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PIWI/piRNA-mediated regulation of signaling pathways in cell apoptosis

Y. TAN, J.-N. QIN, H.-Q. WAN, S.-M. ZHAO, Q. ZENG, C. ZHANG, S.-L. QU

Pathophysiology Department, Institute of Cardiovascular Disease, Key Laboratory for Arteriosclerology of Hunan Province, Hunan International Scientific and Technological Cooperation Base of Arteriosclerotic disease, University of South China, Hengyang, China

Abstract. – OBJECTIVE: This study aims to summarize the role of PIWIs/piRNAs in cell apoptosis through multiple signaling pathways. The PIWI-interacting RNAs (piRNAs) are among the small non-coding RNAs (sncRNAs) and are mainly expressed in germline cells. PIWI protein is the key to the biogenesis of piRNA. With the deepening of research in recent years, the PIWIs/piRNAs are expressed in a tissue-specific way in somatic cells outside the germline. In addition, researchers have found that the PIWIs/piRNAs play a regulatory role in cell apoptosis, proliferation, and necrosis by regulating key signaling pathways, such as PI3K/Akt signaling pathway, STAT signaling pathway, TGF-β signaling pathway, and Fas signaling pathway at the transcriptional or post-transcriptional level. However, the PIWIs/piRNAs' role in cell apoptosis and its underlying mechanisms are still not fully understood. This study reviews the regulatory functions of PIWIs/piRNAs in apoptosis from the perspective of the signal pathway.

MATERIALS AND METHODS: This study is a narrative review. PubMed and MEDLINE were used as the primary sources to search the following keywords: PIWI/piRNAs, signal pathway, pro-apoptotic, anti-apoptotic, and signaling pathway.

RESULTS: PIWIs/piRNAs modulated pro-apoptotic or anti-apoptotic effects in a variety of cells: PIWIs/piRNAs through PI3K/Akt signaling pathway, STAT signaling pathway, TGF-β signaling pathway, and Fas signaling pathway for pro-apoptotic or anti-apoptotic effects in cells.

CONCLUSIONS: Apoptosis is a basic biological phenomenon of cell death, and it also has a great significance and complex molecular biological mechanisms. PIWI/piRNAs are closely related to various types of diseases and play a pro-apoptotic or anti-apoptotic role through the following pathways: PI3K/Akt signaling, STAT signaling, TGF-β signaling, and Fas signaling pathways.

Key Words:
PIWI/piRNAs, Signal pathway, Apoptosis.

Introduction

About 2% of the human genome can encode proteins and the rest are non-coding RNAs (ncRNAs) as shown in previous related studies. Various types of non-coding RNAs include microRNAs (miRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), small nucleolar RNAs (snoRNAs), short interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs)[1-3]. PiRNAs are a new member of this extended family. In addition, piRNAs are a class of RNAs approximately 26-30 nt long similar to miRNAs that can specifically bind to the PIWI proteins family. Its main function is silencing the transposable genes, which were discovered in the gonadal cells of Drosophila in 2006[4,5]. The PIWI sub-family is a classic branch of the Argonaute family, which involved four members: PIWIL1, PIWIL2, PIWIL3, and PIWIL4, based on the sequence comparison in humans, there are named HILI, HIWI1, HIWI2, and HIWIL3, respectively[6,7]. In one search[8], about 23000 piRNA genes were found in the human genome and a mass of genes suggest that piRNAs may take part in gene regulation. In recent years, researchers have not only discovered PIWIs/piRNAs in germ line cells but also found that it is expressed in a tissue-specific way in somatic cells. PIWIs/piRNAs directly or indirectly participate in key signaling pathways of cell activities at the transcriptional or post-transcriptional level, including apoptosis, proliferation, necrosis, etc[7,9].

Apoptosis is an active process of programmed cell death (PCD). Cell apoptosis plays an important role in the process of biological evolution because it stabilizes the biological internal environment and the diversity of organ systems. Apoptosis is involved in the activation, expression, and regu-

Corresponding Author: Shunlin Qu, MD; e-mail: qushunlin78@163.com

lation of a sequence of genes that include endogenous mitochondrial pathway, endogenous endoplasmic reticulum pathway, and exogenous death receptor pathway[10]. Apoptosis can be divided into four stages: external stimulation induces apoptosis signal transduction, activation of apoptosis genes, subsequently entry of the executive stage of apoptosis, and finally, elimination of apoptotic cells[11,12]. In this review, the signal transduction of apoptosis was mainly introduced here.

The concept of "signal pathway" was first proposed in 1972, it was then called "signal transducers"[13]. The signaling pathway is a series of enzymatic reaction pathways that can transfer extracellular molecular signals (called ligand) through the cell membrane to influence the cell, including hormones, growth factors, cytokines, neurotransmitters, and other small molecular compounds[14-17]. Apoptosis is among the many biological changes that occur when ligands specifically bind to cell membranes or intracellular receptors, and step by step amplify and transmit extracellular signals. Apoptosis is a spontaneous and programmed cell death strictly controlled by multiple genes. PIWIs/piRNAs are also involved in the key signaling pathways mentioned above, such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway[18], signal transducer, and activator of transcription (STAT) signaling pathway[19], transforming growth factor-beta (TGF-β) signaling[20], and Fas (also known as CD95) signaling pathway[21] of apoptosis. However, its specific mechanism remains to be unknown. This paper reviews the research progress of PIWIs/piRNAs regulating apoptosis from the perspective of the signaling pathway.

PIWIs/piRNAs Modulated Cell Apoptosis

Apoptosis is the most basic biological phenomenon in life and a fundamental process to maintain the homeostasis of cell numbers in the body. Abnormality in the apoptosis process may be directly or indirectly related to the occurrence of various diseases, such as cardiovascular disease[22], autoimmune disease[23], or cancer[24]. Apoptosis is involved in the precise regulation of various types of genes and activates multiple signaling pathways. In recent years, a growing number of researchers have shown that PIWIs/piRNAs are taken part in the process of apoptosis.

Pro-Apoptotic Effect

Arun et al[25] demonstrated that transient expression of HIWI (a human homolog of the Drosophila gene PIWI) in the human leukemia cell line KG1 results in decreased cell proliferation caused by apoptosis in cell populations containing the HIWI gene. The endoplasmic reticulum (ER) is an important organelle in cells and ER dysfunction leads to ER stress. The long-term ER stress activates the unfolded protein response (UPR)[26] and induces the apoptosis of ER-related cells. Related research findings[27] show that PIWIL2 and PIWIL4 proteins are involved in apoptosis during UPR in human airway epithelial cells. Meanwhile, piRNAs and PIWIs also facilitated cell apoptosis. A report confirmed that *piRNA DQ594040* (piRA-BC) induces bladder cancer cell apoptosis by complementing the 3'-untranslated region (3'UTR) of CD134L, which is a binding partner of CD134 from the tumor necrosis factors[28]. Another report[29] has shown that *piR-1245* inhibits induction and activation of the p53 pathway in colorectal cancer, thereby leading to cell apoptosis, necrosis, and inhibition of cell migration and invasion. Other stcholars[30] also demonstrated that downregulation of *piRNA-004800* in multiple myeloma (MM) cells induces cell apoptosis and autophagy both *in vivo* and *in vitro*. Jia et al[31] regulated the levels of dissociative *piRNA-36026* by supplementing the endogenous *piRNA-36026* with regulatory sequences (RS) on nanoprobes and subsequently inducing cell apoptosis. A study[32] demonstrated that upregulation of *piRNA NU13* inhibited cell proliferation, migration, invasion, and apoptosis of human Wilm's tumor cells. Conversely, downregulating the *piRNA NU13* reduces apoptosis. Jacobs et al[33] found that *piRNA-8041* inhibits cell survival pathways and induces cell apoptosis in mice and glioblastoma multiforme. In addition, it has been found that overexpression of *piR-30188* promotes apoptosis in glioma cells[34]. In recent years, environmental pollution causes diversity among cyanobacteria species, hence, increasing the microcystin (MC) in animals, thereby posing a threat to human health. Zhang et al[35] found that microcystin-leucine-arginine (MC-LR) in the testicular cells of the reproductive system might downregulate thymoma viral proto-oncogene 3 (Akt3) by increasing the expressions of *piR-102923*, *piR-109481,* and *piR-139907*, consequently decreasing the proliferation and increase of apoptosis of testicular cells. Similarly, another study[18] has found that MC-LR decreased the expression of *piR -DQ7222010* and PIWI proteins,

thus promoting the activation of the PI3K/Akt signaling pathway and reducing prostate cell apoptosis in offspring of mice exposed to MC-LR. Recently, a report[36] showed that low expression of *piR-mmu-32362259* in male mouse testis affected the expression of key proteins in the middle and downstream molecules in the PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway, thereby affecting the cell cycle and reducing the apoptosis rate.

Anti-Apoptotic

A growing number of research have confirmed that HIWI, PIWIL1, PIWIL2, and PIWIL4 are expressed in various cell lines and protect cells in the human body from undergoing apoptosis, such as breast cancer, lung cancer, rhabdomyosarcoma, and medulloblastoma[20,37-40]. PiRNAs also exhibit resistance in the apoptotic response. CDKN2B is one of the inhibitors of cyclin-dependent kinase 4 (CDK4) family members. Wu et al[41] reported that *HSA_piR_011186*, several CDKN2B-related piRNA mediate DNA and histone H3 methylation in the CDKN2B promoter region, which affects CDKN2B gene expression, thereby reducing apoptosis. Some reports have shown that inhibition of *piR-651* in lung cancer can change the expression of apoptosis-related proteins CyclinD1 (Ccnd1) and CDK4. This significantly increases the apoptosis rate of cancer cells, thereby potentially regulating the tumor genesis behavior[42,43]. In the study of chemical resistance to cisplatin drugs, Wang et al[44] found that *piR-L-138* can inhibit the apoptosis of lung squamous cell carcinoma. They also found that *piR-L-138* may be a potential strategy to overcome chemotherapy resistance among cancer patients. Similarly, in oral squamous cell carcinoma, Li et al[45] found that *piR-1037* inhibits the apoptosis of oral squamous cell carcinoma after cisplatin chemotherapy and it may be also involved in the metastasis of oral squamous cell carcinoma. This provides a new idea for diseases to be treated with piRNA. In fact, piRNA is associated with the development, invasion, metastasis of the disease, and prognosis. It has been found that apoptosis of the colorectal cancer cells (CRC) can be inhibited by overexpressing the *piR-54265* and promoting the proliferation of CRC cells by inhibiting the apoptosis, which is related to the drug resistance and poor prognosis among colorectal cancer patients. Serum *piR-54265* detection provides more extensive application prospects for colorectal cancer screening, early detection, and clinical monitoring of CRC[19,46]. Das et al[47] found that the upregulated *piR-39980* induced cell proliferation, migration, invasion, and inhibition of cell apoptosis in human osteosarcoma (OS) cells. Roy et al[48] also found that *piR-39980* inhibited drug-induced apoptosis. Das et al[49] interestingly found the opposite effect in human fibrosarcoma by suppressing the transient overexpression of the ribonucleotide reductase subunit M2(RRM2), *piR-39980*, which is similar to piRNA, however, it induces apoptosis. Meng et al[50] downregulating the *mmu_piR_027558* or inhibiting the *mmu_piR_027558* in testis induces testicular cell apoptosis, however, the testicular function was not affected.

It has been shown that *piRNA-823* regulates apoptosis in a diversity of cells. In CRC tissues, inhibiting the *piRNA-823* decreases the proliferation of colorectal cancer cells, arrest the cell cycle in the G1 phase, and induces cell apoptosis. Interestingly, the expressions of heat shock proteins 27, 60, and 70 were inhibited by inhibiting the *piRNA-823*. The increased expression of heat shock protein could partially eliminate the effect of *piRNA-823* on cell apoptosis[51]. In addition, Feng et al[52] conducted a mechanism study and showed that *piRNA-823* may inhibit the ubiquitination of hypoxia-inducible factor-1α (HIF-1α) by upregulating the expression of functional genes related to the glucose metabolism pathway, and consequently upregulate the glucose consumption of cancer cells, thereby affecting the proliferation, invasion, and apoptosis of colorectal cancer cells. On the one hand, Yan et al[53] found that in MM cells, silencing the *piRNA-823 in vivo* disrupted the expression of apoptosis-related proteins and promoted cell apoptosis. On the other hand, silencing the *piRNA-823 in vitro* reduced the secretion of vascular endothelial growth factor (VEGF) and decreased the pro-angiogenic activity, thereby promoting apoptosis. Similarly, Li et al[54] found that the *piRNA-823* may promote MM cell proliferation by promoting angiogenesis and inhibiting apoptosis, thus, leading to the occurrence and development of MM.

In conclusion, the pathogenic mechanisms of piRNAs in different diseases are complicated and further studies are necessary to understand their value. The apoptotic effects of piRNAs on different kinds of cells in different diseases are shown in Table I.

PIWIs/piRNA-Mediated Signaling Pathways in Apoptosis

Phosphorylation or dephosphorylation in the signaling pathways is the main process for the

Table I. Summary of the regulation of PIWl-interacting RNAs (piRNAs) in apoptosis.

	piRNAs	Disease/Tissue	Publication Year	Reference
Pro-apoptotic	HIWI	CD34+hematopoietic progenitor	2001	25
	PIWIL2 PIWIL4	airway epithelial	2018	27
	piRNADQ594040	bladder cancer	2014	28
	piR-1245	colorectal cancer	2018	29
	piRNA-004800	multiple myeloma	2020	30
	piRNA-36026	breast cancer, hepatocarcinoma cervical carcinoma	2019	31
	piRNA NU13	Wilms tumor	2021	32
	piRNA-8041	Glioblastoma multiforme	2018	33
	piR-30188	glioma	2018	34
	piR-102923 piR -109481 piR -139907	testis	2017	35
	piR -DQ7222010	prostate hyperplasia	2019	18
	piR-mmu-32362259	testis	2021	36
	piR-39980	fibrosarcoma	2018	49
Anti-apoptotic	HIWI	breast cancer tissues	2016	37
	PIWIL1	testes	2017	38
	PIWIL2	testis and variety of tumors	2005	39
	PIWIL4	breast cancer	2016	40
	HSA_piR_011186	leukemia	2015	41
	piR-651	lung cancer	2018 2016	42 43
	piR-L-138	lung squamous cell carcinoma	2017	44
	piR-1037	oral squamous cell carcinoma	2019	45
	piR-54265	colorectal cancer	2020	46
	piR-39980	osteosarcoma	2020	47
		neuroblastoma	2020	48
	mmu_piR_027558	testis	2019	50
	piRNA-823	colorectal cancer	2017	51
		colorectal cancer	2020	52
		multiple myeloma	2014	53
		multiple myeloma	2019	54

upstream protein to regulate the downstream protein. Therefore, protein kinases and phosphatase are the main components of the signal pathway, such as PI3K, mTOR, STAT, TGF-β, and so on. These protein kinases also constitute various key factors in signal pathways, such as PI3K, Akt, STAT, TGF-β, and Fas signaling pathways. Emerging evidence suggests that PIWIs/piRNAs are also involved in the regulation of apoptotic signaling pathways.

PI3K/AKT Signaling Pathway

Phosphatidylinositol kinase (PIKs) is a lipid kinase that phosphorylates inositol in Phosphatidylinositol (PI). According to their phosphorylation sites, PIKs are divided into PI3Ks, PIP4Ks, and PIP5Ks. Among them, PI3Ks are vital enzymes of inositol and phosphatidylinositol[55]. Activated PI3K activates protein kinase B extracellular in almost all cells and tissues. Protein kinase B (Akt) is a serine/threonine kinase, mainly found in the cytoplasm, including three homo types of Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ)[56]. The PI3K/Akt pathway widely exists in cells, and it is an important signal transduction pathway that regulates apoptosis[57]. Previous studies[58] have focused on the regulation of miRNA on this pathway. With the development of piRNA research, various piRNAs have been found to regulate apoptosis *via* PI3K/Akt pathway.

MC-LR is a kind of cyclic heptapeptides with strong reproductive toxicity, which is produced by cyanobacteria[58]. Zhang et al[35] found that maternal exposure to MC-LR during pregnancy and lactation can upregulate *piR-102923*, *piR-109481,* and *piR-139907* expressions, while downregulating the protein phosphoinositide-3-kinase regulatory subunit 3 (Pik3r3), AKT3, and Ccnd1, which

are the most important components of PI3K-Akt pathway, respectively. In addition, CDK4, cyclin-dependent kinase 6 (CDK6) and murine double minute2 (Mdm2) were downregulated while upregulating P53 and Bax, thereby resulting in apoptosis. Interestingly, Han et al[18] found that, after maternal mice were exposed to MC-LR, which reduced the expressions of PIWI4 and PIWI2 in progeny mice prostate cells, thereby downregulating the *piRNA-DQ722010* and promoting the expression of Pik3r3, thus, stimulating PI3K/Akt signaling pathway and inhibiting progeny prostate cell apoptosis. mTOR is a typical serine/threonine-protein kinase and a member of the PI3K, it is also an important downstream regulator of PI3K/Akt[59]. PI3K/Akt/mTOR signaling pathway is involved in a variety of life activities *in vivo*, and under the regulation of the piRNA, it can also participate in the process of cell apoptosis. Ma et al[30] studied the MM and found that cell division cycle protein 42 (CDC42) could be downregulated by inhibiting the sphingosine-1-phosphate (S1P)/sphingosine-1-phosphate receptor (S1PR)/G protein signaling pathway, which further downregulated the expression of *piR-004800*, thereby inducing apoptosis of MM cells by regulating the PI3K/Akt/mTOR signaling pathway. Kong et al[36] studied the toxic effects of nickel nanoparticles on testicular cells of male mice and they found that the *piR-mmu-32362259* downregulates the PI3K/Akt/mTOR signaling pathway, and then, subsequently affects its key proteins and downstream molecules, such as CDK4, P21, Bcl-2, Bax and caspase-3, and it also jointly promote cell apoptosis by shortening the S phase of the cell cycle.

STAT Signaling Pathway

STAT signal pathway is one of the most important signal pathways related to apoptosis, proliferation, and differentiation of cells[60]. STAT plays a key role in the transcription of factors and cytokines of cell membrane receptors[61], which can receive signals to the nucleus to influence the transcription and expression of corresponding genes. STAT includes STAT 1–7 with different functions[62], among which is the STAT3, it is widely used in cancer research and is under the regulation of PIWIs/piRNAs involved in cell apoptosis[63]. Sousa-Victor et al[64] found that increasing the PIWI protein expression prevents apoptosis in somatic stem cells under regenerative pressure in the JAK/STAT signaling pathway. STAT3 activation is well-known required tyrosine kinases activity. One study[65] demonstrated that PIWIL2 plays a role in anti-apoptosis in P53-involved tumor cells through the STAT3 signaling pathway, but not in the Akt signaling pathway which has tyrosine kinase activity, but in form of a PIWIL2/STAT3/c-Src triple protein-protein complex with Src, a family of non-receptor tyrosine kinases. This indicates that PIWIL2 has tyrosine kinase activity. Related studies have shown that the *piR-54265* is involved in the development of colorectal cancer, and it can specifically bind with PIWIL2 to form a PIWIL2/STAT3/phosphorylated SRC (p-SRC) complex, which activates the STAT3 signal pathway. Overexpressing the *piR-54265* inhibits colorectal cancer cell apoptosis and is associated with drug resistance and poor prognosis among colorectal cancer patients[19]. This provides us a with new clinical approach to the PIWIs/piRNA as a therapeutic target. Another study has found that stem-cell protein PIWIL2 inhibits apoptosis by activating the STAT3/Bcl-XL pathway, meanwhile, stimulating proliferation by activating the STAT3/cyclin D1 signaling pathway[39]. In addition, Wang et al[40] found in the research on glioma that PIWI4 can bind to miRNA and directly play a role in cell apoptosis. They showed that high expression of miR-384 reduced the phosphorylation of STAT3 by downregulating the PIWIL4, which can provide a good therapeutic target in treating human glioma.

Another Signaling Pathway

TGF-β family was first discovered about four decades ago[66,67]. One of its biological hallmarks and wide range of functions, including cell-cycle control, extracellular matrix formation, angiogenesis, the regulation of early development, differentiation, induction of cell apoptosis, and so on[68]. There are two membrane-bound receptor serine/threonine kinases in the TGF-β signaling pathway, these are the TGF-β type I receptor (TGFβRI) and TGF-β type II receptor (TGFβRII), which form a complex relationship with the TGF-β ligand and initiate TGF-β signaling cascade. Various studies have shown that the TGF-β signaling pathway plays an important role in cell apoptosis. Wang et al[20] indicated that PIWIL4 is highly expressed in human breast cancer and reducing its expression significantly increases apoptosis through the TGF-β signaling pathway. Cao et al[37] suggested that overexpressing the HIWI reduces the levels of TGFβRI, TGFβRII, and anti-apoptosis by regulating the cell cycle by TGF-β signaling. Smad proteins are significant transducers in TGF-β signaling, meanwhile, the Smad2/3 phosphorylation

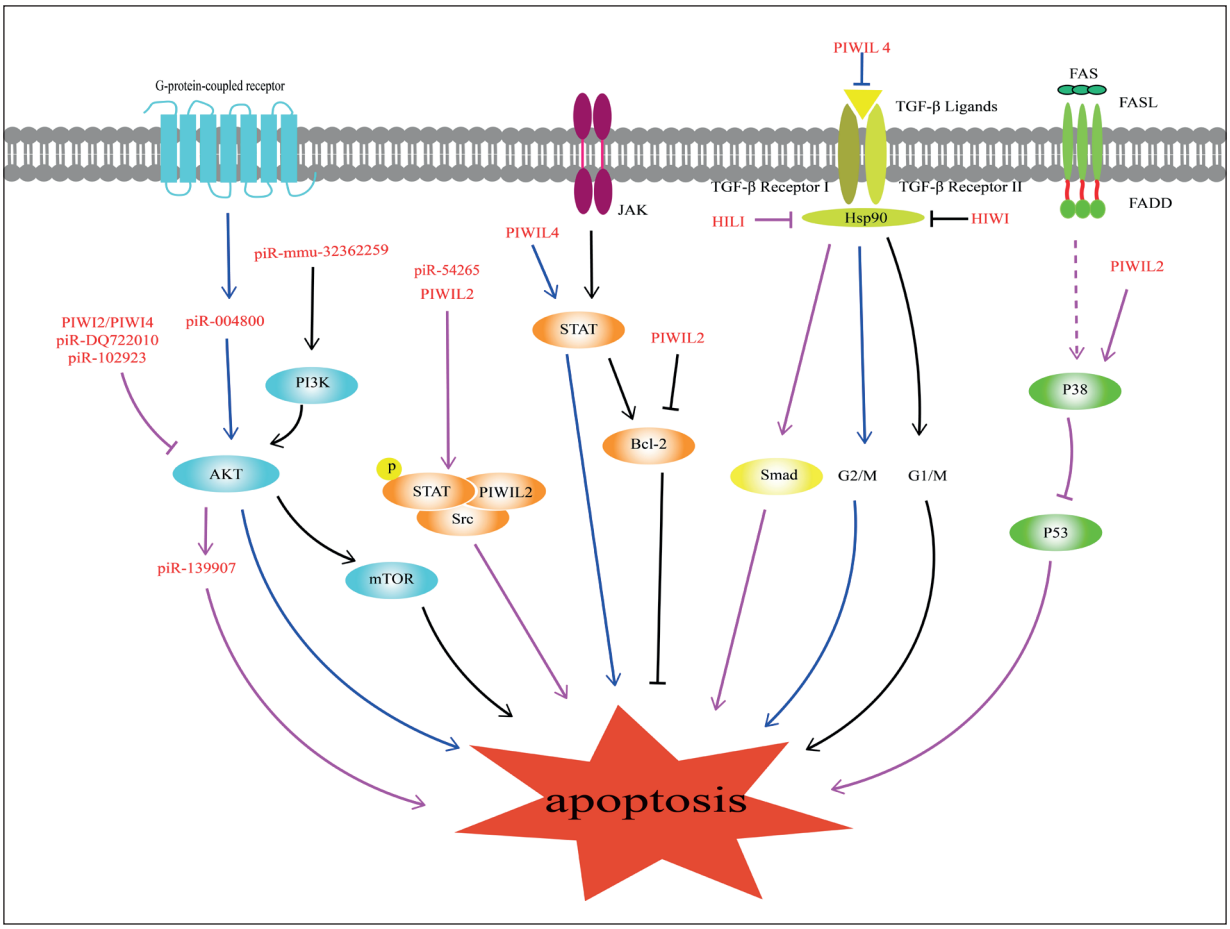

Figure 1. PIWIs/piRNAs promote or inhibit apoptosis by regulating signaling pathways, such as the PI3K/Akt, STAT, TGF-β, and Fas, and downstream molecules.

is induced by TGF-β to regulate a series of downstream events[69]. Zhang et al[70] uncovered that HILI (PIWIL2) inhibits cell apoptosis at the level of Smad phosphorylation by abrogating the TGF-β signaling.

Fas, a type I transmembrane glycoprotein, is a member of the tumor necrosis factor (TNF) superfamily. The Fas signaling pathway is greatly necessary for cell apoptosis, which is dominated by Fas receptor FasL (Fas Ligand). In addition, Fas activates the downstream proteins by binding with trimerized FasL[71]. K8 protects cells against Fas-mediated apoptosis and damage. Jiang et al[21] demonstrated that the increased susceptibility of PIWIL2 knockdown cells to Fas-mediated apoptosis is associated with K8 downregulation.

Accordingly, when the body is stimulated and interfered with by various internal and external pathogenic factors, PIWIs/piRNAs promote or inhibit apoptosis by regulating signaling pathways, such as the PI3K/Akt, STAT, TGF-β, and Fas, and downstream molecules (Figure 1). Consequently, the promotion or inhibition of cell apoptosis causes an imbalance of cell or tissue homeostasis, thereby leading to the occurrence of a variety of diseases.

Conclusions

The research on PIWIs/piRNAs has been gradually deepened with the increasing application of high-throughput sequencing and bioinformatics. As a new member of the non-coding RNA family, studies have found that it is highly expressed not only in the germ line but also in somatic cells, with tens of thousands of piRNAs, suggesting that it may be involved in regulating a variety of biological processes.

Apoptosis is a basic biological phenomenon, which plays an essential role in multicellular organisms in removing superfluous or already completed

missions. Apoptosis is not only a special type of cell death but also of great biological significance with complex molecular biological mechanisms. In recent years, increasing evidence has shown that PIWIs/piRNAs are closely related to a variety of diseases, and play a role in pro-apoptotic and anti-apoptotic effects on cells. This review summarizes the pro-apoptotic or anti-apoptotic effects of PIWIs/piRNAs through PI3K/Akt, STAT, TGF-β, and Fas signaling pathways. In addition, studies have found in some diseases that PIWIs/piRNAs are closely related to drug resistance and disease prognosis. This also provides a new idea for the clinical treatment and prognosis of PIWIs/piRNAs. However, the study of PIWIs/piRNAs is not fully understood and there are still various problems waiting to be further explored. For example, there are also mitogen-activated protein kinase (MAPK) signaling pathways and the Wnt-beta-catenin signaling pathway that are involved in the process of cell apoptosis. Do PIWIs/piRNAs play a role in these pathways? Some PIWIs/piRNAs can play a pro-apoptotic or anti-apoptotic role in the cell cycle by regulating DNA methylation. What is their specific mechanism?

Conflict of Interests

Authors declare no conflict of interests.

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Authors' Contributions

Yao Tan: contributed to the conception of the study and wrote the manuscript; Jianning Qin, Hengquan Wan, Simin Zhao and Qian Zeng contributed to critical revision (writing review and editing); Chi Zhang: made the final approval of the version to be submitted; Shunlin Qu: made the final approval of the version to be submitted; Oversight and leadership responsibility for the research activity planning and execution.

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