

# Regulation of let-7 and its target oncogenes (Review)

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Received October 20, 2011; Accepted February 1, 2012

DOI: 10.3892/ol.2012.609

**Abstract.** MicroRNAs (miRNAs) are highly evolutionarily-conserved non-coding small RNAs, which were first identified in *Caenorhabditis elegans*. Let-7 miRNA is involved in the regulation of gene expression in cells. Several novel factors and feedback loops involved in the regulation of the synthesis of let-7 have been identified and additional let-7 target genes have been found. Let-7 has also been shown to be significantly correlated with the occurrence and development of cancer and the results of preliminary studies suggest that it is involved in the regulation of oncogenic pathways in numerous types of tumors. Let-7 is, therefore, a potential molecular target for tumor therapy. Thus, this review examined let-7 and the correlation between let-7 and oncogenic pathways in cancer.

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## 1. Introduction

MicroRNAs (miRNAs) are short single-stranded RNA molecules approximately 22 nucleotides in length. They are involved in the post-transcriptional regulation of gene expression, through binding to complementary sequences in the 3'

untranslated regions (3' UTRs) of target mRNA transcripts (1). miRNAs exist in three forms during their synthesis: primary miRNAs (pri-miRNAs), precursor miRNAs (pre-miRNAs) and mature miRNAs. Pri-miRNA contains a 5' cap and poly(A) tail and is first transcribed by RNA polymerase II as a long transcript. Following the processing of pri-miRNA by the RNase III enzyme Drosha in the nucleus, pre-miRNA is released, exported to the cytoplasm by exportin-5 and processed by the RNase III enzyme Dicer to produce a mature miRNA-miRNA duplex (2). Single-stranded mature miRNA is incorporated from the duplex into an RNA-induced silencing complex (RISC) where it mediates the silencing of target genes (3,4).

In 2000, Reinhart *et al* identified a novel miRNA, let-7, which was able to implement and change the nematode phenotype during the later development of *Caenorhabditis elegans* (5). Numerous members of the let-7 family have been identified in various species and different members are found in different species (6). At present, 10 mature subtypes of the let-7 family have been identified in humans, including let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-98 and miR-202, in which mature let-7a and let-7f were produced by precursor sequences (let-7a-1, let-7a-2, let-7a-3; let-7f-1, let-7f-2). In normal physiology, let-7 is primarily involved in development, muscle formation, cell adhesion and gene regulation. The regulation of let-7 synthesis has been the subject of increasing attention and numerous proteins and factors have been shown to be correlated with let-7 family expression, including certain complete regulatory loops. Progress has also been made in understanding the mechanisms of action of let-7 miRNA. A number of studies have reported that let-7 is downregulated in numerous types of cancer, including lung cancer (7), gastric tumors (8), colon cancer (9) and Burkitt's lymphoma (10). Low levels of let-7 expression have been associated with the clinical postoperative survival of patients with lung cancer (7). The let-7 miRNA family is involved in the proliferation, apoptosis and invasion of cancer cells. Oncogenes and their signaling pathways are a potential connection between the level of expression of let-7 and the biological characteristics of tumor cells. This review explored the regulation of let-7 expression and its mechanisms of action, as well as its correlation with oncogenic pathways in tumors.

## 2. Regulation of let-7 expression

Let-7 is found in *Caenorhabditis elegans* and appears to act as a key regulator of temporal patterning, coordinating

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**Abbreviations:** miRNA, microRNA; 3' UTRs, 3' untranslated regions; NF, nuclear factor; SNP, single nucleotide polymorphism; RNase, ribonuclease

**Key words:** microRNA, let-7, regulation, cancer, regulatory circuit

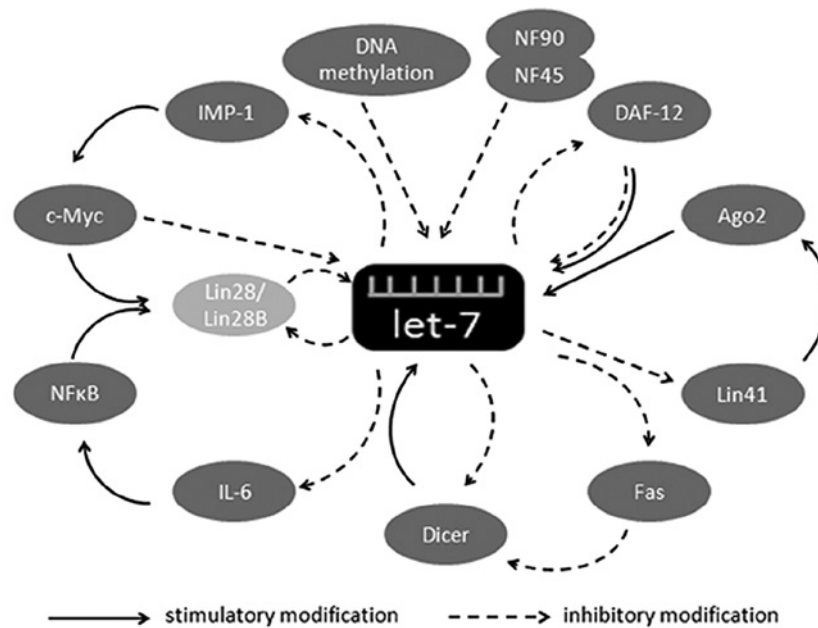


Figure 1. Regulation of let-7 expression.

developmental timing across tissues (11). As a conserved sequence, its expression and function as a regulator of cell differentiation and proliferation have been confirmed in animal and human cells (12-15). In addition to regulating the differentiation and proliferation of normal cells, let-7 has also been found to be involved in inhibiting the growth of tumor cells (16,17). The regulation of let-7 expression has become increasingly important in light of its involvement in tumor suppression. The expression of let-7 is regulated at various stages of its biogenesis and the regulation process involves numerous factors and regulatory circuits (Fig. 1). However, although some regulatory circuits have been demonstrated, others remain theoretical.

**Lin28 and Lin28B.** Reinhart *et al* found that the activity of let-7 was affected by mutations in Lin28 (5). Lin28 and Lin28B have been shown to act as post-transcriptional repressors of let-7 biogenesis, by binding to the loop portion of the pri-let-7 hairpin and the stem of pre-let-7 to inhibit the binding of Drosha or Dicer, thereby inhibiting its processing (18,19). The mechanism of Lin28 binding to the terminal loop region of let-7g has been elucidated (20) and the cold-shock and zinc-finger domains in Lin28 were shown to be required for pre-let-7 binding, resulting in >90% inhibition of let-7g processing upon the upregulation of Lin28. Lin28 is also considered to be necessary and sufficient to block the microprocessor-mediated cleavage of pri-let-7 miRNAs (18), as the ectopic expression of Lin28 completely prevented the processing of pri-let-7a and pri-let-7g, whereas the processing of pri-miR-15a and pri-miR-122 were largely unaffected. In addition, the transfection of Lin28 cDNA decreased the endogenous levels of mature let-7 family members, but had no effect on the levels of endogenous mature miR-21 (18).

A novel mechanism whereby Lin28/Lin28B blocks let-7 processing, other than by Drosha/Dicer inhibition, has been identified. Lin28 was shown to be able to directly regulate let-7 synthesis by targeting pre-let-7 and affecting its terminal

uridylation (21); Lin28 mediates the terminal uridylation of pre-let-7, thus diverting Dicer processing and irreversibly re-routing pre-let-7 to a degradation pathway. Three enzymes have been reported to be involved in the terminal uridylation progress. Zcchc11 is a terminal uridylyl transferase (TUTase) and is required by Lin28 for the mediation of pre-let-7 uridylation and the subsequent blockade of let-7 processing in mouse embryonic stem cells (22). TUT4 is recruited by Lin28 to pre-let-7 via the recognition of a tetranucleotide sequence motif (GGAG) in the terminal loop. TUT4 in turn adds an oligouridine tail to pre-let-7 to block Dicer processing (23). PUP-2 interacts directly with Lin28 to regulate the stability of Lin28-blockaded let-7 pre-miRNA to suppress the action of Dicer and contribute to the Lin28-stimulated uridylation of let-7 pre-miRNA, preventing its maturation in *Caenorhabditis elegans* (24).

**DNA methylation.** It has been suggested that DNA methylation is correlated with human tumorigenesis. The hyper- or hypomethylation of CpG islands, often observed in tumor cells, results in the silencing of genes and is significant in tumor development (25-27). The results of certain studies suggest that some miRNA genes are methylated, with consequent effects on miRNA expression (28-30). The expression of let-7 has been reported to be regulated by DNA methylation. The human let-7a-3 gene is located on chromosome 22q13.31 and is associated with a CpG island, which is methylated by the DNA methyltransferases DNMT1 and DNMT3B. Let-7a-3 is methylated in normal lung samples, with a similar methylation pattern to that observed in other human tissues. The hypomethylation of let-7a-3 was found to increase the level of expression of miRNA and inhibit the growth of tumor cells in lung adenocarcinomas (31). Hypermethylation resulted in the downregulation of let-7a-3 in epithelial ovarian cancer and this low level of expression of let-7a-3 in epithelial ovarian cancer was associated with a poor prognosis (32).

**Nuclear factor 90 and nuclear factor 45.** Nuclear factor (NF) 90 and NF45 belong to the larger Drosha complex family, which is needed for the production of pre-miRNA from pri-miRNA. The overexpression of NF90 and NF45 has been shown to be associated with the level of pri-miRNA. The NF90-NF45 complex, rather than either factor alone, binds the majority of pri-miRNAs with a higher affinity for pri-let-7a-1 than the DGCR8-Drosha complex, which also binds to pri-miRNAs. The NF90-NF45 complex may thus inhibit the processing of pri-miRNA by the DGCR8-Drosha complex. NF90-NF45 also showed a higher binding affinity for pri-let-7a-1 than for pri-miR-21 (33).

**Regulatory circuits involved in let-7 expression.** The regulation of let-7 biogenesis is complex and involves numerous regulatory factors, including a number of factors that control let-7 expression via regulatory loops. These loops may be divided into two categories: Lin28-dependent and Lin28-independent. The regulatory feedback loops that depend on Lin28 are all positive and include the NF $\kappa$ B-Lin28-let-7-interleukin (IL)-6-NF $\kappa$ B, Lin28-let-7-insulin-like growth factor II mRNA-binding protein 1 (IMP1)-c-Myc-Lin28 and Lin28-let-7-Lin28 loops.

Inflammation is clinically linked to cancer and certain inflammation-related molecules, including NF $\kappa$ B, have been demonstrated to be key in this association. A regulatory feedback loop controlling let-7 expression and including NF $\kappa$ B has been identified and may aid the elucidation of the mechanistic link between inflammation and cancer. NF $\kappa$ B directly activates Lin28 transcription and rapidly reduces let-7 levels. Let-7 may then directly inhibit IL-6 expression, which may activate NF $\kappa$ B, thereby completing a positive feedback loop (34).

As a target oncogene of let-7, c-Myc may be directly regulated by IMP1, which has been shown to be directly and negatively regulated by let-7 (35,36). In addition, c-Myc was shown to be able to directly transactivate Lin28B, a Lin28 homolog, to inhibit let-7 expression. The activation of Lin28B was observed to be necessary and sufficient for Myc-mediated let-7 expression (37,38). It is also possible, however, that Myc regulates let-7 directly (39).

Besides indirectly regulating the expression of Lin28, let-7 may also affect Lin28 directly; the binding of let-7 to the 3' UTR of Lin28/Lin28B transcripts represses the expression of Lin28/Lin28B (38). As discussed, Lin28 is a classical direct inhibitor of let-7, creating a double-negative regulatory loop for let-7.

Certain loops, however, do not require Lin28, including the DAF-12-let-7-DAF-12, Ago2-let-7-Lin41-Ago2, Dicer-let-7-Dicer and Fas-Dicer-let-7-Fas loops.

The DAF-12-let-7-DAF-12 loop consists only of DAF-12 and let-7. DAF-12 is able to directly regulate the transcription of let-7 either positively (as liganded DAF-12) or negatively (as unliganded DAF-12). At the same time, let-7 can mediate the expression of DAF-12 by binding its 3' UTR (40).

Ago2, as the RISC slicer providing the RNase activity required to cleave target mRNAs for miRNA, may also directly bind let-7a or generate an additional miRNA precursor to stabilize and enhance mature let-7a (41). Lin41, a target gene of let-7, has been reported to negatively regulate Ago2 levels (42).

Dicer is a classical enzyme which is essential for all miRNA synthesis processes. Let-7 has been reported to directly

target the miRNA-processing enzyme Dicer within its coding sequence, thus establishing another autoregulatory negative feedback loop for let-7 (43). However, let-7b is downregulated when Dicer is overexpressed in oral cancer cells (44), indicating a double-negative regulatory loop for let-7b and Dicer in oral cancer cells.

Fas (also termed APO-1 or CD95) was first identified by Yonehara *et al* in 1989 and was considered to be a novel cell surface protein antigen that differed from the tumor necrosis factor (TNF) receptor, but was able to mediate the cytolytic activity of TNF (45). Fas was found to induce apoptosis by binding to specific ligands, including Fas ligand (FasL), TNF- $\alpha$  and Fas-specific monoclonal antibody CH11 (mAb CH11) in sensitive cells (46,47). The apoptosis-inducing ability of Fas has since been used to inhibit the growth of tumor cells (48). Fas has been reported to be regulated by let-7/miR-98 in T cells (49) and this observation had been confirmed in human colon carcinoma. Activated Fas is able to inhibit Dicer activation, reducing the levels of mature let-7 miRNA (50).

These regulatory circuits maintain the balance of the let-7 levels in a normal organism. The level of expression of let-7 is affected when factors which take part in the regulatory circuits are altered. Changes in the level of expression of let-7 may induce normal and abnormal responses. Therefore, the single nucleotide polymorphisms (SNPs) of tumor suppressor miRNA biogenesis genes are considered to be high-risk factors for cancer (51,52). An SNP of the Lin28 gene, rs3811463, located near the let-7 binding site, resulted in the weakened repression of Lin28 mRNA induced by let-7 and the downregulation of let-7 expression via the let-7-Lin28 double negative feedback loop. This SNP was therefore considered to raise the risk of tumorigenesis in breast cancer (53).

### 3. Mechanisms of action

The classical mechanism of let-7 action involves its binding to the 3' UTR of target mRNAs to regulate their expression. However, in addition to this classical mechanism, let-7 may act in other ways.

Let-7a caused identical responses when it was targeted to the 5' or 3' UTRs of mRNAs containing internal ribosome entry sites (54), indicating that let-7 is able to act through binding to sites other than the 3' UTR of target mRNAs. Furthermore, let-7 binds not only to untranslated or non-coding regions, but is also able to bind directly to coding regions to restrict target mRNAs (43).

The mechanisms of let-7 action on mRNAs remain under debate. Let-7a may repress the translation of target mRNAs by binding to and inhibiting translating polyribosomes (55). Deadenylation is another route whereby let-7 may inhibit translation and contribute to mRNA decay, with the translational repression function being more prominent than mRNA decay. However, deadenylation alone is not sufficient to effect full mRNA repression (56).

### 4. Target oncogenic signaling pathways of let-7

c-Myc, ras, high-mobility group A (HMGA), Janus protein tyrosine kinase (JAK), signal transducer and activator of tran-

scription 3 (STAT3) and NIRF are oncogenes that are critical in tumorigenesis, proliferation and invasion and which are targeted by let-7. These oncogenes activate or upregulate the expression of their downstream target proteins, which directly regulate the cell cycle, apoptosis and cell adhesion. Let-7 may thus act as a tumor suppressor through the inhibition of these oncogenic signaling pathways.

**Regulation of the ras oncogene.** Ras is associated with numerous downstream signaling pathways, including Ras/Raf/mitogen-activated protein kinase, Ras/phosphoinositide 3-kinase/Akt and Ras/Rho/Ral. Following activation by complex signaling cascades, Ras is able to bind to numerous effectors to trigger further signaling cascades, which in turn modulate cell processes including growth, survival, migration, differentiation and death. Let-7 is expressed in normal adult lung tissues (35), but has a lower level of expression in lung tumors that express high levels of Ras protein (7). This inverse correlation between let-7 and Ras suggests a causal relationship. The three human ras genes (H-ras, K-ras and N-ras) have let-7-complementary sites in their 3' UTRs (57). SNPs in the let-7 complementary sites in the 3' UTR of K-ras resulted in increased levels of K-ras and poor prognosis of patients with lung and breast cancer (58-60). Chin *et al* found that tumors which contained a variant allele had lower let-7 levels than those without a variant allele and hypothesized that SNPs in the let-7 binding site of the K-ras 3' UTR reduce the levels of let-7 through a feedback loop (59). The overexpression of let-7 resulted in a lower level of expression of the Ras protein, a reduction in cell proliferation and migration in glioma and lung cancer cells and a decrease in the size of gliomas and lung tumors in nude mice (61,62). These results suggest that let-7 directly and negatively mediates the expression of the Ras protein (H-Ras, K-Ras and N-Ras) by binding to 3' UTR complementary sites. The downstream proteins in Ras signaling pathways should thus be downregulated when let-7 is overexpressed in cancer cells, resulting in changes in cell function.

**HMGA as a target oncogene of let-7.** HMGA proteins (HMGA1a, HMGA1b and HMGA2) are polypeptides of ~100 amino acid residues characterized by a modular sequence organization, with three highly positively-charged regions, termed AT hooks, that bind the minor groove of AT-rich DNA stretches. These proteins regulate gene expression by altering the structure of chromatin or by direct protein-protein interactions with transcription factors (63). As an oncogene, the overexpression of the HMGA2 protein has been observed in a number of tumors (64-68). It has been suggested that HMGA2 expression is controlled by negatively-acting regulatory elements within the 3' UTR. The overexpression of HMGA2 in tumors may thus occur due to this regulatory element being unable to bind effectively to the 3' UTR of HMGA2, which is reduced or absent in tumor cells (69). The level of let-7 expression is significantly higher in gastric cancer patients with a high HMGA2 expression than in those with low a HMGA2 expression (70). Furthermore, HMGA2 expression was found to be downregulated when let-7 was overexpressed (71). These results demonstrate that let-7 negatively mediates the expression of the oncogene HMGA2 by binding to its 3' UTR and directly suppressing its expression (72).

**Myc oncogene and pathway.** The proto-oncogene Myc is frequently activated in tumors (73). Activated Myc increases cell growth, division and survival by increasing the synthesis of its target proteins, some of which are involved in the regulation of the cell cycle and apoptosis (74). The overexpression of let-7a was found to downregulate Myc mRNA and protein (9,10) and it has been suggested that let-7 regulates Myc expression directly by binding to its 3' UTR. However, let-7 also affects the cell cycle by directly mediating certain downstream proteins [cyclin D1 and cyclin-dependent kinase (CDK) 6] in the Myc oncogene signaling pathway (75,76).

**JAK-STAT3 pathway.** JAK is a member of the protein tyrosine kinase family. Activated JAK activates STATs, which are commonly expressed in all tissues and cells in the human body. STAT activation is involved in the genesis, differentiation and apoptosis of cells and inflammation, cancer and the immune response (77). STAT3 is a member of the STAT family. The JAK-STAT3 pathway is activated in a number of types of tumors and has been confirmed to be correlated with cancer (78-80). STAT3 was found to be a target of let-7a, mediating cell proliferation in HepG2 cells (81). Let-7 may thus also regulate the activity of tumor cells by targeting the JAK-STAT3 pathway.

**NIRF oncogene and let-7.** The nucleic protein NIRF is encoded by the UHRF2 gene, which is a member of the UHRF oncogene family. NIRF contains a ubiquitin-like domain and a ring-finger structural domain which are essential for UHRF E3 ligase activity to regulate DNA methylation (82,83). DNA damage may activate a p53-dependent checkpoint pathway resulting in the induction of p21WAF1 expression and subsequent cell cycle arrest at the G1/S phase, through the inhibition of CDK activity and DNA replication. A reduction in the levels of UHRF family members has been reported to correlate with p21WAF1 expression (84). Let-7a was found to suppress NIRF expression via binding to the 3' UTR of NIRF mRNA and enhance p21WAF1 expression in A549 lung cancer cells (85), indicating that let-7a may regulate the cell cycle via a NIRF/p53/p21/CDK signaling pathway.

## 5. Conclusions

Let-7 miRNA has been the subject of increasing interest as a potential therapeutic target in cancer. Let-7 is able to suppress tumor proliferative activities and survival by negatively mediating a number of oncogenes and by affecting key regulators of the cell cycle, cell differentiation and apoptotic pathways. The upregulation of let-7 is expected to provide an effective cancer therapy. Therefore, although significant progress has been made in understanding the regulation of let-7 synthesis and its role in oncogenic signaling pathways, its regulation in normal and malignant cells and the mechanisms whereby it controls cell proliferation and survival require further elucidation. Additional investigations are needed to enable the clinical application of let-7 regulation to cancer suppression.

## Acknowledgements

This study was supported by the China Natural Science Foundation (81072078, 81101901 and 81172389), the Natural



Science Foundation of Jiangsu Province (201123808 and 2010580), Jiangsu Province's Key Provincial Talents Program (RC2011051), Jiangsu Province's Key Discipline of Medicine (XK201117), Program for Development of Innovative Research Team in the First Affiliated Hospital of NJMU, a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, and National High Technology Research and Development Program 863 (2012AA02A508).

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