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MicroRNA – Mediated Regulation of Apoptosis in Osteosarcoma

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Abstract

Osteosarcoma is the most common primary bone cancer that predominantly affects children and young adolescents. Despite the progress in understanding the pathophysiology of osteosarcoma, it remains gravely influencing mobility and mortality of those young patients. A balance between cell proliferation and apoptosis is often dysregulated in cancer; tumor cells often show accelerated proliferation and reduced apoptosis, resulting in uncontrolled cancer cell growth. The recent discovery of microRNAs has added an exciting new layer of research interest in the fields of medical research in general including cancer. miRNAs are a class of 22~25 nucleotide non-coding RNAs that play a critical role in epigenetic modification of physiological functions of their target genes. Various pathway-specific and tumor-specific miRNAs have been identified and the mechanisms, by which they regulate pathological characteristics of cancer cells, have been elucidated intensively during the past several years. In this review, we summarize current knowledge of apoptosis-related miRNAs in osteosarcoma and their future therapeutic potentials.

Keywords: Osteosarcoma; MicroRNA; Apoptosis

Introduction

Osteosarcoma is the most common primary bone tumor that occurs predominantly in adolescents and young adults [1,2]. Advances in osteosarcoma therapy have improved patient outcomes; the most effective current therapeutic regimens include neoadjuvant and adjuvant chemotherapy combined with local limb-preserving surgery [3]. However, overall clinical outcomes remain poor especially for patients with metastasis and recurrent osteosarcoma. Therefore, it is important to address the unmet needs and continue searching for more effective therapeutic strategies. Apoptosis is the process of programmed cell death regulated by extrinsic and intrinsic inducers. In cancer cells, a balance between cell proliferation and apoptosis is often altered; we observe accelerated proliferation and reduced apoptosis of tumor cells, resulting in uncontrolled cancer cell growth. Two direct initiation pathways for apoptosis have been suggested; Tumor Necrosis Factor (TNF) [4] and Fas-Fas ligand (FasL) [5] pathways. Activation of those receptors signals to proapoptotic (Bax, Bid, Bak, and Bad) and anti-apoptotic (Bcl-Xl and Bcl-2) members of the Bcl-2 family [6], and leads to stimulation of initiator and effector caspases [7]. Other key players in apoptosis in tumor cells include the tumor–suppressor protein, p53, which has been shown to be associated with various cancers including osteosarcoma [8]. p53 regulates the expression of various genes such as cyclin-dependent kinase inhibitor 1A (p21) and modulates 4 cell proliferation, apoptosis, and senescence [9], and its reduced expression are also known to be closely associated with cancer. microRNAs (miRNAs) are a class of 22~25 nucleotide non-coding RNAs that play a critical role in regulation of various biological functions [10,11]. miRNAs bind to complementary sequences in the 3'-untranslated region (3'-UTR) of the target gene transcripts and regulate their gene expression and function including apoptosis of tumor cells [12]. miRNA can function as tumor suppressors and oncogenes [13]. Dysregulation of miRNA expression has been reported in various human cancers including colon cancer [14], breast cancer [15,16], prostate cancer [17-19], hepatocellular carcinoma [20-22], and osteosarcoma. Intensive studies during the last several years have identified numerous affected miRNAs in association with apoptosis, their target genes and biological functions, and possible drug interventions. Here, we will review recent progress of research on miRNA-mediated regulation of apoptosis in osteosarcoma and its future therapeutic applications.

p53-related miRNAs

In normal cells, p53 is inactivated by binding to its antagonist, mdm2; however, upon the DNA damage induced by irradiation, hypoxia, and osmotic stress, p53 dissociates from p53-specific E2ubiquitin protein ligase, mdm2, resulting in activation of p53. Activated p53 then induces cell cycle arrest and apoptosis to repair and discard the damaged cells. More than 20% of osteosarcoma showed mutation of the p53 gene [23]. Thus, to identify p53-responsive miRNAs associated with osteosarcoma, Braun et al. treated osteosarcoma cell lines with p53-agonist (nutlin-3) and –antagonist (mdm2), followed by miRNA array analysis, and identified miRNA-34a, miRNA-194, and miRNA-130 as p53-responsive miRNAs [24]. Further analysis revealed that miRNA-194, not miRNA-130, is consistently induced by p53 and is originated from two different RNA clusters, miRNA-194-1/miRNA-192 on chromosome 11 and miRNA-194-2/miRNA-214 on chromosome 1. Overexpression of miRNA-192 and miRNA-215 together with miRNA-194, increases expression of p53 and p21 when compared with miRNA-34a as a positive control in osteosarcoma cell lines. These results demonstrated that p53-responsive miRNA-192 and miRNA-215 can act as effectors and regulators of p53, and suppress carcinogenesis by cell cycle arrest mediated by p21. They characterized that miRNA-34s are also direct targets of p53 in osteosarcoma using two osteosarcoma cell lines, p53-positive U2OS and p53-negative SaOS2 [25]. They found that both U2OS and SaOS-2 cells express miRNA-34s at similar levels in a basal condition; however, irradiation- and 6 adriamycin-induced miRNA-34s expression was observed in p53-

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positive U2OS cells but not in p53-negative SaOS-2 cells. Thus, miRNA-34s reduce the expression of cell cycle and apoptotic regulators, CDK6, E2F3, Cyclin E2, and Bcl-2, at least in part, in a p53-dependent manner, and result in miRNA-34s mediated G1 arrest and apoptosis. Moreover, they showed that expression of miRNA-34a, 3b, and 3c is reduced and that increased levels of miRNA-34b/c DNA methylation are found in human osteosarcoma samples. Taken together, miRNA-34s are p53-regulated miRNAs and undergo genetic and epigenetic alterations in osteosarcoma. Lastly, it was shown that miRNA-31 targets various genes including serine threonine kinase (STK) 40, E2F2, and CEBPA, and synergistically regulates cell proliferation and apoptosis of tumor cells of ovarian cancer through the E2F and p53 pathways [17]. The similar mechanism was also confirmed in an osteosarcoma cell line; U2OS cells, which exhibit normal p53 but inactivated mutant *CDK2A* gene encoding p14 and p16. U2OS cells also showed that miRNA-31 expression inhibits cell proliferation likely due to the impaired p53 pathway [17]. Collectively, miRNAs-31, 34s, 192, 194, and 215 are microRNAs, which can affect p53-associated apoptotic pathway in osteosarcoma cells.

miRNA-17-92 cluster at chromosome 13 and chromosome 14q32 miRNAs

The human miRNA17-92 cluster encodes 6 miRNAs, miRNA-17, miRNA-18a, miRNA-19a, miRNA-19b, miRNA-20a, and miRNA-92 in chromosome 13 (*C13Orf25*) [26]. Overexpression of the cluster miRNA has been shown to promote cell proliferation [27-29], inhibit apoptosis [28,29] and oncogene induced senescence [30]. Huang et al. showed that miRNA-20a encoded by miRNA-17-92 cluster has an anti-apoptotic effect and it is also expressed in metastatic osteosarcoma [31]. Expression of miRNA-20a is negatively correlated with Fas expression and results in reduction of Fas/FasL-stimulated apoptosis in osteosarcoma.

Baumhoer et al. showed, using 6 well-established osteosarcoma cell lines, that miRNA-17-92 cluster deregulates various genes involved in cell differentiation (RGMB, LRRC17), cell cycle (CCNE1), and apoptosis (LIMA1, CAMK2N1) in osteosarcoma [32].

Next, Thayvanithy et al. reported that a subset of miRNAs at the chromosome 14q32 including miRNAs-382, 369-3p, 544, and 134, is down-regulated in osteosarcoma and that those miRNAs could target cMYC transcript [33]. Inversely, restoration of those miRNAs, decreases cMYC expression and induces apoptosis in SaOS2 cells with lower miRNA-17-92 expression on the chromosome 14q32. Thus, some of the miRNAs encoded by the chromosome 14q32 can modify cell proliferation and apoptosis by regulating cMYC.

Furthermore, osteosarcoma samples do not show consistent changes in methylation pattern in the 14q32 miRNA locus; however, down-regulation of the 14q32 miRNAs is associated with histon deacetylation in osteosarcoma cells [34]. Treatment of osteosarcoma cells with a drug, which increases acetylation, partially restores the 14q32 miRNA expression. An additional use of a drug, which increases methylation, interestingly, fully restores the gene expression pattern similar to that of normal osteoblastic cells in osteosarcoma cells.

Conversely, the combined usage of the DNA acetylation inhibitor and the DNA methylation inhibitor induces more aggressive cytotoxicity in osteosarcoma cells. These results suggest that 14q32 miRNA expression is dysregulated in osteosarcoma cells partially through its epigenetic modification including DNA acetylation and, at less extent, methylation.

Thus, the two cluster miRNAs at chromosome 13 and 14 also regulate cell cycle, apoptosis, metastatic potentials, and epigenetic modifications in osteosarcoma cells.

Other miRNAs

miRNA-15a and miRNA-16-1: Cyclin D1 encoded by *CCND1* is a target of miRNA-15a and miRNA-16-1 [35]. Those miRNAs suppress *CCND1* transcription by directly binding to the CCND1 3'-UTR and reduces cell proliferation in osteosarcoma.

miRNA-29s: Expression levels of miRNA-29a and miRNA-29b are down-regulated in most of osteosarcomas. Zhang et al. showed that miRNA-29a, unlike miRNA-29b and miRNA-29c, induces apoptosis independent of p53 expression by silencing Bcl-2 and Mcl-1 and increasing E2F1 and E2F3 expression [36].

miRNA-93: Higher miRNA-93 expression was found in 143B and primary osteosarcoma cells. Up-regulation of mRNA and protein expression of E2F1, one of the potential targets of miRNA-93, is detected in osteosarcoma [37].

miRNA-143: miRNA-143 was reported to be down-regulated in some cancer. Zhang et al. showed that 1) miRNA-143 is also down-regulated in human osteosarcoma samples and cell lines, and that 2) its overexpression promotes cell apoptosis in human osteosacome cell lines, MG63 and U2OS cells. 3) Using luciferase reporter assay, they then displayed that miRNA-143 binds directly to two Bcl-2 3'-UTR sites as predicted by TargetScan prediction and inhibits expression of Bcl-2 in osteosarcoma [38]. Collectively, miRNA 143 negatively regulates expression of anti-apoptotic protein, Bcl-2.

miRNA-221: Expression of miRNA-221 is up-regulated in osteosarcoma cell lines. miRNA-221 expression increases cell survival and cisplatin-resistance and reduces apoptosis [39]. Further, PTEN is determined to be a direct target of miRNA-221. miRNA-221 directly binds to PTEN 3'-UTR, inhibits PTEN translation, and activates the Akt pathway. Several downstream targets of the Akt pathway including CCND1, p27, and Bcl-2, were also regulated by miRNA-221. Inverse correlation between miRNA-221 and PTEN levels has been shown in osteosarcoma. Thus, miRNA-221 induces cell survival and cisplatin-resistance at least partially by modulating the PI3K/PTEN/Akt pathway.

miRNA-223: Heat shock protein 90B1 (HSP90B1) is a direct target of miRNA-223. miRNA-223 may have tumor suppressor function through the PI3K/Akt/mTOR pathway in osteosarcoma [40].

Conclusions

Recent evidence has shown various apoptosis-related miRNAs and their molecular targets in osteosarcoma (Table 1). Some miRNAs serve as oncogenes, which are found up-regulated, and others do as tumor suppressors, which are often down-regulated, in osteosarcoma cells. Several lines of studies have indicated that targeting specific miRNAs could have therapeutic potentials because of their tumor and pathway specificity. Therefore, the next possible therapeutic approaches could be developed to block the expression of oncogenic miRNAs using, for example, antisense-oligonucleotides targeted for the specific miRNAs, and restore the expression of tumor suppressor miRNAs by using miRNA mimics in treatment of osteosarcoma. Moreover, delivery methods of those antisense-oligonucletides and miRNA mimics could be further developed from systemic administration [13,41] to local targeted delivery to the tumors using the latest technology such as nanoparticles [41] in the future.

microRNAs	Target genes	Chromosome	Function	Cell lines	Citations
miR-34a miR-192 miR-215	Cdkn1A/p21	miR-192/194; 11 miR-215/194; 1	Increase apoptosis Cell cycle arrest Suppress carcinogenesis		[24]
miR-34s	CDK6, E2F3 Cyclin E2, Bcl-2	miRNA-34a; 1p36 miRNA-34b/34c; 11q23	Cell cycle arrest Apoptosis	U2OS SaOS2	[25]
miR-31	E2F2, p14 p16	9p21.3	Cell cycle, Promote p53 activity, Induce apoptosis	U2OS	[30]
14q32 miRs (miR-382, 369- 3p, 544, 134)	cMyc miR-17-92	14q32	Pro-apoptotic effect	SaOS2	[33]
miR-20a	Fas	*C13orf25*	Induce metastasis Reduce Fas-mediated apoptosis	LM7 SaOS2	[31]
miR-17-92	LRRC17, RGMB, LIMA1, CCNE1, VAMK2N1	*C13orf25*	Oncogenic & tumor suppressor properties, Differentiation, Apoptosis, Cell cycle regulation	HOS58, U2OS, SaOS2, SJSA1, MG63	[32]
14q32 miRs	HDAC	14q32	Elevate histone deacetylase activity	SaOS2	[34]
miR-15 miR16-1	CCND1		Apoptosis, Cell cycle arrest	SOSP-9607	[35]
miR-29a	Bcl-2, Mcl-1, E2F1, E2F3		Down-regulated in Osteosarcoma, Induce apoptosis (p53-independent) Down-regulate Bcl-2, Mcl-1, Increase tumor suppressor genes, E2F1 and E2F3	U2OS SaOS2	[36]
miR-93	E2F1		Reduce cell viability, Promote apoptosis, Suppress tumorigenesis	MG63, 143B	[37]
miR-143	Bcl-2			MG63, U2OS	[38]
miR-221	PTEN		Increase cell survival and cisplatinresistance, Decrease apoptosis	SOSP-9607, MG63	[39]
miR-223	Heat shock protein 90B1(HSP90B1)		Suppress HSP90B1 and cell growth, Cell cycle arrest and apoptosis. Decrease PI3K/Akt/mTOR/ Bcl-2. Increase Bid	MG63	[40]

Table 1: Apoptosis-related microRNAs in osteosarcoma.

Acknowledgements

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