Research Progress of Micro-RNAs in Apoptosis of Osteosarcoma

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Research Article

Keywords: Osteosarcoma, microRNAs, apoptosis

Posted Date: September 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-922088/v1

Abstract

Osteosarcoma is a primary malignant bone tumor with no effective treatment. Apoptosis, one of the programmed cell death, is any pathological form of cell death mediated by intracellular processes. Under the pathological state, the unregulated regulation of apoptosis can disrupt the balance between cell proliferation and death, causing osteosarcoma proliferation and metastasis. As carcinogenic or tumor suppressor factors, microRNAs (miRNAs) regulate apoptosis of osteosarcoma cells by regulating apoptosis-related signaling pathways and apoptosis-related genes. This review provides the current knowledge of miRNAs and their target genes related to the apoptosis of osteosarcoma.

1. Introduction

Osteosarcoma is a malignant tumor originating from mesenchymal tissue, accounting for 20% of primary malignant bone tumors. It is the most common primary malignant bone tumor in children and adolescents, with 70–80% of patients aged 10 to 25 years and an annual incidence rate of 1 to 3 cases per million [1]. Osteosarcoma is a solid tumor that produces osteoid, which occurs in fast-growing epiphysis (such as proximal humerus, distal femur, and proximal tibia), but rarely in the spine, pelvis, and sacrum. It is often accompanied by amputation, lung metastasis, death, and other consequences, and the cure rate of simple operation is only 15–20% [2]. At present, the clinical treatment of osteosarcoma is unsatisfactory. Although some progress has been made in multi-drug chemotherapy and surgical resection, for patients with osteosarcoma who underwent radical amputation combined with chemotherapy, the five-year survival rate can be increased to 50%-70%, 10% of the patients still have a local recurrence [3]. Therefore, it is essential for osteosarcoma research to find new treatment methods to further improve the patients' survival rate.

MicroRNAs (miRNAs) is a kind of single-stranded RNAs, with long 19 ~ 25 nt, which does not have an open reading frame and does not encode any protein [4]. MiRNAs has been implicated in the occurrence and progression of cancer in a growing number of studies that miRNAs in malignant tumor cells present abnormal, which can positively or negatively regulate cancer progression, such as maintaining proliferative signal transduction, anti-apoptosis, inducing angiogenesis, and cancer cell invasion and metastasis [5]. Recent studies have shown that the expression pattern of miRNAs in osteosarcoma has changed. Lulla et al. showed that differential expressions of twenty-two kinds of miRNAs between osteosarcoma cells and osteoblasts [6], which can regulate the proliferation and apoptosis of osteosarcoma cells in a variety of ways, play a role in promoting or inhibiting cancer in the occurrence and development of osteosarcoma. Although the molecular regulatory mechanism of miRNAs on osteosarcoma cells has attracted widespread attention, its specific biological effect is still not very clear (see in Table 1).

Table 1
Abnormal expression and regulation of microRNAs (miRNAs) in osteosarcoma

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-216b	up	FoxM1/Bax/Bcl-2 OR JMJD2C/HIF1α/HES1	Induction of apoptosis
miR-204-5p	up	EBF2	Induction of apoptosis
miR-144	up	Bax/caspase-3/Bcl-2 OR Ezrin	Induction of apoptosis
miR-1236-3p	up	KLF8	Induction of apoptosis
miR-449a	up	BCL2	Induction of apoptosis
miR-218	up	BIRC5/caspase-5	Induction of apoptosis
miR-194	up	CDH2	Induction of apoptosis
miR-34	up	TGIF2/caspase-3	Induction of apoptosis
miR-9	up	CXCR4	Induction of apoptosis
miR-369	up	SOX4	Induction of apoptosis
miR-1301	up	TRIAP1/Bcl-2/Bax	Induction of apoptosis
miR-124	up	TRAF6 OR AKT/P53/Bcl-2	Induction of apoptosis
miR-29	up	COL3A1/MCL1 OR STAT3	Induction of apoptosis
miR-211	up	EZRIN	Induction of apoptosis
miR-221	up	CDKN1B/p27 OR PI3K/Akt	Induction of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-133b	up	AKT/ERK OR FBXW11/Wnt FGFR1/Ras/MAPK/PI3K/Akt OR BCL2L2/MCL-1/Akt	Induction of apoptosis
miR-18a	up	MED27/p27/Bcl-2	Induction of apoptosis
miR-100	up	mTOR/Beclin-1 OR IGFIR/PI3K/AKT/MAPK/ERK	Induction of apoptosis
miR-206	up	AKT/ANXA2/PARP OR PAX3-MET/Akt1/Erk1/2	Induction of apoptosis
miR-203	up	Runx2	Induction of apoptosis
miR-192	up	MMP-11 OR TCF7	Induction of apoptosis
miR-141-3p	up	GLI2	Induction of apoptosis
miR-34a	up	DUSP1 OR DNMT1/miR-34a/Bcl-2 OR Stathmin1/βIII-Tubulin OR SIRT1/BCL2/c-MET/CDK6 OR C-IAP2/Bcl-2/Caspase-3/9	Induction of apoptosis
miR-127-3p/miR-376a-3p	up	JNK	Induction of apoptosis
miR-133a	up	Bcl-xL/Mcl-1	Induction of apoptosis
miR-451	up	MIF	Induction of apoptosis
miR-340	up	CTNNB1/Notch/Bim OR ZEB1	Induction of apoptosis
miR-23b-3p	up	CCNG1	Induction of apoptosis
miR-30a	up	FOXA1 OR HMGA2/Beclin1/LC3B	Induction of apoptosis
miR-328-3p	up	H2AX OR MMP-16	Induction of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-139	up	SNHG20/RUNX2/Caspase-9/3 OR FOXP2	Induction of apoptosis
miR-495	up	HMGN5/Bcl-2/Caspase-9	Induction of apoptosis
miR-409-3p	up	IGFBP3	Induction of apoptosis
miR-491-5p	up	FOXP4	Induction of apoptosis
miR-138	up	EZH2 OR TNFAIP8/MMP-2/-9	Induction of apoptosis
miR-365	up	KRAS	Induction of apoptosis
miR-127	up	ZEB1/JNK/Wnt	Induction of apoptosis
miR-661	up	CYC1/TRAIL/Caspase-9	Induction of apoptosis
miR-150	up	RUNX2 OR Sp1	Induction of apoptosis
miR300	up	Twist1/NFκB	Induction of apoptosis
miR-22	up	Caspase-3/Bax/Bcl-2/Beclin1 OR Bax/Bcl-2 OR ACLY	Induction of apoptosis
miR-190b	up	Bcl-2/Bax/Caspase-3	Induction of apoptosis
miR-613	up	CXCR4	Induction of apoptosis
miR183	up	Ezrin	Induction of apoptosis
miR-98-5p	up	CDC25A	Induction of apoptosis
miR-127-3p	up	ITGA6	Induction of apoptosis
miR-34b	up	PAK1/ABCB1	Induction of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-376a	up	FBXO11	Induction of apoptosis
miR-145-3p	up	HDAC4	Induction of apoptosis
miR-493-5p	up	KLF5/PI3K/AKT	Induction of apoptosis
miR-143	up	Bcl-2 OR Bcl-2/Caspase-3	Induction of apoptosis
miR-30c	up	SOX9	Induction of apoptosis
miR-326	up	Bcl-2	Induction of apoptosis
miR-182	up		Induction of apoptosis
miR-134	up		Induction of apoptosis
miR-491	up	CRYABOS	Induction of apoptosis
miR-216a-5p	up	SOX5	Induction of apoptosis
miR-338-3p	up	RUNX2/CDK4/MAPK	Induction of apoptosis
miR-542-3p	up	ILK	Induction of apoptosis
miR-452	up	BMI1	Induction of apoptosis
miR-101	up	mTOR	Induction of apoptosis
miR-490-3p	up	HMGA2	Induction of apoptosis
miR-330-5p	up	Survivin	Induction of apoptosis
miR-381	up	ZEB1/JNK/Wnt/β-catenin OR LRH-1/Wnt/β-catenin/p53	Induction of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-9	up		Induction of apoptosis
miR-199a-3p	up	MCL11/PARP	Induction of apoptosis
miR-302b	up	Caspase-3/Akt/Bcl-2/Bim	Induction of apoptosis
miR-217	up		Induction of apoptosis
miR-15a/miR-16-1	up	CCND1	Induction of apoptosis
miR-126	up	EGCG OR VEGF-A/PI3K/AKT	Induction of apoptosis
miR-497	up	MAPK/Erk/P213	Induction of apoptosis
miR-202	up	Gli2 OR PDCD4	Induction of apoptosis
miR-1247	up	NRP1/Wnt/β-catenin	Induction of apoptosis
miR-199a/miR-34a	up	p53	Induction of apoptosis
miR-335	up	Survivin	Induction of apoptosis
miR-34a/miR-203	up	Survivin	Induction of apoptosis
miR-136	up	circRNA_100876/miR-136	Induction of apoptosis
miR-125a	up	ERRα	Induction of apoptosis
miR-93/106b	up	p21	Induction of apoptosis
miR-495-3p	up	CTRP3	Induction of apoptosis
miR-422a	up	TGFβ2/p-smad2/smad3	Induction of apoptosis
miR-370	up	FOXM1	Induction of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
let-7	up	JW74	Induction of apoptosis
miR-342-5p	up	Wnt7b	Induction of apoptosis
miR-152	up	DKK1/Wnt/ β-catenin/LDH/p53	Induction of apoptosis
miR503	up	FGF2	Induction of apoptosis
miR-96	up	EZRIN	Induction of apoptosis
miR-29b	up	MMP-9	Induction of apoptosis
miR-466	up	CCND1	Induction of apoptosis
miR-708-5p	up	URGCP	Induction of apoptosis
miR-638	up	Caspase-3/PLD1/VEGF	Induction of apoptosis
miR-99a	up	TNFAIP8	Induction of apoptosis
miR-497-5p	up	ARL2/p53	Induction of apoptosis
miR-874	up		Induction of apoptosis
miR-188	up	SOX4	Induction of apoptosis
miR-506	up	AEG-1/Wnt/β-catenin	Induction of apoptosis
miR-17	up	SASH1	Inhibition of apoptosis
miR-103	up	p57/JNK/STAT/mTOR	Inhibition of apoptosis
miR-1307	up	AGAP1	Inhibition of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-19b	up	KLF13 OR SOCS3/STAT3	Inhibition of apoptosis
miR-21	up	PTEN/Akt	Inhibition of apoptosis
miR-433	up	PDCD4	Inhibition of apoptosis
miR-663a	up	ZBTB7A/LncRNA/GAS5	Inhibition of apoptosis
miR-181a	up	CFIm25 OR Bcl-2/MMP-9/3	Inhibition of apoptosis
miR-142	up		Inhibition of apoptosis
miR-488	up	Bim	Inhibition of apoptosis
miR-130b	up	NKD2/Wnt	Inhibition of apoptosis
miR-17-5p	up	SRCIN1/E-cadherin/N-cadherin	Inhibition of apoptosis
miR-1248	up	AGTR1	Inhibition of apoptosis
miR-4779	up	PLK1	Inhibition of apoptosis
miR-155	up	PTEN/PI3K/AKT/mTOR	Inhibition of apoptosis
miR-92a	up	PTEN/AKT/mTOR	Inhibition of apoptosis
miR-10b	up	KLF4	Inhibition of apoptosis
miR-26a-5p	up	HOXA5	Inhibition of apoptosis
miR-708	down	CUL4B	Induction of apoptosis
miR-18a-5p	down	SOCS5/FER1L4/PI3K/AKT	Induction of apoptosis
miR-542-3p	down	CDK4/Bcl-2/Bax	Induction of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-21	down	PTEN/PI3K/AKT	Induction of apoptosis
miR-663b	down	TP73/Bcl-2/Bax	Induction of apoptosis
miR-19a	down	PTEN/PI3K/AKT	Induction of apoptosis
miR-29	down	TGF-β1/PUMA	Induction of apoptosis
miR-128	down	Bcl-2/SASH1/Bax/Caspase-3	Induction of apoptosis
miR106a	down	VNN2	Induction of apoptosis
microRNA-586	down		Induction of apoptosis
miR-143-3p	down	FOSL2	Induction of apoptosis
miR29a	down	DNMT3B/SOCS1/NF-κB	Induction of apoptosis
miR-301a	down	CXCR4/Wnt/β-catenin/NF-κB	Induction of apoptosis
microRNA-181a	down	PTEN	Induction of apoptosis
miR-375	down	Bcl-2/Bax	Induction of apoptosis
miR-95-3p	down	CDKN1A/p21	Induction of apoptosis
miR-1200	down	HOXB2	Induction of apoptosis
miR-211	down	TUSC7	Induction of apoptosis
miR-421	down	LTBP2/Caspase3/Bax/Bcl-2	Induction of apoptosis
miR-23b-3p	down	SIX1/VEGF-C/Caspase-3	Inhibition of apoptosis
miR-491-5p	down	CyclinD1/p21	Inhibition of apoptosis

MicroRNAs	Expression (up or down)	Regulated gene/axis	Functions
miR-132-3p	down	SOX4	Inhibition of apoptosis
miR-212-3p	down	FOXA1	Inhibition of apoptosis
miR-16	down	ATG4B	Inhibition of apoptosis
miR-382-5	down	YB-1	Inhibition of apoptosis
miR-340	down	SNHG16/miR-340	Induction of apoptosis
miR-34c	down	BCL-2/CCND1	Inhibition of apoptosis
miR-34a-5p	down	ERK/AKT/Wnt/β-catenin	Inhibition of apoptosis
miR-144-3p	down	EZH2/Wnt/β-catenin	Inhibition of apoptosis
miR-126	down	DDP/MTX	Inhibition of apoptosis
miR-134-5p	down	MBTD1	Inhibition of apoptosis

The whole life process of the body is accompanied by cell death, which also occurs in the pathological process of tumor growth. Therefore, it is imperative to determine how to induce cell death in treating human osteosarcoma effectively [7, 8]. Cell death can be divided into programmed cell death (PCD) and unprogrammed death according to the mode of death. Amongst these, PCD, an active cell death controlled by specific genes and regulated by a unique signal transduction pathway, plays a vital role in the occurrence and development of osteosarcoma. As a polygene-controlled death process, apoptosis is the most typical and well-studied amongst the forms of PCD. The body can remove aging or abnormal cells through apoptosis so that the homeostasis of the internal environment is maintained in the normal physiological state, while in the pathological state, the disorder of apoptosis regulation will destroy the balance between cell proliferation and death, which has a destructive effect on the body and causes a series of diseases including osteosarcoma. Current studies have found that miRNAs can participate in the apoptosis process of osteosarcoma cells by regulating apoptosis-related proteins and pathways [9–11]. It has gradually become a hotspot to explore the mechanism of miRNAs that regulates apoptosis in osteosarcoma

Based on the regulatory process of apoptosis and the biological roles of miRNAs, this review focuses on the regulation of apoptosis by miRNAs in recent years, and discuss remaining problems and likely prospects associated with curing osteosarcoma by promoting miRNAs-induced apoptosis, with a view of more comprehensively exploring the important role of miRNAs in the occurrence and development of osteosarcoma, and then provide new ideas for molecular targeted therapy of osteosarcoma.

2. Mirnas And Apoptosis-related Proteins

There are many proteins involved in the regulation of apoptosis, such as the Bcl-2 family, the Caspase family, the matrix metalloproteinase family (MMPS), and other apoptosis-related proteins. These factors can influence the apoptosis process of osteosarcoma cells by regulating the three major apoptosis pathways: death receptor pathway, mitochondrial pathway, and endoplasmic reticulum pathway (see in Fig. 1, and Fig. 2).

2.1 Bcl-2 family

B-cell lymphoma/leukemia-2 (Bcl-2) family plays a vital role in the regulation of apoptosis in the signal transduction of apoptosis pathway, which is also one of the most noticed genes amongst many genes related to apoptosis. A study related to human follicular lymphoma has been first discovered the Bcl-2 gene [12]. Bcl-2 protein, the first discovered protein among many Bcl-2 family members, is also the first proved anti-apoptotic protein and plays an essential role in the cell apoptotic pathway. Chen et al. found that miRNA449a may exert its pro-apoptotic function by inhibiting the expression of Bcl-2 [13]. The CCK-8 and apoptosis assays results suggested that cell proliferation could be inhibited while apoptosis was promoted by restoring of miRNA449a expression in osteosarcoma U2OS cell lines and Saos-2 cell lines. In addition, Bcl-2 was identified as the target of miRNA449a by using TargetScan prediction technology and luciferase reporter gene detection technology, and the final experimental results also showed that the expression of Bcl-2 was negatively correlated with miRNA449a. Studies have shown that both miRNA326 and miRNA143 promoted cell apoptosis by targeting the down-regulation of Bcl-2 [14, 15]. Bax (Bcl-2 associated X protein), a pro-apoptotic protein closely related to the function of Bcl-2. Overexpressed Bax tends to follow increased apoptosis, on the contrary, Bcl-2 acts as an anti-apoptotic protein. Zhang et al. showed that transfection of miRNA144 analogs inhibited cell proliferation and significantly increased apoptosis in U2-OS cells. Furthermore, the detected results showed that the expression of Bax and caspase-3 was increased, while the expression of anti-apoptotic protein Bcl-2 was decreased [16]. The interaction relationship between Bax and Bcl-2 can be described: increased Bax can numerously and significantly lead to the production of Bax/Bax homodimers, while overpressed Bcl-2 can depart many Bax/Bax dimers so that generates a more stable Bcl-2/Bax heterodimer, and eventually play a role in inhibiting apoptosis. All of these demonstrate that the ratio of Bax/Bcl-2 can affect cell apoptosis.

The Mitochondrial pathway is one of the molecular regulatory pathways of apoptosis, which is also called the Bcl-2 regulatory pathway because it is regulated by Bcl-2 family [17]. It can be regulated by the interaction among Bcl-2 family members. As an effector, Bax can form pores in the outer mitochondrial

membrane through oligomerization, eventually leading to MOMP, and then triggering the Caspase cascade Response to promote cell apoptosis because of the release of pro-apoptotic proteins such as cytochrome C and Smac located in the mitochondrial membrane space into the cytoplasm. Wang et al. showed that knocking out the LncRNA SNHG20, which is the target of miRNA139, can up-regulate Bax's expression and promote the apoptosis of osteosarcoma cells through the mitochondrial apoptotic pathway [18]. Protectors such as Mcl-1 and Bcl-xL can inhibit the activity of effectors. MiRNA29 family can down-regulate the mRNAs and protein expression of Mcl-1 in osteosarcoma cells, promoting cell apoptosis and inhibiting their resistance to methotrexate [19]. MiRNA133b and miRNA133a can also down-regulate the expression of Bcl-xl and Mcl-1 to promote osteosarcoma cell apoptosis [20, 21]. The initiator can promote the permeation of the mitochondrial outer membrane by regulating effectors or protectors. For example, Bim can directly activate the effector to promote apoptosis. Sun et al. observed that down-regulating the expression of miRNA24 can target up-regulating the expression of Bim, and promoting DOX-induced apoptosis through the mitochondrial pathway [22]. In addition, miRNA340 can increase the expression of Bim to promote osteosarcoma cell apoptosis by inactivating CTNNB1 mediated Notch signaling pathway [23]. MiRNA488 can target Bim to inhibit osteosarcoma cell apoptosis, miRNA302b can up-regulate the expression of Bim by regulating the AKT signaling pathway in osteosarcoma cells [24, 25].

2.2 Caspase family

Caspase family is a group of proteases that can regulate cell apoptosis and be activated sequentially through a protease cascade reaction [26]. The occurrence of most cell apoptosis depends on caspases, which induced cell apoptosis by selectively cleaving substrate proteins, which leads to the structural change and the loss of function of the substrate protein. The occurrence and development of tumors can be induced by the dysfunction of cell apoptosis in the body, which is caused by the inhibition of caspase functionally. Caspase family consists of caspase-2, 3, 6, 7, 8, 9 and 10, among which caspase-9 is an upstream initiating factor in the caspase factor family, which is the initiating factor of apoptosis and is important for the regulation of cell apoptosis process [27]. Caspase-3 is the most critical factor in the apoptotic cascade, located downstream of the apoptotic cascade. It can be activated by activating of caspase-9 in the endogenous apoptosis pathway, inducing the repair of related genes and the inactivation of cell cycle-related enzymes, then leading to cell apoptosis [28]. Related studies have shown that the high expression of caspase3 and caspase9 can be detected in both miRNA130 and miRNA34a transfected osteosarcoma cells, while cell invasion and metastasis are inhibited, and apoptosis is promoted [18, 29]. Wang et al. confirmed that miRNA139 increased the expression of caspase3 and caspase9 through targeting the regulation of SNHG20. MiRNA381, on the other hand, increases caspase3/9 activity by inhibiting the expression of LRH-1[30].

Compared with normal osteoblast cell lines, miRNA221 and miRNA196a were increased in osteosarcoma cell lines. Overexpression of miRNA221 and miRNA196a inactivated caspase3. Transfection of the two inhibitors into osteosarcoma cell lines, respectively, showed a significant increase in caspase3 levels. It significantly inhibited cell proliferation, migration and invasion, and cell cycle stagnation in G0/G1 phase

[31, 32]. The expression of miRNA638 and miRNA190b was down-regulated in patients with osteosarcoma, and the overexpressed miRNA638 and miRNA190b could up-regulate caspase3 and induce apoptosis [33, 34]. MiRNA34 increases the content of caspase3 by targeting the expression of TGIF2, thus promoting the apoptosis of osteosarcoma cells [35]. These findings suggest that miRNA221, miRNA196a, miRNA638, miRNA190b, and miRNA34 can promote the apoptosis of osteosarcoma cells by acting on caspase3, a common downstream key factor of different apoptosis pathways. The role of MiRNAs in regulating caspase3 is also related to the mitochondrial pathway. Related studies have shown that miRNA143 can activate caspase3 by targeting Bcl-2 and induce apoptosis of osteosarcoma cells [15]. The activation of miRNA302b on caspase3 can regulate the expression of Bcl-2/Bim, inhibit the proliferation of osteosarcoma cells, and increase apoptosis [24]. Inhibition of miRNA421 can promote cell apoptosis rate, caspase3 activity and Bax/Bcl ratio [36]. Fan et al. showed that the overexpression of miRNA661 could activate caspase9, inhibit the growth of osteosarcoma cells, and promote apoptosis [37], indicating that miRNA661 can activate the signal transduction of death receptor pathway and lead to the apoptosis of osteosarcoma cells by promoting the activity of caspase9, the initiation effector of apoptosis.

2.3 MMPs

Because the progression of osteosarcoma is closely associated with matrix metalloproteinases (MMPs) that degrade the extracellular matrix (ECM), MMPs play an important role in cancer survival and invasion and the development of tumor vascular networks [38]. MMPs as zinc-dependent endopeptidase is secreted mainly by fibroblasts, leukocytes, vascular smooth muscle cells, and rapidly proliferating tumor cells. MMPs biological properties are present in various physiological and pathological processes, such as the degradation of collagen and elastin, the formation of endothelial cells during angiogenesis, migration of vascular smooth muscle, and proliferation of tumor cells migration [39]. Currently, in humans, there are 23 recognized MMPs that stimulate cancer survival and spread, and they represent the target group of anticancer drugs [40].

As one of the MMPs family, the degradation substrates of MMP9 mainly include gelatin, type IV, and V collagen, elastin, glass adhesive protein, etc. Because the basement membrane is mainly composed of type IV collagen, glycoprotein and proteoglycan, MMP9 is the most intensely studied and important amongst the MMP family. The overexpression of MiRNA495 can inhibit the proliferation and invasion of osteosarcoma cells and induce their apoptosis, which is related to the inhibition of the expressions of HMGN5, Cyclin B1, Bcl-2 and MMP9 [41]. In addition, both the overexpressed miRNA29b and miRNA181a can target down the expression of MMP9 and promote cell apoptosis [42, 43]. MiRNA138 can reduce the invasion of osteosarcoma cells and promote apoptosis by down-regulating the expression of MMP2 and MMP9 [44]. In addition, miRNAs can also affect the apoptosis of osteosarcoma cells by acting on other MMPs. The expression of miRNA2682-3p was significantly decreased in osteosarcoma tissues and cell lines, and the overexpressed miRNA-2682-3p could inhibit cell proliferation and promote apoptosis by downregulating the expression levels of CCND2, MMP8 and Myd88 [45]. Transfection of miRNA192

mimics into osteosarcoma cell lines showed that overexpressed miRNA192 could down-regulate MMP11 content, reduce cell proliferation, migration and invasion, and promote apoptosis [46].

2.4 Other apoptosis-related proteins

Due to regulating cell apoptosis is a complex network, the simple way to study osteosarcoma cells apoptosis often cannot well explain the tumor biological behavior, so the related factors of osteosarcoma cells apoptosis in more in-depth research is likely to reveal preliminarily in its role in the development of osteosarcoma cells apoptosis mechanism, and to provide more broad prospects in the treatment of osteosarcoma. Survivin gene belongs to the inhibitor of apoptosis (IAP) family, and its expression can regulate cell cycle, inhibit apoptosis, promote cell proliferation and angiogenesis [47]. Studies have shown that overexpressed miRNA206 can inhibit cell [48] Krüppel like factors (KLFs) play an important regulatory role in cell proliferation, cell differentiation, cell apoptosis and other aspects by regulating the expression of genes rich in GC and CACCC promoters [49]. Wang et al. [50] revealed that miRNA10b promotes the development of osteosarcoma cells by downregulating KLF4 expression, while inhibiting miRNA10b in osteosarcoma cells can promote cell apoptosis. KLF5 is a transcription factor closely related to KLF4 in the KLF family. Several studies have shown that overexpression of KLF5 can promote tumor formation and lead to tumor deterioration [51]. In the study of osteosarcoma, Zhang et al. [48] confirmed that mirNA-493-5p could target down KLF5 and block the PI3K/Akt signaling pathway, reduce the activity, migration and invasion of osteosarcoma U20S cells, and promote the apoptosis of osteosarcoma cells.

In addition to the regulation of cell growth and proliferation, SOX family can also participate in the regulation of cell apoptosis [52]. The three domains contained in Sox4 protein, the high mobility group (HMG) DNA-binding domain (DBD), and the glycine-rich domain (AA152-227) can all participate in the regulation of apoptosis [53]. Pan et al. [54] studied the function of pediatric osteosarcoma and found that the expression of miRNA188 was negatively correlated with SOX4, and restoring the expression of SOX4 could eliminate the pro-apoptotic effect of miRNA188 on OS cells. SOX5 is involved in chondrogenesis and promotes chondrocyte differentiation, and can directly bind DNA or regulate gene expression through binding with other proteins [55]. Literature has shown [56] that P53 inhibits tumorigenesis by regulating cell apoptosis, metabolic network, free radicals, and senescence. MiRNA34a can reduce FOXP1, known as a B-cell oncogene, through the p53 network [57]. By targeting FOXP2, the overexpression of miRNA139 can promote apoptosis of osteosarcoma cells [58].

3. Mirnas And Apoptosis-related Signal Pathway

MiRNAs affect downstream related factors and thus participate in regulating the proliferation, invasion, and metastasis of tumor cells and influencing tumor cells' biological behavior through the activation status of related signaling pathways, thus affecting tumor development and outcome regression. Currently, miRNAs have been found to regulate classical signaling pathways such as MAPK/ERK signaling pathway, PI3K/Akt signaling pathway, Wnt/β-catenin signaling pathway, Notch signaling

pathway, and NF-κB signaling pathway, etc. In osteosarcoma, miRNAs can interact with signaling pathways to exert their oncogenic or oncogenic effects (see in Fig. 1 and Fig. 2) [59].

3.1 MAPK/ERK signal pathway

Many oncogenic tyrosine kinases' activity is directly correlated with the activation status of the MAPK signaling pathway. MAPK remains quiescent in unstimulated cells but is activated upon receipt of activation signals from MAPK kinase (MKK) and MKK kinase and is progressively phosphorylated in growth factor-stimulated cells [60, 61]. When MAPK is activated, it translocates to the nucleus and activates certain oncogenes, thus exerting a role in stimulating cell proliferation and inhibiting apoptosis. Related experiments have shown that the expression of miRNA-338-3p is down-regulated in osteosarcoma cells compared with normal bone tissue, and the inhibition of miRNA-338-3p significantly increases cell viability, promotes cell migration and invasion, and inhibits apoptosis. At the same time, these effects are reversed after overexpression of miRNA-338-3p, which suggests that up-regulation of miRNA-338-3p can effectively inhibit osteosarcoma progression. miRNA-338-3p can act as an oncogene in osteosarcoma cells by targeting Runx2 and CDK4 and inhibiting the MAPK pathway [62]. MiRNA100 overexpression inhibits the MAPK/ERK signaling pathway, which in turn inhibits the proliferation, migration, and invasion of osteosarcoma cells ability and promotes apoptosis [63]. MiRNA497 can activate P21 by inhibiting MAPK/Erk signaling pathway, thus promoting apoptosis of osteosarcoma cells [64]. It was shown that upregulation of miRNA340 could reduce MAPK and ERK phosphorylation levels, reducing osteosarcoma cell proliferation and inducing apoptosis and autophagy by inhibiting MAPK/ERK pathway [65].

3.2 PI3K/Akt signal pathway

Phosphatidylinositol 3 - kinase/protein kinase B (PI3K / Akt) signaling pathway is essential in regulating osteosarcoma development. Some important extracellular signaling stimuli, such as growth factors, cytokines, hormones, hypoxia, etc., can activate PI3K. activated PI3K can catalyze PIP2 on the inner surface of the cell membrane to generate PIP3 as a second messenger and activate Akt, which regulates apoptosis through various pathways [66].

It has been shown that upregulation of miRNA133b, miRNA18a, miRNA206, miRNA21, miRNA19a, miRNA124, and miRNA-493-5p in osteosarcoma cells inhibited the Akt signaling pathway to exert oncogenic effects. For example, transfection of osteosarcoma cells with miRNA133b inhibitor followed by treatment with procaine showed that miRNA133b inhibitor reversed the pro-apoptotic effect of procaine on osteosarcoma cells while increasing the level of Akt, suggesting that procaine may exert its oncogenic effect by inactivating Akt through upregulation of miRNA133a [67]. Activation of PI3K-dependent AKT phosphorylates the Ser126/Serl12 residue of Bad, and the phosphorylated Bad depolymerizes with Bcl-2 or Bcl-xl, while the free Bcl-2 exerts anti-apoptotic effects [68]. Studies have showen that miRNA18a targets MED27 and downregulates the expression levels of Bcl-2 and p-Akt, while upregulation of caspase3 and Bax protein expression promotes apoptosis in MG63 and Saos-2 cells [69]. As a negative regulator of PI3K-dependent Akt signaling, gene of phosphates and tensin homolog deleted

on chromosome 10 (PTEN) deletion can lead to PI3K/Akt hyperactivation. In a study of osteosarcoma, thicket (HNK) was found to decrease miRNA21 expression in a dose-dependent manner while upregulation of PTEN levels and PI3K/Akt signaling inhibition pathway were detected [70]. miRNA19a, miRNA21, and miRNA221 also exhibited similar effects [71–73].

3.3 Wnt/β-catenin signal pathway

Several studies have proposed that the Wnt signaling pathway is aberrantly activated in malignant bone tumors as well as tumor bone metastases, such as multiple myeloma, Ewing's sarcoma, osteosarcoma, and bone metastases from breast or prostate cancer [74]. A growing number of studies have shown that the Wnt/β-catenin signaling pathway is closely related to osteosarcoma development [75]. wnt1, wnt4, wnt5a, wnt7a, and wnt14 were expressed in osteosarcoma cell lines. wnt/β-catenin signaling can upregulate oncogenes (e.g., c-Myc, CCND1, c-MET), leading to osteosarcoma development [76]. Osteosarcoma contains tumor stem cells, and the Wnt/β-catenin signaling pathway also has an important role in osteosarcoma cancer stem cells [77]. In bone tissue, the Wnt/β-catenin signaling pathway can stimulate the differentiation of mesenchymal stem cells into osteoblasts, promote osteoblast proliferation and inhibit their important apoptosis pathway for new bone formation [78]. In osteosarcoma tissues, miRNA-144-3p, miRNA-342-5p, miRNA152, miRNA381, and miRNA506 can regulate the Wnt/β-catenin signaling pathway and thus affect apoptosis in osteosarcoma cells by targeting EZH2, Wnt7b, DKK1, LRH-1, and AEG-1, respectively [30, 79–82]. The massive accumulation of β-catenin in cells is a hallmark of the classical Wnt signaling pathway's activation, and thus it is also a core molecule of the classical Wnt signaling pathway. It has been shown that knockdown of β-catenin increases methotrexate-induced mortality in Saos-2 cells and that inhibition of β-catenin protein enhances the apoptotic effect of methotrexate on Saos-2 cells [83].

4. Discussion

Osteosarcoma is a malignant bone tumor that is very common in adolescents or children, and its treatment is currently based on surgery and neoadjuvant chemotherapy. Although the current treatment protocols for osteosarcoma can improve patients' survival rate, most patients still suffer from local recurrence or pulmonary metastasis after treatment, which seriously affects the prognostic outcome of the patients involved. In recent years, with the continuous research on the molecular mechanism of osteosarcoma, studies have shown that miRNAs play a crucial role in the biological processes of proliferation, metastasis, invasion, and drug resistance of osteosarcoma. Amongst them, the regulation of miRNAs on apoptosis has become a research focus in recent years.

With the continuous improvement in the study of apoptosis mechanism and the elucidation of the relationship between apoptosis and osteosarcoma occurrence, it has been found that promoting apoptosis of tumor cells is gradually becoming a new strategy for osteosarcoma treatment. Through in-depth research on the regulation of apoptosis by apoptosis-related proteins and apoptosis signaling pathway, inducing apoptosis in osteosarcoma cells become a new popular osteosarcoma treatment option. In radiotherapy, which triggers DNA damage in osteosarcoma cells by radiation, p53 gene

expression initiates apoptosis and removes osteosarcoma cells, a process that is also mediated by the death receptor ligand Fas/FasL pathway of the apoptosis pathway and involves the Bcl-2 gene family. Many antitumor drugs (e.g., DNA damaging agents, antimetabolites, topoisomerase inhibitors, etc.) can kill tumor cells by inducing apoptosis, and their mechanisms of action include (1) damaging the DNA of osteosarcoma cells and initiating the p53 gene; (2) regulating the Bcl-2/Bax ratio or inhibiting the activity of Bcl-2; and (3) regulating mitochondrial membrane permeability and promoting the release of cytochrome C. In contrast, heat therapy involves p53, Bcl-2, and Bax in addition to heat shock proteins and calcium ions. Apoptosis-related gene therapy involves introducing apoptosis-related genes into osteosarcoma cells to enhance the sensitivity of osteosarcoma cells to apoptosis or their resistance to apoptosis induction by radiotherapy. In addition, therapeutic measures targeting apoptosis-related receptor ligands, caspases family, and NF-κB are also available. Although comprehensive international therapeutic measures for osteosarcoma have improved survival rates, patients often die of complications, such as pulmonary metastases, shortly after surgery.

MiRNAs can regulate their target genes by degrading miRNAs or inducing miRNAs translation silencing. Abnormal miRNAs expression can be detected in almost all human malignancies. Since miRNAs have been found to regulate the expression levels of more than 90% of protein-coding genes, indirectly influencing the biological behavior of tumors by interfering with miRNAs expression, which is an extremely promising therapeutic approach. Mature miRNAs can play an essential role in the pathogenesis of osteosarcoma as tumor suppressors or promoters, as miRNAs expression is significantly associated with cell proliferation, adhesion, invasion, migration, metastasis, and apoptosis. MiRNAs are stable in circulation because they are resistant to degradation of endogenous circulating RNA enzymes thus quantitatively detected in plasma, serum and whole blood. It seems that circulating miRNAs reflect the pathological changes of miRNAs spectrum in tissues. Circulating miRNAs have great promise as diagnostic, prognostic or predictive biomarkers in the clinical treatment of patients with osteosarcoma. Besides, compared with other bone malignant tumors insensitive to radiotherapy and chemotherapy, radiotherapy and chemotherapy play a vital role in the treatment of osteosarcoma. Many miRNAs regulate the sensitivity of osteosarcoma to chemotherapy and radiotherapy, affecting the therapeutic effect of osteosarcoma. Thus, reducing the expression of pro-oncogenic miRNAs, such as miRNAs silencing, antisense blocking, and miRNAs modifications, may be regarded as a potential therapeutic option. However, there also exist many limitations for treating osteosarcoma, for instance, the sequence error of miRNAs sequence library, inferior RNA extraction methods, the variability of detection and analysis, the diversity of biological information data analysis, and the non-standard clinical testing of miRNAs. In this paper, we review that the miRNAs family plays a role in inhibiting or promoting osteosarcoma cell death by regulating apoptosis, suggesting that miRNAs regulation of programmed cell death in osteosarcoma may be an emerging therapeutic option for osteosarcoma treatment.

In recent years, exosomes have become the focus of extensive attention of scholars. Almost all cells in the human body can secrete exosomes, and exosomes can carry miRNAs and exist stably in peripheral blood and body fluids. Since abnormal expression of the corresponding miRNAs can be detected in the early development stage of osteosarcoma, detecting the content of tumor cell-derived exosomes carrying

miRNAs associated with osteosarcoma progression in early peripheral blood is expected to provide early diagnosis and disease progression monitoring for osteosarcoma. Previous studies have shown that the level of miRNA-25-3p in blood and secrete can be used as a prognostic indicator for patients with osteosarcoma. In addition, mesenchymal stem cell-derived exosomes (MSC-EXO) have attracted extensive attention due to their low immunogenicity, easy access, and storage and are considered to have good potential as targeted gene therapy vectors. Using MSC-EXO as vectors to transfect corresponding miRNAs analogs or inhibitors can regulate the apoptosis of osteosarcoma cells more precisely and stably.

In summary, given the key role of miRNAs in the regulation of apoptosis in osteosarcoma cells, future therapeutic measures targeting apoptosis-related miRNAs genes are expected to play a crucial role in treating osteosarcoma. In addition, the better utilization of exosomes and miRNAs will facilitate the early diagnosis of osteosarcoma and targeted therapy as drug carriers.

Declarations

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript and approved of the final version. Xueyang Cai: Formal analysis; writing-original draft. Wei Yin: Formal analysis; writing-original draft. Chao Tang: Writing-original draft. Yubao Lu: Writing-review and editing. Yuqi He: Supervision and revising the manuscript.

Declaration of competing interest:

None.

Ethical approval:

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

Funds:

This study was funded by China Scholarship Council (Grant Number: 202008520051).

Conflict of Interest:

All author declare that they have no conflict of interest.

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Figures

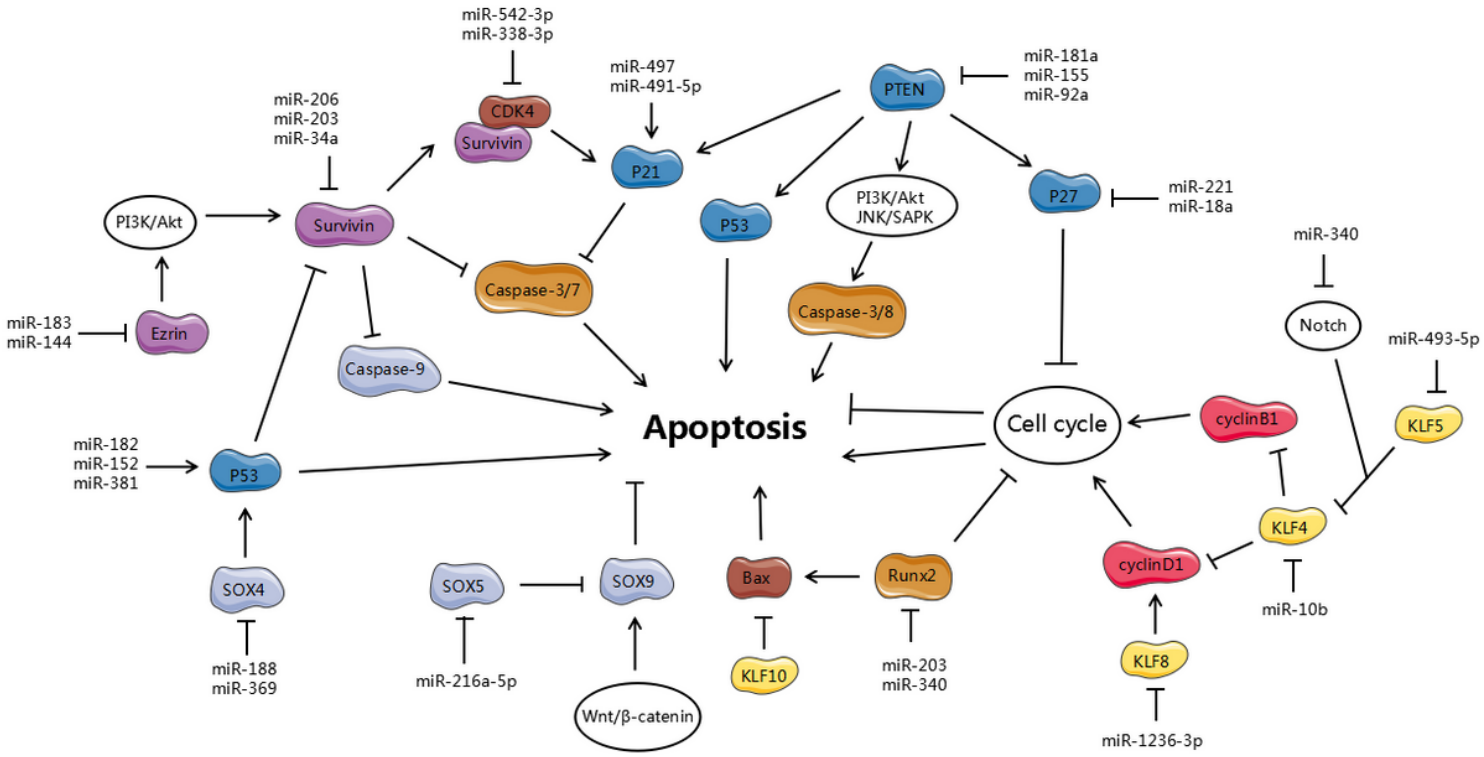

Figure 1

Apoptosis-related proteins and miRNAs. Many proteins affect cell apoptosis by regulating apoptosis-related signal pathways, cell cycle and mutual regulation networks between proteins. The KLF family (yellow), such as KLF4, acting on cell cycle-related proteins (pink) to arrest the cell cycle and promote cell apoptosis. KLF5 can inhibit this effect of KLF4. In addition, KLF8 and 10 can also regulate cell apoptosis. Survivin, Ezrin, PTEN and SOX family（blue）other apoptosis-related proteins can act on the caspase cascade signaling pathway and finally regulate cell apoptosis. Different miRNAs can indirectly regulate cell apoptosis by targeting these proteins, or directly act on signaling pathways to play a regulatory role.

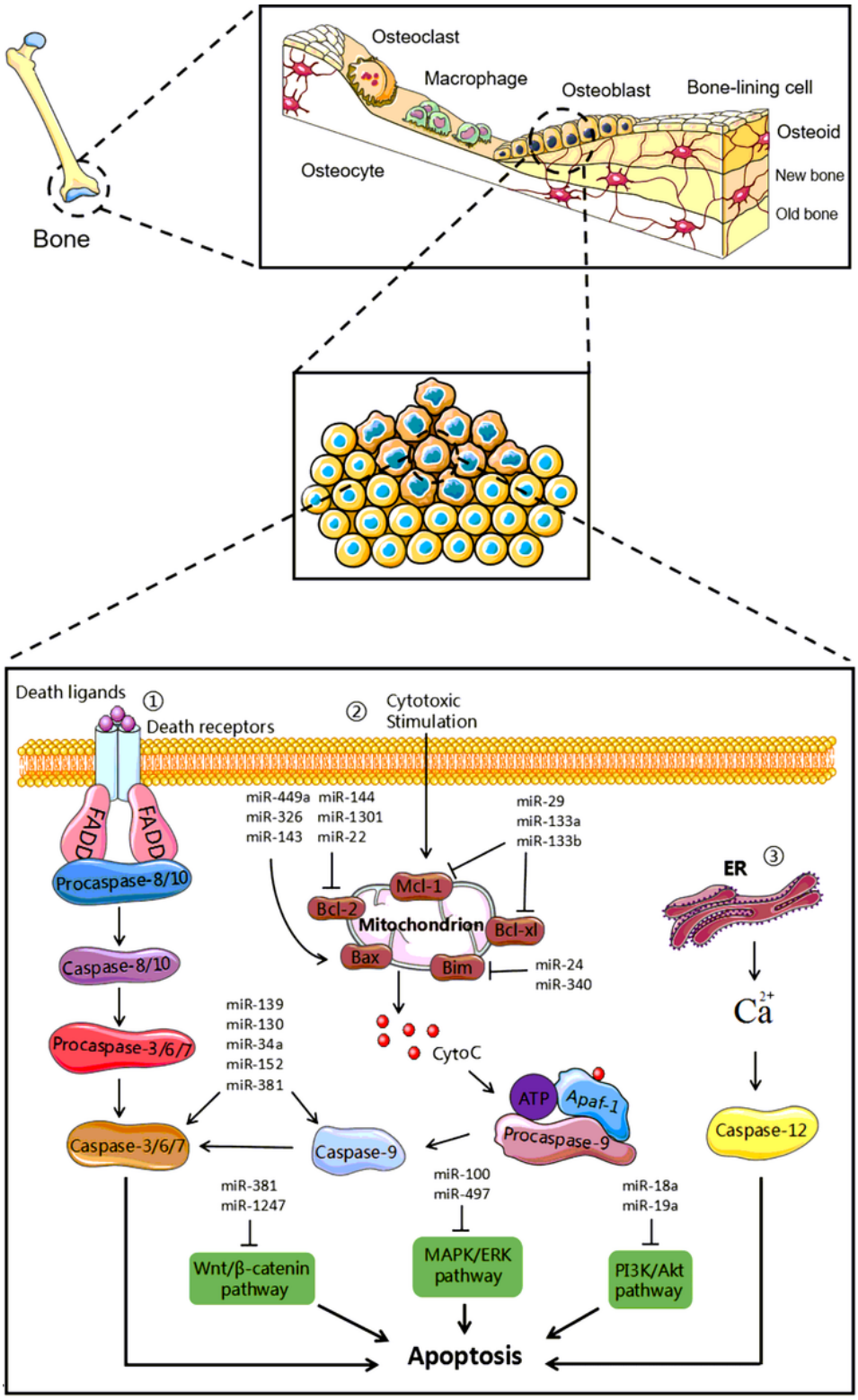

Figure 2

Apoptotic signaling pathways and miRNAs. Death receptor pathway: The extracellular death signals (death ligands) bind to the death receptors and induce the cytoplasmic protein to bind to the death domain of the death receptors. Fas-related death domain protein (FADD) acts as a connexin to activate Caspase-8 and further activate Caspase-3/6/7, thereby starting the Caspase pathway to induce apoptosis. MiR-139, 130, 34a, 152 and 381 can activate Caspase-3/6/7/9 to induce apoptosis.

Mitochondrial pathway: Intracellular death signals activate Bcl-2 family members (such as Bcl-2, Mcl-1, Bcl-xl, Bim, etc.) which can interact with Bax subfamily members, causing changes in mitochondrial membrane permeability and the release of cytochrome C (Cytc). Under the action of ATP/cATP, Cytc forms a multimeric complex with the apoptotic protease activator (Apaf-1), recruits the precursor of Caspase-9 (Procaspase-9) in the cytoplasm and initiates the Caspase cascade reaction, finally activates the Caspase-3/6/7 that located in the downstream of caspase pathway then induces apoptosis. MiR-144/1301/22, miR-29/133a/133b and miR-24/340 can inhibit Bcl-2, Mcl-1, Bcl-xl and Bim, respectively, inhibit cell apoptosis, while miR-449a/143/326 can promote the expression of Bax. Endoplasmic reticulum pathway: When the calcium ion balance in the endoplasmic reticulum is disturbed or the endoplasmic reticulum protein is excessively accumulated, the expression of Caspase-12 can be activated and cell apoptosis can be induced. Apoptosis-related signaling pathways: Other apoptosis-related signaling pathways such as MAPK/ERK, PI3K/Akt, Wnt/β-catenin and other signaling pathways can induce apoptosis through different pathways. MicroRNAs such as miR-381/1247, miR-100/497 and miR-18a/19a can inhibit the three signal pathways respectively to inhibit apoptosis.