# Chapter 5
# Noncoding RNAs in Growth and Death of Cancer Cells

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**Abstract** The mammalian genomes are mostly comprised of noncoding genes. And mammalian genomes are characterized by pervasive expression of different types of noncoding RNAs (ncRNAs). In sharp contrast to previous collections, these ncRNAs show strong purifying selection evolutionary conservation. Previous studies indicated that only a small fraction of the mammalian genome codes for messenger RNAs destined to be translated into peptides or proteins, and it is generally assumed that a large portion of transcribed sequences—including pseudogenes and several classes of ncRNAs—do not give rise to peptides or proteins. However, ribosome profiling suggests that ribosomes occupy many regions of the transcriptome thought to be noncoding. Moreover, these observations highlight a potentially large and complex set of biologically regulated translational events from transcripts formerly thought to lack coding potential. Furthermore, accumulating evidence from previous studies has suggested that the novel translation products exhibit temporal regulation similar to that of proteins known to be involved in many biological activity processes. In this review, we focus on the coding potential of noncoding genes and ncRNAs. We also sketched the possible mechanisms for their coding activities. Overall, our review provides new insights into the word of central dogma and is an expansive resource of functional annotations for biomedical research. At last, the outcome of the majority of the translation events and their potential biological purpose remain an intriguing topic for future investigation.

**Keywords** Cancer cells • Noncoding RNA • Proliferation • Cell cycle • Necrosis • Apoptosis • Autophagy

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## 5.1 Introduction

A striking finding about the production of numerous noncoding RNAs (ncRNAs) from mammalian genomes in the past decade has completely shifted our view of gene expression programs, which have historically been based on the assumption that only protein-coding genes could specify cellular functions. Increasing evidence suggests that while it is entirely possible that many of those ncRNAs are transcription noises or by-products of RNA processing, a large fraction of them provide diverse regulatory functions in the cell, and regulatory RNA networks in general represent a crucial interphase between genomics and proteomics [1, 2], impacting both on physiology and disease. The era of ncRNA research has resulted in and benefited from the rapid advance in genomics technologies and informatics approaches [1] that have been developed in recent years and thus has experienced tremendous progress in elucidating the function and mechanism of various ncRNAs.

Classic ncRNA transcripts have been categorized into two groups: housekeeping ncRNAs and regulatory ncRNAs (see Table 5.1). Housekeeping ncRNAs include small ncRNAs such as transfer RNAs (tRNAs) for carrying amino acids, small nucleolus RNAs (snoRNAs) for RNA modifications, and small nuclear RNAs (snRNAs) for RNA splicing, and large ones, such as ribosomal RNAs (rRNAs) for protein synthesis [1]. Regulatory ncRNAs can be divided into two classes based on an arbitrary size cutoff of 200 nt to separate small ncRNAs from long noncoding RNAs (lncRNAs), including the well-annotated microRNAs (miRNAs), which are 18–25 nt noncoding regulatory RNAs that mediate posttranscriptional regulation of gene expression by inhibiting the translation or promoting the degradation of target

**Table 5.1** The categorization of ncRNAs

| | Abbreviation | Full name | Role |
|---|---|---|---|
| Housekeeping ncRNAs | rRNA | Ribosomal RNA | mRNA reading and decoding |
| | tRNA | Transfer RNA | Amino acid carriers |
| | snRNA | Small nuclear RNA | RNA splicing |
| | snoRNA | Small nucleolar RNA | RNA modification |
| | TR | Telomere RNA | Chromosome end synthesis |
| Regulatory ncRNAs | miRNA | Micro RNA | RNA stability and translation control |
| | siRNA | Endogenous RNA | RNA silencing |
| | piRNA | Piwi RNA | Gene silencing |
| | eRNA | Enhancer-derived RNA | Regulation of gene expression |
| | lncRNA | Long noncoding RNA | Imprinting, epigenetics, nuclear structure |
| | circRNA | Circular RNA | Regulation of miRNA |
| | xRNA | X-inactive RNA | RNA silencing |
| | gRNA | Guide RNA | RNA editing |

mRNAs [3], and lncRNAs, which account for approximately 81.8 % of all ncRNAs [4] and have a role in physiologic aspects of cell-type determination and tissue homeostasis [5]. However, miRNA and lncRNA are not alone—there are many other families of ncRNAs that are necessary for regulatory roles in the cell, such as enhancer RNA (eRNA, 50–2000 nt) for transcribing enhancers, guide RNA(gRNA, 60–80 nt) for editing site selection in RNA editing, small interfering RNAs (siRNAs, 19–23 nt) which interfere with the expression of specific genes with complementary nucleotide sequences, and piwi-interacting RNAs (piRNAs, 26–30 nt), which are involved in germline development, silencing of selfish DNA elements, and maintaining germline DNA integrity. piRNAs form complexes with piwi proteins, members of the Argonaute family, and unlike other small RNAs, they are created without RNase Dicer participation [6]. They represent a large group of ncRNAs with many diverse functions, many of which are only just beginning to be understood. Indeed, the number of ncRNAs per genome correlates far better with organism complexity than the number of coding genes, suggesting that RNA-based regulatory mechanisms are critical in the evolution of developmental complexity [2] and tumorigenesis.

The development of human cancer is mediated by both genetic and epigenetic alterations of the cell [7, 8], and ncRNAs play a crucial role in maintaining genomic stability which is essential for cell survival and preventing tumorigenesis [9]. ncRNAs and coding genes cooperatively mediate pathway dysregulation during the development and progression of cancers [10]. It has become increasingly clear that misexpression of ncRNAs is recognized as a hallmark feature in cancers. Studies have shown that tumor tissue and normal tissue exhibit distinct ncRNA expression profiles, and the aberrant ncRNAs can play an important role in cancer development [9], metastasis, prognosis, and patient survival rates [11]. There is mounting evidence of miRNAs acing as either potent oncogenes or tumor suppressor genes, linking to cancer initiation and progression [12]. lncRNAs involved in different biological processes occur in a space- and time-dependent manner [13], and many of them are dysregulated in a wide range of cancers [14]. siRNA-based therapies are known for offering great potential for cancer treatments. RNA interference (RNAi) is a well-conserved, naturally occurring process of posttranscriptional gene silencing initiated by double-strand siRNA. A RNAi-based approach can effectively silence any disease-causing gene in a given cell type or tissue/organ of interest [15]. piRNA pathway is shown active in various cancers. For example, the expression of PIWIL1 and PIWIL2 has been found in a wide range of human cancers such as stomach, breast, gastrointestinal tract, and endometrium [16–18] and recently also in ovarian carcinoma [19]. Among effects of estrogen signaling on the transcriptome of breast cancer cells, induced eRNA contributes to the dynamic generation/stabilization of enhancer: promoter looping between the regulated coding transcription units and these ERα-bound enhancers [20]. In most advanced cancers, telomerase is reactivated and serves to maintain telomere length, and emerging data have also documented the capacity of telomerase to directly regulate cancer-promoting pathways [21]. A circRNA, ciRS-7, is closely coupled to miR-7, and fine-tuning of the miR-7/miR-671/ciRS-7 axis will likely play profound roles in diseases such as

**Table 5.2** NcRNAs involved in cancer cell biological process

| Cancer cell biological process | ncRNAs | Notes |
|---|---|---|
| Apoptosis | lncRNA, miRNA, piRNA, snRNA, snoRNA | 1–5 |
| Cell cycle | lncRNA, miRNA, siRNA, piRNA | 6–9 |
| Proliferation | lncRNA, miRNA, piRNA, rRNA, tRNA, snoRNA, circRNA | 10–16 |
| Autophagy | lncRNA, miRNA, rRNA | 17–19 |
| Necrosis | lncRNA, miRNA | 20–21 |

Notes
1. Transforming growth factor-β-induced lncRNA-Smad7 inhibits apoptosis of mouse breast cancer
2. TEL-AML1 regulation of surviving and apoptosis via miRNA-494 and miRNA-320a
3. An lncRNA (GAS5)/SnoRNA-derived piRNA induces activation of TRAIL gene by site-specifically recruiting MLL/COMPASS-like complexes
4. Hyperstable U1snRNA complementary to the K-ras transcripts induces cell death in pancreatic cancer cells
5. GAS5, a noncoding protein, controls apoptosis and is downregulated in breast cancer
Cell cycle
6. Long noncoding RNA UCA1 regulates cell cycle distribution via CREB through PI3-K
7. Inverse correlation of miRNA and cell cycle-associated genes suggests influence of miRNA on benign thyroid nodule tumorigenesis
8. Effects of HMGB1 expression suppressed by siRNA on cell cycle and proliferation of human cervical cancer cell line HeLa
9. Overexpression of Hiwi inhibits the cell growth of chronic myeloid leukemia K562 cells and enhances their chemosensitivity to daunomycin
10. lncRNA-LALR1 accelerates hepatocyte proliferation during liver regeneration by activating Wnt/beta-catenin signaling
11. miRNA-26b inhibits proliferation by targeting PTGS2 in breast cancer
12. piRNA, the new noncoding RNA, is aberrantly expressed in human cancer cells
13. Changes in rRNA transcription influence proliferation and cell fate within a stem cell lineage
14. tRNA-derived microRNA modulates proliferation and the DNA damage response and is downregulated in B-cell lymphoma
15. snoRNA U50 levels are regulated by cell proliferation and rRNA transcription
16. Correlation of circular RNA abundance with proliferation—exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues
17. APF lncRNA regulates autophagy and myocardial infarction by targeting miR-188-3p
18. Oncophagy—harnessing miRNA regulation of autophagy in cancer therapy
19. Noncoding rRNA-mediated preferential killing in cancer cells is enhanced by suppression of autophagy in non-transformed counterpart
20. miR-15b/16 protects primary human retinal microvascular endothelial cells against hyperglycemia-induced increases in tumor necrosis factor alpha and suppressor of cytokine signaling
21. Altered expression of long noncoding RNAs during genotoxic stress-induced cell death in human glioma cells

cancer [22]. However, there are still many gaps in our current understanding of the functional roles for the vast majority of these unique ncRNAs.

In this chapter, we pay attention to the relationship between the two mostly studied ncRNAs and related cancer diseases: miRNA and lncRNA (see Table 5.2).

The biogenesis, targeting, and function of these classes of ncRNAs have been extensively studied and reviewed. These ncRNAs are largely involved in biological processes of cancer cells including proliferation, cell cycle, apoptosis, necroptosis, and autophagy. Recent progress suggests that the involvement of ncRNAs in cancers' cellular regulation can be far more prevalent than previously appreciated. Deciphering the role of ncRNAs in cancer benefits not only understanding the molecular basis of this disease but also developing novel clinical tools and treatments. The field of applications for these ncRNAs in cancer harbors great promise.

In this chapter, we will attempt to organize some of the rapidly expanding information, with a focus on roles of ncRNAs involved in the growth and death biological processes of cancer cells, including proliferation, cell cycle, apoptosis, necrosis, and autophagy. Furthermore, we highlight on potential clinical applications of ncRNAs as predictive biomarkers and therapeutic targets for anticancer treatments.

## 5.2 miRNA in Growth and Death of Cancer Cells

miRNAs comprise a class of endogenously expressed small ncRNAs which are approximately 22 nucleotides in length. The canonical miRNA biogenesis pathway consists of at least four steps: transcription, nuclear and cytoplasmic processing, loading into RNA-induced silencing complex (RISC), and decay. Transcription of miRNAs usually involves RNA polymerase II (Pol II), meaning that miRNA genes share the transcriptional machinery of protein-coding genes, including transcription factors, enhancers, and epigenetic regulation [2]. They exert their function via base pairing with complementary sequences within mRNA molecules. Upon sequence-specific binding of miRNAs, mRNA molecules are destabilized through shortening of their poly(A)tails, or degraded by cleavage of the mRNA strand, or less efficiently translated into proteins by ribosomes [23]. Recent evidences suggest that miRNAs can also recognize specific target sites in gene promoters, modulating gene expression likely by recruiting similar Ago proteins and chromatin-remodeling enzymes in the vicinity of promoters [24]. miRNA regulates diverse biological processes, and bioinformatic data indicates that each miRNA can control hundreds of gene targets, underscoring the potential influence of miRNAs on almost every genetic pathway [25]. In fact, miRNA-mediated regulation is one of the most widespread posttranscriptional regulatory mechanisms in eukaryotes and is estimated to affect the majority of human transcripts [26].

miRNAs have been implicated in cancer diseases by regulating the cellular levels of specific oncogenes or tumor suppressor genes. About half of the annotated human miRNAs map within fragile regions of chromosomes, which are areas of the genome that are associated with various human cancers [25]. Gene therapies that use miRNAs are proven to be an effective approach to blocking tumor progression. Accumulating evidence highlights the crucial role of miRNA-mediated regulation

in virtually all cellular processes that modulate malignant transformation of cells including cell growth, differentiation, proliferation, and apoptosis, to name a few. In normal tissues, proper miRNA transcription, processing and binding to complementary sequences on the target mRNA, results in the repression of target gene expression through a block in protein translation or altered mRNA stability, leading to the overall result as normal rates of cellular growth, proliferation, differentiation, and cell death [25]. Alterations in the miRNA balance in the cell can lead to dysregulation of tumor suppressor genes and/or oncogenes controlled by aberrantly expressed miRNAs [26], resulting in the overall outcome involving increased proliferation, promoted cell cycle or cell cycle arrest, decreased levels of apoptosis and necroptosis, or delayed autophagy, ultimately leading to tumor formation [25].

Hereafter, we will summarize the functions and mechanisms of miRNAs in regulating the proliferation, cell cycle, apoptosis, necroptosis, and autophagy of cancer cells.

### *5.2.1 Proliferation*

Proliferation is an important part of cancer development and progression as the cancer cell embodies characteristics that permit survival beyond its normal life span and to proliferate abnormally [27]. The relevance of miRNAs expression correlating with tumor proliferation has been analyzed in detail in the last decade.

miRNAs are thought to function as both tumor suppressors and oncogenes through regulating their target genes and related signal pathway in tumor proliferation. For example, the expression level of miR-31 is significantly higher in cervical cancer patients than in normal individuals (P\0.05). The target genes such as SATB2, TIAM1, and RASA1 have been found to be involved in the biological functions of miR-31 [28]. The miR-34 family is directly transactivated by tumor suppressor p53, which is frequently mutated in various cancers. The MTT assay reveals significant cell proliferation inhibition in miR-34a transfectant compared with the control from HO8910 and SKOV3 cells, which displays lowest expressions of miR-34a [29]. miR-203 is downregulated in renal cancer cell lines ($P < 0.05$). Mechanistic investigations confirm FGF2 as a direct target of miR-203, and upregulation of miR-203 can decrease expression of FGF2 [30]. In lung cancer cell line A549 cell line, overexpression of microRNA-126 inhibits the proliferation rate. VEGF is the target gene of microRNA-126, with the latter exerting its function via regulating the former's protein level [31].

Tumor hypoxia is one of the features of tumor microenvironment that contributes to chemoresistance. Early steps in tumor development are associated with a fibrogenic response and the development of a hypoxic environment which favors the survival and proliferation of cancer stem cells [27]. Moreover, hypoxia also regulates the expression of a series of miRNAs. IGF2BP1 is identified as a potential target of miR-196b. miR-196b overexpression decreases IGF2BP1 RNA expression

and protein level. The IGF2BP1downregulation by either miR-196b or IGF2BP1 siRNA leads to a decrease in cell viability and proliferation in normal culture conditions [32]. Former studies indicate that c-Myc-mediated repression of miR-15-16 in hypoxia is induced by increased HIF-2α and promotes tumor angiogenesis and metastasis by upregulating FGF2.

In addition, mounting evidence has shown that miRNAs take important roles in self-renewal of cancer cells. One such example is linc-RoR, which has already been shown to control self-renewal and maintain pluripotency of human embryonic stem cells by acting as a miR-145 "sponge" and thus controlling OCT4, NANOG, and SOX2 expression. Similarly, linc-RoR is found to regulate expression of the same transcription factors in endometrial cancer stem cells, and inhibit their differentiation, in a miR-145-dependent manner [26]. In the mouse, members of the let-7 family are shown to be expressed at low levels in self-renewal progenitors (ALDH+/Sca-1+) and induce upon differentiation, suggesting that low levels of let-7 mark the self-renewal compartment and can be used to prospectively isolate normal mammary stem cells (MaSCs). Accordingly, the let-7 family emerges as the most induced group of miRNAs upon estradiol treatment in human luminal cells. Independent studies further suggest that miRNAs of this family are implicated in the self-renewal of cancer stem cells (CSCs) in breast and other cancers, by multiple mechanisms. One of these mechanisms involves the pluripotency gene known as LIN28/LIN28B, which inhibits the function of let-7 by interfering with its biogenesis. In breast cancer, LIN28 expression confers CSC traits and impinges on signaling mechanisms involved in self-renewal of normal and CSCs, such as the Wnt/beta-catenin pathway, NF-κB signaling, and inflammatory cytokine signaling [2].

### *5.2.2 Cell Cycle*

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. Mitotic cell cycle is accepted as a constantly reproducible sequence of events, which includes four phases: G1 phase, S phase, G2 phase, and M phase. The expression and activation of cyclin-dependent kinases (CDKs) and cyclin play a pivotal role in proceeding through the G1 into S phase (DNA replication) and from the G2 to M phase (mitosis) [33]. These proteins regulate the cell's progression through the stages of the cell cycle and are in turn regulated by numerous proteins, including p53, p21, p16, and cdc25. Downstream targets of cyclin-CDK complexes include pRb and E2F. The cell cycle often is dysregulated in neoplasia due to alterations either in oncogenes that indirectly affect the cell cycle or in tumor suppressor genes or oncogenes that directly impact cell cycle regulation, such as pRb, p53, p16, cyclin D1, or mdm-2. The cell cycle has become an intense subject of research in recent years. The complexity of the regulation of the cell cycle is reflected in the different alterations leading to aberrant cell proliferation and

development of cancer. A large number of researchers have supposed that miRNA can regulate cell cycle proteins and induce cell cycle arrest [34].

Tumor-associated cell cycle defects are often mediated by alterations in the CDK activity. Misregulated CDKs induce unscheduled proliferation as well as genomic and chromosomal instability [35]. miRNAs can control cell cycle progression after DNA damage by targeting CHK1, p53, retinoblastoma1 serine phosphates from human chromosome3 (RBSP3), cyclin D, CDC25a, p21, CDK2, WEE1, LK1, and so on [9]. miR-223 affects the G1/S transition of cell cycle, so that the expression of CDK2, CDK4, CDK6, CCND1, CCND2, and CCND3 is significantly downregulated in miR-223 inhibitor. miR-31 is a direct regulator of endogenous expression CDK2 in liver cancer cells [36]. In leukemic cells, CDK2 is found to be a target gene of miR-638 that CDK2 inhibition phenotypically mimics the overexpression of miR-638 [37]. miR-449a is able to regulate the expression of the CDK6 protein, and a lower expression level of miR-449 and a higher expression level of CDK6 may contribute to the occurrence and development of gastric cancer [38].

Cell cycle arrest, also referred to as delay, is produced by a variety of factors that may be intrinsic or extrinsic and may affect several checkpoints [34]. miR-449a inhibits neuroblastoma cell survival and growth through two mechanisms, inducing cell differentiation and cell cycle arrest. Its function in inducing cell cycle arrest involves downregulating its direct targets CDK6 and LEF1 [39]. miR-34a is importantly competent in p53 tumor suppressor network, where p53-dependent activation of miR-34a is widely validated and its upregulation induces cell cycle arrest. miR-34a has many potential target genes, with several of these regular cell cycles, including NMYC, CCND1, CCNE2, CDK4, CDK6, and MET, having been experimentally validated, which lead to a significant reduction in the number of cells in the S phase of the cell cycle and an increase in the percentage of cells in theG0/G1 phase [33]. miR-302/367 cluster can dually regulate cell cycle in gene dose-dependent manner that knockdown of the endogenous miR-302/367 cluster causes cell cycle arrest 7. miR-27a is part of the miR-23a/27a/24-2 cluster. The impact of miR-27a silencing or overexpression on the cell cycle of U251 and U87MG cells is examined in vitro. Treatment with miR-27a-mimics oligonucleotides suppresses U251 cell proliferation, promotes apoptosis by inducing G2/M phase arrest, and impairs the invasive potential of U251 cells in vitro [40].

### *5.2.3 Apoptosis*

Apoptosis is characterized by specific morphological and biochemical changes of dying cells, including cell shrinkage, nuclear condensation and fragmentation, dynamic membrane blebbing, and loss of adhesion to neighbors or to extracellular matrix. Biochemical changes include chromosomal DNA cleavage into internucleosomal fragments, phosphatidylserine externalization, and a number of intracellular substrate cleavages by specific proteolysis [41]. The field of apoptosis research has been moving forward at an alarmingly rapid rate as many of the key

apoptotic proteins have been identified. Inappropriate apoptosis is a hallmark in many cancer diseases as the most striking feature of the cancer cells is that they do not undergo apoptosis. This fact is due to the involvement of dynamic interplay between oncogenes and/or inactivated tumor suppressor genes. Both of them have key roles in generation of a tumor [42]. To date, research indicates that there are two main apoptotic pathways in cancer: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. Besides, studies show that the two pathways are linked and that molecules in one pathway can influence the other. Multiple lines of evidence indicate that miRNAs can modulate apoptotic pathways.

The intrinsic (or mitochondrial) apoptotic pathway is triggered by a variety of intracellular stimuli, including DNA damage, cytotoxic drug treatment, growth factor deprivation, and/or oxidative stress. This pathway relies on the formation of a complex termed the apoptosome, which is composed of procaspase-9, apoptotic protease-activating factor 1 (Apaf-1), and cytochrome c. A series of Bcl-2 family members including Bax, Bak, Bcl-2, Bcl-xL, Mcl-1, Bid, and Bim control the release of cytochrome c by regulating mitochondrial membrane permeabilization [43]. It has been reported that miR15-a and miR-16-1 target Bcl-2 to induce apoptosis, while Bcl-2 inhibits mitochondrial-mediated apoptosis, by influencing oligomerization of Bax and Bak at the posttranscriptional level. Additionally, overexpression of miR-15a-miR-16-1 in vitro can decrease Bcl-2 operation and then promote apoptosis [41]. miR-181c can target the 3′ untranslated region of Bcl-2, and the increased level of Bcl-2 caused by the decrease in miR-181c protects mitochondrial morphology from the tumor necrosis factor alpha-induced apoptosis [44]. miR-608 is reported to target Bcl-xL to regulate chordoma malignancy [45]. The tumor-suppressive activity of miR-133a is likely due to the targeted suppression of Bcl-xL and Mcl-1 expression [46]. Besides, the nuclear transcription factor p53 can govern main apoptotic signals that mitochondria receive in the intrinsic pathway of apoptosis. miR-34a is a tie molecule between the p53 and SIRT1 and is composed of a p53/miR-34a/SIRT1 signal feedback loop, which can enhance apoptosis signal and significantly promote cell apoptosis [47].

The extrinsic pathway of apoptosis is initiated by the binding of death ligands such as Fas ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), TNF-α, and TNF-like weak inducer of apoptosis (TWEAK) to death receptors in the TNF receptor (TNFR) superfamily [43]. TRAIL is an attractive therapeutic target in cancer because it directly induces tumor cell apoptosis. miR-221 and miR-222 induce TRAIL resistance by directly targeting 30-UTRs of phosphatase and tensin homolog, tissue inhibitor of metalloproteinase-3 (TIMP3), and the cyclin-dependent kinase inhibitor p27kip1 [12]. miR-145, miR-216, miR-182, and miR-96 can interact with DR4/5 and Fas-associated death domain protein. Overexpression of these miRNAs reduce caspase-3 activation through TRAIL-induced apoptosis signaling [12]. miR-20a downregulates Fas expression in osteosarcoma cells, thus enhancing the metastatic capacity of osteosarcoma cells by promoting cell survival in the FasL-positive lung microenvironment [48].

There are also other miRNA-mediated apoptosis pathways. For example, miR-125b-mitochondria-caspase-3 pathway plays a role in doxorubicin resistance in

human breast cancer [49]. And previous researches have shown that miRNA-221 and miRNA-222 induce apoptosis via the KIT/AKT signaling pathway in gastrointestinal stromal tumors [50].

### 5.2.4 Necrosis

Necrosis has always been considered to be almost "accidental" cell death, a random, uncontrolled process. However, emerging evidence has shown that necrosis can be induced and regulated in a similar manner to apoptosis. Regulated necrosis is termed "programmed necrosis" or "necroptosis" to distinguish this process from necrosis induced by physical trauma [43]. Necroptosis can be induced by the activation of the TNF receptor superfamily, T-cell receptors, interferon receptors, toll-like receptors (TLRs), cellular metabolic and genotoxic stresses, or various anticancer agents [51]. Receptor-interacting protein kinases (RIPK), poly(ADPribose) polymerase-1(PARP1), NADPH oxidases, and calpains have also been identified as signaling components of necroptosis pathways in cancer [41]. Ensuing local inflammation induced by necroptosis may promote tumor growth. However, at present, study on how miRNAs regulate necroptotic cell death is still in its infancy.

Tumor necrosis factor-α (TNF-α) triggers necroptotic cell death through an intracellular signaling complex containing RIPK1 and RIPK3 called the necrosome. RIPK1 phosphorylates RIPK3, which phosphorylates the pseudokinase mixed lineage kinase domain-like (MLKL), driving its oligomerization and membrane-disrupting necroptotic activity [52]. miR-155 has been reported to prevent necroptosis in human cardiomyocyte progenitor cells by directly targeting RIP1 [43], and overexpressing miR-155 in cardiomyocyte progenitor cells (CMPCs) reveals that miR-155 attenuates necrotic cell death by $40 \pm 2.3\%$ via targeting receptor-interacting protein 1 (RIP1) [53].

Caspase-8 is a cysteine protease that is critically involved in regulating cellular apoptosis and has recently been shown to be important in regulating necrotic cell death. The RIP3 is a key signaling molecule in the necroptosis pathway. Caspase-8 has been demonstrated to be able to cleave and inactivate RIP3 and prevent the pathway of necroptosis. Caspase-8 is a target of miR-874 in the necrotic pathway. Foxo3a regulates caspase-8 expression and the consequent myocardial necrosis through targeting miR-874 [54].

To date, no report has demonstrated how miRNAs regulate other key necroptotic factors, including RIP3, MLKL, and PGAM5 [43].

### 5.2.5 Autophagy

Autophagy is a catabolic process that allows cellular macromolecules to be broken down and recycled into metabolic precursors. It is a highly conserved, critical process, allowing cells to gain survival advantages under various stress situations due

to growth and environmental changes. The process involves enwrapping fractions of the cytoplasm until a double-membrane autophagic vacuole (autophagosome) is formed. The pathways and genes, such as multiple autophagy-related genes (ATGs), involved in autophagy that have been identified in detail in recent years include ATG1, ATG4, LC3/ATG8, and beclin-1. Autophagy serves a dichotomous role in cancer, and recent advances have helped delineate the appropriate settings where inhibiting or promoting autophagy may confer therapeutic efficacy in patients [55]. Silencing Dicer1, an essential processor of miRs, increases levels of ATG protein and formation of autophagosomes in cells, indicating that miRs regulate autophagy [56]. Accumulating evidence indicates that miRNAs contribute significantly to autophagy in cancer mainly through the three following pathways.

miRNAs regulates the expression of key autophagy-related proteins. miR-30a is a potent inhibitor of autophagy by downregulating Beclin-1. miR-30a in human renal cell carcinoma (RCC) interferes with the effectiveness of sorafenib-mediated apoptosis by an autophagy-dependent pathway. Expression of miR-30a is significantly downregulated in several RCC tissues and in RCC cell lines. Accordingly, its targeted gene Beclin-1 is upregulated. Sorafenib activates autophagy in RCC cells (786-0 and A489 lines), evidenced by p62 degradation, Beclin-1/autophagy protein 5 (ATG-5) upregulation, and light chain (LC)3B-I/-II conversion. Exogenously expressing miR-30a in 786-0 or A489 cells inhibits Beclin-1 expression and enhances sorafenib-induced cytotoxicity [57]. The ability of miR106B and miR93 to regulate ATG transcripts in human cancer cell lines (HCT116, SW480, HeLa, and U2OS) is studied using luciferase report assays and bioinformatics analyses. Results show that miR106B and miR93 target ATG16L1 messenger RNA which they reduce levels of ATG16L1. In contrast, miR106B and miR93 antagonists increase formation of autophagosomes [56]. What's more, miR-375 has been reported to inhibit autophagy through its target, ATG7, in hepatocellular carcinoma (HCC) cells [58]. miR-376b expression targets ATG4C and beclin-1, which, in turn, downregulate autophagy induced by nutrition starvation and rapamycin in breast cancer cells [59]. Meanwhile, ectopic expression of miR-7 in human lung cancer and esophageal cancer cell lines enhances autophagy by suppressing epidermal growth factor receptor (EGFR) expression [60].

miRNAs mediate autophagy in genotoxic stress and hypoxia. Hypoxia plays an important role in the tumor microenvironment by allowing the development and maintenance of cancer cells. Studies have shown that hypoxia-inducible factor 1 (HIF-1) regulates a panel of microRNAs, whereas some of microRNAs target HIF-1. For example, miR-210 has emerged as the predominant miRNA regulated by hypoxia. Hypoxia-induced miR-210 can repress glycerol-3-phosphate dehydrogenase 1-like (GPD1L), which, in turn, stabilizes HIF-1α by reducing hyperhydroxylation [61]. miR-96 can promote or inhibit autophagy by principally inhibiting MTOR or ATG7 depending on the expression levels of miR-96. Inhibition of miR-96 abolishes hypoxia-induced autophagy [62]. Under hypoxia, HIF-1α induces miRNA-210 which in turn enhances autophagy and reduces radiosensitivity by downregulating Bcl-2 expression in colon cancer cells [63]. miR-137, which targets the expression of two mitophagy receptors NIX and FUNDC1, markedly inhibits mitochondrial degradation by autophagy without affecting global autophagy.

Impaired mitophagy in response to hypoxia caused by miR-137 is reversed by re-expression of FUNDC1 and NIX expression vectors lacking the miR-137 recognition sites at their 3′ UTR [64].

miRNA is involved in p53-mediated autophagy. A number of previous studies have reported the direct involvement of p53 in autophagy. The cytoplasmic pool of p53 suppresses autophagy under nutrient deprivation [60]. P53 also functions as a key regulator of autophagy, a catabolic pathway for degradation and recycling of proteins and cellular organelles, which has been shown to be dysregulated in cancers [65]. Previous studies suggest that some of the p53-mediated miRNAs may play important roles in autophagy. miR44 induces autophagy in lung cancer cells by targeting the p53-induced glycolysis and apoptosis regulator TIGAR [66]. In colon cancer, miR-502 inhibits autophagy by suppression of Rab1B, a critical mediator of autophagy. The expression of miR-502 is regulated by p53 via a negative feedback regulatory mechanism [65].

## 5.3 lncRNA in Growth and Death of Cancer Cells

Although studies of small regulatory RNAs, in particular miRNAs, have dominated the field of RNA biology during the past decade, a surprisingly broad spectrum of biological processes is also associated with lncRNAs. Over the past several years, accumulated data have begun to advance the idea that lncRNAs are not just transcriptional noise or cloning artifacts but important supplements to proteins and other effectors in complex regulatory networks. Thus, the focus of scientists is now shifting to one of the most poorly understood yet most common products of transcription from genomes: lncRNAs [13]. The most commonly used definition of lncRNA is an RNA molecule that is longer than 200 nucleotides and that is not translated into a protein. However, this definition may be too simple and does not take into account certain issues. For example, the same RNA can contain both protein-coding genes (PCGs) and noncoding functions. Furthermore, a PCG is usually defined as a transcript that contains an open reading frame (ORF) longer than 100 amino acids, while lncRNAs can contain ORFs longer than 100 amino acids and not necessarily synthesize polypeptides [67]. Although the nomenclature is still evolving, lncRNA typically refers to a polyadenylated long ncRNA that is transcribed by RNA polymerase II and associated with epigenetic signatures common to protein-coding genes, such as trimethylation of histone 3 lysine 4 (H3K4me3) at the transcriptional start site (TSS) and trimethylation of histone 3 lysine 36 (H3K36me3) throughout the gene body [68]. Besides, advances in the depth and quality of transcriptome sequencing have revealed many new classes of lncRNAs. The existing classifications of lncRNAs rest on their descriptive and distinctive properties including their size, their localization, and their function, which have been discussed in previous reviews [69].

lncRNA genes are interspersed in the genome in various possible locations in relation to protein-coding transcripts, such as overlapping, intergenic, or divergent

transcripts. Transcription of lncRNAs follows the same rules as for protein-coding genes and is executed by RNA Pol II. Functions of lncRNAs are executed by multiple modes of action and can occur both in the nucleus and in the cytosol. In contrast to miRNAs, which are highly conserved and mainly involved in negative regulation of gene expression at the posttranscriptional level, lncRNAs are poorly conserved and can regulate gene expression (either positively or negatively) at numerous levels by a variety of mechanisms, some of which are yet to be characterized [2]. It is now becoming evident that lncRNAs are important transcriptional outputs of the genome. Generally, lncRNAs have been implicated in gene-regulatory roles, such as chromosome dosage compensation, imprinting, epigenetic regulation, cell cycle control, nuclear and cytoplasmic trafficking, transcription, translation, splicing, cell differentiation, and others. Multiple lines of evidence demonstrate that a number of characterized lncRNAs are implicated in a spectrum of biological processes and that misregulated lncRNA expression can cause various human diseases and cancers [13]. Transcription of individual lncRNAs involved in different biological processes occurs in developmental stage-, tissue-, and organ-specific patterns. Thus, lncRNAs can function as molecular markers that signal the space, time, and expression of gene transcription, specifically reflecting the integrative biological outcome of transcription factors and signaling pathways controlling gene expression in space and time [10].

In this section, we will focus on summarizing the functions and mechanisms of lncRNAs that have been linked to cancer through regulating the proliferation, cell cycle, apoptosis, necroptosis, and autophagy of cancer cells.

### *5.3.1 Proliferation*

As lncRNAs are crucial regulators of gene expression, it is expected that their misregulation will lead to abnormal cellular function and growth defects and cause cancer diseases [14]. lncRNAs have been detected in cancer by various techniques including expression microarrays, tiling arrays, next-generation sequencing, and methylation analysis. These approaches have led to the identification of a wide range of lncRNAs whose expression is significantly associated with cancerous tissues [70]. The abundance of these transcripts in cancer suggests that lncRNA-mediated biology occupies a central place in cancer proliferation. lncRNAs are abundant during embryogenesis and reactivation, or non-suppression of some of these fetal lncRNAs may critically regulate pluripotency and uninhibited cellular growth, thus giving rise to adult or developmental cancers [14]. lncRNAs act through a variety of mechanisms in cancer cell proliferation such as remodeling of chromatin, transcriptional co-activation or co-repression, and protein inhibition, as posttranscriptional modifiers or decoy elements [71].

Accumulating evidence suggests that a major role of lncRNAs is to guide the site specificity of chromatin-modifying complexes to effect epigenetic changes in cancer cell proliferation. The well-characterized lncRNAs ANRIL, XIST, HOTAIR,

and KCNQ1OT1 are able to recruit epigenetic modifiers to specific loci to reprogram the chromatin state [71]. ANRIL is significantly upregulated in gastric cancer, and it can promote cell proliferation and inhibit cell apoptosis by silencing of miR99a and miR449a transcription [72]. XIST expression is upregulated in glioma tissues and human glioblastoma stem cells (GSCs). Knockdown of XIST exerts tumor-suppressive functions by reducing cell proliferation, migration, and invasion as well as inducing apoptosis. Mechanistic investigations have defined that XIST and miR-152 are in the same RNA-induced silencing complex (RISC) so that miR-152 mediates the tumor-suppressive effects that the knockdown of XIST exerts [73]. HOTAIR promotes the proliferation of serous ovarian cancer cells by at least partly regulating certain cell cycle- and apoptotic-related genes, including cyclin E, Bcl-2, caspase-9, caspase-3, and BRCA1 [74]. A novel short tandem repeat (STR) polymorphism within KCNQ1OT1 contributes to hepatocellular carcinoma (HCC) cell proliferation, possibly by affecting KCNQ1OT1 and CDKN1C expression through a structure-dependent mechanism, but further functional studies are needed to validate the hypothesis [75].

Other lncRNAs have been found to be key regulators of the protein signaling pathways underlying cancer cell proliferation. The ability of lincRNA-p21 to promote tumor growth is validated in mouse xenograft models. After loss of lincRNA p21, hnRNP-K is inappropriately localized at the promoters of p53-repressed genes, which results in deregulated expression and altered chromatin states of polycomb target genes, a defective G1/S checkpoint, increased proliferation rates, and enhanced reprogramming efficiency [9]. Estrogen plays a critical role in cancer development and progression. H19 is an estrogen-inducible lncRNA and mediates 17β-estradiol-induced cell proliferation in MCF-7 breast cancer cells through the mechanism in which 17β-estradiol produces a dose- and time-dependent induction of H19 expression in MCF-7 cells, which is mediated via ERα as evident by the blockade of this 17β-estradiol effect with ICI 182780, a specific ER antagonist and knockdown of ERα using specific RNAi [76]. Steroid receptor RNA activator (SRA) noncoding RNA and SRA protein together regulates estrogen receptor signaling pathways and plays a critical role in breast cancer cell proliferation [77].

Some lncRNAs are constituents of macromolecular with roles in RNA processing during cancer cell proliferation. MALAT1, a highly conserved long noncoding RNA, which is thought to act at a posttranscriptional level by controlling alternative splicing of pre-mRNAs [71], is deregulated in several types of cancers. Emerging evidences have shown that MALAT1 has been linked to an increase in cancer cell proliferation. MALAT1 contributes to proliferation and metastasis in esophageal squamous cell carcinoma [78], and silencing of MALAT1 by miR-101 and miR-217 inhibits proliferation of esophageal squamous cell carcinoma cells [79]. Other lncRNAs can also act as decoys, sequestering biomolecules and preventing them from fulfilling their cellular functions. An example of this mechanism is represented by the PTENP1 restricting cell proliferation by acting as a microRNA decoy for the tumor suppressor PTEN [71]. miR-21 regulates the potential anticancer effects of icariin on cell proliferation and apoptosis by targeting PTEN, RECK, and Bcl-2 in the ovarian cancer A2780 cells [80]. In HCC, the overexpressed PTENP1 represses

the oncogenic PI3K/AKT pathway and inhibits the HCC proliferation in vivo, which is accompanied by enhanced apoptosis, autophagy, and dampened angiogenesis/neovasculature maturation [81].

In addition, there is evidence that lncRNA is involved in the self-renewal maintenance of cancer stem cells (CSCs). Using transcriptome microarray analysis, lncTCF7 is identified as being required for liver CSC self-renewal and tumor propagation through Wnt signaling. Mechanistically, lncTCF7 recruits the SWI/SNF complex to the promoter of TCF7 to regulate its expression, leading to activation of Wnt signaling [82]. MALAT1 is reported to increase the proportion of pancreatic CSCs, maintain self-renewing capacity, decrease the chemosensitivity to anticancer drugs, and accelerate tumor angiogenesis in vitro. The potential mechanism may be that MALAT1 functions as endogenous sponge RNA to interact with miR-200c and miR-145, and upregulates the expression of their target gene Sox2, leading to enhanced stem cell-like phenotypes [83].

### *5.3.2 Cell Cycle*

The connection between the cell cycle and cancer is obvious: cell cycle machinery controls cell proliferation, and cancer is a disease of inappropriate cell proliferation. At least two types of cell cycle control mechanisms are recognized: a cascade of protein phosphorylations that relay a cell from one stage to the next and a set of checkpoints that monitor completion of critical events and delay progression to the next stage if necessary. The first type of control involves a highly regulated kinase family, and the progress through the cycle is accomplished in part by the regulated activity of numerous cyclin-CDK complexes [84]. The cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases controlling progression through the cell cycle. The regulatory subunits of the CDKs, known as cyclins, form complexes with their catalytic partner to function as checkpoint kinases of specific proteins that regulate progression through the cell cycle. The cyclin-CDK complexes govern a linear progression of events that lead cells from a resting state (G0), growth phase (G1), through DNA replication (S), and finally to cell division (M) [85]. The second type of cell cycle regulation is not an essential part of the cycle progression machinery. Cell cycle checkpoints sense flaws in critical events such as DNA replication and chromosome segregation. When checkpoints are activated, for example, by underreplicated or damaged DNA, signals are relayed to the cell cycle progression machinery [84]. Previous work has shown that many lncRNAs have been involved in cell cycle regulation of cancer cells, but many lncRNAs that have a functional role in cell cycle regulation remain to be identified because the functions of only a small percentage of the total lncRNA population are understood. To clarify the roles of lncRNAs in cell cycle regulation, it should be determined how they regulate the target cell cycle regulators and which signaling pathways induce these lncRNAs.

Mutations of checkpoint proteins are frequent in all types of cancer. The tumor suppressor protein p53 is a sequence-specific DNA-binding protein that is able to induce either cell cycle arrest or apoptosis at the cell cycle checkpoints [86]. Although p53 is unstable, it is stabilized and activated via phosphorylation mediated by the ATM/ATR pathway in response to DNA damage. Moreover, p53 is also regulated via phosphorylation at various sites by specific kinases [87]. A prominent p53-induced lncRNA termed lncRNA activator of enhancer domains (LED) is required for p53-induced cell cycle arrest and is involved in the activation of a subset of p53-bound and p53-unbound enhancers by inducing an epigenetic change. Chromatin-binding and eRNA expression analyses show that LED associates with and activates strong enhancers. One prominent target of LED is located at an enhancer region within CDKN1A gene, a potent p53-responsive cell cycle inhibitor. LED knockdown reduces CDKN1A enhancer induction and activity and cell cycle arrest following p53 activation [88]. MALAT1, an abundant nuclear-retained lncRNA, is overexpressed in several cancers, and its elevated expression has been associated with hyper-proliferation and metastasis. Recent studies show that MALAT1 modulates the expression of cell cycle genes and is required for G1/S and mitotic progression. Depletion of MALAT1 leads to activation of p53 and its target genes. The cell cycle defects observed in MALAT1-depleted cells are sensitive to p53 levels, indicating that p53 is a major downstream mediator of MALAT1 activity. Furthermore, MALAT1-depleted cells display reduced expression of B-MYB (Mybl2), an oncogenic transcription factor involved in G2/M progression, due to altered binding of splicing factors on B-MYB pre-mRNA and aberrant alternative splicing [89]. Moreover, there are more lncRNA-regulated cell cycle proteins. The transcription factor MYC is known to regulate lncRNAs and has been implicated in cancer cell proliferation and tumorigenesis. MYC-regulated lncRNAs, named MYCLos, function in cell proliferation and cell cycle by regulating MYC target genes such as CDKN1A (p21) and CDKN2B (p15) [90]. A recently identified lncRNA mapped to chromosome 8q24 is found to be generally upregulated in colon cancer tissues compared to their neighboring normal tissues. CARLo-5 knockdown inhibits GC cell growth through inducing G0/G1 arrest and apoptosis. That p27, p21, and p16 protein expression levels are increased with inhibition of CARLo-5 expression shows CARLo-5 has a role in regulating cell cycle genes, but the precise underlying mechanism still needs to be revealed [91].

lncRNAs also play a role in the cell cycle arrest of cancer development. lncRNAs are induced in a p53-dependent manner, suggesting that they are induced by DNA damage. Also, these reported lncRNAs may participate in cell cycle arrest [87]. There are other lncRNA-related cell cycle arrest pathways. BRAF-activated noncoding RNA (BANCR), an lncRNA, is crucial for cell migration in melanoma cells and non-small cell lung cancer (NSCLC) cells. Downregulation of BANCR contributes to the induction of G0/G1 cell cycle arrest of colorectal cancer cells, at least in part, through the regulation of p21 protein [92]. AK001796, the lncRNA with the most clearly altered expression, is overexpressed in lung cancer tissues and cell lines, but its expression is downregulated in resveratrol-treated lung cancer cells. AK001796 knockdown causes a cell cycle arrest, with significant increases in

the percentage of cells in G0/G1 in lung cancer cells. The cell cycle-associated genes up- or downregulated directly or indirectly by lncRNA AK001796 knockdown include (i) TFDP2, which encodes a protein that forms heterodimers with the E2F transcription factors, resulting in the transcriptional activation of cell cycle-regulated genes; (ii) CDC6, which encodes a protein that functions as a regulator of the early steps of DNA replication; (iii) ATR, a cell cycle-checkpoint gene required for cell cycle arrest and DNA damage repair in response to DNA damage; (vi) and CCNB1, which encodes a regulatory protein involved in the cell cycle [93].

### *5.3.3 Apoptosis*

The mechanisms of apoptosis are highly complex and sophisticated, involving an energy-dependent cascade of molecular events. To date, research indicates that there are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there is now evidence that the two pathways are linked and that molecules in one pathway can influence the other [94]. While the role of miRNAs in apoptosis control is well established, lncRNAs have received less attention, especially in extrinsic apoptosis pathway. Further studies about the lncRNA-mediated apoptosis pathways are needed.

P53, the gene most commonly mutated in human cancer, is a major regulator of mitochondrial apoptosis. P53 is essential for the cellular response to DNA-damaging stimuli to maintain genomic integrity of cells, mainly by activating a gene expression program that leads to cell cycle arrest or elimination of the damaged cells through programmed cell death, including apoptosis [95]. Of significant interest, recent studies suggest that lncRNAs participate in the p53 apoptosis pathway. P53-regulated lincRNA-p21 expression plays an important role in triggering apoptosis. HuR is a ubiquitous RBP that influences cell proliferation, survival, carcinogenesis, and the stress and immune responses. Association of the RNA-binding protein HuR with lincRNA-p21 favors the recruitment of let-7/Ago2 to lincRNA-p21, leading to lower lincRNA-p21 stability. Under reduced HuR levels, lincRNA-p21 accumulates in human cervical carcinoma HeLa cells, increasing its association with JUNB and CTNNB1 mRNAs and selectively lowering their translation [96], resulting in suppressed apoptosis. The expression of lnc_bc060912 is repressed by p53. In human lung carcinoma cells, researches using a recently developed method for RNA pulldown with formaldehyde cross-linking found that lnc_bc060912 interacted with the two DNA damage repair proteins PARP1 and NPM1. The results suggest that lnc_bc060912, via PARP1 and NPM1, affects cell apoptosis and plays important roles in tumorigenesis and cancer progression [97]. Maternally expressed gene 3(MEG3), an lncRNA, represents as a tumor suppressor gene, and its ectopic expression can inhibit cell proliferation and promote cell apoptosis in human glioma cell line. Accumulation of p53 (TP53) protein and its target gene expression partly contribute to cell growth inhibition induced by MEG3. MEG3 is significantly downregulated in non-small cell lung cancer (NSCLC) tissues that can be affected

by DNA methylation and regulates NSCLC cell proliferation and apoptosis, partially via the activation of p53 [98]. DNA damage can induce five lncRNAs from the CDKN1A promoter, and one such lncRNA, named promoter of CDKN1A antisense DNA damage-activated RNA (PANDAR), is induced in a p53-dependent manner. Moreover, PANDAR interacts with the transcription factor NF-YA to limit the expression of pro-apoptotic genes. PANDAR is a direct transcriptional target of p53 in NSCLC cells. PANDAR-mediated growth regulation is in part due to the transcriptional modulation of Bcl-2 by interacting with NF-YA, thus affecting NSCLC cell apoptosis [99].

There are also other mitochondrial apoptosis pathways. BCL-X is a key apoptotic member of the BCL-2 gene family that modulates tumor cell death and growth. Alternative splicing of exon 2 in the BCL-X pre-mRNA produces two isoforms, BCL-XL and BCL-XS, which have been shown to exert antagonistic functions in the apoptotic pathway. A lncRNA named INXS, transcribed from the opposite genomic strand of BCL-X, is five- to ninefold less abundant in tumor cell lines from the kidney, liver, breast, and prostate and in kidney tumor tissues compared with nontumors. Three apoptosis-inducing agents increase INXS lncRNA endogenous expression in the 786-O kidney tumor cell line, increase BCL-XS/BCL-XL mRNA ratio, and activate caspase-3, caspase-7, and caspase-9 in the mitochondrially mediated apoptosis pathway. These effects are abrogated in the presence of INXS knockdown. Similarly, ectopic INXS overexpression causes a shift in splicing toward BCL-XS and activation of caspases, thus leading to apoptosis. The overexpression of INXS lncRNA causes a significant activation of caspase-3, caspase-7, and caspase-9, the major caspases in the mitochondrially mediated apoptosis pathway, but has no effect on initiator caspase-8 of the death receptor extrinsic pathway [100]. Growth arrest-specific 5 (GAS5) encodes multiple snoRNAs within its introns, while exonic sequences produce lncRNA which can act as a riborepressor of the glucocorticoid and related receptors. GAS5 negatively regulates the survival of lymphoid and breast cells and is aberrantly expressed in several cancers. In prostate cancer, high levels of GAS5 lncRNA expression promote basal apoptosis and enhance the action of a range of apoptotic stimuli [101]. The HOX transcript antisense RNA (HOTAIR) gene is located within the homeobox C (HOXC) gene cluster on chromosome 12 and encodes a 2.2 kb lncRNA molecule. In serous ovarian cancer (SOC), HOTAIR is found to regulate apoptosis-related protein such as cyclin E, bcl-2, caspase-9, caspase-3, and BRCA1, but further studies are required to explore the underlying molecular mechanism [74].

There are few studies about lncRNA-mediated extrinsic apoptosis pathway. In gastric cancer, the expression of MRP1 and P-glycoprotein is founded decreased significantly in SGC7901/DDP and BGC823/DDP cells using siRNA to interfere with lncRNA AK022798 expression, and their apoptosis and the expressions of caspase-3 and caspase-8 obviously increase [102]. HOXA cluster antisense RNA 2 (HOXA-AS2) is a long noncoding RNA located between the HOXA3 and HOXA4 genes in the HOXA cluster. Its transcript is expressed in NB4 promyelocytic leukemia cells and human peripheral blood neutrophils, and the expression is increased in NB4 cells treated with all-trans-retinoic acid (ATRA). Knockdown of

HOXA-AS2 expression by transduced shRNA decreases the number of viable cells and increases the proportion of apoptotic cells, measured by annexin V binding and by activity and cleavage of caspase-3, caspase-8, and caspase-9. The increase in death of HOXA-AS2 knockdown cells is accompanied by an elevated TNF-related apoptosis-inducing ligand (TRAIL) levels, but ATRA-induced NB4 cells treated with TRAIL show an increase in HOXA-AS2 expression. These results demonstrate that ATRA induction of HOXA-AS2 suppresses ATRA-induced apoptosis, possibly through a TRAIL-mediated pathway. HOXA-AS2-mediated negative regulation thus contributes to the fine-tuning of apoptosis during ATRA-induced myeloid differentiation in NB4 cells [103].

### *5.3.4 Necrosis*

Regulated necrosis is termed "programmed necrosis" or "necroptosis" to distinguish this process from necrosis induced by physical trauma. Necroptosis can be induced by the engagement of the TNF receptor superfamily, T-cell receptor (TCR), interferon receptors (IFNRs), toll-like receptors (TLRs), cellular metabolic and genotoxic stresses, and a number of anticancer agents [43]. While still lacking enough investigation, emerging studies have shown that lncRNAs participate in controlling necrosis of cancer cells.

The aberrant expression of lncRNAs is detected during genotoxic stress-induced necrosis in human glioma cells. A change in the expression of specific candidate lncRNAs (neat1, GAS5, TUG1, BC200, MALAT1, MEG3, MIR155HG, PAR5, and ST7OT1) is detected during DNA damage-induced apoptosis in human glioma cell lines (U251 and U87) using doxorubicin (DOX) and resveratrol (RES). The expression pattern of these lncRNAs is also detected in human glioma cell lines under necrosis induced using an increased dose of DOX. The results reveal that the lncRNA expression patterns are distinct between genotoxic stress-induced apoptosis and necrosis in human glioma cells. The sets of lncRNA expressed during genotoxic stress-induced apoptosis are DNA-damaging agent specific. Potentially, these lncRNAs are involved in an apoptotic signaling pathway rather than a necrotic signaling pathway. But TUG1 and BC200 are only involved in necrosis rather than apoptosis [104]. Future studies are necessary to elucidate the function and mechanisms of the regulation of individual lncRNAs in necrosis.

### *5.3.5 Autophagy*

The role of autophagy in cancer cells is still under investigation. It seems that autophagy function depends on several factors, for example, step of tumor formation, tissue origin, and gene mutations existing in specific cancer type. Some cancer types like human pancreatic cancers with constitutive Ras activation have elevated levels

of autophagy that contributes to their growth and survival. Conversely, other tumor types like human breast, ovarian, and prostate cancers have allelic deletions of the essential autophagy regulator Beclin 1, indicating that decreased autophagy may promote tumor development [105]. In general, autophagy is activated in cancer cells and contributes to tumor cell survival. Autophagy is first linked to human cancer through the identification of Beclin 1, and later a number of studies show that ATG genes and others are also oncogenically associated [106]. lncRNA controls autophagy through regulating a series of autophagy-related proteins in cancer cells.

lncRNA PTENP1 is a pseudogene of the tumor suppressor gene PTEN. The molecular mechanisms of how PTENP1 represses the tumorigenic properties of hepatocellular carcinoma (HCC) cells are related with lncRNA-mediated autophagy pathway. A recent study shows that PTENP1 and PTEN are downregulated in several HCC cells. Sleeping Beauty (SB)-based hybrid baculovirus (BV) vectors are constructed for sustained PTENP1 lncRNA expression. Cotransduction of HCC cells with the SB-BV vector expressing PTENP1 elevates the levels of PTENP1 and PTEN, which suppresses the oncogenic PI3K/AKT pathway-induced autophagy. The overexpressed PTENP1 decoys oncomirs miR-17, miR-19b, and miR-20a, which would otherwise target PTEN, PHLPP (a negative AKT regulator), and such autophagy genes as ULK1, ATG7, and p62, indicating that PTENP1 modulates the HCC cell behavior and gene networks by miRNA regulation. Injection of the PTENP1-expressing SB-BV vector into mice bearing HCC tumors effectively mitigates the tumor growth, suppresses intratumoral cell proliferation, elicits apoptosis and autophagy, and inhibits angiogenesis [81].

A frontier study shows that an lncRNA named autophagy promoting factor (APF) can regulate autophagic cell death by targeting miR-188-3p and ATG7. The results show that miR-188-3p suppresses autophagy and myocardial infarction by targeting ATG7. Further, APF lncRNA regulates miR-188-3p and thus affects ATG7 expression, autophagic cell death, and myocardial infarction. The study reveals a novel regulating model of autophagic program, which comprises APF, miR-188-3p, and ATG7 in cardiovascular diseases [107].

High oncogenic BRAF levels have been shown to initiate autophagy, and it has also been speculated that BANCR can be involved in the regulation of autophagy, which is involved in tumor progression. BRAF-activated lncRNA (BANCR) is overexpressed in melanoma and has a potential functional role in melanoma cell migration. BANCR levels are significantly higher in the papillary thyroid carcinoma (PTC) tissues and PTC IHH-4 cells compared with the normal controls. Knockdown of BANCR in the IHH-4 cells inhibits proliferation and increases apoptosis of the cells in vitro. Further investigation of the underlying mechanisms reveals that BANCR markedly activates autophagy. Overexpression of BANCR inhibits apoptosis in the IHH-4 cells, whereas inhibition of autophagy stimulates apoptosis in the BANCR-overexpressed cells. BANCR overexpression also increases cell proliferation and the inhibition of autophagy abrogates BANCR overexpression-induced cell proliferation. In addition, the overexpression of BANCR results in an increase in the ratio of LC3-II/LC3-I, a marker for autophagy, while the knockdown

of BANCR decreases the ratio of LC3-II/LC3-I. Additionally, BANCR increases PTC cell proliferation, which can activate autophagy [108].

Maternally expressed gene 3 (MEG3) is an imprinted gene that encodes an lncRNA associated with tumorigenesis. MEG3 levels are significantly reduced in bladder cancer tissues compared with normal controls, and autophagy activity is increased in bladder cancer tissues. A significant negative correlation is observed between MEG3 levels and LC3-II (autophagy marker) levels in vivo. MEG3 is further demonstrated to markedly suppress autophagy activation, whereas MEG3 knockdown activates autophagy in human bladder cancer cell lines. Downregulated expression of MEG3 inhibits cell apoptosis, whereas autophagy inhibition increases MEG3-knockdown cell apoptosis. MEG3 knockdown also increases cell proliferation. More importantly, autophagy inhibition abrogates MEG3-knockdown-induced cell proliferation. Overall data has demonstrated that downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer [109].

## 5.4 Other ncRNAs in Growth and Death of Cancer Cells

By sitting at the intersection of complex circuitries that integrate transcriptional, posttranscriptional, and epigenetic control, ncRNAs exert a pervasive function on cell regulation [2]. Studies have shown that tumor tissue and normal tissue exhibit distinct noncoding RNA expression profiles, and the aberrant noncoding RNAs can play an important role in cancer development [9]. However, the biological functions of most ncRNAs remain largely unknown. Recently, evidence has begun to accumulate, describing how ncRNAs are dysregulated in growth and death of cancer cells. In addition to miRNAs and lncRNAs, other ncRNA species are also gaining a greater appreciation for their role in carcinogenesis, although further investigation is needed to unveil the underlying mechanisms. To date, except the most studied miRNAs and lncRNAs, ncRNAs which have been reported involved in growth and death of cancer cells include piRNA, siRNA, circRNA, rRNA, tRNA, snRNA, and snoRNA.

rRNA, tRNA, snRNA, and snoRNA belong to the housekeeping noncoding RNA family. rRNA is the RNA component of the ribosome and essential for protein synthesis in all living organisms. It constitutes the predominant material within the ribosome. The ribosomal RNAs form two subunits, the large subunit (LSU) and small subunit (SSU). The LSU rRNA acts as a ribozyme, catalyzing peptide bond formation. The regulation of rRNA transcription is physiologically important because the rate of rRNA transcription is coupled tightly to ribosome biogenesis, which subsequently determines the capacity of cells to grow and proliferate [110]. rRNA has been reported in controlling proliferation and autophagy of cancer cells. tRNA is an adaptor molecule composed of RNA, typically 76–90 nucleotides in length, which serves as the physical link between the nucleotide sequence of nucleic acids (DNA and RNA) and the amino acid sequence of proteins by carrying an amino acid to the protein synthetic machinery of a cell as directed by a three-

nucleotide sequence in a mRNA. As such, tRNAs are a necessary component of protein translation, the biological synthesis of new proteins according to the genetic code [111]. tRNA has been reported in controlling proliferation of cancer cells. snRNA is a class of small RNA molecules that are found within the splicing speckles and Cajal bodies of the cell nucleus in eukaryotic cells. The length of an average snRNA is approximately 150 nucleotides. They are transcribed by either RNA polymerase II or RNA polymerase III, and studies have shown that their primary function is in the processing of pre-messenger RNA (hnRNA) in the nucleus. They have also been shown to aid in the regulation of transcription factors (7SK RNA) or RNA polymerase II (B2 RNA) and maintaining the telomeres [112]. snRNA has been reported in controlling apoptosis of cancer cells. snoRNAs are a large class of small noncoding RNAs present in all eukaryotes sequenced thus far. They play a conserved role in ribosome biogenesis and are components of well-characterized ribonucleoprotein complexes referred to as snoRNPs. They can be divided in two main classes, the box C/D and the box H/ACA snoRNAs, which differ in terms of their characteristic motifs and structure as well as in their protein binding preferences and the chemical modifications they catalyze [113]. snoRNA has been reported in controlling proliferation and apoptosis of cancer cells.

piRNA, siRNA, and circRNA belong to the regulatory noncoding RNAs family. piRNAs are a novel class of noncoding single-strand RNAs. They are involved in germline development, silencing of selfish DNA elements, and maintaining germline DNA. The complex piRNA pathway can have regulatory functions in mammalian spermatogenesis in the timing of meiotic and postmeiotic events through transcriptional and translational repression or by supporting the search for homologous chromosomes and chromosome pairing integrity [114]. piRNA has been reported in controlling proliferation, cell cycle, and apoptosis of cancer cells. siRNAs are a class of double-stranded RNA molecules, 20–25 base pairs in length, which play many roles but are most notable in the RNAi pathway, where they interfere with the expression of specific genes with complementary nucleotide sequences. siRNA functions by causing mRNA to be broken down after transcription, resulting in no translation. It also acts in RNAi-related pathways, such as an antiviral mechanism or in shaping the chromatin structure of a genome. The complexity of these pathways is only now being elucidated [115]. siRNAs have been reported in controlling cell cycle and apoptosis of cancer cells. circRNAs are a special class of endogenous RNAs featuring stable structure and high tissue-specific expression. They are common in mammalian cells and regulate gene expression at the transcriptional or posttranscriptional level by interacting with microRNAs (miRNAs) or other molecules [116]. Unlike linear RNAs terminated with 5′ caps and 3′ tails, circular RNAs are characterized by covalently closed loop structures with neither 5′ to 3′ polarity nor polyadenylated tail. With the advent of specific biochemical and computational approaches, a large number of circRNAs have been identified in various cell lines and across different species [117]. circRNAs have been reported in controlling proliferation of cancer cells.

## *5.4.1 Proliferation*

### 5.4.1.1 rRNA

Ribosome biogenesis drives cell growth and proliferation, but mechanisms that modulate this process within specific lineages remain poorly understood. A *Drosophila* RNA polymerase I (Pol I) regulatory complex is identified to be composed of underdeveloped (Udd), TAF1B, and a TAF1C-like factor. Disruption of Udd or TAF1B results in reduced ovarian germline stem cell (GSC) proliferation. Female GSCs display high levels of rRNA transcription, and Udd becomes enriched in GSCs relative to their differentiating daughters. Increasing Pol I transcription delays differentiation, whereas reducing rRNA production induces both morphological changes that accompany multicellular cyst formation and specific decreased expression of the BMP pathway component Mad. These findings demonstrate that modulating rRNA synthesis fosters changes in the cell fate, growth, and proliferation of female *Drosophila* GSCs and their daughters [118].

### 5.4.1.2 tRNA

Diverse and abundant small RNAs may be derived from tRNA, but the function of these molecules remains undefined. One such tRNA-derived fragment, cloned from human mature B cells and designated CU1276, is found to possess the functional characteristics of a microRNA, including a DICER1-dependent biogenesis, physical association with Argonaute proteins, and the ability to repress mRNA transcripts in a sequence-specific manner. Expression of CU1276 is abundant in normal germinal center B cells but absent in germinal center-derived lymphomas, suggesting a role in the pathogenesis of this disease. Furthermore, CU1276 represses endogenous RPA1, an essential gene involved in many aspects of DNA dynamics, and consequently, expression of this tRNA-derived microRNA in a lymphoma cell line suppresses proliferation and modulates the molecular response to DNA damage [119].

### 5.4.1.3 snoRNA

The snoRNA U50, mediating the methylation of C2848 in 28S rRNA, has been suggested as a potential tumor suppressor-like gene playing a role in breast and prostate cancers and B-cell lymphoma. The downregulation of U50 is observed in colon cancer cell lines as well as tumors. The relationship between U50 and proliferation is investigated in lymphocytes stimulated by phytohemagglutinin (PHA) and observed to have a strong decrease in U50 levels associated with a reduced C2848 methylation. This reduction is due to an alteration of U50 stability and to an increase of its consumption. Indeed, the blockade of ribosome biogenesis

induces only an early decrease in U50 followed by a stabilization of U50 levels when ribosome biogenesis is almost completely blocked. In conclusion, the results link U50 to the cellular proliferation rate and ribosome biogenesis [120].

#### 5.4.1.4 circRNA

Circular RNAs are a recently (re)discovered abundant RNA species with presumed function as miRNA sponges, thus becoming part of the competing endogenous RNA network. The expression of circular and linear RNAs and proliferation is analyzed in matched normal colon mucosa and tumor tissues. Interestingly, the ratio of circular to linear RNA isoforms is always lower in tumor compared to normal colon samples and even lower in colorectal cancer cell lines. Furthermore, this ratio correlates negatively with the proliferation index. The correlation of global circular RNA abundance (the circRNA index) and proliferation is validated in a noncancerous proliferative disease, idiopathic pulmonary fibrosis, ovarian cancer cells compared to cultured normal ovarian epithelial cells, and 13 normal human tissues. A global reduction of circular RNA abundance is found in colorectal cancer cell lines and cancer compared to normal tissues, and a negative correlation of global circular RNA abundance and proliferation is discovered [121].

#### 5.4.1.5 piRNA

The hiwi gene is a human member of the piwi family, which represents the first class of genes known to be required for stem cell self-renewal in diverse organisms. The hiwi gene, located in 12q24.33, was originally isolated from a human testis cDNA library and encoded a 98.5 kDa basic protein. Like other piwi family members, the HIWI protein contains a conserved architecture with a PAZ motif in the middle and Piwi motif in the C-terminal region. Recent discoveries have shown that hiwi may participate in germ cell proliferation, and its overexpression may cause germ cell malignancy development [122]. Upregulation of Hiwi has been demonstrated to promote tumor cell proliferation in breast and cervical cancers, while its downregulation has been noted to suppress tumor cell proliferation in glioma and lung cancer [123]. HIWI expression is higher in gastric cancers than in normal mucosa or in mucosa with atrophic gastritis or intestinal metaplasia. The expression of hiwi in atrophic gastritis and intestinal metaplasia is also upregulated when compared to the normal state. Moreover, the proliferation of AGS gastric cancer cells is significantly inhibited when the endogenous hiwi is blocked with either recombinant antisense adenovirus or RNA interference. Consistent with previous studies of the piwi gene, a homolog of hiwi in *Drosophila* indicates that a certain level of somatic piwi activity is required to establish or maintain the stem cell identity, but a higher level of somatic piwi expression will not increase the number of germ stem cells. These findings reveal that endogenous HIWI is essential for the proliferation of gastric cells, but the exogenous HIWI does not significantly

influence cell growth. The pattern of HIWI protein expression is similar to that of Ki67 expression, which suggests that hiwi participate in the proliferation of gastric cancer cells [17]. Hiwi is confirmed to overexpress at both the mRNA and protein level, in HCC specimens, as well as in MHCC97L and MHCC97H HCC cell lines. Hiwi downregulation mediated by shRNA reduces the proliferation of HCC cells [124]. In hematopoietic cancer (CML), Hiwi protein expression is undetectable in CML K562 cells and that lentivirus-mediated ectopic expression of Hiwi markedly suppresses the proliferation [125]. Hiwi expression suppressed by an RNA interference-based strategy has been demonstrated to inhibit tumor cell proliferation in a xenograft mouse model, which is generated by subcutaneously inoculating with lung cancer stem cell SSCloAldebr cells. Plasmids containing U6 promoter-driven shRNAs against Hiwi or control plasmids have been successfully established. In nude mice, intravenous delivery of Hiwi shRNA plasmids significantly inhibits tumor growth compared to treatment with control scrambled shRNA plasmids or the vehicle PBS. Moreover, delivery of Hiwi shRNA plasmids results in a significant suppressed expression of Hiwi and ALDH-1 in xenograft tumor samples, based on immunohistochemical analysis [126]. The effect of HiWi gene silencing on lung cancer tumor stem cell proliferation has been demonstrated using gene transfection and RNA interference in lung cancer tumor stem cells (TSCs). shRNA eukaryotic expression vectors, pGenesil-2-HiWi1, pGenesil-2-HiWi2263, and pGenesil-2-control, targeting the HiWi gene are constructed. PBS serves as the control group. The expression vector of the target HiWi gene shRNA is transfected into lung cancer TSCs with PEI as the medium. The results suggest that HiWi gene silencing decreases proliferation in lung cancer TSCs [127].

### *5.4.2 Cell Cycle*

#### 5.4.2.1 siRNA

The effects of HMGB1 expression on cell cycle of the human cervical cancer cell line HeLa is evaluated by RNA interference. Effective eukaryotic expression vectors carrying PGCsi3.0-1/HMGB1 siRNA and PGCsi3.0-3/HMGB1 siRNA have been previously constructed and screened, and then the vectors are transfected into HeLa cells. The introduction of PGCsi3.0-1/HMGB1 siRNA and PGCsi3.0-3/HMGB1 siRNA inhibits the expression of HMGB1 mRNA and protein efficiently and specifically. There is a significant difference between the siRNA groups and the control groups ($P<0.05$). Flow cytometry shows that the content of DNA in G2 phase in PGCsi3.0-1 group and PGCsi3.0-3 group is obviously more than those in PGCsi3.0-Neg group and non-transfected group, but the content in S phase is less ($P<0.01$). The progression of cell cycle is arrested from G2 to S phase. PGCsi3.0-1/HMGB1 siRNA and PGCsi3.0-3/HMGB1 siRNA can specially suppress the expression of HMGB1 gene and arrest the progression of cell cycle from G2 to S phase [128].

#### 5.4.2.2 piRNA

piR-651, one member of the piRNA family, has been shown to be involved in carcinogenesis. The piR-651 inhibitor is transfected into gastric cancer cells to assess its influence on cell growth. Cell cycle analysis is used to reveal the cellular mechanisms of piR-651 in the genesis of gastric cancer. piR-651 expression is upregulated in gastric cancer tissues compared with paired noncancerous tissues. The levels of piR-651 are associated with TNM stage ($P = 0.032$). The expression of piR-651 in gastric, colon, lung, and breast cancer tissues is higher than that in paired noncancerous tissues. The upregulated expression of piR-651 is confirmed in several cancer cell lines including gastric, lung, mesothelium, breast, liver, and cervical cancer cell lines. The growth of gastric cancer cells is inhibited by a piR-651 inhibitor and arrested at the G2/M phase. Results indicate that a piR-651 inhibitor blocks gastric cancer cells at the G2/M phase [114]. Cell cycle analysis reveals that the suppression of hiwi results in significant G2/M arrest in gastric cancer. On the basis of the inhibition of growth of AGS gastric cancer cells by antisense hiwi and 106shRNA, their cell cycle progression is investigated in response to either overexpression or suppression of hiwi. The DNA content analysis shows the cell cycle of the cells treated with Ad-antisense hiwi, or Ad-106shRNA is arrested in G2/M phase [17]. Hiwi upregulation inhibits leukemic cell growth and induces cell cycle arrest at G0/G1 and G2/M phases in K562 cells [125]. Cells treated with betulinic acid (BA) show increased cell population in G(2)/M phase, with decreases in S phase population. The expression of Hiwi and cyclin B1 is downregulated in BA-treated AGS cells in a dose-dependent manner. G(2)/M cell cycle arrest and induction of apoptosis in AGS cells in vitro. The result suggests that BA exerts potent effect on G(2)/M cell cycle arrest possibly associated with the downregulation of Hiwi and its downstream target cyclin B1 expression [129].

### *5.4.3 Apoptosis*

#### 5.4.3.1 snRNA

A recent study utilizes U1 small nuclear RNA (snRNA) that binds physiologically to the 5′ splice site (5′ss) of pre-mRNA, to develop a novel vector system that permits imposed binding of antisense RNA to its target. The 5′ free end of U1snRNA is replaced with the antisense sequence against the K-ras gene to generate a hyperstable U1snRNA, whose binding stability to 5′ss of the K-ras transcript is tenfold higher than that of wild-type U1snRNA. The efficacy of such hyperstable U1snRNA is examined by transducing the expression plasmids into human pancreatic cancer cell lines. It reveals that two of the hyperstable U1snRNAs induce cell death after gene transduction and significantly reduce the number of G418-resistant colonies to less than 10% of the controls. Furthermore, hyperstable U1snRNA suppresses intraperitoneal dissemination of pancreatic cancer cells in vivo [130].

#### 5.4.3.2 snoRNA

GAS5 has been identified as critical to the control of mammalian apoptosis and cell population growth using functional expression cloning. GAS5 transcripts are subject to complex posttranscriptional processing, and some, but not all, GAS5 transcripts sensitize mammalian cells to apoptosis inducers. In some cell lines, GAS5 expression induces growth arrest and apoptosis independently of other stimuli. GAS5 transcript levels are significantly reduced in breast cancer samples relative to adjacent unaffected normal breast epithelial tissues. The GAS5 gene has no significant protein-coding potential, but expression encodes small nucleolar RNAs (snoRNAs) in its introns [131].

#### 5.4.3.3 piRNA

Bcl-like family includes a series of gene products that either suppress apoptosis such as Bcl-2 or promote apoptosis such as Bax. Interfering with Bcl-2 expression or function and/or activation of Bax may present therapeutic strategies against myeloid leukemias. Caspase-9 is a crucial regulator of cell apoptosis, the activation of which triggers the activation of the executioner caspase-3, leading to cell death. Promoting the activation of caspase-9 and caspase-3 could induce apoptosis in leukemia cells. Hiwi upregulation induces an obvious apoptosis in K562 cells, which is accompanied by decreased Bcl-2 expression and increased Bax, activated caspase-3 and caspase-9, and cleaved PARP. Hiwi overexpression decreases anti-apoptotic Bcl-2 expression and increases pro-apoptotic Bax, activated caspase-9 and caspase-3, and cleaved PARP in K562 cells. These results at least partly demonstrate that Hiwi can promote CML apoptosis by mediating these apoptosis-related proteins [125]. In glioma cells, silencing HIWI inhibits cell proliferation by promoting apoptosis and increases cell cycle arrest. The expression of proteins related to apoptosis and the cell cycle, including p21, cyclin D1, Bcl-2, and Bax is significantly altered [132]. In lung cancer tumor stem cells (TSCs), the effect of HiWi gene silencing on lung cancer tumor stem cell apoptosis has been demonstrated using gene transfection and RNA interference. 24 h after transfection, the apoptotic ratios in the pGenesil-2-HiWi1 and pGenesil-2-HiWi2263 groups are $26.16 \pm 1.21$ and $28.06 \pm 1.78\,\%$, respectively, which are higher as compared to those in the pGenesil-2-control group $2.86 \pm 0.09\,\%$ ($P < 0.01$). The results suggest that HiWi gene silencing promotes apoptosis of lung cancer TSCs [126].

### *5.4.4 Necrosis*

There are no reports about ncRNA-related necrosis in cancer cells except miRNA and lncRNA.

### *5.4.5 Autophagy*

#### 5.4.5.1 rRNA

Several agents known to interfere with rRNA transcription and processing are first-line anticancer therapy, such as cisplatin for advanced non-small cell lung carcinomas and 5-fluorouracil for colon cancers. The list of agents inhibiting various stages of rRNA biogenesis continues to grow. Cis-noncoding rRNAs, alternative to primary rRNA, have been shown to regulate rRNA biogenesis. Bidirectional noncoding rRNAs have recently been detected carrying ribozyme-like properties. Anti-antisense oligonucleotides complementary to antisense noncoding rRNAs markedly stabilize the bidirectional transcripts and induce cell death in mouse lung cells. Autophagic activation is largely undifferentiable between the anti-antisense and other oligonucleotides and accounts for the undesired cytotoxicity in noncancer cells. Co-treatment with chloroquine, an autophagy inhibitor, reduces cytotoxicity in the noncancer cells, but retains the anti-antisense-mediated killings in cancer cells. The 86S oligonucleotide is more effective than the 84AS in the induction of apoptotic cell death in human lung cancer cells. Furthermore, the anti-antisense oligonucleotide stabilizes bidirectional noncoding rRNAs predominantly in human cancer cells and perturbs rRNA biogenesis [133].

## 5.5 Conclusion

Historically, both proteins and protein-coding RNAs have tended to dominate our view of the cell and the human diseases because of their abundance and the relative ease with which protein-coding genes and their gene products can be identified and studied. However, this paradigm has been undermined in recent years with the development of whole-genome and transcriptome sequencing technologies. Over the past several years, accumulated data have begun to advance the idea that ncRNAs are not just transcriptional noise or cloning artifacts but important supplements to proteins and other effectors in complex regulatory networks [13]. In this review, we present an updated vision of ncRNAs and summarize the mechanism of regulation by ncRNAs, which can control a wide range of biological functions such as cellular proliferation, cell cycle, apoptosis, necroptosis, and autophagy, providing new insight into the functional cellular roles that they may play in cancers, primarily focusing on the two most commonly studied ncRNA biotypes, lncRNAs and miRNAs.

Given the critical role miRNAs play in tumorigenesis processes and their disease-specific expression, they hold potential as therapeutic targets and novel biomarkers. Expression profiling of miRNAs has been shown to be a more accurate method of classifying cancer subtypes than using the expression profiles of protein-coding genes [25]. The differential expression of certain miRNAs in various tumors is a powerful tool to aid in the diagnosis and treatment of cancer. Different strategies

based on blocking miRNA function or specific miRNA delivery to the tumor cells have already been used. Several preclinical approaches have been developed in order to block miRNAs, including anti-miRNA oligonucleotides, miRNA sponges, miRNA masks (target protectors), and small molecule inhibitors [134]. However, it is worth noting that further studies are needed to unveil the functions and mechanisms of miRNAs in the necroptosis of cancer cells. The discovery of dysregulated lncRNAs involved in cancer cellular biology represents a new layer of complexity in the molecular architecture of oncology. However, there are still many gaps in our current understanding of lncRNA function. lncRNAs can be useful as novel biomarkers for diagnosis, prognosis, and prediction of response to therapy. Better understanding of lncRNA functions will help clarify the real impact of genomic pervasive transcription on cell biology, on evolution, and, eventually, on the clinical setting for oncology. Furthermore, many more types of ncRNAs exist but their underlying mechanisms are still in infancy. For example, a growing number of reports have revealed the aberrant expression of PIWI proteins in various cancers, and it appears highly plausible that PIWI proteins are involved in tumorigenesis, in part because a considerable portion of the experimental results is derived from studies of patient samples, with limited opportunities for experimental manipulation. Further basic studies with more manipulable materials, such as cell lines and experimental animals, are urgently needed to address the possibility of PIWI as a therapeutic target. However, data for elucidating the detailed molecular role of PIWI proteins in tumorigenesis is very limited. An immediate focus is to identify PIWI protein-associating RNAs to determine whether piRNAs and/or other RNA species specifically interact with PIWI proteins in cancer cells. It is also imperative to investigate how PIWI proteins are involved in the biological functioning of cancer cells, such as transposon silencing, transcriptional or posttranscriptional regulation, DNA repair, and chromosome condensation and segregation, and to determine the roles of PIWI proteins in tumorigenesis [135].

Given the versatile, critical, and surprising regulatory functions of ncRNAs uncovered so far, forming a better understanding of the precise molecular mechanisms by which ncRNAs function in various cancers will be an exciting journey and also critical for exploring new potential strategies for early diagnosis and therapy. In the future, the field of ncRNAs research will certainly be in spotlight. Clearly, a deeper understanding of the biological effects of the various ncRNAs will need to be acquired before this possibility can be actualized into real therapeutic strategies.

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