEN in osteosarcoma.

8. Conclusion

The stagnation of current chemotherapy regimens has forced us to look beyond the usual players in oncogenesis. The mentality behind the recent targeted developments against OS involving PI3K and PTEN could be expanded to benefit many tumor types. We have been able to see through the genetic chaos of OS, finding predictability in the form of kataegis and the seemingly random mutations that converge on the PI3K/Akt pathway. Significant inroads still need to be made to clinically validate what has been proven on the bench and in animal models, but sarcomatologists are optimistic that potential therapies and improved patient survival lie within the PI3K and PTEN axis.

Author details

Matthew G. Cable and R. Lor Randall*

*Address all correspondence to: Lor.Randall@hci.utah.edu

Huntsman Cancer Institute, The University of Utah, Salt Lake City, Utah, USA

References

[1] Smith MA, Altekruse SF, Adamson PC, Reaman GH, Seibel NL. Declining childhood and adolescent cancer mortality. Cancer. 2014 Aug 15;120(16):2497–2506. doi:10.1002/cncr.28748

[2] Meyers PA, Schwartz CL, Krailo M, Kleinerman ES, Betcher D, Bernstein ML, et al. Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. J Clin Oncol. 2005 Mar 20;23(9):2004–2011.

[3] 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 Apr 10;7(1):104–112. doi:10.1016/j.celrep.2014.03.003

[4] 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 USA. 2014 Dec 23;111(51):E5564–E5573. doi:10.1073/pnas.1419260111

[5] Baserga R. The IGF-I receptor in cancer research. Exp Cell Res. 1999 Nov 25;253(1):1–6.

[6] Vadas O, Burke JE, Zhang X, Berndt A, Williams RL. Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. Sci Signal. 2011 Oct 18(4):195:re2. doi:10.1126/scisignal.2002165

[7] Zhang J, Yu XH, Yan YG, Wang C, Wang WJ. PI3K/Akt signaling in osteosarcoma. Clin Chem Acta. 2015 Apr 15;444:182–192. doi:10.1016/j.cca.2014.12.041

[8] Cantley LC. The phosphoinositide 3-kinase pathway. Science. 2002 May 31;296(5573): 1655–1657.

[9] Georgescu MM. PTEN tumor suppressor network in PI3K-Akt pathway control. Genes Cancer. 2010 Dec;1(12):1170–1177. doi:10.1177/1947601911407325

[10] Chang W, Wei K, Ho L, Berry GJ, Jacobs SS, Chang CH, et al. A critical role for the mTORC2 pathway in lung fibrosis. PLoS One. 2014 Aug 27;9(8):e106155. doi:10.1371/journal.pone.0106155

[11] Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol. 2007 Mar;9(3):316–323.

[12] Shin I, Yakes FM, Rojo F, Shin NY, Bakin AV, Baselga J, et al. PKB/Akt mediates cell-cycle progression by phosphorylation of p27Kip1 at threonine 157 and modulation of its cellular localization. Nat Med. 2002 Oct;8(10):1145–1152.

[13] Grandage VL, Gale RE, Linch DC, Khwaja A. PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kB, MAPkinase and p53 pathways. Leukemia. 2005 Apr;19(4):586–594.

[14] Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell. 1997 Oct 17; 91(2):231–241.

[15] Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, et al. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J Biol Chem. 2000 Apr 14;275(15):10761–10766.

[16] Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999 Mar 14; 96(6):857–868.

[17] Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell. 1998 Oct 2;95(1):29–39.

[18] Steck PA, Pershouse MA, Jasser SA, Yung WA, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23. 3 that is mutated in multiple advanced cancers. Nat Genet. 1997 Apr;15(4):356–362.

[19] Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem. 1998 Mar 29; 273(22):13375–13378.

[20] Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, et al. The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc Natl Acad Sci USA. 1998 Nov 10;95(23):13513–13518.

[21] Yim EK, Peng G, Dai H, Hu R, Li K, Lu Y, et al. Rak functions as a tumor suppressor by regulating PTEN protein stability and function. Cancer Cell. 2009 Apr 7;15(4):304–314. doi:10.1016/j.ccr.2009.02.012

[22] Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell. 2000 Feb 18;100(4):387–390.

[23] Weng LP, Brown JL, Eng C. PTEN coordinates G1 arrest by down-regulating cyclin D1 via its protein phosphatase activity and up-regulating p27 via its lipid phosphatase activity in a breast cancer model. Hum Mol Genet. 2001 Mar 15;10(6):599–604.

[24] Hopkins BD, Fine B, Steinbach N, Dendy M, Rapp Z, Shaw J, et al. A secreted PTEN phosphatase that enters cells to alter signaling and survival. Science. 2013 Jul 26; 341(6144):399–402. doi:10.1126/science.1234907

[25] Gong L, Govan JM, Evans EB, Dai H, Wang E, Lee SW, et al. Nuclear PTEN tumor-suppressor functions through maintaining heterochromatin structure. Cell Cycle. 2015;14(14):2323–2332. doi:10.1080/15384101.2015.1044174

[26] 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 Sept 15;113(6):1453–1461. doi:10.1002/cncr.23782

[27] Choy E, Hornicek F, MacConaill L, Harmon D, Tariq Z, Garraway L, et al. High-throughput genotyping in osteosarcoma identifies multiple mutations in phosphoinositide-3-kinase and other oncogenes. Cancer. 2012 Jun 1;118(11):2905–2914. doi:10.1002/cncr.26617

[28] Ji H, Ding Z, Hawke D, Xing D, Jiang BH, Mills GB, et al. AKT-dependent phosphorylation of Niban regulates nucleophosmin-and MDM2-mediated p53 stability and cell apoptosis. EMBO Rep. 2012 Jun1;13(6):554–560. doi:10.1038/embor.2012.53

[29] Song R, Tian K, Wang W, Wang L. P53 suppresses cell proliferation, metastasis, and angiogenesis of osteosarcoma through inhibition of the PI3K/AKT/mTOR pathway. Int J Surg. 2015 Aug;20:80–87. doi:10.1016/j.ijsu.2015.04.050

[30] Salmena L, Carracedo A, Pandolfi PP. Tenets of PTEN tumor suppression. Cell. 2008 May 2; 133(3):403–414. doi:10.1016/j.cell.2008.04.013

[31] Levine RA, Forest T, Smith C. Tumor suppressor PTEN is mutated in canine osteosarcoma cell lines and tumors. Vet Pathol. 2002 May;39(3):372–378.

[32] Yamaguchi T, Toguchida J, Yamamuro T, Kotoura Y, Takada N, Kawaguchi N, et al. Allelotype analysis in osteosarcomas: frequent allele loss on 3q, 13q, 17p, and 18q. Cancer Res. 1992 May 1;52(9):2419–2423.

[33] Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. Proc Natl Acad Sci USA. 1999 May 25;96(11):6199–6204.

[34] Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, et al. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res. 1997 Oct 1;57(19):4183–4186.

[35] Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet. 1997 May;16(1):64–67.

[36] Cristofano AD, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. Nat Genet. 1998 Aug;19(4):348–355.

[37] Lahtz C, Stranzenbach R, Fiedler E, Helmbold P, Dammann RH. Methylation of PTEN as a prognostic factor in malignant melanoma of the skin. J Invest Dermatol. 2010 Feb;130(2):620–622. doi:10.1038/jid.2009.226

[38] Kang YH, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. Lab Invest. 2002 Mar;82(3):285–291.

[39] García JM, Silva J, Peña C, Garcia V, Rodríguez R, Cruz MA, et al. Promoter methylation of the PTEN gene is a common molecular change in breast cancer. Genes Chromosomes Cancer. 2004 Oct;41(2):117–124.

[40] Salvesen HB, MacDonald N, Ryan A, Jacobs IJ, Lynch ED, Akslen LA, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int J Cancer. 2001 Jan;91(1):22–26.

[41] Kawaguchi KI, Oda Y, Saito T, Yamamoto H, Takahira T, Kobayashi C, et al. DNA hypermethylation status of multiple genes in soft tissue sarcomas. Mod Pathol. 2006 Jan; 19(1):106–114.

[42] Gao Y, Luo LH, Li S, Yang C. miR-17 inhibitor suppressed osteosarcoma tumor growth and metastasis via increasing PTEN expression. Biochem Biophys Res Commun. 2014 Feb 7;444(2):230–234. doi:10.1016/j.bbrc.2014.01.061

[43] Shen L, Chen XD, Zhang YH. MicroRNA-128 promotes proliferation in osteosarcoma cells by downregulating PTEN. Tumour Biol. 2014 Mar;35(3):2069–2074. doi:10.1007/s13277-013-1274-1

[44] Namløs HM, Meza-Zepeda LA, Barøy T, Østensen IH, Kresse SH, Kuijjer ML, et al. Modulation of the osteosarcoma expression phenotype by microRNAs. PLoS One. 2012;7(10):e48086. doi:10.1371/journal.pone.0048086

[45] 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 Jun;47(6):615–624. doi:10.1038/ng.3293

[46] Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 2009 Aug;9(8):550–562. doi:10.1038/nrc2664

[47] Cahill CM, Rogers JT. Interleukin (IL) 1β induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase-dependent AKT/IκB kinase α pathway targeting activator protein-1. J Biol Chem. 2008 Sep 19;283(38):25900–25912. doi:10.1074/jbc.M707692200

[48] Weiss KR, Cooper GM, Jadlowiec JA, McGough RL 3rd, Huard J. VEGF and BMP expression in mouse osteosarcoma cells. Clin Orthop Relat Res. 2006 Sep;450:111–117. doi:10.1097/01.blo.0000229333.98781.56

[49] Xi Y, Chen Y. Oncogenic and therapeutic targeting of PTEN loss in bone malignancies. J Cell Biochem. 2015 Sep;116(9):1837–1847. doi:10.1002/jcb.25159

[50] Moon JB, Kim JH, Kim K, Youn BU, Ko A, Lee SY, et al. Akt induces osteoclast differentiation through regulating the GSK3β/NFATc1 signaling cascade. J Immunol. 2012 Jan 1;188(1):163–169. doi:10.4049/jimmunol.1101254

[51] Sugatani T, Alvarez U, Hruska KA. PTEN regulates RANKL-and osteopontin-stimulated signal transduction during osteoclast differentiation and cell motility. J Biol Chem. 2003 Feb 14;278(7):5001–5008.

[52] Filtz EA, Emery A, Lu H, Forster CL, Karasch C, Hallstrom TC. Rb1 and Pten co-deletion in osteoblast precursor cells causes rapid lipoma formation in mice. PLoS One. 2015 Aug 28;10(8):e0136729. doi:10.1371/journal.pone.0136729

[53] Blüml S, Friedrich M, Lohmeyer T, Sahin E, Saferding V, Brunner J, et al. Loss of phosphatase and tensin homolog (PTEN) in myeloid cells controls inflammatory bone destruction by regulating the osteoclastogenic potential of myeloid cells. Ann Rheum Dis. 2015 Jan;74(1):227–233. doi:10.1136/annrheumdis-2013-203486

[54] Guntur AR, Reinhold MI, Cuellar J, Naski MC. Conditional ablation of Pten in osteoprogenitors stimulates FGF signaling. Development. 2011 Apr;138(7):1433–1444. doi:10.1242/dev.058016

[55] Zhao J, Zhang ZR, Zhao N, Ma BA, Fan QY. VEGF silencing inhibits human osteosarcoma angiogenesis and promotes cell apoptosis via PI3K/AKT signaling pathway. Cell Biochem Biophys. 2015 Nov;73(2):519–525. doi:10.1007/s12013-015-0692-7

[56] Zhang A, He S, Sun X, Ding L, Bao X, Wang N. Wnt5a promotes migration of human osteosarcoma cells by triggering a phosphatidylinositol-3 kinase/Akt signals. Cancer Cell Int. 2014 Feb 14;14(1):15. doi:10.1186/1475-2867-14-15

[57] Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T, et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. Nat Med. 2012 Feb 19;18(3):405–412. doi:10.1038/nm.2653

[58] Dissanayake SK, Wade M, Johnson CE, O'Connell MP, Leotlela PD, French AD, et al. The Wnt5A/protein kinase C pathway mediates motility in melanoma cells via the inhibition

of metastasis suppressors and initiation of an epithelial to mesenchymal transition. J Biol Chem. 2007 Jun 8;282(23):17259–17271.

[59] Weeraratna AT, Jiang Y, Hostetter G, Rosenblatt K, Duray P, Bittner M, et al. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell. 2002 Apr;1(3):279–288.

[60] Mulholland DJ, Kobayashi N, Ruscetti M, Zhi A, Tran LM, Huang J, et al. Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. Cancer Res. 2012 Apr 1;72(7):1878–1889. doi:10.1158/0008-5472.CAN-11-3132

[61] Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. Cancer Res. 2007 Apr 1;67(7):2922–2926.

[62] Chen J, Yan D, Wu W, Zhu J, Ye W, Shu Q. MicroRNA-130a promotes the metastasis and epithelial-mesenchymal transition of osteosarcoma by targeting PTEN. Oncol Rep. 2016 Jun; 35(6):3285–3292. doi:10.3892/or.2016.4719

[63] Lin YM, Chang ZL, Liao YY, Chou MC, Tang CH. IL-6 promotes ICAM-1 expression and cell motility in human osteosarcoma. Cancer Lett. 2013 Jan 1;328(1):135–143. doi:10.1016/j.canlet.2012.08.029

[64] Wang J, Loberg R, Taichman RS. The pivotal role of CXCL12 (SDF-1)/CXCR4 axis in bone metastasis. Cancer Metastasis Rev. 2006 Dec;25(4):573–587.

[65] Conley-LaComb MK, Saliganan A, Kandagatla P, Chen YQ, Cher ML, Chinni SR. PTEN loss mediated Akt activation promotes prostate tumor growth and metastasis via CXCL12/CXCR4 signaling. Mol Cancer. 2013 Jul 31;12(1):85. doi:10.1186/1476-4598-12-85

[66] Tamura M, Gu J, Matsumoto K, Aota SI, Parsons R, Yamada KM. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science. 1998 Jun 5; 280(5369):1614–1617.

[67] Ren K, Lu X, Yao N, Chen Y, Yang A, Chen H, et al. Focal adhesion kinase overexpression and its impact on human osteosarcoma. Oncotarget. 2015 Oct 13;6(31):31085–31103. doi:10.18632/oncotarget.5044

[68] Hu Y, Xu S, Jin W, Yi Q, Wei W. Effect of the PTEN gene on adhesion, invasion and metastasis of osteosarcoma cells. Oncol Rep. 2014 Oct;32(4):1741–1747. doi:10.3892/or.2014.3362

[69] Robl B, Pauli C, Botter SM, Bode-Lesniewska B, Fuchs B. Prognostic value of tumor suppressors in osteosarcoma before and after neoadjuvant chemotherapy. BMC Cancer. 2015 May 9;15:379. doi:10.1186/s12885-015-1397-4

[70] Zhou Y, Zhu LB, Peng AF, Wang TF, Long XH, Gao S, et al. LY294002 inhibits the malignant phenotype of osteosarcoma cells by modulating the phosphatidylinositol 3-kinase/

Akt/fatty acid synthase signaling pathway in vitro. Mol Med Rep. 2015 Feb;11(2):1352–1357. doi:10.3892/mmr.2014.2787

[71] Gobin B, Huin MB, Lamoureux F, Ory B, Charrier C, Lanel R, et al. BYL719, a new α-specific PI3K inhibitor: single administration and in combination with conventional chemotherapy for the treatment of osteosarcoma. Int J Cancer. 2015 Feb 15;136(4):784–796. doi:10.1002/ijc.29040

[72] Anderson JL, Park A, Akiyama R, Tap WD, Denny CT, Federman N. Evaluation of in vitro activity of the class I PI3K inhibitor buparlisib (BKM120) in pediatric bone and soft tissue sarcomas. PLoS One. 2015 Sep 24;10(9):e0133610. doi:10.1371/journal.pone.0133610

[73] Sarker D, Ang JE, Baird R, Kristeleit R, Shah K, Moreno V, et al. First-in-human phase I study of pictilisib (GDC-0941), a potent pan–class I phosphatidylinositol-3-kinase (PI3K) inhibitor, in patients with advanced solid tumors. Clin Cancer Res. 2015 Jan 1;21(1):77–86. doi:10.1158/1078-0432.CCR-14-0947

[74] Liang W, Gao B, Xu G, Weng D, Xie M, Qian Y. Possible contribution of aminopeptidase N (APN/CD13) to migration and invasion of human osteosarcoma cell lines. Int J Oncol. 2014 Dec;45(6):2475–2485. doi:10.3892/ijo.2014.2664

[75] Yao C, Wei JJ, Wang ZY, Li D, Yan SC, Yang YJ, et al. Perifosine induces cell apoptosis in human osteosarcoma cells: new implication for osteosarcoma therapy? Cell Biochem Biophys. 2013 Mar;65(2):217–227. doi:10.1007/s12013-012-9423-5

[76] Jiang H, Zeng Z. Dual mTORC1/2 inhibition by INK-128 results in antitumor activity in preclinical models of osteosarcoma. Biochem Biophys Res Commun. 2015 Dec 4–11;468(1):255–261. doi:10.1016/j.bbrc.2015.10.119

[77] Bagatell R, Norris R, Ingle AM, Ahern C, Voss S, Fox E, et al. Phase 1 trial of temsirolimus in combination with irinotecan and temozolomide in children, adolescents and young adults with relapsed or refractory solid tumors: a Children's Oncology Group Study. Pediatr Blood Cancer. 2014 May;61(5):833–839. doi:10.1002/pbc.24874

[78] 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. J Clin Oncol. 2012 Jan 1;30(1):78–84. doi:10.1200/JCO.2011.35.6329

[79] Grignani G, Palmerini E, Ferraresi V, D'Ambrosio L, Bertulli R, Asaftei SD, et al. Sorafenib and everolimus for patients with unresectable high-grade osteosarcoma progressing after standard treatment: a non-randomised phase 2 clinical trial. Lancet Oncol. 2015 Jan;16(1):98–107. doi:10.1016/S1470-2045(14)71136-2

[80] Zhu YR, Zhou XZ, Zhu LQ, Yao C, Fang JF, Zhou F, et al. The anti-cancer activity of the mTORC1/2 dual inhibitor XL388 in preclinical osteosarcoma models. Oncotarget. 2016;Jul 2. doi:10.18632/oncotarget.10389 [Epub ahead of print].

[81] Zhu YR, Min H, Fang JF, Zhou F, Deng XW, Zhang YQ. Activity of the novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 against osteosarcoma. Cancer Biol Ther. 2015;16(4):602–609. doi:10.1080/15384047.2015.1017155

[82] Dolly SO, Wagner AJ, Bendell JC, Kindler HL, Krug LM, Seiwert TY, et al. Phase I study of Apitolisib (GDC-0980), dual phosphatidylinositol-3-kinase and mammalian target of rapamycin kinase inhibitor, in patients with advanced solid tumors. Clin Cancer Res. 2016 Jun 15;22(12):2874–2884. doi:10.1158/1078-0432.CCR-15-2225

[83] Lv C, Hao Y, Tu G. MicroRNA-21 promotes proliferation, invasion and suppresses apoptosis in human osteosarcoma line MG63 through PTEN/Akt pathway. Tumour Biol. 2016 Jul;37(7):9333–9342. doi:10.1007/s13277-016-4807-6

[84] Leone E, Morelli E, Di Martino MT, Amodio N, Foresta U, Gullà A, et al. Targeting miR-21 inhibits in vitro and in vivo multiple myeloma cell growth. Clin Cancer Res. 2013 Apr 15;19(8):2096–2106. doi:10.1158/1078-0432.CCR-12-3325

[85] Zhao G, Cai C, Yang T, Qiu X, Liao B, Li W, et al. MicroRNA-221 induces cell survival and cisplatin resistance through PI3K/Akt pathway in human osteosarcoma. PLoS One. 2013;8(1):e53906. doi:10.1371/journal.pone.0053906

[86] Mirantes C, Dosil MA, Eritja N, Felip I, Gatius S, Santacana M, et al. Effects of the multikinase inhibitors Sorafenib and Regorafenib in PTEN deficient neoplasias. Eur J Cancer. 2016 Aug;63:74–87. doi:10.1016/j.ejca.2016.04.019

[87] He C, Dong X, Zhai B, Jiang X, Dong D, Li B, et al. MiR-21 mediates sorafenib resistance of hepatocellular carcinoma cells by inhibiting autophagy via the PTEN/Akt pathway. Oncotarget. 2015 Oct 6;6(30):28867–28881. doi:10.18632/oncotarget.4814

[88] Grignani G, Palmerini E, Dileo P, Asaftei SD, D'Ambrosio L, Pignochino Y, et al. A phase II trial of sorafenib in relapsed and unresectable high-grade osteosarcoma after failure of standard multimodal therapy: an Italian Sarcoma Group study. Ann Oncol. 2012 Feb;23(2):508–516. doi:10.1093/annonc/mdr151

[89] Song D, Ni J, Xie H, Ding M, Wang J. DNA demethylation in the PTEN gene promoter induced by 5-azacytidine activates PTEN expression in the MG-63 human osteosarcoma cell line. Exp Ther Med. 2014 May;7(5):1071–1076.

[90] Srinivasan U, Reaman GH, Poplack DG, Glaubiger DL, Levine AS. Phase II study of 5-azacytidine in sarcomas of bone. Am J Clin Oncol. 1982 Aug;5(4):411–416.

[91] Thayanithy V, Park C, Sarver AL, Kartha RV, Korpela DM, Graef AJ, et al. Combinatorial treatment of DNA and chromatin-modifying drugs cause cell death in human and canine osteosarcoma cell lines. PLoS One. 2012;7(9):e43720. doi:10.1371/journal.pone.0043720

[92] Loftus JP, Cavatorta D, Bushey JJ, Levine CB, Sevier CS, Wakshlag JJ. The 5-lipoxygenase inhibitor tepoxalin induces oxidative damage and altered PTEN status prior to apoptosis in canine osteosarcoma cell lines. Vet Comp Oncol. 2016 Jun;14(2):e17–30. doi:10.1111/vco.12094

[93] Meng ZJ, Wu N, Liu Y, Shu KJ, Zou X, Zhang RX, et al. Evodiamine inhibits the proliferation of human osteosarcoma cells by blocking PI3K/Akt signaling. Oncol Rep. 2015 Sep;34(3):1388–1396. doi:10.3892/or.2015.4084

[94] Sui W, Zhang Y, Wang Z, Wang Z, Jia Q, Wu L, et al. Antitumor effect of a selective COX-2 inhibitor, celecoxib, may be attributed to angiogenesis inhibition through modulating the PTEN/PI3K/Akt/HIF-1 pathway in an H22 murine hepatocarcinoma model. Oncol Rep. 2014 May;31(5):2252–2260. doi:10.3892/or.2014.3093

[95] Liu J, Wu J, Zhou L, Pan C, Zhou Y, Du W, et al. ZD6474, a new treatment strategy for human osteosarcoma, and its potential synergistic effect with celecoxib. Oncotarget. 2015 Aug 28;6(25):21341–21352.

[96] Miwa S, Sugimoto N, Shirai T, Hayashi K, Nishida H, Ohnari I, et al. Caffeine activates tumor suppressor PTEN in sarcoma cells. Int J Oncol. 2011 Aug;39(2):465–472. doi:10.3892/ijo.2011.1051

[97] Al-Ansari MM, Aboussekhra A. Caffeine mediates sustained inactivation of breast cancer-associated myofibroblasts via up-regulation of tumor suppressor genes. PLoS One. 2014 Mar 3;9(3):e90907. doi:10.1371/journal.pone.0090907

[98] Das S, Dixon JE, Cho W. Membrane-binding and activation mechanism of PTEN. PNAS. 2003 Jun;100(13):7491–7496. doi:10.1073/pnas.0932835100