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Let-7 microRNA as a potential therapeutic target with implications for immunotherapy

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Abstract

Introduction: MicroRNAs (miRNA) are a class of small non-coding RNA that play a major role in various cellular processes by negatively regulating gene expression. In the past decade, miRNA dysregulation has been reported to be closely linked to inflammatory diseases. The immune response modulates cancer initiation and progression; miRNAs including *let-7* family members have been shown to act as key regulators of the immune responses in various diseases and cancers. Notably, the *let-7* miRNA has been reported to be closely associated with immunity, specifically with Toll-like receptors that mediate cytokine expression during pathogen infection and with the regulation of various other immune effectors.

Areas covered: In this review, the authors describe the discovery of *let-7* as the starting point of the RNA revolution and highlight *let-7* as an efficient tool for cancer and immune therapy.

Expert opinion: *let-7* miRNA has emerged as a key player in cancer therapy and immune responses and it has potential role as a new immunotherapeutic target. However, while there are challenges regarding miRNA delivery, the exciting emergence of personalized medicine for cancer and immunotherapy could be beneficial for the development of *let-7* therapeutics.

Keywords

let-7; miRNAs; RNA therapy; immune response; immunotherapy

1. Introduction

MicroRNAs (miRNA or miRs) are small non-coding RNA, which were first discovered in 1993 in *Caenorhabditis elegans* (*C. elegans*), followed 7 years later by the discovery of the first mammalian miRNA [1,2]. Their discovery opened a new paradigm for gene regulation

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[3]. MiRNAs are transcribed by RNA polymerase-II, are 18–25 nucleotides-long, and play a major role in various cellular processes. miRNAs negatively regulate gene expression by acting through imperfect complementary base pairing to the 3′-untranslated region (UTR) of target mRNAs, regulating their translational inhibition or decay. *let-7*, the second miRNA discovered in *C. elegans* is extremely conserved among species including humans. Since its discovery, *let-7* studies have substantially expanded and paved the way for the ncRNA revolution, allowing the emergence of the concept of miRNAs acting as tumor suppressors, and later as oncomiRs.

MiRNAs, including *let-7* are dysregulated in a plethora of diseases, including cancers, and *let-7* is currently implicated in a variety of approaches for therapy and diagnosis. *let-7* has been largely described as a tumor-suppressive miRNA because it is under-expressed in multiple cancers. The emergence of the use of small chemically modified nucleic acid compounds as miRNA mimics or antimiRs allows one to manipulate *let-7* expression for therapeutic approaches. *let-7* reintroduction, using mimics, in cancer has led to repressed tumor growth and metastasis in numerous tumor types. *let-7 dysregulation has been reported to be closely linked to inflammatory diseases and immune response modulates cancer initiation and progression. In this review the authors will summarize how, since its discovery, the let-7 miRNA, has emerged as a key player in cancer therapy and immune responses and we highlight its potential role as a new immunotherapeutic target*.

2. *let-7* discovery

The first miRNA, *lin-4*, discovered in the *C. elegans* model paved the way to a new gene regulation paradigm where short non-coding RNAs of 22 nucleotides long have the ability to repress mRNA expression by specific binding to the 3′UTR [1, 4]. Reinhart et al. subsequently demonstrated that another small non-coding RNA named *let-7* (for *lethal-7*) encoded for a 21-nucleotide RNA that is complementary to the 3′ UTR regions of *lin-14, lin-28, lin-41, lin-42* and *daf-12*, and was a key regulator of the temporal sequence of stem cell development in *C. elegans* development [2,5]. An important aspect of *let-7's* role during *C. elegans* late-stage development has been demonstrated through the use of mutant *let-7* worms. The authors showed that the mutants die because some of their cells cannot successfully complete terminal differentiation. Specifically, the mutant seam stem cells fail to exit the cell cycle and fail to express determinants required for proper formation of adult cell fates [2]. This evidence bolstered the idea that a small RNA gene is able to control the temporal sequence of development in *C. elegans* and more generally across animal phylogeny [6]. In 2000, the same group reported the expression of *let-7* RNA in human cells and that expression levels of the human *let-7* are different among various tissues, indicating a possible cell-type regulation of *let-7* expression [6]. In 2004, a study investigating miRNA expression level by northern blot from 159 non-small cell lung carcinoma (NSCLC) tissues and cell lines showed for the first time a reduced expression of *let-7* in tumor tissues [7]. Later in 2006, by using LNA microarrays to compare and profile miRNA expression in tumor breast samples compared to normal, it was shown that high expression of miRNAs including *let-7* occurs in various adult tissues but more predominantly in luminal epithelial cells than malignant cells [8,9]. Later *let-7* expression level was found to be downregulated in many human cancers [10].

let-7 and many other miRNAs are extremely well conserved across animal species and have multiple orthologs in each species, including in human and mouse [6]. In humans, mature *let-7* members are encoded by 13 genomic loci. The *let-7* family is composed of 10 different *let-7* sequences annotated from *let-7-a* to *let-7-i* that share the same seed sequence (nucleotides 2–8) for target recognition. Only the *let-7*-a sequence UGAGGUAGUAGGUUGUAUAGUU is fully conserved across the following species: mouse, human, nematode, fly, frog, chicken, zebrafish, and dog; whereas the other isoforms differ in some nucleotide positions [11].

Since the discovery of *lin-4* and *let-7*, 439 mature *C. elegans* miRNA sequences (cel-miR) have been discovered. From that starting point, the miRNA revolution has led to the discovery of 2694 new mature sequences for human miRNAs (hsamiRNAs), 2014 mature sequences for mouse (mmu-miRNAs), and 430 in *Arabidopsis thaliana* (ath-mIR), the majority of which have not yet been extensively studied, used, developed, and modified for therapy (http://miRBase.org).

3 *let-7*: the starting point of the RNA revolution

Since the discovery of human *let-7*, which sparked the ncRNA revolution and led to a variety of impressive discoveries, miRNA has emerged as an innovative tool for therapy [12]. Briefly, from early 2000, by studying miRNAs as a new class of gene products in chronic lymphocytic leukemias, the dogma that miRNAs can be dysregulated in cancer and associated with a cancer signature emerged [13]. In 2003, the discovery that one miRNA can target multiple genes by its specific miRNA seed sequence revolutionized our understanding of the regulation of the mammalian transcriptome [14]. The emergence of advanced techniques and methods of analysis led to the first report of down-regulation of *let-7* in lung tumor samples, paving the way to using alteration of miRNAs with high clinical potential as biomarkers [7]. Concomitantly, by analyzing the increase of mature miRNAs from the mir-17–92 locus in B-cell lymphoma samples compared to normal tissues, the new concept that miRNA could act as an ‘oncomiR’ was developed [15]. One strong piece of evidence of oncomiR addiction was demonstrated by our group in the model of miRNA-21-induced pre-B-cell lymphoma in 2010 [16]. By generating a mouse model that allows tissue-specific and doxycycline-controlled expression of miR-21 we obtained mice that develop hematological malignancies. To highlight the role of miR-21 addiction to tumor development, we switched off miR-21 expression in established tumors by feeding mice with doxycycline-impregnated food and observed tumor regression after 48 h. This strong evidence demonstrated a central role of miR-21 expression in tumor initiation and maintenance and confirmed the dogma of miRNA addiction in tumors [16]. The complete comprehension of miRNA expression in disease has allowed identification of misregulation of miRNA in other sites like tumor metastases [17,18], blood circulation [19,20] and later in tumor Cancer Cell-Derived exosomes and the microenvironment [21].

To treat oncomiR-addicted tumors, researchers have used a number of innovative tools including the first generation of an inhibitor of miRNA function in *C. elegans* that consists of *2′-O*-methyl oligonucleotides. To test it, researchers injected this *let-7-c* oligonucleotide modified compound into *C. elegans* larvae and observed an efficient derepression of lin-41, a

well-known *let-7* target [22]. From that point, a variety of innovative tools for inhibition or reintroduction of miRNAs have been developed, including locked-nucleic acid (LNA)-inhibitor for various miRNA with oncogenic functions like miR-221 or miR-155 [23,24] see Rupaimoole and Slack for a review [25].

Because it is known that naked miRNA oligonucleotides are rapidly degraded in blood circulation, optimization of new drug delivery systems allowing potent and safe delivery of RNA-based therapy including miRNAs are needed for *in vivo* applications. One of them, first described in 2008, highlights a reduction of orthotopic KRAS G12D lung tumors after intranasal *let-7* administration. Then, enhancement of anti-miRs stability using 2′-O-methyl-modified nucleotides and phospho rothioate bonds lead to significantly reduces tumor burden in another model of lung cancer [26,27]. These *let-7* therapeutics has led to a generation of modified mimic formulations, including delivering the miRNA in a lipid-based delivery vehicle designed for systemic delivery of the miR-34a to lung tumor [28]. In the meantime, the first miRNA antagomir targeting miR-122 entered clinical phase I trials, followed by a miR-34a mimic in 2012. The RNA revolution continues to progress with development of various innovative engineering tools for RNA-based delivery, including use of peptide nucleic acid antimiRs with a low pH-induced transmembrane structure (pHLIP) targeting the acidic tumor microenvironment [29], and the tumor-penetrating-nanoparticles miR-21 (TPN-21) that pave the way to using miRNAs for personalized medicine in pancreatic cancer [30].

Meanwhile, deep sequencing has uncovered lncRNAs, circRNAs and various epigenetic regulations of non-coding RNA elements that are now extensively studied for further fundamental comprehension and therapeutic applications [31,32]. To date, http://Clinicaltrial.gov, a website that collects data on privately and publicly funded clinical trials, shows that there are 516 clinical trials ongoing or recruiting for miRNA research, 21 for lncRNAs and 2 for circRNAs.

Let-7 sparked the start of this RNA revolution and continues to play a central role in RNA therapeutic fields with more than 1479 publications to date. Currently, six clinical trials are recruiting patients, active, or terminated for studying *let-7* in various diseases including obesity, cancer, and diabetes, where it can be detected in a variety of samples from serum to tissues. For example, in one of these trials, they are using *let-7* for disease prognosis by investigating one *let-7* family member (*let-7i*) in the patient's serum to detect intracranial traumatic lesions [33]. A second study hopes to detect the level of various *let-7* members before and after radiotherapy to study neurological complications after radiotherapy in the brain, and in that case, *let-7* will be studied to predict chemoresistance [34]. For a clinical trial regarding obesity, they are measuring *let-7e* in the blood after a specific diet to follow diet loss and search for a biomarker for obesity.

Altogether, these examples of *let-7* utilization in different clinical trials highlight that *let-7*, and more generally miRNAs, are extensively used to classify and identify disease/cancer tissue origin, determine prognosis and disease progression, predict chemoresistance, monitor therapy, and screen for diseases – demonstrating huge promise for human therapy.

4. *Let-7* as a tool for therapy

A widely employed approach used to study miRNA gain or loss of function is the use of chemically modified oligonucleo-tide sequestering or reintroducing miRNAs. The first *in vivo* miRNA inhibitor was tested by microinjection of 2′-*O*-Me oligonucleotide complementary to *let-7* in *C. elegans*, promoting a loss-of-function phenotype in larvae [22]. Then, to allow a better contact with the cell membrane, 2′-*O*-Me oligonucleo-tides were modified by conjugation of a 3′ cholesterol motif, called antagomirs and tested first by intravenous injection of miR-16 in normal mice [35]. Chemical inhibition using an anti-*let-7* 2′OMe oligonucleotide underlined that *let-7* miRNA is able to promote the repression of cell proliferation pathways in human cells [36]. From this evidence, various therapeutic approaches have emerged for the targeting or re-introduction of *let-7* for therapy.

Our group showed one of the first pieces of evidence that *let-7* plays a key role in cancer. By showing that RAS is regulated by the *let-7* miRNA family in *C. elegans* and by highlighting *let-7* complementary sites in human RAS 3′UTRs, we first validated that the *let-7* miRNA family negatively regulates RAS in two different *C. elegans* tissues and human cell lines and lung tissue. This was the first report of the mechanism of action of a miRNA acting as a tumor suppressor [37].

Later, *let-7* was associated with lung cancer by showing that ectopic expression of *let-7g* in *Kras* driven lung cancer cells promoted both cell cycle arrest and cell death [38]. More generally, all the *let-7* family members are dysregulated in cancer with the majority of them found downregulated. Loss of *let-7* family members also predicts poor survival in various types of cancer [39]. For therapy, numerous innovative approaches using tumor-suppressive activity of *let-7* have been used for cancer therapy. For example, systemic mimic delivery using neutral lipid emulsions to reintroduce *let-7* in lung tumors. This delivery system showed that systemic delivery of *let-7b* promoted its accumulation into the tumor site and induced tumor-inhibitory effects in an autochthonous $KRAS^{G12D}$ transgenic mouse model of lung cancer [40]. Table 1, highlights which specific *let-7* family member expression levels (high or low) is associated with a significant low overall survival from a variety of cancers patient databases [41]. This table suggests not only which tumor types might benefit most from *let-7* therapy, but also suggests which *let-7* family members have to be targeted or reintroduced for therapy. This analysis suggests that Kidney renal clear cell carcinoma and Brain lower grade glioma tumor types may benefit most from *let-7* therapy (respectively mimics and anti-miRs) (Figure 1).

Inhibition of the oncogene c-MYC by the reintroduction of the same *let-7b* mimic has been shown to reverse multidrug resistance in gastric cancer and similarly, *let-7* sensitizes cells to chemotherapy in a model of *KRAS* mutant cells [42,43]. Favorable outcomes in other diseases, such as preventing obesity-induced glucose intolerance or improving cardioprotection and cardiac function, using anti-*let-7* strategies have also been observed [44–46]. Diverse mimics have also been used to reintroduce *let-7* family members to counteract HCV, brain injury, endometrioses or inflammation [47–50].

Interestingly, a variety of approaches have also targeted key upstream effectors of *let-7* for therapy. The most notable way is to target Lin28b, a well-known regulator of the *let-7* family member expression/accumulation [51]. Lin28b targeting has been used for pancreatic cancer therapy where downregulation of oncofetal protein Lin28b through SIRT6 led to increased expression of *let-7* family member and acted as a method to promote growth inhibition of PDAC cells models [52].

5. *let-7* interacts with immune effectors during infections

MiRNAs have been reported to contribute to the formation and progression of diseases like cancer, but also to inflammatory disorders [53]. The body's initial defense against external pathogen infection is called the innate immune response. This immune response is well organized and starts with detection of the external pathogen by macrophages or dendritic cells and their specific receptors like (Toll-like). Activation of Toll-like receptors triggers many biological processes including apoptosis and cytokine expression that help counteract infections. In 2004, a *Science* paper showing that the production of mature B and T lymphocytes was modified after ectopic expression of miRNAs in hematopoietic stem cells and immature lymphocytes supported the idea that miRNAs represent a class of molecules that regulate hematopoiesis and are important in the immune system [54]. One of the first pieces of evidence linking the *let-7* miRNA and the immune response emerged in the model of SV40-transformed human cholangiocytes derived from the normal liver that were screened using a semi-quantitative microarray for 385 human miRNAs. In this study, using computational prediction analysis, they found that three members of the *let-7* family (*let-7b*, *let-7i*, *let-7g*) have complementarity to the 3′-UTR of toll-like receptor 4 (TLR4) mRNA [55]. In an *in vitro* model, modulation of *let-7i* caused alterations in immune response to *C. parvum* infection through modulation of TLR4 expression [55]. Additionally, *let-7* miRNA has been linked to the TLR-mediated inflammatory response in many other diseases such as coronary artery disease and in an oxygen-glucose deprivation model [56,57]. In dilated cardiomyopathy, levels of *let-7i* are negatively correlated with TLR4 protein levels. Furthermore, low levels were associated with poor clinical outcome [58]. As miRNAs take part in the immune response against viral, bacterial, fungal, and parasitic infections, studies have also highlighted a relationship between *let-7* family members and the regulation of the innate immune response to pathogenic infection and stress. They all show that *let-7* family members target *TLR4* to regulate innate immunity and inflammation in response to *P. aeruginosa* or *Helicobacter pylori* infection [59–61]. It has also been reported that use of loss-of-function *let-7* mutants impaired the colony-forming units of a type of *P. aeruginosa* in the nematode model. This change of *let-7* expression drove increased transcriptional expression of specific antimicrobial genes that enhance the innate immune response [59].

By performing a functional anti-HCV (Hepatitis C Virus) luciferase reporter screen using mimic and inhibitors, a study demonstrated an important role for *let-7b* during HCV infection. They showed that deletion of the wild-type seed region of *let-7b* plays a crucial role in its antiviral activity via targeting of the HCV genome and host gene [62].

Another key effector of immune response and inflammation is the transcription factor NF-κB, which promotes immunity by driving expression of target genes that mediate cell

proliferation and the release of antimicrobial molecules and cytokines to activate the immune response. During *Mycobacterium tuberculosis* infection (Mtb), *let-7f* has been shown to target A20, a feedback inhibitor of the NF-κB pathway that regulates the innate immune response. Moreover, downregulation of *let-7f* is associated with concomitant upregulation of a number of its putative targets. These results reveal a role for *let-7f* and its target A20 in regulating immune responses to Mtb and is directly associated with enhanced NF-κB activity [63].

In other infectious diseases, *let-7* has been linked to the regulation of NF-κB. First, a significant decrease in *let-7i* was observed by northern blot in H69 cells after *C. parvum* infection. Moreover, reintroduction or inhibition of *let-7i* precursor in H69 cell line can modify SIRT1 protein expression and could modulate NF-κB activation [64]. Taken together, these examples highlight a strong role for *let-7* in the immune response and inflammation, and suggest that *let-7* may also regulate cancer cell immune evasion.

6. *Let-7* as a new tool for immunotherapy

Cancer development and progression have been extensively studied and a variety of hallmarks of cancer are now extremely well-known, allowing researchers to propose innovative therapies. Among them, cancer immunity, i.e. immune cell infiltration into the tumor site and tumor microenvironment, regulates the equilibrium between pro or anti-tumoral behavior [65]. Various molecular players are known to regulate cancer immune response, including miRNAs [66]. *Let-7* has been reported to control maintenance and development of immature and mature immune cells [66] but also to control differentiation and function of natural killer T cells, a subset of T cells [67,68]. The role of *let-7* in the suppressive function of Tregs has also been reported [3] as well as that *let-7* family members suppresses B cell activation [69].

MiRNA and specifically, *let-7c* has been reported to play a role in regulating macrophage plasticity. Briefly, macrophages have the ability to switch between different functional pheno-types that are called M1 or M2. M1 macrophages are critical to counteract external infections by bacteria or viruses whereas M2 macrophages are found in the inflammatory zone and participate in many remodeling biological process such as angiogenesis and tumor progression. In a fibrotic mouse model, isolated macrophages showed significantly increased *let-7c* levels. By loss and gain of function studies, the authors show that *let-7c* is a negative regulator of M1 macrophage phenotypes and that *let-7c* overexpression promotes the GMBMM transition to the M2 phenotype [70].

Another study reported that *let-7* facilitates T-cell anergy. mTOR and Rictor, two members of MTOR, are miRNA targets in cancer. By using miRNA deficiency approaches, it has been shown that ability of CD4 T cells to decide between an activating and anergizing state requires post-transcriptional regulation of the mTOR components by *let-7* and miR-16. These results show that expression of mTOR components in T cells is regulated by *let-7* miRNAs [71].

As described earlier, *let-7* family members generally function as tumor suppressors by targeting multiple oncogenes, including *RAS*, *HMGA2*, and *c-Myc*, but also act as immune regulators (Figure 2) [72]. Multiples lines of evidence described earlier suggest that the *let-7* family can distort or inhibit immune responses and promote tumor immune evasion. A study performed in 2016 in a colorectal cancer model highlighted the inverse association between *let-7a* expression levels and the densities of CD8+ cells and CD45RO+ T cells in the tumor site, associated with a favorable prognosis. They also highlighted a correlation between *let-7a* expression and colorectal cancer mortality. This work nicely supports the idea that *let-7a* can suppress antitumor immunity in this cancer and suggests that *let-7a* could inhibit the T cell immune response against colorectal cancer, highlighting *let-7a* as a good target for immunotherapy [73].

CD8 T cells promote rapid clearance of cancer cells or virus-infected-cells. In a study from 2017, the authors identified a new role for *let-7* in CD8 T cell differentiation and function. They demonstrated that a high level of *let-7* miRNAs is required to maintain the naive phenotype of CD8 T cells. Moreover, they show that overexpression of *let-7* copy number impairs the proliferation and differentiation of CD8 T cells by modulating the expression of genes involved in the regulation of the cell cycle and metabolism, such as Myc [74].

Cytokines are soluble substances of 8 to 50 kDa, synthesized and secreted by various tissues or cells, including immune cells, to regulate the functions other cells. Cytokines include different subgroups like interferons, interleukins or chemokines. Their production is mediated in response to foreign antigen presentation by the immune system. One of the first reports linking *let-7* to cytokine expression was published in 2008. By computational approaches, the authors reported a putative miRNA binding site for *let-7* in the interleukin 6 sequence (*IL-6*) [75]. In another study using RAW 264.7 cells that have been infected with the *bacteria Salmonella*, the authors observed a downregulation of *let-7* family members. By monitoring cytokine expression using a Renilla luciferase ORF in a reporter vector that also expresses firefly luciferase in mouse embryonic fibroblast (MEF) cells, they observed that *let-7* family members post-transcriptionally repress the production of cytokines interleukin-10 and 6 (IL-10, IL-6). This study was the first to provide molecular evidence of how the *let-7* family was implicated in anti-bacterial defense during *Salmonella* infection and to show that

Repression of *let-7* family alleviates the negative post-transcriptional regulation of IL-6 and IL-10, which are the key players of the immune response. This work establishes a strong link between *let-7* expression and the modulation of activation of inflammatory factors during pathogen invasion [76]. Similarly, in HIV-1 infection, downregulation of *let-7* miRNAs promotes IL-10 expression from CD4(+) T cells. As elevated IL-10 occurs during HIV-1 infection, and because *let-7* regulates IL-10 expression in CD4 T cells, the decrease of *let-7b* and c expression after infection drives an immune change favorable for the virus survival [77].

It has been also reported that IL-10, an anti-inflammatory cytokine, has a well-established role in neuroprotection. One paper demonstrated that extracellular miRNA *let-7*, which is highly expressed in the central nervous system, induces neuronal cell death [78].

Let-7 family members have been also shown to target another interleukin (IL-13) *in vivo* and *in vitro* in a lung model of experimental asthma [79]. Interferon-γ (IFN-γ) is an important member of the interferon family that regulates various biological purposes like the antiviral response, and immunomodulatory responses. Interferon-γ is an essential player in the modulation of anti-tumor effector because it sensitizes Fas-related apoptosis in colon cancer cells. Using a luciferase expression assay, it has been reported that *let-7* directly inhibits expression of Fas by binding to a defined target sequence in the 3′UTR. By combining *let-7* inhibitor and FAS mABs they also determined that *let-7* suppression sensitizes Fas-induced apoptosis of HT29 cells *in vitro* as well as *in vivo* [80]. As FAS and *let-7* are inversely correlated in various other cancer cells these findings could notably influence new clinical approaches for tumor therapy.

In another recent study, a group has studied the relationship between *let-7* expression, *Fas* and *FasL* and bone marrow-derived mesenchymal stem cells (MSCs) for the treatment of inflammatory diseases. It is already known that Fas and FasL are essential for MSCs to induce T cell apoptosis. Using an *in vitro* transwell system and an experimental colitis mouse model, they confirmed that knockdown of *let-7a* in MSCs significantly promoted MSC-induced T cell migration and apoptosis *in vitro* and *in vivo*. In addition, they observed a strong change in immune response by suppression of the inflammation reaction in MSCs from a *let-7a* knocked down mouse model. Altogether, by identifying *let-7a* as a negative modulator of FasL/Fas they highlight a nice way to improve MSC immunotherapy by enhancing FasL/Fas expression [81]. Finally, these studies show that *let-7* modulation can be a tool to suppress tumor immune reactions.

7. Conclusion

miRNAs and specifically *let-7* are very attractive targets for cancer therapy but have also emerged now as key players in immune responses that modulate cancer initiation and progression. As described earlier, many studies suggest that the *let-7* family can regulate or inhibit immune responses and promote tumor immune evasion, and multiple strategies have emerged to develop therapeutic approaches for the targeting or re-introduction of *let-7* for therapy. Taken together, this review highlights *let-7* as a new target for immunotherapy.

8. Expert opinion

let-7 is the most studied miRNA in biology and the *let-7* family plays a major role in the regulation of pluripotency and differentiation in many species. It plays a major role in various process including development, lifespan, cell proliferation, differentiation, signaling pathways, apoptosis and metabolism but also in aging-associated diseases, viral infections, genetic disorders and cancer [82]. Here we have collated a non-exhaustive list of examples and scientific reports which highlight that *let-7* family members are an important node in the miRNA regulatory network that governs the immune response. More specifically, through regulation of Toll-like receptors, immune cell activation, T-cell anergy, and cytokine regulation, *let-7* appears to be a great tool for immunotherapy. Specifically, *let-7* therapy could be beneficial for some specific immune cell types including macrophages [70], lymphocytes T cells (NK, CD8+, CD4+, Treg) [67,68,73,77], lymphocytes B cells [69] and

Bone Marrow-Derived Mesenchymal Stem Cells [81]. As novel systems of drug delivery for RNA medicine have emerged [25,29,30], the reintroduction of *let-7* could be a strategy for the modulation of immune reactions and immunotherapy in cancer. However, as *let-7* is closely linked to immune response in various cancer cell models, utilization of RNA medicine for human therapy has to be handled with care and precision.

The first-in-human miRNA therapeutic trial (MRX-34 Phase I clinical trial) demonstrated some immune abnormalities including lymphopenia, neutropenia, and thrombocytopenia and side effects leading to five immune-related serious adverse events, causing the cessation of the phase I trial [83]. Because *let-7* family members can also display strong immune response upon various immune mediators (myeloid compartment, T cell, or Toll-like receptors) that we previously highlighted: utilization of *let-7* family members for therapy has to carefully developed to avoid unexpected and non-specific immune response. To this end, utilization of specific drug delivery systems that will target only tumor cells and relevant stroma has to be selected for human therapy. In addition, targeting specific cell surface receptors and adoption of next-generation oligonucleotide chemistry that will not initiate unwanted immune responses have to be developed for *let-7* RNA delivery. Finally, as current anti-cancer strategy has shown better efficiency by combining two or multiple therapeutic approaches (such as chemotherapy and immunotherapy), combo-therapy between the *let-7* family member and any other cancer modulator has to carefully studied to avoid non-specific cancer-related immune responses.

Another interesting point is the emergence of personalized medicine for therapy with use of personalized avatar models that help guide precision medicine for individual patients that show different genetic or immune patterns. In our previous study, through the utilization of Patient-Derived-Organoids as a rapid screening platform for our RNA-based-therapeutic (TPN-21) we showed for the first time, the potential of personalized models for RNA-based precision medicine therapy in PDAC [30]. By working with patient avatars, we have shown the potential to predict clinical outcomes of patients and thus align pre-clinical work to patient response to our RNA therapy. This major change of therapeutically screening is changing the landscape of cancer therapy and immunotherapy and are a valuable approach to deepen our knowledge regarding the roles and impact of *let-7* as a new target for immunotherapy.

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Article Highlights

- *let-7* discovered as the first mammalian miRNA
- *let-7* discovery was the starting point of the RNA revolution
- Emergence of chemically modified oligonucleotide sequestering or reintroducing miRNAs as a novel therapeutic modality
- *let-7* family members are an important node in the miRNA regulatory network that governs cancer progression and the immune responses

This box summarizes key points contained in the article.

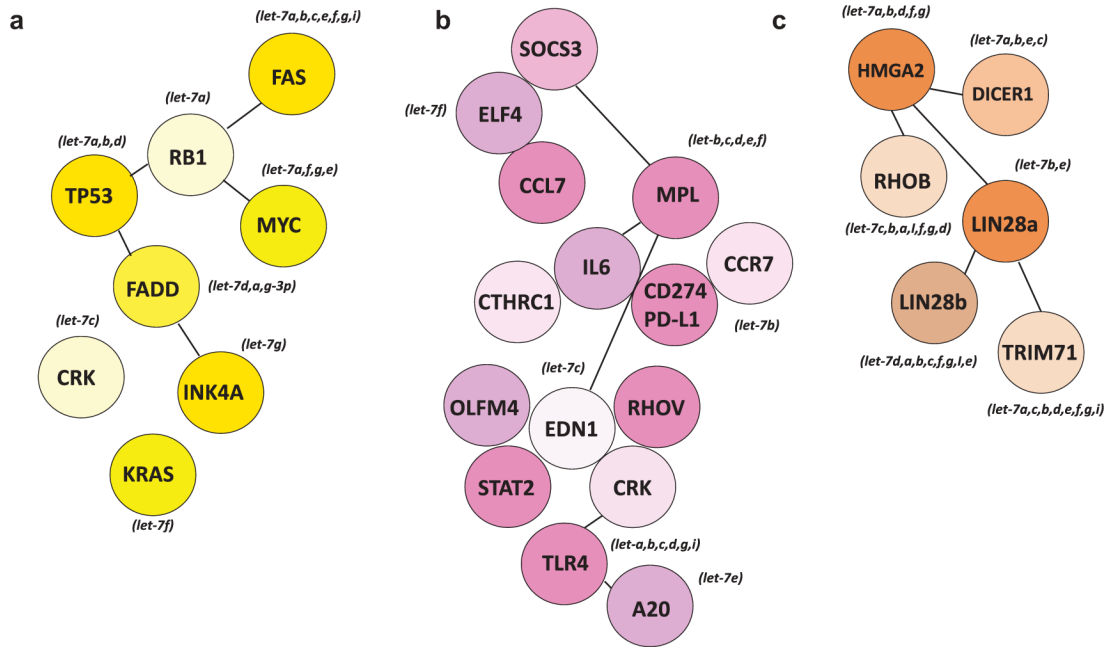

Figure 1.

miRNA prognostic plots from two *let-7* family member that may benefit most from *let-7* therapy. (a) Overall survival of hsa-*let-7*b miRNA expression in Kidney renal clear cell carcinoma. (b) Overall survival of hsa-*let-7*i miRNA expression in Brain lower grade glioma.

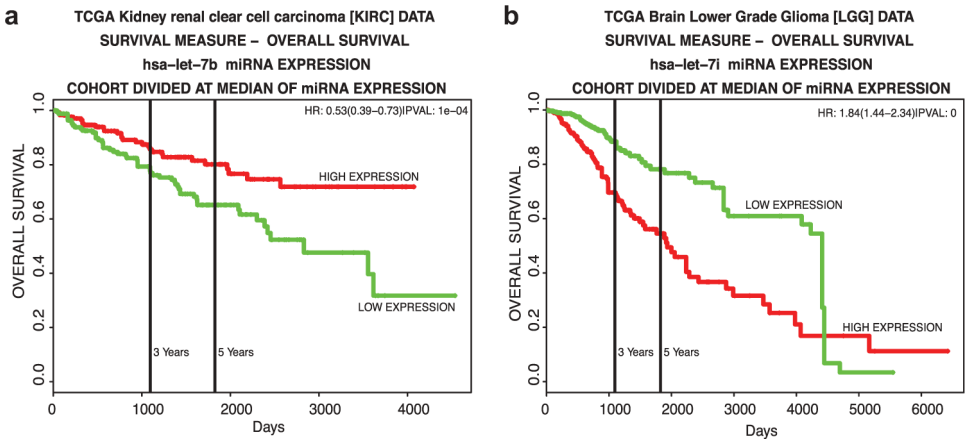

Figure 2.

let-7 targets with oncogene/tumor suppressor properties or immune-related properties. Validated targets gene were identified in DIANA-TarBase v8 considering the top candidates (1 ~ 40) of validated targets for each *let-7* family member. Shown are key *let-7* family target genes with oncogene or tumor suppressor properties (a) or immune-related properties (b) or general *let-7* family targets conserved among family members (c).

Table 1.

Let-7 family member expression associated with poor overall survival in cancers. Table 1 has been generated from the PROGmiR tool by querying each *let-7* family member's expression level and correlating this with overall survival (*p*-value ≤0.1) among 33 human cancer databases. Unsignificant result (*p*-value ≥0.1) from Cholangiocarcinoma [CHOL] Glioblastoma multiforme [GBM], Thymoma [THYM], Breast invasive carcinoma [BRCA], Diffuse Large B-cell Lymphoma, Cervical squamous cell carcinoma, and endocervical carcinoma, Rectum adenocarcinoma [READ] are not presented in the table.

Cancer types	Low miRNA expression associated to low survival		High miRNA expression associated to low survival	
Adrenocortical carcinoma [ACC]	*let-7* family member *let-7c*	*p*-Value 0.016948897	*let-7* family member *let-7b* *let-7d* *let-7g* *let-71*	*p*-value 0.030754687 0.081720566 0.052540823 0.033604282
Kidney renal papillary cell carcinoma [KIRP]	*let-7* family member *let-7g*	*p*-Value 0.006148305	*let-7* family member *let-71*	*p*-value 0.006714306
Lung adenocarcinoma [LUAD]	*let-7* family member *let-7b* *let-7c*	*p*-Value 0.053530532 0.020505798	*let-7* family member	*p*-value
Pancreatic adenocarcinoma [PAAD]	*let-7* family member *let-76* *let-7g*	*p*-Value 0.000419855 0.007191681	*let-7* family member *let-7b*	*p*-value 0.000614112
Sarcoma [SARC]	*let-7* family member	*p*-Value	*let-7* family member *let-7d* *let-71*	*p*-value 0.01726064 0.019594756
Thyroid carcinoma [THCA]	*let-7* family member *let-7d*	*p*-Value 0.034278599	*let-7* family member *let-7a-1* *let-7a-2* *let-7a-3* *let-7f1* *Iet-7f2* *let-7g*	*p*-value 0.04003319 0.040378847 0.039595321 0.038909032 0.023087711 0.008662061
Uveal melanoma [UVM]	*let-7* family member *let-7c* *let-7g*	*p*-Value 0.000107251 0.104325918	*let-7* family member *let-7b* *let-7e*	*p*-value 0.002430699 0.04168769
Bladder urothelial carcinoma [BLCA]	*let-7* family member *et-7f1* *let-7g*	*p*-Value 0.019933527 0.015729926	*let-7* family member *let-7c*	*p*-value 4.72E-05
Colon adenocarcinoma [COAD]	*let-7* family member	*p*-Value	*let-7* family member *let-7c* *let-7e* *let-7g* *let-7i*	*p*-value 0.086099976 0.063650106 0.032521736 0.073548534
Head and neck squamous cell carcinoma [HNSC]	*let-7* family member *let-7c*	*p*-Value 0.004211464	*let-7* family member	*p*-value

Cancer types	Low miRNA expression associated to low survival		High miRNA expression associated to low survival	
Acute myeloid leukemia [LAML]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
			let-7a-1	0.068066729
			let-7a-2	0.110382351
			let-7a-3	0.069096686
			let-7b	0.000228442
			let-7c	0.03908679
Lung squamous cell carcinoma [LUSC]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7f1	0.028973805		
	let-7f2	0.098768735		
	let-71	0.099783464		
Pheochromocytoma and paraganglioma [PCPG]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7a-1	0.006725665		
	let-7a-2	0.006375698		
	let-7a-3	0.00709643		
	let-7b	0.090803967		
	let-7c	0.082592342		
	let-7e	0.032377698		
	let-7f2	0.013186697		
	let-7g	0.109684624		
Skin cutaneous melanoma [SKCM]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7a-1	0.00202646		
	let-7a-2	0.002124937		
	let-7a-3	0.001942797		
	let-7b	0.014905228		
	let-7f1	0.088167942		
	let-7g	0.009275654		
Kidney chromophobe [KICH]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7c	0.001515206	*let-7a-1*	0.038444651
			let-7a-2	0.039861791
			let-7a-3	0.037566956
			let-7d	0.032730936
			let-712	0.086293043
			let-71	0.070056101
Brain lower grade glioma [LGG]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
			let-7a-1	0.037891823
			let-7a-2	0.038907961
			let-7a-3	0.039434459
			let-7c	0.006373482
			let-7e	6.17E-05
			let-7g	0.002189787
			let-7i	9.02E-07
Mesothelioma [MESO]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7f1	0.059549538		
	let-7i	0.101150698		
Prostate adenocarcinoma [PRAD]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7c	0.028908849	*let-7d*	0.006208984
			let-7g	0.05622126

Cancer types	Low miRNA expression associated to low survival		High miRNA expression associated to low survival	
Stomach adenocarcinoma [STAD]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7f2	0.078859686	*let-7c*	0.033118777
Uterine corpus endometrial carcinoma [UCEC]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7a-1	0.005929407		
	let-7a-2	0.005870681		
	let-7a-3	0.005808579		
	let-7b	0.013321126		
	let-7f2	0.029634711		
Esophageal carcinoma [ESCA]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7a-1	0.058000018		
	let-7a-2	0.054346774		
	let-7a-3	0.054789831		
	let-7b	0.119825178		
Kidney renal clear cell carcinoma [KIRC]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7a-1	0.009771075	*let-7i*	0.005679393
	let-7a-2	0.01042424		
	let-7a-3	0.009462964		
	let-7b	9.10E-05		
	let-7c	0.001029453		
	let-7e	0.082738752		
Liver hepatocellular carcinoma [LIHC]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7c	0.019666702	*let-7d*	0.086695391
			let-7i	0.083386848
Testicular ggerm ccell tumors [TGCT]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
			let-7f1	0.108508216
			let-7i	0.055108285
Uterine carcinosarcoma [UCS]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
			let-7f1	0.081338369
Ovarian serous cystadenocarcinoma [OV]	*let-7* family member	*p*-Value	*let-7* family member	*p*-value
	let-7d	0.091576628	*let-7a-1*	0.0770986
			let-7a-2	0.078229956
			let-7a-3	0.078569352