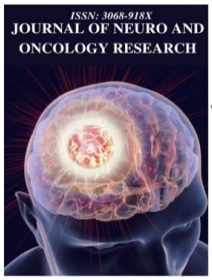

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Review Article

Epigenetic Modifications are Instrumental for Improving Overall Research and Management of Osteosarcoma in the Framework of 3P Medicine

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Abstract

Osteosarcoma is characterized as the most aggressive malignant bone cancer in infants and adolescents. Due to advanced therapeutic outcomes, the survival rate of 5 years is still nearly 60% if it is diagnosed early and can prescribe enervating treatments, e.g. amputations. A complete knowledge of the disease can assist in enriching clinical consequences for osteosarcoma patients. One propitious approach of osteosarcoma investigation is the discipline of epigenetics. Epigenetics, a key discipline that mainly describes the distinctive countenance of phenotypes emerging from the constant sequence of genes and the congenital of epigenetic modification, has acquired much devotion in prescription. Epigenetic modifications e.g. DNA methylation, histone alteration, ubiquitination, noncoding RNAs and m6A are widely intricate in the growth and progression of human cancers. Current study highlights how epigenetic profiles modified proteoforms to determine osteosarcoma prediction, prevention and prescribe personalized medicine to improve patient outcomes. Underlines many epigenetic target genes and molecular networks mechanisms that regulate different angles of osteosarcoma, including metastasis, propagation, infiltration and chemotherapy resistance. Current review emphasized the recent advancement in epigenetic regulation targeting the proteoforms of osteosarcoma and elaborated the clinical outcomes in the discipline of Predictive, Preventive as well as Personalized Medicine (PPPM; 3PM).

Keywords: Epigenetic Modification; Proteoforms; Proteoformics; Biomarkers; DNA Methylation; Histone Modification; Ubiquitinatioin; Noncoding RNAS; M6A; Predictive; Preventive; Personalized Medicine; Osteosarcoma

Abbreviations

DNMT: DNA methyltransferase; PTM: Post translation modification; RISCs: RNA-induced silencing complexes; M6A: N6-methyladenine; TAL1: T cell acute lymphocytic leukemia; HAT: Histone acetyltransferase; HDAC: Histone deacetylase; HMT: Histone methyltransferase; RA: Retinoic acid; RARα: Retinoic acid receptor alpha; DDB1: DNA damage binding protein 1; RBX1:

Ring box protein 1; CDK: Cyclin dependent kinase; SUMO: Small ubiquitin-like modifier; PML: Promyelocytic leukemia; NB: Nuclear bodies; H3T3ph: Histone 3 threonine 3 phosphorylation; NF-κB: Nuclear factor kappa beta; TNFα: Tumor necrosis factor alpha; ECM: Extracellular matrix; MMPs: Matrix metalloproteinases; TGFα: Transforming growth factor alpha; BANCR: BRAF-activated noncoding RNA; DR3: Death receptor 3; EMGs: Modification-related genes; EZH2: Enhancer of zeste homolog 2; FBP1: FUSE binding protein 1; FGFR3-AS1: Fibroblast growth factor receptor 3 antisense transcript 1; GSK343: GlaxoSmithKline 343; HOTAIR: HOX antisense intergenic RNA; IMPs: Immune-related DNA methylation patterns; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; PARPi: Poly (ADP-ribose) polymerase inhibitors; POU2F1: POU domain class 2 transcription factor 1; SE: Super enhancer molecule; SNHG12: Small nuclear RNA host gene 12; TARGET: Therapeutically applicable research to generate effective treatment; TUG1: Taurine upregulated gene 1; UCA1: Urothelial carcinoma associated 1; VEGI: Vascular endothelial growth inhibitor

Introduction

Osteosarcoma is the most aggressive malignant bone tumor and the second leading cause of cancer related death among children and adolescents globally. This highly mineralized tissue constitutes an osteoid matrix in multinucleated cells [1,2]. Approximately 10-25% of bone cancer patients develop lung metastasis with pulmonary complications being the root causes of osteosarcoma-related mortality [3]. Comprehensive diagnosis procedures including surgery resection and chemotherapies have expressively improved patient outcomes and survival rate [4]. Presently, the five-year survivance percentage of osteosarcoma patients has improved to 70%. In addition, some patients acquire resistance to chemotherapeutics agents leading to disease progression, metastasis and recurrence, which is a vital obstacle for thriving successful long-term treatment [5]. Advancement in technology and furnished research to pinpoint some inventive therapeutic methodologies, most of the diagnosed percentage of osteosarcoma patients have shown limited improvement over the past three decades [6].

A lack of clear disease progression highlights the prerequisite for new approaches to study and diagnosis of this disease, necessitating the development of innovative approaches. In modern medicine, cancer is closely associated with genetics. Scientists have acknowledged communication between certain cancers and specific genetic mutations, inherited patterns and exposure to cancer-causing agents. The precise genetic causes of osteosarcoma remain unclear, the majority of cases sporadically. However, osteosarcoma has been linked to factors such as radiation exposure, hereditary cancer predisposition syndromes (like familial retinoblastoma and Li-Fraumeni syndrome) and certain bone abnormalities, mainly Paget's disease [7,8]. In osteosarcoma, the genetic complexity and variability of cancer have partially the clinical impact of genetic discoveries as no specific molecular target has significantly reduced mortality [9]. While genetics has provided some insights, it doesn't fully explain the disease's diverse behaviors and phenotypes. This gap has led researchers to explore epigenetics, which studies heritable variations in gene transcription profiles that are not involved in alteration to the DNA sequence itself. Epigenetic alterations play critical role in cancer development, influencing processes like tumor growth, development, progression, metastasis and conflict to chemotherapy [10]. These changes can help in explaining the wide range of osteosarcoma behaviors, such as aggressive metastasis or treatment resistance.

Recent research has focused on profiling epigenetic changes in osteosarcoma, aiming to better understand their role in the disease and identify potential therapeutic targets or prognostic markers [11]. However, existing reviews often lack a comprehensive integration of the various epigenetic mechanisms involved. This review aims to fill that gap by providing a detailed overview of recent advances in osteosarcoma epigenetics, focusing on three key areas: genomic DNA Methylation, histone alterations and RNA modifications (non-coding RNA (ncRNA)). Addition of methyl group (CH3) to DNA sequence by DNA Methyltransferases (DNMTs) known as DNA methylation.

In the human genome, DNA methylation targets Cytosine-Guanine nucleotides (CpG) that mostly exist in the promoter sequence of genes. DNA methylation alters the methylation pattern of promoter sequence to regulate the transcription profile of the gene. In case of human malignancy, methylation can either promote or inhibit the gene expression [12]. Histones are proteins that DNA wraps around, forming chromatin and chemical changes to histones (like addition or eradicating methyl or acetyl groups) can untie or constrict chromatin, making genes more or less active. These changes play a key role in cancer progression [13]. ncRNA is a widely expression RNA molecules that do not encode proteins but possess several additional functions such as microRNA (miRNAs), mainly intricate in silencing the Post-Translational Modification (PTM) of mRNA functions. Circular RNAs (cirRNA) and long noncoding RNAs (lncRNA) are involved in regulating gene expressions and other various regulatory

processes, including epigenetic control [14].

Emerging research highlights the important contribution of epigenetic alterations including DNA methylation, histone modifications, as well as ncRNAs in osteosarcoma development, progression and treatment resistance. These changes provide opportunities for Predictive, Preventive and Personalized Medicine (PPPM; 3PM) approaches. It will be supportive for investigators/scientists to explore the novel role of epigenetic modification in osteosarcoma as well as a potential target that enhances the potency of therapeutic treatment modalities.

Epigenetic Regulation of Osteosarcoma

In 1942, Waddington introduced the concept of epigenetics in biology to explore the relationship between phenotypes and genotypes [15]. Over the last two decades, epigenetics has grown into a fast-evolving field, with a significant increase in research publications [16]. Epigenetic regulation is crucial in various diseases, such as autoimmunity and cancer [17,18]. These epigenetic changes can lead to heritable and reversible shifts in phenotypes without altering the DNA sequence [19]. Epigenetic modifications mediate the biological molecules that regulate the entire biological system mechanism. These biological molecules are DNAs, RNAs and proteins that form the complete structure and function of biological system called proteoform (Fig. 1). The key mechanisms include histone modifications, DNA methylation, ncRNAs, as well as N6-methyladenine (m6A) [20,21]. MicroRNAs (miRNAs), which modify RNA molecules rather than encoding proteins, have been extensively studied. They work together with other miRNAs to finely regulate protein production [22]. Anti-miRNA oligo nucleotides can block miRNA activity by disrupting their interaction with RNA-Induced Silencing Complexes (RISCs) [23].

DNA methylation, influenced by sequences beyond CpG sites, can vary based on the presence or absence of specific DNA bases [24]. Histone modifications are often controlled by small molecules like histone deacetylase inhibitors, which target enzymes responsible for these changes. Additionally, targeting transcription factors and exploring the interplay between RNAs and histone-binding proteins can influence processes like alternative splicing which connects histone modifications to stem cell behavior [25,26]. As epigenetic research advances, it offers promising tools for disease prevention, diagnosis and treatment [27].

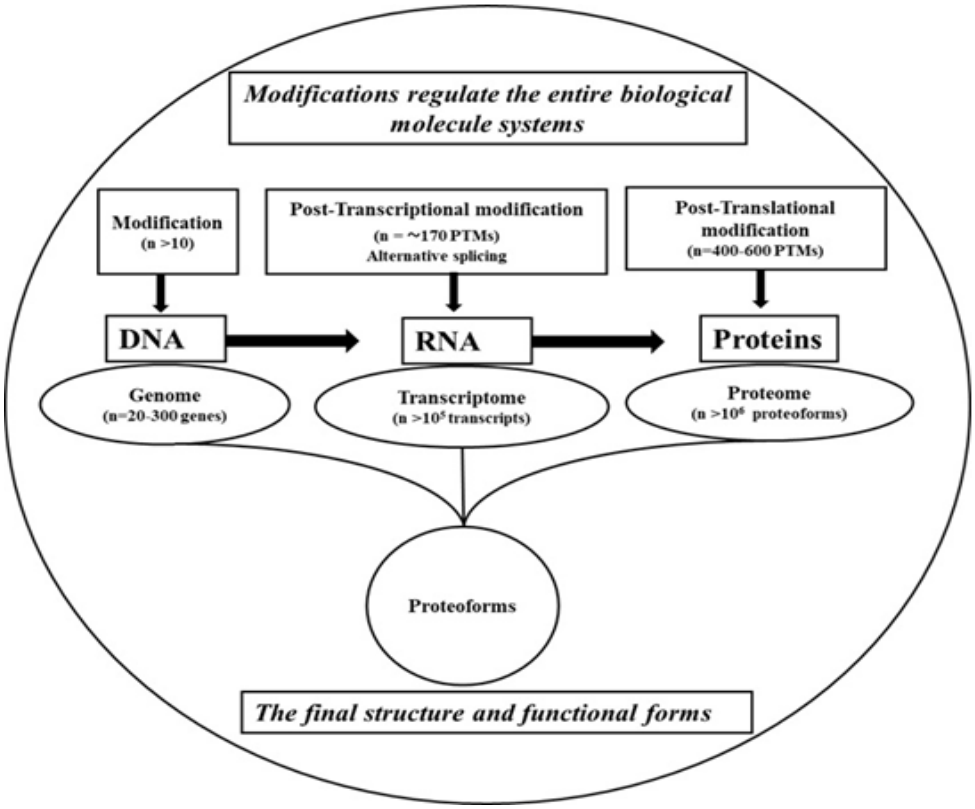

Figure 1: Epigenetic modification regulates the inclusive biological system at DNA, RNA and protein level, which mainly result in the formation of proteoforms that are the possible structure and function of a gene or protein. Refined and reconstructed figure with copyright permission from Wiley publisher [28].

DNA Methylation in Osteosarcoma

DNA methylation is the most significant epigenetic modification concerns with hindering expression of gene permanently or aimed at long term. It is primarily defined by the attachment of methyl group (CH3) to the cytosine bases of DNA molecule, which occurs frequently in CpG islands that are not completely spread in the entire genome [29]. The human genome comprises of prolong bounces of CpG regions, with abundance of CpG dinucleotides, mostly located in large numbers in the promoter region of a gene. Under normal conditions, the CpG islands located within gene promoter regions naturally remain unmethylated and letting for active transcription [30]. During mammalian development, methylation status of these region is established and preserved throughout entire organism's lifespan. This epigenetic modification is mediated by DNMT enzymes which regulate the methylation pattern of genes. Abnormal expressions of DNMTs might deregulate the methylation form that result in genomics uncertainty and promote tumorigenesis or continues the malignancy of cancer cells [31, 32]. Eukaryotic DNMTs are classified as Dnmt1, Dnmt2, Dnmt3a and Dnmt3b [33].

DNMT1 activaly contributes in methylation process and is particularly accountable for upholding DNA methylation function at developmental stages while DNMT2 enzymes supports transfer RNAs (tRNAs) methylation. In embryogenesis/germ cell development process, DNMT3a/DNMT3b regulate DNA methylation status [34, 35]. Typically, sarcoma arises due to the transcription inactivation of a gene as a result of hypermethylation in the promoter region. Aberrant DNA methylation mediates initiate or block gene may affect various signaling communications including cell cycle regulation, DNA restoration and activation of apoptosis associated with the tumor cell development [36]. The inactivation of tumor repressor genes due to methylation process occurs in osteosarcoma. Even though blocking of RB and TP53 genes do not often occur due to methylation, where variation in p53 and pRB pathways identified as virulent methylation objects, particularly at CDKN2A gene position, which encode proteins that inhibit the expression of Mdm2, p14ARF, p16INK4a and cyclin-dependent kinase inhibitor [37].

In osteosarcoma/xenograft cell lines, numerous p53-responsive genes such as HIC1, CDKN1A and GADD45 validate epigenetic blocking through promoter hypermethylation, signifying this as a mutual mechanism of tumor suppressor inactivation [38]. Furthermore, promoter hypermethylation is involved in silencing the expression of some additional tumor repressor genes in osteosarcoma cell lines, such as TIMP3, DAPK1 and RASSF1A [39]. Another study investigated that GADD45 gene expressed proteins [5-hydroxymethylcytosine (5hmc)] regulate the pattern of methylation in osteogenic differentiation is predominantly suppressed through c-Myc and Akt/PI3K signaling in osteosarcoma cells [40]. IRX1 gene recognized as a pro-metastatic allied gene in osteosarcoma once it possesses low methylation status. IRX1 gene codes a protein of the Iroquois homeobox protein 1 family, a transcript aspect involved in embryo development and formerly known as a tumor silencer in gastric malignancy [41]. It has been determined that IRX1 hypomethylation enhances lung metastasis of osteosarcoma and hence the enhanced expression of this gene is mainly involved with the promoter hypomethylation in both clinical tumor samples as well as in osteosarcoma-derived cell lines.

Methylation scrutiny of approximately 1.1 million loci has been investigated on the tissue samples taken from osteosarcoma patients concluded that patients having cancer relapse possess large percentage of methylation. Several oncogenes such as PAK1, RAF1 and SEMA4D are hypomethylated and are extremely articulated in osteosarcoma [42]. Nevertheless, almost these epigenetic alterations such as abnormal stimulation or dominance are interrelated with the absence of trancriptions capability at object loci in osteosarcoma [42,43].

A complete research analysis of DNA methylation patterns both in osteosarcoma tissue samples and normal tissue samples clearly validated that the promoter region of specific genes is highly methylated in the cancer tissue samples. The indispensable functions and pathways embattled by these genes are defined by protein-protein interaction (PPI) [44], which results in the detection of cancer related genes on the basis of their methylated promoters [39]. Toll receptors signaling communications controlled enhance level of distinction hypomethylation. The pattern of hypermethylation is suggestively superior in the transcription activator STAT3, the MAXI interactor signals transducer 1 (MXI1) and the T-cell Acute Lymphocytic leukemia 1 (TAL1) [39]. The transcription of HOTAIR gene has also been closely involved in the regulation of osteosarcoma and its inhibition results in decreasing the expression of DNMT1 which leads to reduce the overall DNA methylation patterns of target genes. Furthermore, it has also been shown that the product of HOTAIR gene decreased CDKN2A level by inhibiting the CDKN2A promoter function via DNA hypermethylation [39,45].

Systematically, HOTAIR gene acts as a significant part in osteosarcoma by silencing miR-126 expression, which turns as a negative regulator of DNMT1. Increased functions of DNMT1, corresponding to the suppression of CDKN2A due to DNA hypermethylation of its promoter, therefore, run the progression of tumor [45]. Moreover, several moderately methylated loci have been concerned with overexpression of genes including CDK4, KIRRL2 and CEP72, which can exert important part in the pathogenesis of osteosarcoma [46].

Investigation of more than 12,000 genes for distinctive methylation status and approximately more than 3,000 genes for divergent manner in the osteosarcoma disclosed that the performance of genes associated to this malignancy are mostly improved in cellular and biological methods correlated to inflammatory response, immune reaction, pertussis pathway and hematopoietic cell ancestry pathway etc. NRF and UBS are identified to be mediated by various genes in osteosarcoma. Kaplan-Meier identification of osteosarcoma-associated genes revealed that the RIPK3, DOCK2 and BHMT2 act as necessary survivance-related condidates. PRAME and SEMA3A are embarrassed in the 40 genes and are especially in the top 10 of the most differentially transcribed genes in osteosarcoma [47]. The detailed description summary of methylation modifications targeting different cellular and molecular network were mentioned in Table 1. Schematic representation of DNA methylation and acetylation regulating osteosarcoma were shown (Fig. 2).

Modification	Modifying Agent	Proposed Downstream Mechanism	Resulting Cellular Changes	References
Hypermethylation	PTB1	enhanced DNMT expression	Increased cell migration, proliferation,	[48]
SOCS3 methylation	THAP9-AS1	Activate JAK2/STAT3	Silenced ROS levels, increased cell migration, metastasis and proliferations	[49]
CDK4 methylation	lncRNA 91	disturbed Cell cycle	Inhibited cell apoptosis, enhanced cell migration, invasion and proliferation	[50]
NNAT methylation,		Bone and Ca2+ homeostasis	Thapsigargin sensitivity increase cell migration and colony-forming potential	[51]
APCDD1 methylation	DNMT3a	Target Wnt/B-Catenin singling pathway	Increased cell metastasis and invasion	[52]
CXCL12 methylation	DNMT1	Lowered CD8+T cell response	Tumor immune response, increased cell migration, metastasis and proliferations	[53]
SPRY2 methylation		Activate MAPK/ERK pathway	enhanced cell proliferation	[54]
miR-149 promoter methylation		Activate NOTCH1-mediated Sonic Hedgehog pathway	Increased cell metastasis and growth	[55]
ANK1 methylation		Suppressed miR-486-5p level	increased cell morphology	[56]
miR-195 promoter methylation		Enhanced FASN expression	Increased cell movement, viability and invasion	[57]
CDKN2A methylation	HOTAIR	Target DNMT1 expression via suppression of miR-126	Inhibited cellular apoptosis	[58]
E-Cadherin methylation	SENP3	Accelerated epithelial-mesenchymal transition	Inhibited cell apoptosis, increased cell invasion, migration and proliferation	[59]
Increased m6a levels	METTL3	Enhanced ATAD2 and DRG1 expression	Suppressed cellular apoptosis, promoted cell invasion and migration	[60]

Table 1: Descriptive summary of methylations targeting different cellular and molecular network.

Histone Modifications in Osteosarcoma

Histone modifications act as one of the vital covalent post-translational modifications that occur at the amino locus of the histone proteins. It plays a significant role in gene transcript either by modifying chromatin framework or by selecting histone modifiers [61]. Histone modification tends to be more complicated compared to DNA methylation and mainly composed of methylation, phosphorylation, acetylation, sumoylation and ubiquitination [62]. The covalent modification at the amino-terminus of histone residues altered the functionality of chromatin-related proteins and promoted the modulation of linear transitions among transcriptionally energetic or implicit chromatin states [39]. Histone proteins are mainly associated with packaging of DNA in the chromosomes and therefore intricate in different biological procedures including transcriptional silencing/initiation, packaging of chromosomes as well as DNA damage/repair. Histone Acetyltransferase (HAT) and Histone Deacetylase (HDAC) mainly concern with the typical state of acetylating histone proteins and other important regulating factors that tethered to the promoter of genes [39].

The association of histone modification that regulates the role of A and B promoters were observed in different osteosarcoma cell lines. It has been recognized that H3K4me3, a biomarker for initiations, possesses enhanced percentage of histone modification in A promoter and a reduced percentage of histone modification in B promoter of WNT5A gene in U2OS osteosarcoma cells indicating that H3K4me3 emerges suppressive role, declining the function of B promoter. Moreover, it has been investigated that the level of active H3K4me3 in A promoter is more than B promoter in both SaOS-2 and U2OS osteosarcoma cells. Hence, the repressive role of H3K4me3 is highly enriched in the B promoter of WNT5A in SaOS-2 osteosarcoma cells and is mainly associated with osteosarcoma characteristics. Suppression of B promoter of the WNT5A gene results in the progression of osteosarcoma and highly involved in histone modifications and DNA methylation. This observation identified that the occurrence of histone modifications in the promoter B of WNT5A gene repressed their expression in osteosarcoma cells [66]. Diagrammatic representation of list of histone modification associated with gene regulation were shown (Fig. 2).

Figure 2: Graphical representation of different types of histone modifications associated with the development and progression of human diseases. The red color letters represent the enzyme that regulate target histone modification. The green color letters represent the target site of mentioning histone modification.

Histone Acetylation in Osteosarcoma

The acetylation of histone occurs due to enzymatic attachment of an acetyl group (COCH3) to the amino terminus of histones. Histone acetylation is particularly involved in the directive of different biological and cellular developments such as transcription activation/silencing of genes, chromatin dynamics, cell cycle procession, DNA copying, DNA restoration, cell differentiation, apoptosis, neuronal repression and nuclear import. Aberrant acetylation of histone residues resulted in anomalous regulation of certain oncogenes or tumor silencing genes, which absolutely support tumorigenesis [63]. The HAT and HDAC enzymes maintained the balance state of histone acetylation in normal cells. The histone hypoacetylation is mostly observed in cancer cells [64]. The HAT enzymes are mainly associated with histone acetylation and plays critical role in regulating the H3 and H4 histone acetylation. In addition, H3 acetylation may be promoted by the repression of HDACs, while reduced by blocking HAT [64].

Histone Deacetylation in Osteosarcoma

Histone deacetylation is the hydrolytic elimination of acetyl groups from histone residues. Histone deacetylase enzymes are mainly involved in controlling the deacetylation state of histone residues. Alteration in the dynamic equilibrium of histone acetylation results in the development and progression of tumor. Like HATs, HDACs also play significant roles in the regulation of different cellular processes. To date, at least 4 different classes of HDACs have been investigated. Class I HDACs are composed of HDAC1, HDAC2, HDAC3 and HDAC8; Class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10; Class III HDACs are also called sirtuins that acquire NAD+ cofactors and are composed of seven members (SIRTs 1-7); and Class IV HDAC, which is composed of only HDAC11, possesses the characteristics of both Class I and II HDACs. The suppression of HDACs significantly inhibits cell growth, differentiation, block cell cycle progression and promotes apoptosis in cancer cells [64].

Histone Methylation in Osteosarcoma

In addition, histone methylation is also an important histone modification that occurs due to allocation of methyl groups from S-adenosyl-L-methionine to arginine or lysine substrates of histone residues through Histone Methyltransferases (HMTs) enzyme. Histone methylation is also tightly concomitant with activation and silencing of gene expression, depending on the origin of methylation to occur. Generally, the methylation of different histones, including H3K36, H3K4 and H3K79 are associated to activate the genes transcript, while H3K9, H4K20 and H3K27 are responsible for gene suppression. Thus, histone modifications along with DNA methylation, act as a acute regulator in the directive of gene transcription [65]. WNT5A is classified as a family of gene which transcript signaling protein called glycoprotein and its aberrant transcription is involved in various kinds of sarcoma. The expression studied of promoter A and B of the WNT5A gene is observed in normal osteoblast cells, osteosarcoma cell lines (U2OS and SaOS-2) and in cancer tissues. Both promoters are identified in normal osteoblasts, but promoter B is highly energetic than the promoter A. Three regions were reported that are highly augmented with CpG islets of exon 1β of promoter B are hypermethylated in U2OS and SaOS-2 osteosarcoma cells [39,66].

It has been determined that cisplatin enhanced the expression of histone demethylase enzymes, KDM6A and KDM6B in osteosarcoma cells involved in the demethylation of H3K27me3. Cisplatin-sensitive cancers have higher level of H3K27me3 as compared to resistant osteosarcoma samples. *In-vitro* suppression of histone methyltransferase (EZH2) in osteosarcoma cells condensed the stages of H3K27me3 as well as resulted in cisplatin counteract clarified that H3K27me3 acting a significant part in lowering the expression of KDM6A as well as KDM6B by promoting tumor cell susceptibility to cisplatin [67]. Detailed summary of different histone modifications targeting different cellular and molecular networks were mentioned (Table 2).

Modification	Modifying Agent	Suggested Downstream Mechanism	Subsequent Cellular Variations	References
Improved H3T3ph	Sirt1	Amplified ATG 5/13/14 expression	enhanced autophagy	[68]
Modified H3K36me3	SETD2		Not studied	[69]
H2A monoubiquitination	ALKBH5	Increased USP22 and RNF 40 expression	Increased cell proliferation, cell invasion and relocation	[70]
Decreased H4K20 me3	decreased	Multiple signaling pathways	Not studied	[71]

	SUV420H2 expression			
Reduced H3K27me3	KDM6B	LDHA overexpression	Increased metastasis	[72]
Decreased H3K27me3	KDM6A/KDM6B	PRCKA upregulation	Cisplatin confrontation and declined apoptosis	[73]
Reduced H3K9me2 and improved PTEN methylation	G9a and DNMT3B	Inhibited PTEN signaling	Malignant BM-MSC	[74]
Decreased HDAC2 and DNAMT3a	Treatment with VPA and 5-Aza	enhanced stem cell aspects (SOX2, OCT4, NANOG and CD133)	Improved stem cell phenotype, cell viability and cellular migration	[75]

Table 2: Descriptive knowledge of histone amendments targeting different cellular and molecular networks.

Histone Phosphorylation in Osteosarcoma

Phosphorylation is an additional class of histone alteration that has been well considered in osteosarcoma. Histone phosphorylation of Sirt1 protein has been recently investigated in osteosarcoma cells. Before Sirt1 was investigated as a histone methylation and deacetylation enzyme, but the Western blot results of current studied showed that high expression of Sirt1 protein could related with enhanced expression of Histone 3 Threonine 3 phosphorylation (H3T3ph). The co-immunoprecipitation study clarified the direct association among Sirt1 and H3 using antibodies against both proteins. The Sirt1 expression is directly associated with H3T3ph phosphorylation. Changing the expression of Sirt1 directly effects H3T3ph on osteosarcoma cell autophagy. Autophagy is basically cell survival process that plays crucial role in reprocessing of cellular constituents as a vigor constituent through development of an autophagosome. In case of osteosarcoma, Cells development in state of starvation environment together with autophagosomes, H3T3ph levels, SirT1, conversion of LC3-1 to LC3-II, the deprivation of highly maintains proteins promoted. Therefore, the countenance levels of the autophagy genes ATG14, ATG5 and ATG13 result to enhance in target cells [76]. Autophagy in osteosarcoma is mostly feedback that develops conflict with chemotherapy management. Deep investigation necessary to discuss that may help to completely understand the treatment strategies and how to pledge the actions of autophagy in osteosarcoma [77].

Control of certain factors associated with apoptosis is mostly regulated through phosphorylation. Nuclear Factor-kappa B (NF-κB) act as a most noteworthy function in controlling cell apoptosis in various cancer cells. Stimulation of IκBα monitored by its degradation persuades transfer of NF-κB from cytoplasm into the cell nucleus where it induces numerous genes. Tumstatin treatment and TNF-α phosphorylate serine on position 536 that result to activated p65NF-κB in Saos-2 cells [78-80]. Up regulation of Akt clarified that IKK promoted p65NF-κB transactivation, whereas alteration of Ser536 eliminates this consequence [81]. Similarly, Ser536 phosphorylation on p65NF-κB can be noticed both in the nucleus and the cytoplasm. TNF-α and Tumstatin treatment repressed IκBα expression and therefore influence the nuclear translocation of NF-κB in Saos-2 cells.

Moreover, PI3K/Akt/mTOR network serves as an important function in osteosarcoma metastasis. Phosphorylation of mTOR is closely related to osteosarcoma cell migration, metastasis and poor prognosis through downstream effectors including 4EBP1, eIF4E and S6K1, which are mostly upregulated in osteosarcoma. Rapamycin is active inhibitor of mTOR and acts as possible therapeutic biomarkers for treatment of osteosarcoma. Research study described that ezrin encourages phosphorylation and manifestation of 4E-BP-1 and S6K1 as well as function of S6K1, which might encourage ezrin-allied metastatic activity in osteosarcoma [82].

Tyrosine phosphorylation enhances STAT3 expression and also frequently stimulates STAT3 in retort to Interleukin-6 (IL-6) as well as Epidermal Growth Factor (EGF) [83]. STAT 3 serves as a central part in facilitating cell distinction, cell growth and subsistence signs of cytokine family (IL-6) through regulation of gp130 receptor subunit [84]. STAT3 significantly contributed in the development, progression of various types of cancers such as leukaemia, lymphomas, multiple myeloma and other solid malignant tumors [85]. Upregulation of STAT3 is promotes progression growth and poor prediction of osteosarcoma and acts as

a latent therapeutic signature for the diagnosis of osteosarcoma [86]. STAT3 is a ubiquitously expressed transcription factor that undergoes transient tyrosine phosphorylation upon stimulation by Epidermal Growth Factor (EGF) and Interleukin-6 (IL-6) [100][83]. It mediates critical cellular processes, including proliferation, differentiation and survival, primarily through signaling via the IL-6 cytokine family and the gp130 receptor subunit [84]. Genetic knockout studies demonstrate its essential role in embryonic development, as STAT3-deficient mice exhibit embryonic lethality [87]. Notably, persistent STAT3 activation is frequently observed in various cancers, such as multiple myeloma, leukemia, lymphomas and solid tumors [85].

In osteosarcoma, raised STAT3 activity is associated with tumor progression and unfavorable clinical outcomes, highlighting its potential as a therapeutic target for OS treatment [86]. Small non-peptide molecule (LLL12), was framework to directly block STAT3 function. Basically, LLL12 inhibit the STAT3 phophorylation at Y705 and to promote cancer cells apoptosis. LLL12 repressed DNA binding activity of STAT3 and reduced the transcription of STAT3 board genes that involved in malignancy, mainly surviving, cyclin D1 and Bcl-2 [87]. LLL12 and FLLL32 block STAT3 directly and indirectly by phosphorylation of IL-6 in osteosarcoma and rhabdomyosarcoma cells [88,89]. SC-1, (as a Structural Correspondent of sarcoma drug, sorafenib) designed to reduce osteosarcoma cell growth and proliferation *in-vivo*. SC-1 reduces STAT3 function by directly mediating Y705 phosphorylation and disturbing STAT3/JAK signaling pathway in a SH2-mediated method [90,91]. Synthetic oleanane triterpenoid such as 12-dioxoolen-1,9-dien-28-oic acid (CDDO-Me) and C-28 methyl ester of 2-cyano-3 efficiently inhibits STAT3 phosphorylation and also blocks STAT3 nuclear translocation that subsequent in apoptosis of osteosarcoma cell lines [92].

Ubiquitination in Osteosarcoma

Malignant tumors are characterized by uncontrolled growth of cells and tissues resulted in intratumoral heterogeneity, enhanced cell mobility, invasion and high recurrence rate [93]. The possible evidence identified the crucial function of ubiquitination-based factors that mediate malignancy and tumorigenesis. Ubiquitination involves SMURF1 to block the SMAD/BMP network, which potentially promotes osteosarcoma cells differentiation. Significantly, the distinguished osteosarcoma cells are highly sensitive to chemotherapeutic resistant [94]. Briefly, SMURF1 interacts with E2 conjugates UBCH5B-UEV1A to modulate the polyubiquitination of SMAD1, targeting SMAD1 for degradation through the 26S proteasome. More importantly, SMURF1 functions as an E3 ligase for RUNX2 controlling its ubiquitination, which is critical process in osteoblast cell differentiation [95]. Therefore, SMURF1 mainly exerts dual effect on osteosarcoma suppression particularly for the subgroups that extremely regulate RUNX2 and SMAD1. Moreover, HECT-type E3 ligase, WWP1 functions as to control RUNX2 expression and activates its polyubiquitination [96]. This activity is essential for mediating mineralization of extracellular matrix during adult bone formation [96]. E3 ligase activity of MDM2 acts as an important contribution in preserving the osteosarcoma stem cell activities by stimulating the ubiquitination of Retinoic Acid Receptor alpha (RAR α) for degradation via proteasome which disrupts the Retinoic Acid (RA) mediated osteosarcoma cell diversity and induces tumor distortion [97]. Therefore, suppression of MDM2 results in repressing the progression and metastasis of osteosarcoma.

However, a large amount of ubiquitination aspects has been determined for their activities in regulating osteosarcoma self-governing cell differentiation. Thereof, the upregulation of CUL4B in osteosarcoma cells resulted in enhancing cell proliferation and suppressed apoptosis [98]. Furthermore, CUL4B is also associated with three extra proteins, DNA damage binding protein 1 (DDB1), RING-box protein 1 (RBX1) and DDB1- and CUL4-associated factor 13 (DCAF13) that leads to develop the CRL4B DCAF13 E3 conjugate. The DCAF13 subunits play a crucial role particularly in the identification of tumor suppressor, phosphates and tensin homolog deleted on chromosome 10 (PTEN) and emerge CUL4/RBX1-regulated PTEN mediated degradation as well as ubiquitination [99]. Additional research identified that CUL4B/RBX1/DDB1 can associate with DCAF11 result in CRL4B DCAF11 E3 conjugate that leads to ubiquitinate the Cyclin-Dependent Kinase (CDK) inhibitor p21, Cip1 and enhance their abasement essential for the osteosarcoma cell proliferation [100]. Its expression is decreased in OS cells and assists in the degradation of Receptor Tyrosine Kinase (RTK) through ubiquitination. However, upregulation of c-Cbl is concerned with the enhance deprivation of RTK, which suppresses the metastasis and proliferation of osteosarcoma cells [101]. In osteosarcoma cells, the E3 conjugate enhanced hypoxia-mediated metastasis as a result of modification of Rho Guanosine Diphosphate Dissociation Inhibitor 2 (RhoGDI2) with poly-Ub networks for proteasome-mediated degradation [64, 102]. Furthermore, various ubiquitination aspects have also been identified for their activities to regulate the pathogenesis of osteosarcoma, including UBE2T, Adaptor Speckle- type Pox virus and Zinc finger protein (POZ), protein (SPOP) and ankyrin-repeat-containing E3 ubiquitin-protein ligase 1 (HACE1) and HECT domain [103,104]. These factors formed a network that widely commands the development as well as progression of osteosarcoma cells.

Sumoylation in Osteosarcoma

Sumoylation is basically another post-translation modification occur in osteosarcoma. Sumorylation mainly based on group of proteins called Small Ubiquitin-Like Modifier (SUMO) proteins, critically intricate in biological molecules Post-Translational Modifications (PTM) [105]. SUMO expression is highly expressed in osteosarcoma and play contributing role in osteosarcoma progression, development and metastasis [106]. In addition, the enhance transcription of SUMO 1 or SUMO 2/3 is closely associated with higher histological grade and an advanced level of SUMO 2/3 stemmed in inferior overall survival. Hence SUMO 2/3 has the probable to develop a predictive marker. Moreover, *in-vitro* investigation also determined the same studies revealed that silencing of SUMO E1 decreased Chondrosarcoma (CS) cell proliferation, metastasis and viability. Dedifferentiated CS cell lines, which significantly contributed in cell aggressiveness, were extremely vulnerable to knocking down of SUMO E1. Finally, the development of drug targeting SUMO might be potential diagnosis strategies of CS [106].

Similar to ubiquitin, SUMO-1 is covalently associated to lysine remains on Promyelocytic Leukemia (PML) and other target proteins in an ATP-dependent process [107]. However, SUMO-1 modification primarily influences protein localization rather than induce degradation. In PML, SUMO-1 modification occur at three main sites: Lys-65 (RING-finger domain), Lys-160 (first B box domain) and Lys-490 (nuclear localization signal domain) [108]. PML sumoylation is crucial for establishment of PML-nuclear bodies (PML-NBs) and the recruitment of related proteins [107]. Sumoylation was initially believed to be mandatory for PML-mediated growth retradation. However, recent finding contradiction, as a sumoylation deficient PML mutant maintained its growth-inhibitory function when overexpressed [109].

Other Epigenetic Modifications in Osteosarcoma

MicroRNA Regulation in Osteosarcoma

MicroRNAs (miRNAs) are categorized as a group of small ncRNA composed of 20-30 nucleotides that play an essential role in controlling different biological sequences such as cell propagation, distinction, cell cycle, embryogenesis, apoptosis, as well as innate immunity [110]. The direct interaction of miRNAs with 3' untranslated regions (3'UTR) of intent mRNAs promoted mRNA degradation and translational inhibition. It has also been reported that miRNAs interact with other active regions such as 5'UTR gene promoter sequence to regulate gene expression. Moreover, miRNAs may also regulate gene expression or promote translation of target gene in particular conditions [111]. Certain miRNAs are directly associated with OS and act as a significant function in cell protection, tumor initiation, growth and advancement. This identification was confirmed both *in-vivo* and *in-vitro* in cancer cell lines revealed that the miR-27a has a pro-metastatic role while the miR-16 serves as tumor suppressor [112].

It has been reported that reduced range of miR-200b transcript is linked with progressive metastasis and clinical phase in osteosarcoma. The expression of miR-200b is mostly downregulated in osteosarcoma cell (U2OS, Saos2, MG63 and HOS) equated to typical osteoblast cells. Up-regulation of miR-200b transcript results in a countless decrease in sarcoma cells migration, propagation, invasion and metastasis. Furthermore, the transcription of Interleukin 2 (IL-2) gene in definite T lymphocytes is repressed by a transcription factor encoded by ZEB1. The target regulators of mir-200b and its transcription is suppressed by miR-200b in osteosarcoma. The upregulation of ZEB1 gene is mostly observed in tumor cells, while its suppression leads to inhibit tumor cells migration, proliferation and invasion. MiR-200b reduced cancer cell propagation, relocation and invasion via decreasing the activity of ZEB1 [113]. Recent studies described that upregulation of miR-101 in MG63 osteosarcoma cells resulted in significant reduction of ROCK1 expression (gene coding serine/threonine kinase signaling protein) compared to SimiR-101 in MG63 cells [113,114].

Upregulation of miR-101 in MG63 cells ensued in reduced cell migration, invasion, propagation and promoted cellular apoptosis. Downregulation of miR-101 and absolute inhibition of ROCK1 leads to promote cell migration, proliferation as well as invasion of MG63 cells and block apoptosis. Moreover, knocking down of ROCK1 in MG63 cells resulted in reverting the miR-101 targeted suppressive effect on cell viability, migration and invasion as well as inhibit apoptosis. These studies demonstrated that miR-101 acts as a cancer repressor in osteosarcoma by inhibiting the ROCK1 gene. Upregulation of miR-101 decreased sarcoma cell movement and cancer growth by inhibiting the JAK/STAT and AKT/PI3K signaling transduction networks via decreasing the transcription profile of ROCK1 gene [114]. Another study with human osteosarcoma-derived cell lines and control osteoblasts determined the relative expression of miR-3928 and showed that overexpression of miR-3928 reduced tumor expansion, promoted cell apoptosis, enhanced the portion of cells arrested in G1 stage and reduced the portion of cells arrested in S phase of the cell cycle, hence its downregulation induced cellular migration, proliferation, metastasis and tumor growth. The outcomes

indicate that miR-3928 act as a tumor repressive and possess the CDK6, IL 6R and ERBB3 gene that encode for the cyclin-dependent kinase 6, IL 6 receptor and tyrosine-protein kinase receptor respectively [115].

Previously, it has been shown that the expression of Fas (a type II transmembrane protein of the TNF family) present on the cell surface is inversely associated with formation of pulmonary metastasis in osteosarcoma. The transcription profile of miR-17-92 group members such as miR-19a and miR-20a is identified to enhance in LM7 lineage metastatic cells presenting truncated level of Fas action as equated to non-metastatic cell lines which give advance function of Fas. The converse relationship among miR-20a and Fas expression was detected in most of the characterized tumor-associated cells. Upregulation of miR-20a in SAOS 2 cells leads to consistent and stable downregulation of Fas expression. Further study revealed that miR 17 92 gene encode miR-20a that serves as a negative regulator of Fas in osteosarcoma and promoted its metastatic potential [116]. In addition, miR-574-3p have strong association with osteosarcoma progression and development, its overexpression has been identified in the tumor tissues and in osteosarcoma derived cell lines including MG63, U2OS and SAOS balanced to osteoblast cells. Brief summary of certain miRNAs, circRNAs and their target mechanisms were shown in Table 3. Lower expression of miR-574-3p via antisense miR-574-3p leads to promote cellular apoptosis and inhibits tumor cell growth. Moreover, upregulation of miR-574-3p because of transfection with mimics' miR-574-3p activates cell propagation in U2OS cells. Furthermore, miR-574-3p negatively regulates the function of decapentaplegic homologue 4 (SMAD4).

Previous study reported that upregulation of SMAD4 abolished the promoter activities of miR-574-3p on the development of tumor cells. Since it has been clarified that miR-574-3p acts as a tumor promoter in osteosarcoma via suppressing the transcription profile of SMAD4 [117]. Further studies of the expression of miRNA in about 40 osteosarcoma tissues showed that miR 140 expression is also greatly abandant. MiR-140 plays essential role in controlling cell proliferation, migration, invasion and promotes apoptosis *in-vitro*, while repress tumor progression *in-vivo*. Computational and Bioinformatics analysis described that miR-140 targets the gene that encode Histone Deacetylase 4 (HDAC4) and plays tumor suppressive role [118]. Another study revealed that the transcript of miR 125b is decreased in human OS tissue. The low expression of miR 125b has been closely allied with advance cancer stages and poor diagnosis in osteosarcoma [119].

MiR-150 acts as a negative regulator of the Ezrin gene which encodes tyrosine kinase protein in F5M2 cells. In addition, upregulation of miR 150 via exogenous transfection of miR 150 mimics leads to reduce the transcription of Ezrin gene which consequences in suppression of tumor cell metastasis, invasion and thus shows that miR-150 act as tumor suppressive in osteosarcoma [120]. Further study reported that the expression of miR-449c is expressively reduced in osteosarcoma cells and showed DNA hypermethylation in the osteosarcoma cells. Upregulation of miR-449c expression suppresses osteosarcoma cell propagation, inhibits colony formation and arrest cell cycle at G1 point. Hence, miR-449c also adversely regulated c-Myc oncogenes. Mostly miR-449c is downregulated in human osteosarcoma cells due to DNA hypermethylation which indicates that miR-449c have tumor suppressive role via blocking the expression of c-Myc oncogenes [121]. Targeting epigenetic mechanisms of miRNAs regulating osteosarcoma progression were shown in Fig. 3.

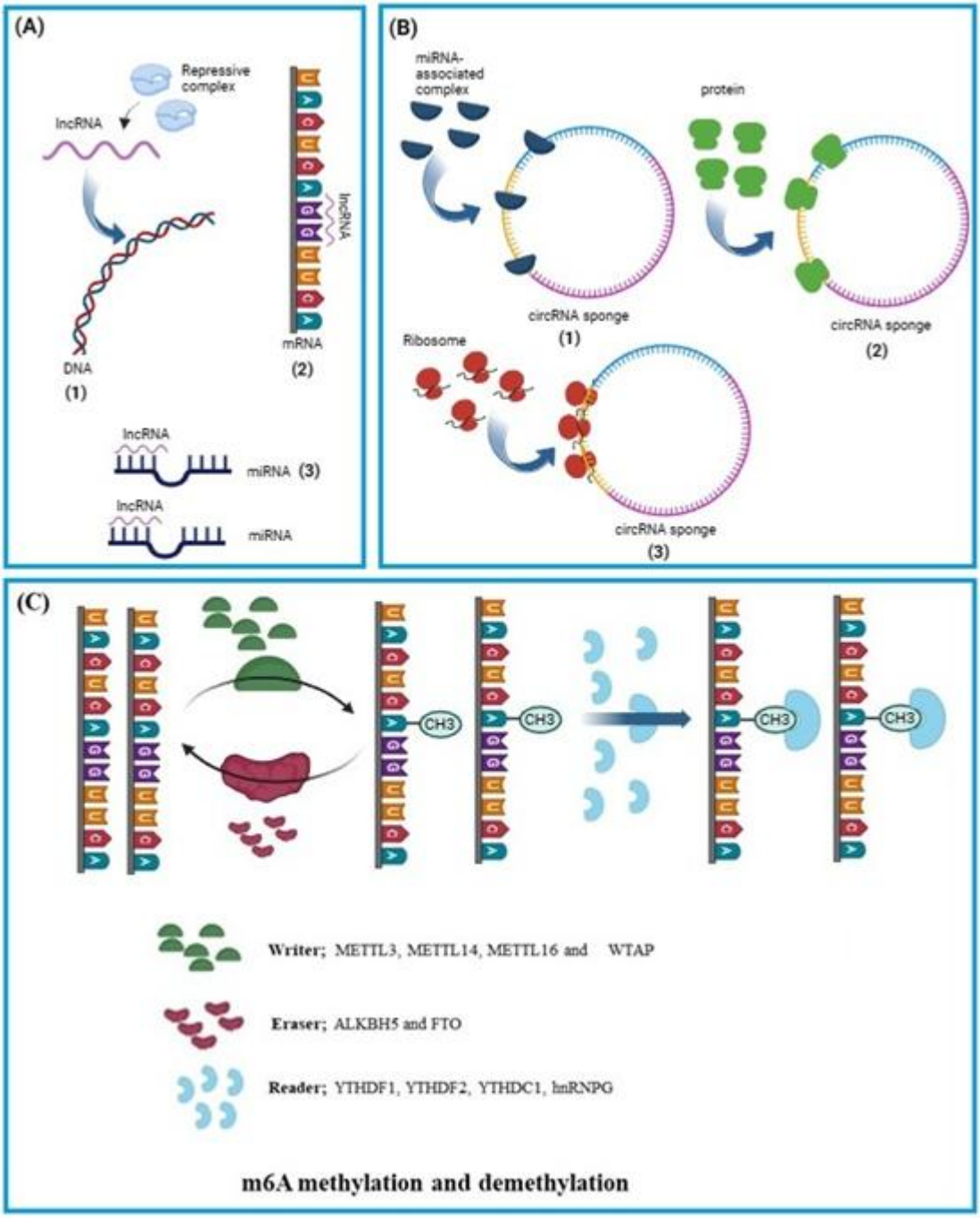

Figure 3: Epigenetic regulatory mechanisms of ncRNA: (A) Three mechanisms for lncRNA-mediated epigenetic regulation. (1) LncRNAs recruit a repressive chromatin-modifying complexes to suppress genes expression. (2) LncRNAs bind mRNA to prevent antisense mediated degradation or translational inhibition. (3) LncRNAs directly binds and deactivated miRNA averting their communication with target mRNAs. (B) Three main mechanisms for circRNAs dependent epigenetic regulation. (1) CircRNAs directly bind miRNAs, protecting their function and upregulating miRNA-targeted genes expression (circMagil and circSnx13 in muscle tissue) and decrease level of circRNA converse this effect. (2) CircRNA regulate protein activity such as circMbl in Drosophila sequesters Muscleblind (MBL) protein, preventing additional circMbl synthesis and encouraging mbl mRNA translation. (3) CircRNAs possessed translation capacity. (C) Main mechanisms for m6A RNA modification. m6A composed of writers, eraser and reader. Writer catalyze m6A methylation on adenine moieties, erasers eliminate m6A scripts, conversing methylation while readers identify m6A-modified RNAs to stimulate their constancy, splicing and translation [122].

Target Modifications	Proposed Downstream Mechanism	Resulting Cellular Changes	References
Upregulation of MALAT and SNHG20	Histone modifications	Under investigation	[123]
Downregulations of SERTAD 1/2/3	Promoted activity of miR-29c	Promoted cell metastasis	[124]
Downregulations of LINC00619	Activated HGF-mediated PI3K/Akt pathway	Inhibited cell apoptosis, enhanced cell invasion and migration	[125]
Upregulation of KCNQ1AT1	Decreased expression of miR-34c-5p-meditated ALDOA	Involved in Aerobic glycolysis	[126]
Upregulation of ARSR	Involved in MRP1 expression	Results in Multidrug resistance	[127]
Upregulation of PVT1	Promoted epithelial-mesenchymal transition and inhibited expression of miR-486	Increased cell proliferation, migration and metastasis,	[125, 128]
Upregulation of MEG3	Enhanced AKT2 expression via inhibited miR-200b-3p expression	Results in Doxorubicin resistance	[129]
Downregulations of PWRN1	Not studied	Results in Chemoresistance and promoted cell proliferation	[130]
Upregulation of HCG9	Inhibited function of miR-34-3p	Enhanced cell invasion and proliferation, Inhibited cell apoptosis and cell cycle arrest,	[131]
Upregulation of PARTICLE	Decreased WWOX expression	Not studied	[132]
Upregulation of HNF1A-AS1	Promoted expression of HMGB1 via suppression of miR-32-5p	Inhibited cell proliferation, migration, invasion and blocked cell apoptosis,	[133]
Downregulations of expression of miR-433-3p	Enhanced FBXO22 expression	Silence cell apoptosis results to enhanced cell migration, proliferation and invasion	[134]
Upregulation of CRNDE	Decreased miR-335-3p expression	Promoted cell migration, proliferation and invasion	[135]
Downregulations of miRNA-223-3p	Enhanced CHD6 expression	Enhanced cell migration and invasion	[136]
Downregulations of miR-451a	Involved in activation of AKT/mTOR pathway	Increased cell migration and colony-forming ability, decreased cell apoptosis,	[137]
Upregulation of cir-ITCHI	Result to activate EGFR pathway via miR-7 suppression	Increased cell growth and metastasis	[138]
Upregulation of circ_001422	Promoted phosphorylation of FGF2 and PI3K/ Akt	Inhibited cell apoptosis, enhanced metastasis, proliferation	[139]
Upregulation of Circ-ECE1	Associated with C-Myc to silence TXNIP transcription	Activation of Warburg effect, Promoted cell metastasis and proliferation	[140]
Downregulations of circ-MTO1	Inhibited expression of KLF6 via increased miR-630 level	Inhibited cell migration, invasion and cell apoptosis	[141]

Table 3: Brief summary of a few lncRNAs, micro-RNA and circ-RNA targeting different cellular and molecular networks.

lncRNA Regulation in Osteosarcoma

Osteosarcoma is an extremely aggressive malignant bone tumor that widely expresses and metastasizes to lung and pulmonary organ with complete progression that results in respiratory failure. Tumor invasion and metastasis are manifold complicated processes in which malignant cells change Extracellular Matrix (ECM) interconnection at the initial tumor site to conquer

adjoining tissues and thus distribute through the vasculature to other systems to form secondary tumors [142]. Matrix Metalloproteinases (MMPs) belong to proteolytic enzymes family that play an important role in tumor invasion and metastasis by flouting the ECM and cellar membrane. It has been explored that during osteosarcoma development and progression, numerous lncRNAs stimulate or conquer cellular metastasis, propagation and cell invading ability through modifying MMP-2/MMP-9 excretion [143].

LncRNAs show important contributions to the growth, advancement, management, prediction and judgment of osteosarcoma [142]. LncRNAs can pretend and accentuate different cellular consequences including cell proliferation, diversity, migration, cell cycle and cell apoptosis [144]. Accordingly, advancement of tumors can be branded by transcriptomics study to discriminate between metastasis, development and relapse of human malignancies such as osteosarcoma. Further examined that metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) / microRNA 376A (MIR376A) / transforming growth factor alpha (TGFα) shows acute role in the development of osteosarcoma. MALAT1 originated osteosarcoma growth by conquering MIR376A and indorsing TGFA transcription [145]. The HOX antisense intergenic RNA (HOTAIR), advance-studied lncRNA, which linked with the development and pathological process of various malignant tumor. HOTAIR is greatly articulated in osteosarcoma and therefore allied with progressive myeloma stages, advance histological ranks and deprived persistence. Consequently, HOTAIR might be a noteworthy object in the conclusion of osteosarcoma [146].

Furthermore, small nuclear RNA host gene 12 (SNHG12) persuaded cell migration and proliferation by activating the function of angiomotin gene in osteosarcoma cells, which mediates MMP-2/MMP-9 expression [6,147]. LncRNA (H19) serve as tumor suppressor or oncogenic in the development of osteosarcoma. Current studies recommended that H19 is associated with the pathological process of osteosarcoma and serves as an oncogenic lncRNA [148,149]. Excitingly, different tumor suppressive and oncogenic lncRNAs have been documented in osteosarcoma pathological process including cell migration, propagation, metastasis, invasion, cell growth and apoptosis. Presently, this study reiterated the thinkable contributions and emphasize the modulations of few lncRNAs that are concomitant with osteosarcoma pathological process as shown (Table 3). Enhance expression of lncRNA Taurine Upregulated Gene 1 (TUG1) in osteosarcoma commenced cell propagation, repressed cellular apoptosis and detained cell cycle at G0/G1 through underscore proposed mediating process with sponges miR-9-5p to decrease the activity of POU domain class 2 transcription factor 1 (POU2F1).

TUG1 is closely associated with disease station turns as a potential therapeutic target and as a self-governing predictive aspect in osteosarcoma [150]. Upsurge transcription of HOTTIP consequences to boosted cellular migration, propagation and invasion that is allied with progressive Enneking stage, high metastasis and weak endurance of osteosarcoma [151]. Enhanced expression of fibroblast growth factor 3 antisense transcript 1 (FGFR3-AS1) in osteosarcoma is thoroughly allied with upsurge sarcoma size, high medical stage and meagre prediction [152]. Increase expression of ZEB1-AS1 in osteosarcoma is closely linked to tumor size, progressed clinical stage and poor prognosis via stimulating expression of ZEB1 gene [153]. LncRNA MEG3 controls p53 transcription and is thoroughly connected with osteosarcoma survival [154]. Similarly, enhanced expression of FOXC2-AS1 is interconnected with promising existence for osteosarcoma patients [155]. LncRNA loc285194 is originally documented in osteosarcoma but the emphasized efficient mechanism is still undefined. A study has described that loc285194 had a latent indicative role in several myeloma such as liver cancer, colon cancer and osteosarcoma [156]. Additionally, some supplementary lncRNAs have been known as sovereign predictive biomarkers in osteosarcoma. Another study primarily exposed BRAF-activated non-coding RNA (BANCR) in sarcoma cells, which is nearly 693bp in length [157]. Upregulation of Urothelial Carcinoma Associated 1 (UCA1) has adjacent link with confident reserve metastasis, huge tumor size and progressed clinical outcomes and serves as a self-sufficient predictive gauge for poor persistence of osteosarcoma [114].

Similarly, activation of antisense H19 transcript (91H) posses' substantial correspondences with great tumor volume, positive clinical outcomes and post-operative chemotherapy and serves as an independent prediction factor with weak consequences of osteosarcoma patients [158]. Enhanced transcription of MALAT1 is allied with distinct metastasis, advanced clinical outcomes and low prediction of osteosarcoma patients and acts as a self-governing prognostic factor of patients' subsistence condition [159]. Target epigenetic mechanism of lncRNA and m6A RNA modification regulating osteosarcoma progression were shown in Fig. 3.

Target Prevention of Osteosarcoma Based on Available Epigenetic Drugs Applicable in Clinical Practices

Tumors is actually regulated by the genetic and epigenetic modifications in the human genome. These modifications are quite critical in the terms of prevention and development of specific target drug. Epigenetic modification is reversible and might be measured by certain biochemical biomarkers and possess wide predictions as a mark for sarcoma diagnosis and medications. Presently, different epigenetic biomarkers directing mechanisms have been established and emphasis for the diagnosis and handling of osteosarcoma. These mechanisms essentially comprise DNA methyltransferase inhibitors, histone deacetylase inhibitors and drug associated approaches (Table 4).

Drugs	Targets	Mechanism of Action	Preclinical/Clinical Rationale	Strength of Evidence	References
5-aza-CdR (decitabine)	CREG1, p14ARF, p21, RASSF1	DNMTi	Numerous genes allied with promoter hypermethylation in osteosarcoma. phase I clinical trials completed and ongoing (www.clinicaltrials.gov recognizer NCT01241162)	Medium-low	[160]
Azacytidine	Nucleosides DNA methyltransferase inhibitors	DNMTI	MDS, AML	Medium-High	[161]
Ibandronate	Ras, DNMT, Fas	Bisphosphonate; upregulates Fas	Repressed Ras role and knockdown DNMT expression, leading to enhanced Fas role; prompted cell apoptosis *in-vitro*	Low	[162]
Zolendronate	Small GTPases	Bisphosphonate; downregulates VEGF	Repressed lung metastasis and sustained overall subsistence in mouse models; phase I medical trial completed	Medium-high	[163, 164, 165]
Tranylcypromine	LSD1	Forms adduct with inactive region of LSD1	Articulated in osteosarcoma tissues ; inhibit osteosarcoma growth *in-vitro*	Low	[166]
Pracinotat (SB939)	HDAC	HDACi	Phase I trial completed	Medium-low	[167]
Vorinostat	Hydroxamic Acids	HDAC Class I,II,IV inhibition	CTCL	Medium-high	[161]
Erinostat (MS-275)	HDAC, Fas HDACi;	Fas upregulation	Enhanced Fas role in Fas-metastatic osteosarcoma; instigated relapse of metastasis in xenograft models via stimulated Fas function in Fas- cells	Medium	[168]

Valproic acid	HDAC	HDACi	Repressed tumor progression *in-vitro* and tumor metastasis models togather with doxorubicin; initiall medical trials is still ongoing (www.clinicaltrials.gov attribute NCT01106872; NCT01010958)	Medium-high	[169]
Panobinost	HDACI	HDAC1,2,3 inhibition	Multiple myeloma	Medium-high	[161]
Antioxidants	Oxidative stress	reduce oxidative stress	Reduced oxidation to stacked osteosarcoma progression	Medium -low	[161]
Folate and B-vitamin	DNA methylation	Support DNA methylation	Promote DNA methylation to suppressed osteosarcoma progression	Medium-high	[161]

Table 4: Detail description of different epigenetic drugs and their target mechanisms in osteosarcoma.

Epigenetics-Based Personalized Therapeutic Approaches of Osteosarcoma

Investigation of molecular processes that driven the progression and development of osteosarcoma can insight reliable in determining predictive markers for personalized medicine. In addition, the discovery of epigenetic alterations that closely related to the initiation, development and metastasis of osteosarcoma patients might result in the advancement of therapeutic efficiency and prognosis. The recently selected epigenetic modifications and signaling pathway for prognosis and therapy of osteosarcoma are shown (Fig. 4). Certain detailed epigenetic alterations could be recognized as predictive biomarkers for diagnosis and treatment of osteosarcoma. According to literature and bioinformatics analysis, utilizing the Therapeutically Applicable Research to Generate Effective Treatment database (TARGET), figure out a list of Epigenetic Modification-Related Genes (EMGs) and lncRNAs in osteosarcoma. Based on the determination of EMGs and their medical outcomes in osteosarcoma, a nomogram was then established for addressing prediction in osteosarcoma patients [148]. MYC, TERT, EIF4E3 and RBM34 are determined as well-characterized EMG-related genes [170]. Another study used the same TARGET database, identified five lncRNAs (RP5-894D12.4, RP11-128N14.5, RP11-346L1.2, RP11- 231/13.2 and LAMA5-AS1) as a useful predictive biomarker for osteosarcoma patients with an AUC (area under the curve) prognosis for the 5-year surveillance proportion at a 0.75 fidelity [171].

DNA methylation has been recognized as a reliable biomarker in progression, development, progression and categorizing algorithms for osteosarcoma. Briefly, a study reported the methylation status of whole genome in primary osteosarcoma tumors, which stated that the range of methylation was highly associated with patient consequences. Samples possess hypomethylation have proven better results and enhanced response to advance chemotherapy treatments [172]. Moreover, bioinformatic analysis focuses on multiomics data to determine how alteration in mRNA expression and DNA methylation of immune-response genes that regulated overall patient prognosis and tumor microenvironment in osteosarcoma. They grouped patient samples according to machine learning and Immune-related DNA Methylation Patterns (IMPs) and developed an associated predictive menace model. This model was associated with varying sensitivities of drug and treatment based on the concurrent cancer microenvironment [173]. Moreover, the probe-based algorithm of DNA methylation was used to categorize osteosarcoma patient trials into groups according to their "BRCAness." BRCAness is a phenotypic feature in tumors with deficiency in homologous recombination reparation parallel to defusing of the BRCA1/2 genes. Therefore, BRCAness-positive tumors possessed high-rate genomic instability [174]. BRCAness in human tumors results in sensitive to ADP-ribose Polymerase inhibitors (PARPi) [175].

The contribution of DNA methylation strategy may serve to categorize patients diagnostically and fruitful to medical conclusion-making and diagnosis outcomes. Further studies identified epigenetic-mediating enzymes require for switching a Super Enhancer (SE) molecule in osteosarcoma development and progression [176]. Osteosarcoma-specific SE control certain important factor such as Leukemia-Inhibitory Factor (LIF). Further study identified that in case of osteosarcoma, the histone 3 lysine 27 tri-methylation (H3K27me3) demethylase UTX was the main promoter of LIF [177]. Moreover, GSK-J4, a UTX inhibitor, resulted in

reducing histone 3 lysine 27 acetylation (H3K27ac), while enhanced in H3K27me3 at the LIF gene locus in osteosarcoma cells. Due to this diagnosis, osteosarcoma cells showed loss of stem cell-like properties by altering histone acetylation that is linked with genes involved in NOTCH signaling pathways [176]. In addition, another study identified the epigenetic modification of ZEB1gene upregulated in osteosarcoma inhibits cell differentiation and promotes the capability of metastatic colonization [177]. Treatment of osteosarcoma cells with the epigenetic drugs that blocks DNA methylation, 5-Aza, this occurred in an enhanced level of miRNA controllers and decreased expression of ZEB1, acting as a diagnostic indicator for ZEB-1-promting osteosarcoma [177]. Another study investigated the N6- methyladenosine modification identified ALKBH5 (or ALKB homolog 5) as an eraser of N6- methyladenosine from RNAs molecules and might be involved in decreasing osteosarcoma myeloma cell development, evolution, proliferation, invasion and migration over epigenetic alterations of YAP (yes-associated protein) and pre-miRNA-181b-1 [178].

Vascular Endothelial Growth Inhibitor (VEGI) also plays a contributing role in osteosarcoma progression. Different epigenetic drugs such as hydralazine Hydrochloride (Hy) and sodium Valproate (VPA) used to treated osteosarcoma cells with the mechanisms of act of a DNA methylation inhibitor and a histone deacetylase inhibitor, ensued in the regulation of death receptor 3 (DR3) and VEGI. DR3 is mainly responsible for activation of VEGI [179]. Similarly, this study also clarified that histone deacetylase inhibitors and DNMTs can enhance transcription of tumor suppressor genes and possess anti-angiogenic effects in osteosarcoma [179]. Finally, certain drugs play positive role to treat osteosarcoma with the mechanism of action through epigenetic modifications. Execution of osteosarcoma cells with GlaxoSmithKline 343 (GSK343) suppressed activity of histone methyltransferase (enhancer of zeste homolog 2 (EZH2)). Downregulation of EZH2 ensued in disturbed cell apoptosis and autophagy allied cell death in osteosarcoma cells. Suppression of EZH2 decrease the function of c-Myc and its activator FUSE Binding Protein 1 (FBP1) [180]. Moreover, alternative study identified that plant extract called alkaloid berberine inhibited the expression of EZH2 by reducing the cell migration, cell viability, wound healing ability and colony formation [181]. Treatment of osteosarcoma cells based on epigenetic modifications provided clues how therapeutics could regulate c-Myc expression and inhibit cell propagation in malicious cells. Similarly, another study reported that the nanoparticle-coated drug mixture of epirubicin and gemcitabine might reduce tumor volume approximately 250% and the allied epigenetic alterations noted were tumor suppressor-associated miR-34a increased transcription profiles while miR-10b and miR-21 with the decreased expression level [182].

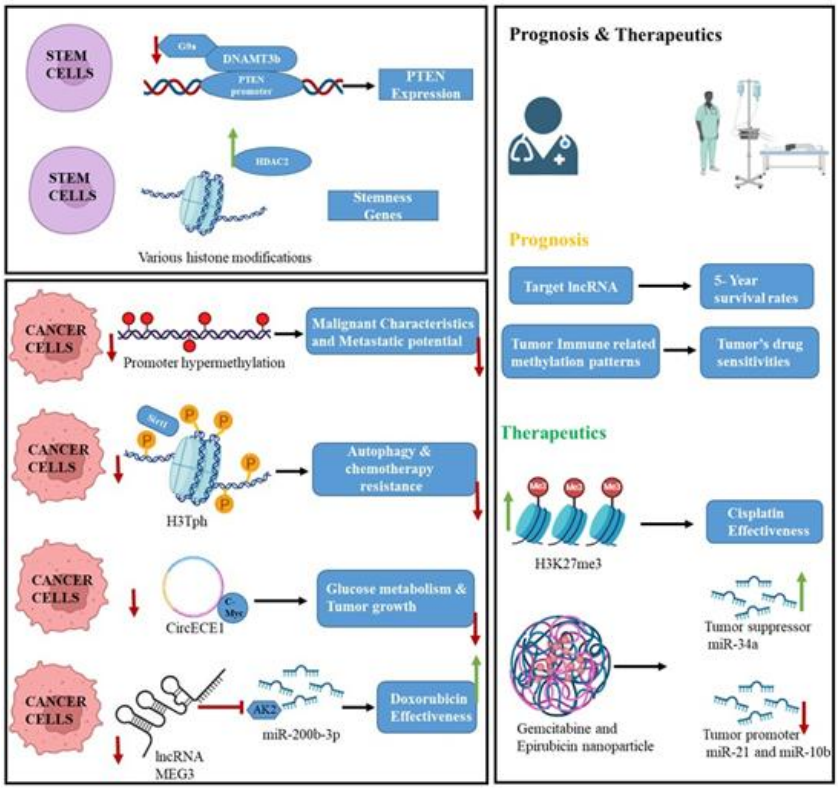

Figure 4: Diagrammatic representation of epigenetic adjustments and its target networks for diagnostic and treatment of osteosarcoma. A. Stem cells: target suppression of G9a lower PTEN promoter methylation, resulted to promote PTEN function.

HDAC2 play crucial role in manifold histone alterations inhibits CSC stemness. B. Cancer Cell: Lowered promoter methylation in certain tumor suppressor's gene inhibits malignancy features. Blocking of Sirt1-regulated H3Tph inhibits OS chemoresistance and cell autophagy. Blocking of CircECE1 inhibits c-Myc-determined growth. Suppression of MEG3 blocks silencing of miR-200b-3p, promoting doxorubicin efficiency. C. Prognosis and therapeutics: target lncRNA and immune-allied models. Increases H3K27me3 level through Cisplatin with GSK-J4 promoted drug effectiveness. Gemcitabine and epirubicin nanoparticle amends levels of tumor-associated miRNAs [122].

Future Perspectives

Expanded Epigenetic Modification Study in Osteosarcoma

Epigenetic modification is basic heritable amendment in the transcript profile of gene that exists in the genome instead of genetic mutation in the genetic code. Epigenetic alteration mainly regulates the physical and cellular mechanism of gene. Deviant epigenetic alteration has frequently observed with all stages of cancer such as development, growth, metastasis, invasion and chemotherapy opposition [13]. Such epigenetic modifications fully explain full range of phenotype in osteosarcoma such as severe malignant cells with advanced metastatic capacity or therapeutic resistance. Osteosarcoma epigenetic mainly categorized into three wide range groups such as alteration of DNA methylation, histone modifications and alteration in expression of non-coding RNA (ncRNA). DNA methylation occurs in CpG islands in the promoter region of a gene and mainly regulated through DNA Methyltransferases (DNMTs). In cancer phenomena, DNA methylation can either promote or inhibit gene expression [14]. Histone is important protein element of chromatin and act as a framework for DNA enfolded. Posttranslational alterations of histone proteins modify chromatin structure which lead to differential gene expression in osteosarcoma. Up to now different histone modifications exists such as methylation, acetylation, deacetylation, phosphorylation, sumoylation and ubiquitination [15]. ncRNA modifications include all range of RNA molecules such as miRNAs, lncRNA, cirRNA that are not responsible for coding of protein but still regulate various cellular functions which is mainly associated with epigenetic regulation of osteosarcoma [16].

Emphasize Proteoformics Study to Drive the Applications of Epigenetic Modifications in Osteosarcoma

Recent advancement in concept of molecular biology, the human genome atlas acknowledges more than 20,300 genes [183]. This surprising discovery showed that variations in biological activity not only arises from number of genes but also depends on the many variations of proteins [183]. This variation comes from multiple mechanisms such as Genetic differences (alleles), epigenetic modifications at RNA and DNA level, alternative splicing at RNA and Post-Translational Modifications (PTMs) [183,184]. Together, these processes made different proteoforms with distinct molecular versions of a protein originating from a single gene, which can influence wide range of key biological functions, such as regulations of gene regulation, energy metabolism and cell signaling pathways in osteosarcoma. The term proteoform concept was first defined by Smith and Kelleher in 2012. Proteoform refers to determine all protein variants resulting from genetic variations, RNA alternative splicing and PTMs [185]. Modern techniques such as Mass Spectrometry (MS) and two-Dimensional Gel Electrophoresis (2DGE) allow researcher to completely study protein's amino acid sequence, 3D structure, PTMs, cofactor binding partners, cellular localization and cellular function [183,184,186]. Protein usually studies all variants of a gene's product while proteoform specifies a study of functionally distinct molecular form. Although proteomics is mainly study of proteome (the whole set of proteins in a cell), while proteoform is recognized as a fundamental unit of proteome. To highlight their significance, the word proteoformics has been suggested to study proteoforms and their contributions in the regulation of epigenetic modifications osteosarcoma [187]. Different computational and bioinformatics software are being established to process and screen out large-scale proteoformics data. Proteoformics play critical role in deep understanding of protein structure, function and associated osteosarcoma mechanism [183,187]. By enlighting protein diversity and regulation, we can better understand their role in epigenetic modification of osteosarcoma, enabling more precise personalized medicine and personalized drug development. Therefore, proteoformics study will pull scientist interest to study protein variants and lay foundation for the development of targeted therapies based on proteoforms not only canonical proteins as biomarkers or drug targets for osteosarcoma.

Integrate Epigenetic Modification Data, Proteoformics Data and Other Multiomics Data to Establish Comprehensive Molecular Landscape and Molecular Models Towards 3P Medical Approaches in Osteosarcoma

Osteosarcoma is malaginant primary bone tumor, mainly occurs in adult and children. In spite of advance treatment strategies, osteosarcoma survival rates still very low. A mutiomics concept assimilating epigenetic modifications, transcriptomic, proteoformics metabolomics as well as clinical record can untie certain molecular mechanisms, classify biomarkers and assist 3P

medicine (Predictive, Preventive, Personalized) approaches. Epigenetic modification such as DNA methylation, Histone modification and non-coding RNA (ncRNA). Integration of DNA methylation data using WGBS, Illumina EPIC arrays that may identify hyper/hypomethylation of gene and can be investigated by analysis of oncogene such as MYC, RUNX2 or tumor suppressors such as TP53, RB1. DNA methylation profiles may also correlate with certain drug resistance e.g., cisplatin, methotrexate [188,189]. Histone modification data may be integrated using ChIP-seq technique to investigate H3K4me3, H3K27me3 H3K27ac, levels in the osteosarcoma genome [190].

Noncoding RNA modifications can be analyzed with miRNA-seq, lncRNA-seq approaches to determine regulatory mechanisms involving miR-21, miR-34a, MALAT1 as well as lncRNA that is involve in epigenetic alteration in chromatin remodeling [191]. Integration of proteomic data using modern techniques such as Mass Spectrometry (MS) and Two-Dimensional Gel Electrophoresis (2DGE). These techniques researcher to completely study protein diversity such as Alternative splicing variants (TP53 isoforms) and Fusions/Truncations (EWSR1-FLI1), protein's amino acid sequence, 3D structure, PTMs (mTOR, MAPK pathways), Protein-Protein Interaction (PPI) Networks (STRING, BioPlex software), cofactor binding partners, cellular localization and cellular function of cancers [184,186]. Integration of Genomics and Transcriptomics data via Whole Exome/Genome Sequencing (WES/WGS) and Single-Cell RNA-seq (scRNA-seq) to determine Somatic mutations in TP53, KRAS, ATRX, CDKN2A gene, Structural variants such as chromothripsis, Tumor heterogeneity, Immune microenvironment, stem-like subpopulations, in osteosarcoma [192-194].

Integration of Metabolomics and Lipidomics profiles of osteosarcoma using LC-MS, GC-MS for Metabolic Profiling to studies Warburg effect, Lipid reprogramming (sphingolipids in metastasis) oncometabolites (2-HG, lactate) [195]. In addition, integration of Radiomics and Clinical using different molecular approaches such as analysis of metastasis free survival (bone vs lung metastasis), MRI/CT tumor consistency analysis, Computational and Systems Biology strategies as well as neoadjuvant chemotherapy [196]. The prospective of 'omics' strategies for the initial diagnosis and treatment of biomarker or drug may assistance to find out the best tolerated and utmost effective treatment approach at optimum dose arrangement according to individual 'omics' fingerprints of osteosarcoma patient.

Conclusion and Expert Recommendation in the Context of 3P Medicine in Osteosarcoma

The emerging epigenetic mechanism insight into treatment strategies is quite improving [197]. Epigenetic alteration in the human genome results in promoting the development and progression of mesenchymal tumors. The mesenchymal tumor has a lot of epigenetic dysregulations, which in most situations are instigated via few genetic alterations. For example, deregulation of methylation resulted in chondrosarcoma [198]. Histone modification resulted in giant cell tumors of bone [199]. Ewin sarcoma fusions result in disorder chromatin organization [200-202] and fusion gene in synovial melanomas change the BAF-framwork, ensuing in a great epigenetic variation of the cell [203]. DNA methylation configurations play a fundamental role in the characterization of central nervous malignant tumor [204]. In addition, the methylation forms might be used in osteosarcoma classification, but the efficacy is still low level. Molecular diagnostics have identified as a significant gadget in bone- and soft-tissue sarcoma group and a noteworthy mechanism to regulate diagnosis management for a rare subtype. With the explosion accessibility of sequencing technologies, it turns into gradually significant to recognize the fortes and flaws of these procedures. Possibly utmost prominently, an advance-skilled pathologist is prerequisite to understand genomic discoveries in the circumstance of tumor histomorphology.

The inspiration of epigenetic modifications on osteosarcoma prognosis, prevention and personalized medicine is a multifaceted and front-line research field, which is closely allied with the entire aspects of PPPM, including predictive approach, targeted prevention and personalization of medical amenities, because epigenetic modification procedures are connected with all-inclusive carcinogenesis and pathophysiological process of osteosarcoma. We intensely recommend disbursing consideration to the investigation and training of epigenetic modification measures in osteosarcoma. This comprehensive study of these epigenetic modification events in osteosarcoma has made countless innovations in the subsequent three aspects for osteosarcoma PPPM.

(i) *Predictive Approach*

Researchers use different computational and lab techniques to find out epigenetic variations (like DNA methylation, histone amendments and non-coding RNAs) that disturb OS development, metastasis, survival or drug resistance. Here are some

predictive approaches. Patient Data-based predictions that includes machine learning and AI to predicts patient outcome such as patient survival, chemotherapy resistance, drug resistance using DNA methylation, histone modifications, miRNA or lncRNA levels etc. Sometime use Algorithms software such as SVM, Random Forest, XGBoost and deep learning to analysis patient data. Epigenetic modification in osteosarcoma can also predicted by analysis of multiple patient data with Methylation Biomarkers targeting certain genes such as RASSF1A, CDKN2A etc as well as determined by certain epigenetic mechanism such as Gene regulation mechanisms and CRISPR and Epigenetic gene editing mechanism. Certain database Such as TCGA-OS, ENCODE/Roadmap (Reference epigenomes) and key tools such as Methylation based survival analysis (MethSurv), methylation effects on genes (MethReg), Enhancer Linking by Methylation/Expression Relationships (ELMER) and ChIP-qPCR test are also used to predict epigenetic modification in osteosarcoma.

(ii) Targeted Prevention

Targeted prevention of epigenetic amendments in osteosarcoma is a gifted therapeutic approach, as epigenetic deregulation emerges a crucial role in tumor development, progression and drug resistance. Distinct genetic mutations, epigenetic alterations are reversible, making them interesting targets for cancer treatment and rehabilitation. Different prevention strategies such as targeting DNA methylation using DNMT Inhibitors (Azacitidine and Decitabine), Guadecitabine Dietary compounds (folate, green tea polyphenols) and certain methylation biomarkers (e.g., MGMT, BRCA1). Target prevention of osteosarcoma targeting histone modification such as using HDAC inhibitor e.g Vorinostat (SAHA), Romidepsin, Panobinostat and Histone Methyltransferase (HMT) and Demethylase (KDM) inhibitors such as Tazemetostat and PARP (EZH2 Inhibitors), Tranylcypromine derivatives (LSD1/KDM Inhibitors). Target prevention of osteosarcoma using RNA-Based Epigenetic Therapy such as Antisense oligonucleotides, miRNA/siRNA and combination therapies such as Epigenetic + Immunotherapy HDAC + DNMT Inhibitors, Epigenetic + Targeted Therapy. Prevention of osteosarcoma using other prevention approaches such as Dietary HDAC inhibitors and Exercise and metabolic regulation.

(iii) Personalization of Medical Services

Personalized medicine does not think to treat whole patient drug resistance mechanism, but to identified and characterized the exact target gene or combination of drug with other biomarkers for each individual patient. Therefore, the inclusive biomarker strategies to clarify the certain resistance mechanisms in each osteosarcoma patient are necessary. Even though, all the available list of drugs and biomarkers will be potentially effective for cancer diagnosis and treatment. The PPPM concept is quite new and interested to identify the exact treatment procedure for osteosarcoma at point time as opposite to the concept of 'one size fit all'. In case of osteosarcoma treatment, the use of different drug and biomarkers need to identify the new treatment procedure, which will quite effective and economical affordable. The emerging osteosarcoma data from generated from certain database proved the severances and full the attraction of research to focus on epigenetic mechanism of osteosarcoma. To construct PPM concept for clinical patient outcome, the competent and quick bioinformatics website and systems biology software are mandatory to screen out the related data convenient for physician and clinicians to proper monitor their treatment decisions. In addition, osteosarcoma patients will receive a complete genomic and proteomic signature of their tumor that will help oncologists to determine exact therapies intended at precise tumor mutation site for significant clinical assistance with negligible treatment-based toxicity. Not each and every outcome will be applicable to all osteosarcoma patient, but time to time, observance and complete understanding of patient molecular profiles will results to the development of proper surgical, radiation and drug-based treatment strategies, truthfully fetching personalized medicine to osteosarcoma attention.

Different mechanisms that modulate the progression of cancer are more complicated and epigenetic modifications provide a novel field of investigation in recent years. Certain novel discoveries have been published recently such as occurrence of DNA methylation, histone alterations and the recently discovered lncRNA, circRNA and M6A alterations. Obviously, the availability of these innovative targeting mechanisms and molecular network is amazing for establishing investigative and medication approaches for osteosarcoma. The applicable regulatory mechanisms of epigenetics in osteosarcomaas mentioned in this study have not been much illuminated.

Several studies reported that aberrant epigenetic changes influence osteosarcoma proliferation, invasion and migration as well as decreased cell apoptosis. Moreover, in *in-vivo* research model, tumor size are also results to decrease due to reverse of epigenetic changes. These inspiring results demonstrate that epigenetic changes and biomarkers are optimistic therapeutic targets for diagnosis and treatment of osteosarcoma.

In addition, further determination of epigenetic biomarkers in osteosarcoma will be proposed to the constant improvement and development of new predictive models for osteosarcoma patients. These new models can be helpful for both physicians and patients at the onset of diagnosis and in the prescription as well as advancement of patients' care plans. As the elaboration of epigenetic modifications and the different signaling pathways they occurred in osteosarcoma lead to more development, there is possibility to enhance current prognostic, diagnostic and therapeutic options, finally guiding to better patient consequences.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement

All data and materials are provided in this article, which can be available publicly.

Ethical Statement

The project did not meet the definition of human subject research under the preview of the IRB according to federal regulations and therefore was exempt.

Informed Consent Statement

Not applicable.

Authors' Contributions

Arshad Ali collected and analyzed literature and wrote the manuscript draft. Ayaz Ali, Y.Z. and Z.F. participated in collection and analysis of literature and visualization. X.Z. conceived the concept, coordinated, critically revised manuscript and was responsible for the corresponding works. All authors approved the final manuscript.

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