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Function and Regulation of MicroRNA-31 in Development and Disease

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SUMMARY

MicroRNAs (miRNAs) are small noncoding RNAs that orchestrate numerous cellular processes both under normal physiological conditions as well as in diseases. This review summarizes the functional roles and transcriptional regulation of the highly evolutionarily conserved miRNA, microRNA-31 (miR-31). miR-31 is an important regulator of embryonic implantation, development, bone and muscle homeostasis, and immune system function. Its own regulation is disrupted during the onset and progression of cancer and autoimmune disorders such as psoriasis and systemic lupus erythematosus. Limited studies suggest that miR-31 is transcriptionally regulated by epigenetics, such as methylation and acetylation, as well as by a number of transcription factors. Overall, miR-31 regulates diverse cellular and developmental processes by targeting genes involved in cell proliferation, apoptosis, cell differentiation, and cell motility.

INTRODUCTION

MicroRNAs (miRNAs) are conserved small non-coding regulatory RNAs that mediate translational repression and/ or induce decay of their target mRNAs in animal cells (Lewis et al., 2005; Lim et al., 2005; Iwama et al., 2007; Bartel, 2009; Hammond, 2015; Wilczynska and Bushell, 2015). Their transcripts form a hairpin secondary structure that is then subjected to a sequential processing by endoribonucleases DROSHA and DICER to yield short double-stranded RNAs (Bartel, 2009). One of the miRNA strands is then loaded onto an RNA-induced silencing complex (RISC). The miRNA in RISC binds to target mRNAs in a sequence-specific manner, primarily through the pairing of its seed sequence (2–8 bp of the miRNA's 5′ end) with the 3′ untranslated region (UTR) of its target mRNA, although it can also bind to the 5′UTR or coding region of its target mRNA (Bartel, 2009). A single miRNA can have multiple gene targets, and a single gene can be regulated by multiple miRNAs (Lewis et al., 2005; Lim et al., 2005; Iwama et al., 2007; Hammond, 2015; Wilczynska and Bushell, 2015).

miRNAs regulate numerous biological processes, and are present in all bilaterian animals or plants. According to the miRNA online database (miRBase.org), 466 mature miRNAs have been annotated in the fly *Drosophila melanogaster*; 70 in the sea urchin *Strongylocentrotus purpuratus*; 1,915 in mice; and 2,588 in humans (Kozomara and Griffiths-Jones, 2014). Many of the miRNAs are evolutionarily conserved. Interestingly, analysis of the sequence

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divergence and miRNA expression among various species revealed that higher expression of miRNA directly correlates with higher miRNA conservation (Liang and Li, 2009). Approximately 15.6% of the human miRNAs appear to be human-specific, and 38.7% are not conserved beyond primates—although only 453 of 834 human miRNAs annotated by 2012, were analyzed in this study (Kiezun et al., 2012; Mor and Shomron, 2013).

Here we review the function and regulation of the highly conserved miR-31 (Fig. 1), which is involved in diverse biological processes including fertility, embryonic development, bone formation, and myogenesis (Table 1). miR-31 has also been shown to be mis-regulated in a number of diseases, including cancer (Table 2) and autoimmune diseases, such as psoriasis and systemic lupus erythematosus.

miR-31 PROMOTES SPERMATOGENESIS AND FACILITATES EMBRYONIC IMPLANTATION

Spermatogenesis is a complex process in which the male germ cells develop into spermatozoa in sequential, well-regulated phases of mitosis, meiosis, and spermiogenesis (Luo et al., 2016). The testes of patients suffering from infertility may contain germ cells arrested at specific stages during development, with the most severe phenotype being the absence of any germ cells (germ cell aplasia) (Muñoz et al., 2015).

Spermatogenesis is regulated at the post-transcriptional level by piRNAs (piwi-interacting RNAs) and miRNAs (reviewed in Luteijn and Ketting, 2013; Luo et al., 2016). miR-31 is expressed in the testis but not in mature spermatozoa (Krawetz et al., 2011; Muñoz et al., 2015), and was found to be among the least-abundant miRNAs in the testes of patients suffering from the germ cell aplasia compared to the testes of healthy men, suggesting its potential role in promoting early sperm development (Muñoz et al., 2015). Yet the molecular mechanism of how miR-31 regulates sperm development is unknown.

In contrast to the testes, human ovaries do not express miR-31, although the level of miR-31 expression was significantly elevated in both the endometrium and serum during the time of embryonic implantation (Kuokkanen et al., 2010; Kresowik et al., 2014). Uterine endometrium receptivity is critical for the success of embryo implantation (Kresowik et al., 2014). The exact role of miR-31 in endometrium receptivity is not yet known; however, the observed increased miR-31 expression during implantation suggest that it participates in the creation of an immune-tolerant maternal environment.

miR-31 may mediate maternal immunotolerance by suppressing *FOXP3* (Forkhead Box P3) and *CXCL12* (C-X-C Motif Chemokine Ligand 12). *FOXP3*, which is directly suppressed by miR-31, encodes a master regulator of the differentiation and activity of T regulatory (T_{reg}) cells (Rouas et al., 2009). T_{reg} cells are a subset of immune T cells that are critical for the maintenance of immune cell homeostasis as well as maternal immunotolerance to the developing fetus (reviewed in Vignali et al., 2008; La Rocca et al., 2014; Li and Zheng, 2015). Down-regulation of miR-31 in the T_{reg} cells is required for the accumulation of FOXP3 to regulate the differentiation and function of T_{reg} cells (Rouas et al., 2009). Interestingly, chromatin immunoprecipitation assays revealed that FOXP3 may bind to and

repress the *miR-31* promoter in these induced T_{reg} cells, thus suggesting a negative regulatory feedback loop (Zhang et al., 2015). An additional mechanism in which miR-31 regulates implantation may be through its suppression of *CXCL12*, a chemokine ligand that establishes proper fetal and maternal connections during pregnancy (reviewed in Hanna et al., 2003, 2006; Erlebacher, 2011). *CXCL12* can be directly regulated by miR-31 in mice (Itkin et al., 2012). An inverse expression pattern between the levels of miR-31 and its target *FOXP3* and *CXCL12* transcripts was observed in endometrium tissue and serum during the window of implantation, suggesting that miR-31 directly suppresses *FOXP3* and *CXCL12* to ensure an immune-tolerant environment for the implanted fetus (Kresowik et al., 2014). Thus, miR-31 is a potential biomarker that can be used to monitor the activity of endometrium to assess the success of embryonic implantation (Kresowik et al., 2014).

miR-31 REGULATES DIVERSE PROCESSES DURING EMBRYONIC DEVELOPMENT

miRNAs are known regulators of embryonic development (reviewed in Pauli et al., 2011). Several studies reported the expression pattern and importance of miR-31 in regulating embryogenesis of various organisms. Depletion of miR-31 in *Drosophila* embryos results in severe segmentation defects (Leaman et al., 2005). The segmented body plan in the *Drosophila* embryo is determined by transcription factors, encoded by gap genes, that control the expression of pair-rule genes. Pair-rule genes, in turn, activate segment-polarity genes that regulate the WNT and HH (Hedgehog) signaling pathways in determining the polarity of the embryonic parasegments. Mutation of pair-rule genes results in loss of the normal developmental pattern of the segmented insect embryos. The direct targets of miR-31 in the *Drosophila* embryo have not been identified; however, miR-31 knockdown induced the mis-expression of pair-rule genes *eve* (*even skipped*), *ftz* (*fushi tarazu*), and *hairy*, as detected by RNA in situ hybridization, indicating pattern-formation defects (Leaman et al., 2005). This observation suggests that miR-31 may indirectly regulate these pair-rule genes, which is consistent with the spatial expression of miR-31 in a pair-rule pattern in the foregut, anterior endoderm, and hindgut of *Drosophila* embryos (Aboobaker et al., 2005).

miR-31 may be one of the most highly abundant miRNAs that is ubiquitously expressed during *Strongylocentrotus purpuratus* (sea urchin) early development (Song et al., 2012; Stepicheva and Song, 2015). The knockdown of miR-31 in the sea urchin embryos resulted in a range of dose-dependent phenotypes, including the formation of extra cells and cell clumps in the blastocoel of the embryo, gut widening and reduction of the embryo size, as well as skeletogenesis defects (discussed below) (Stepicheva and Song, 2015).

In *Danio rerio* (zebrafish) embryos, the knockdown of miR-31 with loss-of-function morpholinos did not result in significant phenotypes, whereas the overexpression of miR-31 resulted in internal lymphatic vascular defects (Pedrioli et al., 2010). Similarly, overexpression of miR-31 in *Xenopus laevis* (frog) embryos resulted in dose-dependent reduction in venous sprouting, but no other developmental defects (Pedrioli et al., 2010). While miR-31 is ubiquitously expressed in the zebrafish embryos (Wienholds et al., 2005), it has a specific function in vascular development.

The regulation of vascular development by miR-31 may involve its direct repression of *PROX1* (Prospero Homeobox 1), which encodes a well-characterized lymphatic transcription factor. Bioinformatics analysis of potential miR-31 targets revealed a high conservation of the binding site of miR-31 in the 3′UTR of *PROX1* in humans, frogs, and zebrafish (Pedrioli et al., 2010). Moreover, *PROX1* can be directly suppressed by miR-31 in primary human lymphatic and blood vascular endothelial cells isolated from neonatal human foreskins (Pedrioli et al., 2010), indicating that the function of miR-31 may be conserved in regulating lymphatic vasculature via *PROX1* in vertebrates. In addition, miR-31 also inhibits vasculature development in the adult by directly suppressing *LATS2* (Large Tumor Suppressor Homolog 2) in rats and *CREG* (Cellular Repressor of E1A-stimulated Genes) in humans, which both promote cell proliferation (Liu et al., 2011; Wang et al., 2013).

miR-31 REGULATES MYOGENESIS

No data are currently available on the effect of systemic *miR-31* knockout or overexpression in the mouse. miR-31 in the mouse brain may be involved in preventing inappropriate accumulation of the key myogenic transcription factor *Myf5* (Daubas et al., 2009). *Myf5* is transcribed at the onset of myogenesis in the somite and limb bud, as well as in some of the restricted domains of the ventral mesencephalon, prosencephalon, and neural tube of the mouse embryo (Daubas et al., 2009). *miR-31* is highly expressed in areas where *Myf5* is transcribed but not translated, as miR-31 directly suppresses the translation of *Myf5* to prevent myogenesis in the developing brain (Daubas et al., 2009).

miR-31 also regulates myogenesis in adult murine tissues by suppressing the activation of muscle satellite cells (Crist et al., 2012). Skeletal muscle satellite cells are the equivalent of myogenic stem cells, and are usually maintained in a quiescent state. They are activated in response to injury, giving rise to regenerated muscle and new satellite cells (Morgan and Partridge, 2003). The activation of muscle satellite cells requires MYF5. In the quiescent satellite cells, *Myf5* mRNA is transcribed, but not translated, due to its sequestration in the messenger ribonucleoprotein granules along with miR-31 (Crist et al., 2012). These granules are dynamic, self-assembling structures containing translationally silent mRNAs bound by various proteins (reviewed in Buchan, 2014). Upon activation of the satellite cells, messenger ribonucleoprotein granules dissociate, leading to a rapid release of translatable *Myf5* mRNA. Importantly, exogenous overexpression of miR-31 suppressed the translation of *Myf5* in activated satellite cells, resulting in the disruption of normal muscle satellite cell activation (Crist et al., 2012). Thus, decreased abundance of miR-31 or increased abundance of MYF5 is necessary for the activation of satellite cells in muscle regeneration.

Skeletal muscles are highly dynamic tissues that adapt to the level of exercise performed. Interestingly, acute endurance exercise resulted in decreased abundance of miR-31 in human muscle biopsies, despite the increased transcript abundance of the key miRNA biogenesis proteins DROSHA, DICER1, and XPOT5 (exportin 5) (Russell et al., 2013). The exact mechanism or role of miR-31 down-regulation has not been elucidated; nevertheless, miR-31 may be involved in regeneration and delaying skeletal muscle atrophy after exercising.

Mis-regulation of miR-31 may lead to myopathies. In wild-type mice, *miR-31* expression was high at the early stages of differentiation of muscle satellite cells, but progressively decreased at later differentiation stages (Cacchiarelli et al., 2011). Persistent accumulation of miR-31 in muscle satellite cells was associated with severe myopathies, as in the case of Duchenne muscular dystrophy (DMD), a genetic disorder caused by the mutations in *DYS (Dystrophin)* (Cacchiarelli et al., 2011). DYSTRO-PHIN is critical for the proper muscle formation, linking the internal cytoskeleton to the transmembrane protein (sarcoglycan complex) at the plasma membrane that interacts with the extracellular matrix (reviewed in Nowak and Davies, 2004). miR-31 directly suppress *DYS* in mice and humans, and its accumulation in regenerating myoblasts in the patients with Duchenne muscular dystrophy resulted in a lower differentiation potential (Cacchiarelli et al., 2011). Thus, repression of miR-31 function in compromised adult muscles may improve the efficiency of therapeutic treatments aimed at the accumulation of DYSTROPHIN in muscles (Cacchiarelli et al., 2011).

miR-31 also plays a role in mouse cardiac myocytes during injury (Wang et al., 2015b). Cardiac ischemia/reperfusion injury results in the accumulation of miR-31 in the myocardium and the down-regulation of its direct target *Pkcε* (Protein Kinase C epsilon) (Wang et al., 2015b). Decreased PKCε down-regulates NFκB, whose activation is important for ischemic late pre-conditioning (a delayed adaptive response to increase heart resistance to ischemia/reperfusion injury) (Xuan et al., 1999; Wang et al., 2015b). Treatment of post-injury cardiac myocytes with miR-31 inhibitor was cardioprotective and reduced myocardial infarct size (Wang et al., 2015b).

Thus, miR-31 has been shown to regulate myogenesis in adult tissues through suppression of *Myf5* and *Dystrophin* during the differentiation of muscle cells and to respond to cardiac myocyte ischemia/reperfusion injury through suppression of PKCε.

miR-31 MAINTAINS BONE HOMEOSTASIS IN THE ADULT VERTEBRATES AND MODULATES SKELETOGENESIS IN THE INVERTEBRATE SEA URCHIN EMBRYOS

The vertebrate skeleton is a complex, metabolically active tissue that is remodeled throughout life in order to maintain the shape, quality, and size of the bone (Hadjidakis and Androulakis, 2006; Fisher and Franz-Odendaal, 2012; Long, 2012). A number of evolutionarily conserved pathways are reportedly involved in proper formation and maintenance of bone in vertebrates. Recent studies also indicate that miRNAs control multiple targets of the vertebrate skeletogenic gene regulatory networks, from initial response of stem/progenitor cells to the structural and metabolic activity of the mature tissue (Lian et al., 2012; Zhao et al., 2014).

Bone remodeling in vertebrates begins with the resorption of mineralized bone by osteoclasts specialized multi-nucleated cells that differentiate from hematopoietic stem cells (Fig. 2) (Boyle et al., 2003; Hadjidakis and Androulakis, 2006). Following adhesion to the fragment of the bone that needs to be remodeled, osteoclasts undergo a significant

polarization and cytoskeleton reorganization in order to form a specialized extracellular compartment where protons and proteases are released to demineralize the bone (Boyle et al., 2003; Hadjidakis and Androulakis, 2006; Itzstein et al., 2011). miR-31 is highly abundant in osteoclasts, and inhibition of miR-31 resulted in impaired osteoclast function (Mizoguchi et al., 2013). One of the direct miR-31 targets involved in osteoclast function is *RHOA* (RAS Homolog Family Member A), which is essential for osteoclast cytoskeleton reorganization, and thus is critical for the ability of osteoclasts to resorb the bone (Destaing et al., 2005; Itzstein et al., 2011; Mizoguchi et al., 2013). The identity of *RHOA* as a miR-31 target is supported by the finding that inhibition of *RHOA* restores osteoclast maturation in bone marrow-derived macrophages treated with miR-31 inhibitor (Mizoguchi et al., 2013).

Following the resorption of bone by osteoclasts, specialized mononuclear cells removing demineralized undigested collagen from the bone surface (Raggatt and Partridge, 2010). These mononuclear cells also produce signals to attract osteoblasts, the specialized cells that are responsible for the formation of the new bone matrix (Hadjidakis and Androulakis, 2006). Osteoblasts differentiate from multi-potent mesenchymal cells in response to two main transcription factors, SATB2 (SATB Homeobox 2) and SP7/OSX (Osterix), whose transcripts are negatively regulated by miR-31 (Baglìo et al., 2013; Deng et al., 2013a; Xie et al., 2014). miR-31 is down-regulated in bone mesenchymal stem cells during osteogenic differentiation in humans and rats (Baglìo et al., 2013; Deng et al., 2013a). Down-regulation of miR-31 in bone marrow mesenchymal stem cells, bone marrow stromal stem cells, and adipose tissue-derived stem cells of the rats and dogs was also necessary for repairing critical-size bone defects, which are the smallest size defects that would not heal without medical intervention, indicating the potential importance of miR-31 in bone injury therapeutics (Deng et al., 2013b, 2014a,b).

Several studies suggest a regulatory loop in the osteogenic differentiation process (Deng et al., 2013a; Ge et al., 2015) (Fig. 3). RUNX2 (RUNT-related Transcription Factor 2) transcriptionally activates *SP7/OSX*, resulting in an accumulation of its downstream target SATB2 in differentiating osteoblasts (Nakashima et al., 2002; Tang et al., 2011). SATB2, in turn, physically interacts with RUNX2 to enhance the transcriptional activation of *SP7/OSX* (Dobreva et al., 2006). In addition, RUNX2 suppresses *miR-31* transcription through direct binding of its promoter, thus removing the miR-31-mediated translational silencing of *SP7/OSX* and *SATB2* (Deng et al., 2013a).

This regulatory loop plays an important role not only in osteogenic differentiation but also in tooth eruption. Patients with cleidocranial dysplasia (delayed tooth eruption) were found to have mutations in *RUNX2*, and thus higher levels of miR-31 and down-regulated SATB2 compared to the healthy individuals (Ge et al., 2015). Knockdown of miR-31 in dental follicle cells from patients with cleidocranial dysplasia led to increased levels of SATB2 and RUNX2, as well as the rescue of osteoclast-inductive and matrix degradation capacities (Ge et al., 2015).

In addition to its role in the bone injury repair and teeth eruption, miR-31 is a critical component of the age-related reduction of osteogenesis (Weilner et al., 2016). miR-31 is significantly elevated in the plasma of elderly people or patients with osteoporosis.

Senescent endothelial cells secrete miR-31 in microvesicles that are taken up by mesenchymal stem cells, where miR-31 may inhibit osteogenic differentiation by suppressing *FZD3* (Frizzled-3), which encodes a receptor for WNT5 signaling (Weilner et al., 2016). Previously increased WNT5A is associated with BMP2 (Bone Morphogenetic Protein 2)-mediated osteoblast differentiation (Nemoto et al., 2012), and *FZD3* mRNA was up-regulated during osteogenesis (Chakravorty et al., 2014). Elevated *FZD3* transcript abundance in age-related reduction of osteogenesis correlated with reduced miR-31 (Weilner et al., 2016).

Thus, miR-31 regulates bone maintenance in vertebrates by modulating both osteoclasts (through *RHOA*) and osteoblasts (through *SP7/OSX*, *SATB2*, and potentially *FZD3*) (Fig. 2A). Further, knockdown of miR-31 resulted in increased bone volume and mineralization due to the suppression of osteoclast function and promotion of osteoblast differentiation (Fig. 2B) (Baglìo et al., 2013; Deng et al., 2013a,b; Mizoguchi et al., 2013).

The role of miR-31 in regulating skeletogenesis is not restricted to vertebrates; indeed, this function seems to be a conserved mechanism in invertebrates. miR-31 is critical for regulation of skeletogenesis in the sea urchin embryo (Stepicheva and Song, 2015), an echinoderm and a sister group to the chordates (McClay, 2011). Sea urchin embryos undergo less complex skeletogenesis than vertebrates. The larval skeleton comes from a single cell type, the primary mesenchyme cells (PMCs) (Oliveri et al., 2003). The larval skeleton supports larval swimming, the shape of the larvae, as well as larval feeding (Pennington and Strathmann, 1990; Hart and Strathmann, 1994; Piacentino et al., 2015). For proper skeletogenesis, the PMCs need to differentiate, undergo an epithelial-to-mesenchymal transition, fuse with each other, and localize into the correct pattern (Sharma and Ettensohn, 2010; Rafiq et al., 2012; Lyons et al., 2014; Saunders and McClay, 2014; McClay, 2016). The complexity of the signals received by the PMCs is not known, but the process of PMC positioning or patterning is, in part, dependent on VEGF (Vascular Endothelial Growth Factor) signaling, ALK4/5/7(Transforming Growth Factor Beta receptors), SLC26A2/7 (Solute Carriers 26a2 and 7), LOX (Lipoxygenase), and BMP5/8 (Bone Morphogenetic Proteins 5 and 8) (Duloquin et al., 2007; Adomako-Ankomah and Ettensohn, 2013, 2014; Piacentino et al., 2015,2016a,b). Knockdown of miR-31 resulted in a significant decrease in the length of dorsoventral connecting rods, formation of extra tri-radiates, as well as PMC patterning defects in the sea urchin gastrulae (Stepicheva and Song, 2015).miR-31 directly suppresses at least three transcription factors (*SpPmar1*, *SpAlx1*, and *SpSnail*) and one effector gene (*SpVegfr7*) within the sea urchin skeletogenic gene regulatory network. Inhibition of *SpAlx1* and/or *SpVegfr7* by miR-31 in the developing embryo results in a less severe, but similar, PMC defect to the phenotype of miR-31 inhibition, suggesting that those targets contribute to the same regulatory pathways in sea urchin embryo. In addition, miR-31 regulates expression of *SpVegf3*, which encodes an ectodermal ligand that is critical for the positioning of the PMCs, by a yet-to-be-identified mechanism (Stepicheva and Song, 2015). The fact that miR-31 regulates both the signal receiving PMCs and signal-sending ectoderm suggests its ability to cross-regulate multiple pathways to ensure proper skeletogenesis.

ROLE OF miR-31 IN CANCER IS CONTEXT-DEPENDENT

miR-31 plays an important role in different types of cancers, including breast (Sossey-Alaoui et al., 2011; Lu et al., 2012; Körner et al., 2013; Mulrane et al., 2014; Viré et al., 2014), ovarian (Anderson et al., 2010; Yu et al., 2010; Hassan et al., 2015), lung (Liu et al., 2010; Meng et al., 2013; Dong et al., 2014; Edmonds et al., 2016; Yu et al., 2016), colon (Cottonham et al., 2010; Cekaite et al., 2012; Xu et al., 2013b; Kim et al., 2014; Li et al., 2015c; Kurihara et al., 2016), and melanoma (Greenberg et al., 2011; Asangani et al., 2012). Intriguingly, even though the expression of miR-31 is consistently altered in various cancers, miR-31 can perform either tumor-suppressive or oncogenic functions, depending on the type of cancer (Table 2) (reviewed in Laurila and Kallioniemi, 2013).

The best-characterized example of differential miR-31 expression in cancer cells is its down-regulation in breast cancer, where miR-31 has been shown to serve as a tumor suppressor miRNA (Augoff et al., 2011). In breast cancer cell lines, miR-31 suppresses translation of genes involved in apoptosis (such as *PKCε*), cell motility (such as actin remodeling genes *WASF3* [WAS Protein Family Member 3] and *RHOA*, and *ITGB1* [Integrin Beta 1]), and cell invasion (*GNA13* [G protein alpha-13]), through activation of RHOA) (Augoff et al., 2011; Sossey-Alaoui et al., 2011; Körner et al., 2013; Rasheed et al., 2015).

miR-31 is also down-regulated in patients with leukemia, in which it suppresses *MAP3K14* (NFκB-inducing Kinase) to repress NFκB signaling (Yamagishi et al., 2012). The loss of miR-31 results in constitutive activation of NFκB that contributes to abnormal cell proliferation as well as inhibition of apoptosis (Yamagishi et al., 2012). miR-31 also has a tumor suppressor function in glioblastomas, which are a fast-growing, aggressive tumor of the central nervous system that form on the supportive tissue of the brain (Bleeker et al., 2012; Hua et al., 2012; Zhou et al., 2015; Zhang et al., 2016). In glioma cells, miR-31 inhibits *RADIXIN*, which encodes a cytoskeletal protein that is essential for cell motility, adhesion, and proliferation, as well as *DOCK1* (dedicator of cytokinesis 1), which encodes a promoter of epithelial-to-mesenchymal transition through NFκB/ SNAIL signaling (Hua et al., 2012; Zhang et al., 2016). The down-regulation of miR-31 during progression of glioblastoma results in increased migration and invasion of these cancer cells (Hua et al., 2012; Zhang et al., 2016). In addition, miR-31 can promote angiogenesis in glioma tumors through the direct suppression of *FIH1* (Factor Inhibiting Hypoxia-inducible Factor 1) (Wong et al., 2015). FIH1 is a multi-functional hydroxylase whose downstream targets include HIF1A (Hypoxia-inducible Factor 1 alpha) and NOTCH. Suppression of *FIH1* by miR-31 up-regulates HIF1A, resulting in the up-regulation of VEGF and promotion of angiogenesis (Wong et al., 2015).

Recently miR-31 was found to be at aberrantly low levels in the patients with hepatocellular carcinoma (Kim et al., 2015). The molecular targets of miR-31 in hepatocellular carcinoma include *HDAC2* (Histone Deacetylase 2) and *CDK2* (Cyclin-dependent Kinase 2), which promote the cell cycle, as well as *CDH1* and *CDH2* (N- and E-Cadherins), *VIM* (Vimentin), and *FN1* (Fibronectin), which are involved in the epithelial-to-mesenchymal transition (Kim et al., 2015). Thus, in hepatocellular carcinoma, miR-31 functions as a tumor suppressor that represses genes that promote cell proliferation and cell metastasis.

In ovarian cancer, loss of miR-31 increases chemoresistance to taxane through the lack of suppression of *STMN1* (Stathamin 1) and *MET* (a receptor tyrosine kinase) (Mitamura et al., 2013; Hassan et al., 2015). Taxane chemotherapy is based on its binding to beta-tubulin, resulting in stabilized microtubules. Microtubule stabilization causes cell cycle arrest in G2/M phase, leading to apoptosis (Hassan et al., 2015). STMN1 is a cytosolic, tubulin-binding protein shown to stimulate microtubule depolymerization. Down-regulation of miR-31 in ovarian cancer cells results in accumulation of STMN1, which counteracts taxane chemotherapy aimed at microtubule stabilization (Hassan et al., 2015). MET, on the other hand, is a transmembrane receptor that contributes to acquired apoptotic resistance to chemotherapy (Tang et al., 2010). One MET-dependent mechanism that contributes to apoptotic resistance is the activation of PI3K (Phosphoinositol-3 Kinase)/AKT (Protein Kinase B) signaling (Tang et al., 2010; Xiao et al., 2001). AKT promotes resistance to apoptosis via multiple mechanisms, including phosphorylation and activation of CHUK (Conserved Helix-Loop-Helix Ubiquitous Kinase/IκB kinase), which results in nuclear localization of NFκB to activate transcription of anti-apoptotic genes, or phosphorylation of BAD to prevent cell death (del Peso et al., 1997; Ozes et al., 1999; Xiao et al., 2001). Down-regulation of miR-31 results in the accumulation of MET and development of chemoresistance due to a block to apoptosis (Mitamura et al., 2013). These findings suggest that miR-31 has a protective effect on these cancers, and may be a potential therapeutics tool for improving the success of taxane-prescribed ovarian cancer treatment.

miR-31 is also up-regulated in some cancers, acting as an oncogenic miRNA (oncomiR). One well-documented oncomiR role of miR-31 was described in colorectal cancers (Cottonham et al., 2010; Cekaite et al., 2012; Xu et al., 2013b; Yang et al., 2013; Ito et al., 2014; Lei et al., 2014; Li et al., 2015c; Tateishi et al., 2015). Some of the miR-31 targets of colon cancer include important tumor suppressors such as *E2F2* (E2F Transcription Factor 2); *SATB2*, *RASA1* (RAS p21 GTPase activating protein 1), which was recently shown to be targeted by miR-31 in pancreatic cancer); *RHOBTB1* (Rho-Related BTB Domain Containing 1); and *TIAM1* (T Lymphoma and Metastasis Gene 1) (Cottonham et al., 2010; Sun et al., 2013; Xu et al., 2013b; Yang et al., 2013; Li et al., 2015c; Kent et al., 2016). Importantly, suppression of miR-31 in colon cancer cells resulted in the increased sensitivity to chemotherapeutic drug fluorouracil, suggesting the potential of using miR-31 as a therapeutic target to enhance the efficacy of chemotherapy treatments (Wang et al., 2010).

miR-31 also acts as an oncomiR in the lung cancer, where it directly targets tumor-suppressing genes, such as *LATS2* (Large Tumor Suppressor 2), *PPP2R2A* (Protein Phosphatase 2 Regulatory Subunit B alpha), and *BAP1* (BRCA1-associated Protein 1) (Liu et al., 2010; Yu et al., 2016). Additional miR-31 targets involved in the progression of lung cancer include negative regulators of RAS/ MAPK signaling *RASA1*, *SPRED1*, *SPRED2* (Sprouty-related EVH1 Domain Containing 1/2), *SPRY1*, *SPRY3*, and *SPRY4* (Sprouty RTK Signaling Antagonist 1/3/4) (Edmonds et al., 2016). The overexpression of miR-31 is proposed to be a predictor of lymph node metastasis, and results in a poor prognosis in patients with lung adenocarcinoma (Meng et al., 2013). Even though miR-31 is generally overexpressed in the lung cancers, some lung cancer tumors were reported to have a decreased miR-31 expression (Okudela et al., 2014a,b).

miR-31 may additionally contribute to chemotherapy-related multi-drug resistance by targeting *ABCB9* (ATP-Binding Cassette B9 Transporter) (Dong et al., 2014). Inhibition of *ABCB9* expression leads to chemoresistance, presumably through decreased drug uptake. miR-31 directly inhibits translation of *ABCB9*, thus contributing to poor treatment outcomes (Dong et al., 2014). Further, in cervical cancer, miR-31 directly inhibits tumor suppressor *ARID1A* (AT-rich Interactive Domain 1A), which activates transcription of genes by chromatin remodeling (Wang et al., 2014). Inhibition of *miR-31* expression in cervical cancer resulted in growth arrest and a decrease in cell migration. Importantly, the anti-tumor effects of miR-31 inhibitor could be reversed by the knockdown of *ARID1A* (Wang et al., 2014).

Taken together, the function of miR-31 in various cancers depends on its local environment where its complex interaction with other factors determine its role as a tumor suppressor or an oncomiR.

miR-31 PROMOTES RADIATION-INDUCED APOPTOSIS

Several independent studies reported miR-31 to be involved in radioresistance, the ability of an organism to withstand ionizing radiation. For example, miR-31 was significantly down-regulated in radioresistant esophageal adenocarcinoma cells (Lynam-Lennon et al., 2012). Over-expression of miR-31 re-sensitized the cells to radiation-induced cell death and down-regulated thirteen DNA repair genes. The exact mechanism of miR-31 in radiosensitivity has not been elucidated (Lynam-Lennon et al., 2012). A similar study demonstrated that inhibition of miR-31-5p protected human colon epithelial cells against ionizing radiation (Kim et al., 2014).

The role of miR-31 in radiation-induced apoptosis seems to be evolutionarily conserved. An interesting study conducted recently unraveled the mechanism behind the unusual resistance to radiation-induced apoptosis of the fall armyworm moth (*Spodoptera frugiperda*) (Kumar et al., 2015). Increased radiation led to increased miR-31 expression, which induced caspase-3-dependent apoptosis. Apoptosis is a complex process dependent on a number of pro- and anti-apoptotic proteins (Elmore, 2007). Upon irradiation, the ratio between the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl2 was not altered, yet Bax was translocated to the mitochondria, thus inducing apoptosis. Importantly, inhibition of miR-31 resulted in decreased translocation of Bax to mitochondria and decreased levels of the pro-apoptotic Bim, thereby reducing apoptosis. Moreover, ectopic overexpression of miR-31 in unirradiated cells resulted in increased apoptosis through mitochondrial Bax translocation and upregulation of *Bim* expression, suggesting that miR-31 may regulate mediators of Bax translocation and *Bim* expression by yet to be identified mechanism (Kumar et al., 2015).

The evidence that miR-31 is important for mediating radiation-induced apoptosis may be important in understanding the underlying mechanism of increased radiation resistance of some cancer cells and contribute to improved cancer therapeutics.

miR-31 IS MIS-REGULATED IN AUTOIMMUNE DISEASES AND ALLERGY

Immune system homeostasis is mediated by regulatory T (T_{reg}) cells (reviewed in Vignali et al., 2008; Li and Zheng, 2015). miR-31 regulates T_{reg} cells through several mechanisms, including the suppression of *FOXP3* involved in T_{reg} cells differentiation (discussed earlier). In addition, miR-31 can repress the generation of peripherally derived T_{reg} cells (Dhamne et al., 2013), which differentiate in secondary lymphoid organs and tissues to control autoimmune responses under certain inflammatory conditions (Yadav et al., 2013). One of the mechanisms for the induction of peripherally derived T_{reg} cells is mediated by retinoic acid, which indirectly induces expression of *Foxp3* (Hill et al., 2008; Zhang et al., 2015). miR-31 directly suppresses *Gprc5a* (G Protein-Coupled Receptor Class C Group 5 Member A/Retinoic Acid-inducible Protein 3), leading to decreased retinoic acid. This in turn indirectly decreases *Foxp3* expression, resulting in the suppression of peripherally derived T_{reg} cell differentiation (Zhang et al., 2015). Suppression of *Gprc5a* by miR-31 in peripherally derived T_{reg} cells is important in mice with experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. miR-31 was significantly overexpressed in both splenocytes and pathogenic CD4-positive T cells in mice with EAE (Zhang et al., 2015). Further, conditional deletion of miR-31 resulted in a significant decrease in the severity of EAE (Zhang et al., 2015). Thus, the abundance of miR-31 correlates with EAE severity.

miR-31 has also been shown to regulate the function of T cells in patients with systemic lupus erythematosus, a chronic autoimmune disease in the connective tissue that affects various organs, such as skin, heart, lungs, kidneys, and the nervous system (Fan et al., 2012). The level of miR-31 is significantly decreased in T cells obtained from lupus patients compared to the healthy controls (Fan et al., 2012). The proposed mechanism of miR-31 in the progression of lupus involves miR-31 directly repressing translation of *RHOA*, which is critical for the proper homing of lymphocytes to sites of infection (Helms et al., 2007; Fan et al., 2012;). RHOA indirectly inhibits *IL2* (Interleukin 2) transcription by interfering with the NFAT (Nuclear Factor of Activated T cells) activity that decreases the abundance of acetylated histone 3 at the *IL2* promoter (Helms et al., 2007). IL2 is a multi-functional cytokine that is crucial for T cell activation, proliferation, and contraction; its abundance is reduced in lupus, resulting in the systemic mis-regulation of immune responses in patients (reviewed in Lieberman and Tsokos, 2010). Decreased levels of miR-31 result in the accumulation of RHOA, leading to decreased production of IL2 and thus progression of lupus. A separate study demonstrated that miR-31 regulates IL2 levels by directly suppressing *KSR2* (Kinase Suppressor of RAS 2), which encodes an upstream kinase suppressor of *IL2* (Xue et al., 2013). Activation of primary lymphocytes through stimulation of T-cell receptor results in the up-regulation of miR-31, followed by increased expression of *IL2* and thus activation of an immune response (Xue et al., 2013).

miR-31 is also overexpressed in keratinocytes of the patients suffering from psoriasis, a chronic autoimmune disease that is characterized by the formation of itchy, silvery scales on the skin (Xu et al., 2013a; Yan et al., 2015). Overexpression of miR-31 has been linked to increased expression of chemokines and cytokines, and thus an increase in the ability of psoriatic keratinocytes to attract leukocytes, which result in chronic inflammation (Xu et al.,

2013a). miR-31 can directly suppress translation of *STK40* (Serine/Threonine Kinase 40), which encodes a negative regulator of the NFκB pathway; the NFκB pathway, in turn, activates the expression of cytokine and chemokines) (reviewed in Pasparakis, 2009). Thus, overexpression of miR-31 can indirectly and constitutively activate the NFκB pathway, contributing to increased inflammation in psoriatic epidermis (Xu et al., 2013a; Yan et al., 2015). Interestingly, activation of NFκB signaling induces miR-31 expression (Yan et al., 2015), creating a potential feed-forward loop that enhances NFκB pathway activity.

In addition to regulating inflammation responses, miR-31 can directly suppress *PPP6C* (Protein Phosphatase 6), which encodes a negative regulator of G1-to-S phase progression, indicating that this miR can regulate keratinocyte proliferation (Yan et al., 2015). miR-31 was previously found to enhance keratinocyte proliferation and migration during the process of wound healing by suppressing *EMP1* (Epithelial Membrane Protein 1), which encodes a tumor suppressor (Li et al., 2015a). The rate of wound healing is increased in psoriasis (Morhenn et al., 2013), so itis possible that that miR-31 promotes keratinocyte proliferation in psoriasis not only through suppression of *PPP6C*, but also through suppression of *EMP1*. Up-regulation of miR-31 in the psoriatic epithelium might also result in increased keratinocyte differentiation through NOTCH signaling as over-expression of miR-31 indirectly activates NOTCH through direct suppression of *FIH1* (Peng et al., 2012b).

Overall, overexpression of miR-31 in psoriatic keratinocytes results in: (i) the ability of keratinocytes to attract leukocytes through direct suppression of *STK40*; (ii) keratinocyte proliferation and epidermal hyperplasia through reduction of *PPP6C* and *EMP1* expression; and (iii) enhanced keratinocyte differentiation through suppression of *FIH1* (Peng et al., 2012b; Xu et al., 2013a; Yan et al., 2015). Inhibition of miR-31 led to decreased epidermal hyperplasia and reduced disease severity, suggesting that miR-31 may be a potential therapeutic target for the treatment of psoriasis (Yan et al., 2015).

Keratinocytes are present not only in the skin, but also in the eyes, where they may contribute to the complications arising from autoimmune diabetes. The miR-31 target FIH1 regulates corneal epithelial glycogen metabolism. Increased levels of FIH1 result in decreased AKT signaling, activation of GSK3β (Glycogen Synthetase Kinase 3 beta), and inactivation of glycogen synthase (Peng et al., 2012a). The exact role of miR-31 in diabetes has not been elucidated, although it might be involved in regulation of corneal epithelial glycogen metabolism by suppressing *FIH1*. Moreover, miR-31 abundance is reported to be elevated in the sera and skin of diabetic patients compared to healthy individuals (Sebastiani et al., 2013; Ramirez et al., 2015).

miR-31 has also been linked to the progression of the allergic airway disease in mice (Rutled Ge et al., 2015). The abundance of miR-31 in the lungs of mice sensitized with immunodominant allergen positively correlated with the degree of neutrophil recruitment to the airways and negatively correlated with the levels of OXRS1 (Oxidative Stress Responsive 1) and NSF (N-ethylmaleimide Sensitive Fusion Protein). NSF is involved in vesicular trafficking (required for the proper immune response) by facilitating disassembly of SNAREs (Stow et al., 2006), whereas OXSR1 protects against oxidative stress, which occurs in many allergic and autoimmune diseases (Ingram et al., 2007). Both *OXSR1* and

NSF trancsripts contain miR-31 binding sites, and their regulation by miR-31 is correlative (Rutledge et al., 2015).

The level and effect of miR-31 thus vary by the type of autoimmune disease. miR-31 is increased in the T cells of mice with EAE and keratinocytes of the patients with psoriasis, where it exacerbates these disease conditions. On the other hand, miR-31 is decreased in T cells, and has a protective effect in patients with lupus. miR-31 may also modulate immune response of the neutrophils. Thus, miR-31 emerges as an important regulator of autoimmune responses that acts through a number of mechanisms.

REGULATION OF miR-31 EXPRESSION

Most studies to date have focused on identifying miR-31 targets, so relatively little is known of how miR-31 expression itself is regulated. Several studies, mostly in the context of cancer, have shown that the transcriptional regulation of *miR-31* is repressed in part by hypermethylation in breast, prostate, liver, leukemia, and melanoma cancer cells (Asangani et al., 2012; Augoff et al., 2012; Yamagishi et al., 2012; Lin et al., 2013; Vrba et al., 2013; Kim et al., 2015). Treatment of breast cancer cells expressing low levels of miR-31 with demethylating agents resulted in increased *miR-31* expression (Augoff et al., 2012). Interestingly, treatment of these cells with a demethylation agent in addition to the deacetylating agent resulted in higher *miR-31* expression compared to the level in the cells treated with the demethylation agent alone, suggesting that the regulation of the *miR-31* gene may involve both promoter methylation and acetylation (Augoff et al., 2012). Usually the abundance of miR-31 is increased in the lung cancer; however, one study found that in some of lung cancer cells, *miR-31* expression was decreased with a methylated promoter. Treatment of these cells with DNA methylation inhibitors did not affect *miR-31* expression, indicating that in these lung cancer tumors, methylation cannot be a source of the reduced *miR-31* expression (Okudela et al., 2014b).

Histone modification was also demonstrated to be involved in the regulation of *miR-31* expression. For example, the Polycomb group protein EZH2 (Enhancer Of Zeste 2), a transcriptional repressor that catalyzes histone H3K27 trimethylation, suppresses miR-31 expression in colorectal cancer, prostate cancer, melanoma, and leukemia (Asangani et al., 2012; Yamagishi et al., 2012; Zhang et al., 2014; Kurihara et al., 2016). Histone deacetylase inhibitors also regulate cell proliferation and senescence in breast cancer cell lines via up-regulation of *miR-31* expression (Cho et al., 2015). In esophageal cancer cells, EZH2 form a co-repressor complex with SOX4 (SRY-box 4) and HDAC3 (Histone Deacetylase 3) to repress miR-31 transcription through an epigenetic silencing and by histone acetylation (Koumangoye et al., 2015).

In addition to epigenetic silencing, miR-31 expression was directly silenced by transcription factors such as the breast cancer oncogene EMSY, which is recruited to the *miR-31* promoter by the transcription factor ETS1 and the histone lysine demethylase KDM5B to repress its transcription (Viré et al., 2014). In vitro findings further demonstrated that expression of EMSY promoted oncogenic cell transformation as well as migration, whereas

the re-expression of *miR-31* reversed those phenotypes, suggesting that miR-31 is an important antagonist of EMSY function in breast cancer (Viré et al., 2014).

In colorectal cancer, miR-31 levels were up-regulated by the overexpression of AEG1 (Astrocyte Elevated Gene 1) (Huang et al., 2014), a multi-functional oncoprotein (reviewed in Ying et al., 2011). Similarly, miR-31 expression was induced by the MAPK/ERK pathway during vascular smooth muscle cell proliferation (Liu et al., 2011). The exact mechanism of how AEG1 or MAPK/ERK regulate *miR-31* has not been elucidated.

In oral carcinoma, up-regulation of *miR-31* was linked to the activation of EGFR (Epidermal Growth Factor Receptor) (Lu et al., 2014). EGFR activation initiates AKT signaling, which, in turn, induces the expression of the basic leucine zipper transcription factor C/EBPβ (CCAAT/enhancer binding protein). C/EBPβ directly binds to the promoter of *miR-31* in a lung cancer cell line (Xi et al., 2010). A strong positive correlation was also observed between the levels of C/EBPβ and *miR-31* expression in an oral carcinoma cell lines (Lu et al., 2014). These data thus suggest a model involving an EGFR-AKT-C/EBPβ-miR-31 regulatory axis (Lu et al., 2014).

In Kaposi's sarcoma, miR-31 was shown to be regulated by the minor form of the K15 protein (K15M) expressed by Kaposi's sarcoma-associated herpesvirus (Tsai et al., 2009). Expression of K15M up-regulated the expression of *miR-31* and *miR-21*, which promoted cell migration and invasion. Down-regulation of *miR-31* and *miR-21* in the infected host cells expressing K15M disrupted K15M-induced cell migration, suggesting that K15M regulates cell motility via these miRNAs (Tsai et al., 2009). miR-31 may increase cell motility in response to Kaposi's sarcoma-associated herpesvirus infection by directly suppressing the tumor suppressor FAT4 (FAT Atypical Cadherin 4), which can reduce cell motility and proliferation (Wu et al., 2011).

Regulation of *miR-31* expression by transcription factors and signaling pathways has been described in cancer, in bone homeostasis (as discussed earlier), and in macrophages during microbial infections (Ghorpade et al., 2013). *miR-31* expression is activated by SHH (Sonic Hedgehog) signaling that is induced in macrophages during *Mycobacterium bovis* infections. This pathogen activates TLR2 (Toll-like Receptor 2), which initiates the expression of the first-responder cytokine TNFα (Tumor Necrosis Factor alpha). TNFα, in turn, activates SHH signaling, which induces the expression of *miR-31*. Interestingly, miR-31 regulates its own transcription by directly suppressing MYD88 (Myeloid Differentiation Primary Response 88), a key anchor adaptor that is recruited to TLR2. Recruitment of TLR2 results in activation of *TNFA* expression. During *M. bovis* infection, miR-31 turns off transcription of *SHH* and its own gene by inhibiting MyD88-medated activation of *TNFA* expression and SHH signaling (Ghorpade et al., 2013). Of note, *M. bovis* is a known causative agent of tuberculosis, and miR-31 is down-regulated in patients with tuberculosis (Wang et al., 2015a; Zhou et al., 2016), suggesting an aberrant immune response that failed to activate *miR-31*.

Lastly, a number of non-protein molecules (hormones and indoles) were demonstrated to regulate *miR-31* expression (Kuokkanen et al., 2010; Busbee et al., 2015). A number of

studies reported that *miR-31* expression is activated by RELA (p65 subunit of NF-κB) and SP1 (a zinc-finger transcription factor) in esophageal cells under conditions that lack zinc (Alder et al., 2012; Fong et al., 2016; Taccioli et al., 2015). These data were correlative, and no molecular mechanism has been elucidated.

In summary, even though the regulation of miR-31 expression is not well understood, these studies suggest multiple regulatory mechanisms that control miR-31 expression.

miR-31 AND lnc-31 MAY BE CO-REGULATED AND SHARE SIMILAR FUNCTIONS

miRNAs are one of several types of non-coding regulatory RNAs that modulate gene expression in the cells. In fact, a large proportion of eukaryotic genome is transcribed into long non-coding RNAs (lncRNAs) (Ponting et al., 2009). The definition of the lncRNA is variable, but in general this refers to RNAs that are >200 base pairs long and have low or no protein-coding potential (Ponting et al., 2009; Pauli et al., 2011; Rinn and Chang, 2012). lncRNAs play a critical role in regulating a number of cellular processes, such as differentiation, development, and disease progression—yet lncRNAs are among the least understood non-coding RNAs, and the exact mechanism of their regulation depends on the specific lncRNA (Ponting et al., 2009; Pauli et al., 2011; Rinn and Chang, 2012; Dey et al., 2014).

A structural relationship between the lncRNAs and miRNAs was recently identified. Some lncRNAs have miRNA sequences embedded within them. In humans and mice, the primary transcript of *lncRNA-31* (*lnc-31*) contains miR-31, and some evidence indicates that they may be co-regulated and share similar functions (Augoff et al., 2012; Ballarino et al., 2015). For example, in triple-negative breast cancer cells, which lack *HER2* (hormone epidermal growth factor receptor 2), *ER* (estrogen receptor), and *PR* (progesterone receptors), both miR-31 and its host lnc-31 are silenced by an epigenetic mechanism involving promoter hypermethylation, suggesting that theses genes might be co-transcribed (Augoff et al., 2012). In a different context, both lnc-31 and miR-31 were down-regulated during myogenic differentiation in mice and humans (Ballarino et al., 2015). Interestingly, treatment with silencing RNAs against the exon sequences of lnc-31 reduced the abundance of mature lnc-31, but not miR-31, suggesting that the cytoplasmic *lnc-31* transcript undergoes biogenesis along a pathway that is independent from that of miR-31 biogenesis (Ballarino et al., 2015). Further studies are required to determine the exact mechanism of lnc-31 biogenesis and its relation to miR-31 biogenesis.

lnc-31 and miR-31 are induced upon activation of the oncogene-induced senescence program, using 4-hydroxytamoxifen, in human diploid fibroblasts (Montes et al., 2015). Both lnc-31 and miR-31 also promote oncogene-induced cellular senescence (Cho et al., 2015; Montes et al., 2015). Cellular senescence can be induced by the expression of several tumor suppressor pathways, including the $p16^{INK4A}$/RB (Retinoblastoma) pathway. The expression of $p16^{INK4A}$ is dependent on the activation of INK4B-ARF-INK4A locus, which is tightly repressed by the Polycomb group proteins. lnc-31 directly interacts with Polycomb group proteins to de-repress the *INK4* locus upon induction of senescence, leading to cell

cycle arrest. Thus, *lnc-31* expression up-regulates the $p16^{INK4A}$-dependent senescence in human diploid fibroblasts (Montes et al., 2015).

Similar to lnc-31 in the oncogene-induced senescent cells (Montes et al., 2015), miR-31 acts as a tumor suppressor in promoting senescence in breast cancer cells, albeit miR-31-mediated activation of cellular senescence may be dependent on p21 rather than on $p16^{INK4A}$ (Cho et al., 2015). The overall evidence indicates that miR-31 and lnc-31 may be co-transcribed (Ballarino et al., 2015; Montes et al., 2015), and that they may perform similar functions, as in the case of their activation of cellular senescence (Cho et al., 2015; Montes et al., 2015).

CONCLUSION

The gene regulatory networks orchestrating gene expression have been under a close investigation for decades, and are conventionally believed to be controlled by transcription factors that work as on/off switches of gene expression. Over the last 15 years, significant progress has been made towards the understanding of the regulatory role of miRNAs in various biological processes. The accepted model is that miRNAs can impact developmental decisions by modulating morphogen gradients or transcription factor levels, and thus contributing to the preciseness of the gene expression programs inside the living cells (Inui et al., 2010; Song et al., 2015).

miR-31 is a highly conserved miRNAs that has been examined in both normal physiological processes as well as in the context of various diseases. Numerous studies demonstrate that the function of miR-31 is context-dependent. This complexity may be caused by its broad spectrum of molecular targets and specific expression in various tissues and organs (Fig. 4). miR-31 regulates genes involved in cell differentiation, cell proliferation, migration, and apoptosis, so its function as a tumor suppressor or oncomiR depends on the combination of factors involved in the onset and progression of a specific disease. Despite the existing experimental data, we are still far from understanding biological pathways that miR-31 cross-regulates and how it is regulated. Identification of miR-31 gene targets in the developing embryos using systems biology approach may reveal a global view of its function.

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Abbreviations

FIH1	factor inhibiting hypoxia inducible factor 1
FOXP3	forkhead box P3
lncRNA	long noncoding RNA

miRNA	microRNA
oncomiR	oncogenic microRNA
PKCε	protein kinase C epsilon
SATB2	special AT-rich sequence-binding protein 2
SP7/OSX	osterix
T_{reg} cells	T regulatory cells
UTR	untranslated region
VEGF	vascular endothelial growth factor

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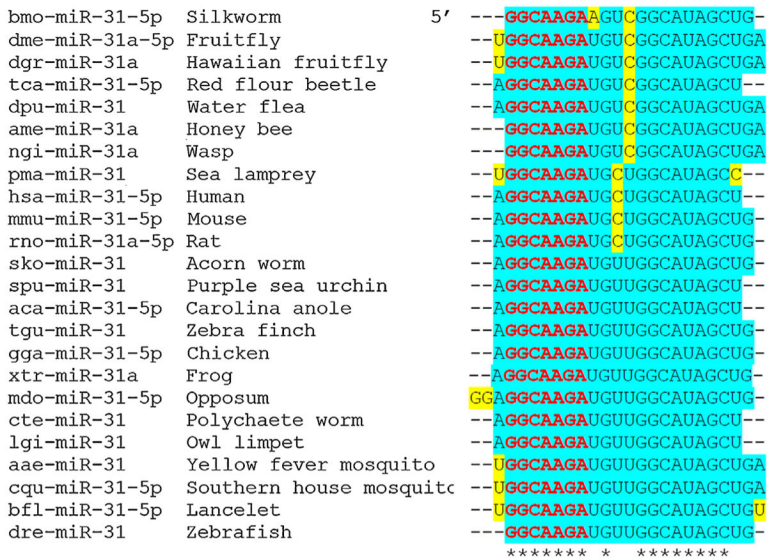

Figure 1.

The evolutionary conservation of miR-31. Alignment of mature miR-31 sequences in metazoan species. miR-31 sequences were obtained from miR Base (Kozomara and Griffiths-Jones, 2014) and aligned using clustal multiple alignment (Sievers et al., 2011). Red nucleotides indicate miR-31 seed sequences. Blue indicates conserved nucleotides.

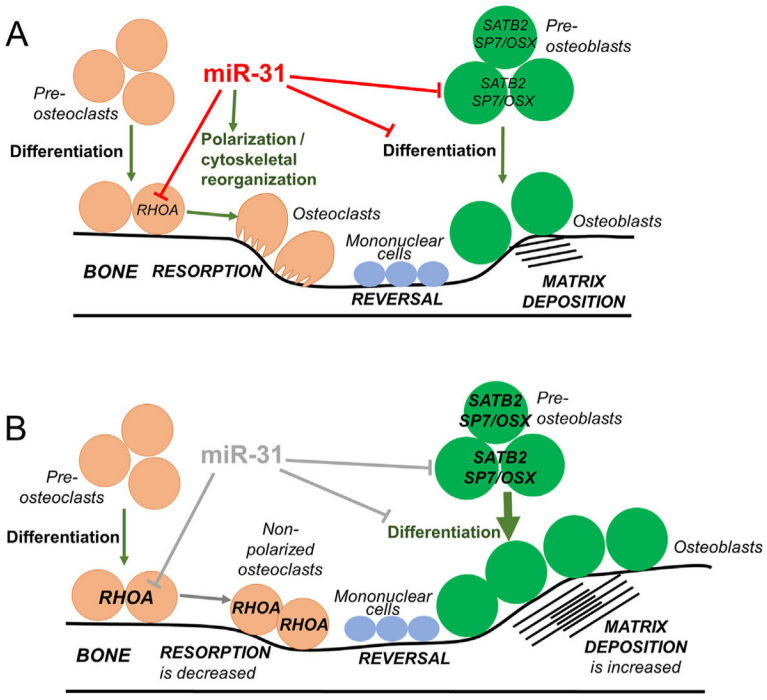

Figure 2.

Modulation of miR-31 during bone remodeling. **A**: Bone remodeling begins with the resorption of mineralized bone by osteoclasts, which must undergo cytoskeletal reorganization, involving the formation of an actin-rich sealing zone followed by apico-basal polarization and formation of the ruffled border (Boyle et al., 2003; Hadjidakis and Androulakis, 2006). Activated osteoclasts adhere to the fragment of the bone that needs to be remodeled / resorbed. One of the miR-31 targets involved in osteoclast function is RHOA, which is essential for osteoclast cytoskeleton reorganization (Mizoguchi et al., 2013). The second phase of bone remodeling involves specialized mononuclear cells that prepare the bone surface and attract osteoblasts (Raggatt and Partridge, 2010). Differentiation of osteoblasts is regulated mainly by SATB2 and SP7/OSX, whose transcripts are suppressed by miR-31 (Baglìo et al., 2013; Deng et al., 2013a; Xie et al., 2014). Differentiated osteoblasts promote bone matrix formation. **B**: Down-regulation of miR-31 increases bone volume and mineralization due to the suppression of osteoclast function (decrease in bone resorption) (Mizoguchi et al., 2013) and promotion of osteoblast differentiation (increase in matrix deposition) (Deng et al., 2013a; Xie et al., 2014).

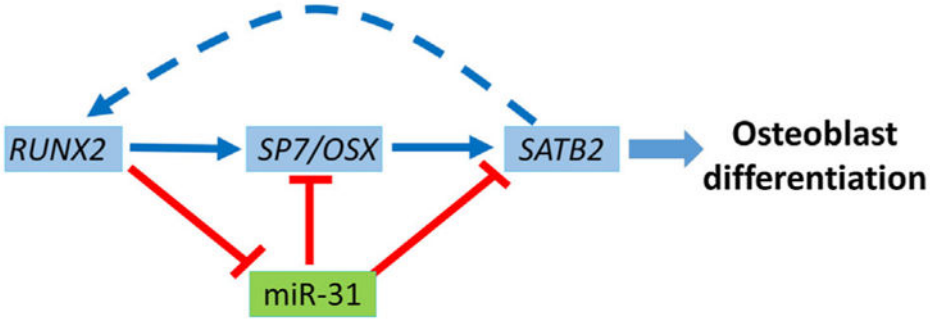

Figure 3.

Regulatory feedback of miR-31 during osteogenic differentiation. RUNX2 transcriptionally activates (solid arrow) *SP7/OSX*, resulting in the accumulation of SATB2 in differentiating osteoblasts (Nakashima et al., 2002; Tang et al., 2011). SATB2, in turn, physically interacts (dashed arrow) with RUNX2 to auto-enhance *RUNX2* expression, thus positively increasing SP7/OSX production (Dobreva et al., 2006). RUNX2 also suppresses expression of miR-31 by directly binding to its promoter, thus removing the miR-31-mediated translational silencing of *SP7/OSX* and *SATB2* transcripts (Deng et al., 2013a).

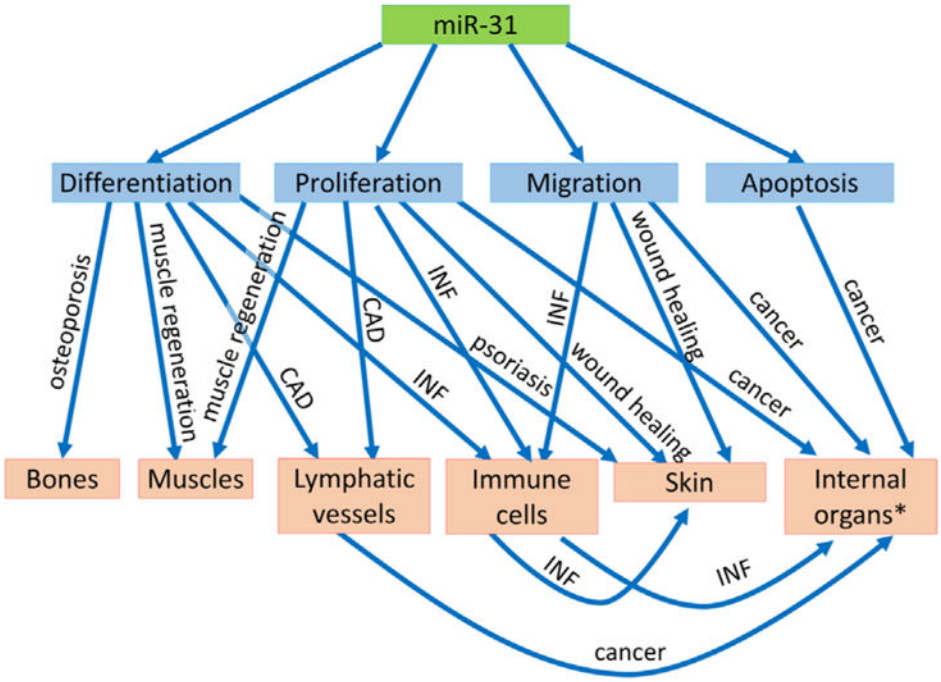

Figure 4.

The complexity of miR-31 regulation. miR-31 regulates many biological processes—including cell differentiation, proliferation, migration, and apoptosis—in various tissues and organs. Depending on a combination of factors involved in the onset and progression of a specific disease, miR-31 abundance can influence the prognosis. Arrows indicate a postiive regulatory relationship. CAD, coronary artery disease; INF, inflammation. *Internal organs include the brain, esophagus, lungs, kidney, stomach, colon, and etc.

TABLE 1

Summary of miR-31 in Development and Disease

Physiological process/disease	miR-31 expression	Direct targets	Function	Model system	Reference
Spermatogenesis	Down-regulated in testis of infertile patients	Unknown	Potential role in promoting early sperm development	Human testis	Munoz et al. (2015)
Embryo implantation	Elevated in both endometrium and serum during the time of implantation	Possibly through *FOXP3* and *CXCL12*	Provides an immune-tolerant maternal environment	Human (embryo implantation); HeLa cells (*FOXP3* regulation); HEK293T cells (*CXCL12* regulation)	Kresowik et al. (2014), Rouas et al. (2009), Itkin et al. (2012)
Embryonic development	Expressed in a pair-rule pattern in the foregut, anterior endoderm and hindgut	Unknown	Promotes proper segmentation	Fruitfly (*Drosophila*) embryos	Aboobaker et al. (2005), Leaman et al. (2005)
	Expressed ubiquitously during early development	*Pmar1*, *Alx1*, *Snail*, *VegfR7*	Promotes skeletogenesis	Sea urchin (*S. purpuratus*) embryos	Stepicheva and Song (2015)
	Expressed ubiquitously in zebrafish embryos	*PROX1*	Suppresses vascular development	Frog (*Xenopus laevis*) and zebrafish (*Danio rerio*) embryos; primary human lymphatic and blood vascular endothelial cells isolated from neonatal human foreskins	Pedrioli et al. (2010), Wienholds et al. (2005)
	Expressed in prosomeres p1/p4 of the brain and in the extremities of the somites, notably the hypaxial dermomyotome	*Myf5*	Prevents inappropriate accumulation of the key myogenic factor MYF5 in the brain	Mouse embryo	Daubas et al. (2009)
Vascular development	Down-regulated in differentiated vascular smooth muscle cells and up-regulated in de-differentiated and proliferative vascular smooth muscle cells	*LATS1*, *CREG*	Increased miR-31 levels results in increased proliferation of vascular smooth muscle cells	Rat vascular smooth muscle cells from the aortic media; human vascular smooth muscle cells from segments of internal thoracic arteries retrieved during coronary bypass surgery	Liu et al. (2011), Wang et al. (2013)
Myogenesis	Highly expressed at the early stages of differentiation of muscle satellite cells; progressively decreases at later differentiation stages.	*MYF5*, *DMD*	Maintains quiescence of muscle satellite cells	Muscle satellite cells isolated from mice and humans	Crist et al. (2012), Cacchiarelli et al. (2011)
	Increased in the cardiomyocytes after ischemia/ reperfusion injury	*Pkcε*	Downregulation of miR-31 increases heart resistance to ischemia/ reperfusion injury	Mice	Wang et al. (2015b)
Bone homeostasis	Highly abundant in osteoclasts	*RhoA*	Promotes osteoclast polarization and bone resorption	Mouse bone marrow-derived macrophages	Mizoguchi et al. (2013)
	Down-regulated during osteoblast differentiation	*SATB2*, *SP7/OSX*	Inhibits osteoblast differentiation	Humans, rats, dogs	Baglìo et al. (2013), Deng et al. (2013a,b, 2014a,b), Xie et al. (2014)

Physiological process/disease	miR-31 expression	Direct targets	Function	Model system	Reference
	Up-regulated in the plasma of patients with osteoporosis		Inhibits osteoblast differentiation	Humans	Weilner et al. (2016)
Radio-resistance	Down-regulated in radioresistant cells	Unknown	Promotes radiation- induced apoptosis. May regulate mediators of BAX translocation and BIM expression	Oesophageal adenocarcinoma cells; human colon epithelial cells; fall armyworm moth (*Spodoptera frugiperda*)	Lynam-Lennon et al. (2012), Kim et al. (2014), Kumar et al. (2015)
Auto-immunity	Overexpressed in both splenocytes and pathogenic CD4+ T cells in mice with EAE	*FOXP3*, *GPCR5A*	Represses generation of T_{reg} cells (both thymus- derived and peripherally-derived)	HeLa cells; NIH3T3 mouse embryo fibroblast cell.	Rouas et al. (2009), Zhang et al. (2015)
	Decreased in the T cells of the patients with systemic lupus erythematosus	*RhoA*	Decreased miR-31 results in accumulation of RHOA, exacerbates lupus progression	Jurkat T cells	Fan et al. (2012)
	Overexpressed in keratinocytes of the patients with psoriasis	*STK40*, *PPP6C*, *FIH1*	Increases the ability of keratinocytes to attract leukocytes and promotes keratinocyte proliferation and differentiation and epidermal hyperplasia	Human primary keratinocytes; NIH3T3 mouse embryo fibroblast cells	Xu et al. (2013a), Yan et al. (2015), Peng et al. (2012b)
Skin and hair	Overexpressed in epidermal keratinocytes during wound healing and in psoriasis;	*EMP1*, FIH1	Enhances keratinocyte proliferation and migration	Human biopsies	Li et al. (2015a), Peng et al. (2012b)
	Increased expression during anagen (hair growth phase), decreased during catagen (apoptosis- driven involution) and telogen (relative quiescence)	*Krt16, Krt17, Dxl3, Fgf10, Tgfb2*	Inhibition of miR-31 activity results in anagen acceleration, alteration in hair shaft structure and outer root sheath hyperplasia	Mice	Mardaryev et al. (2010), Kim and Yoon (2015)

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TABLE 2

Role of miR-31 in Various Cancers *

	Cancer type	Validated target	Function of the target	References
Tumor suppressor (downregulated)	Breast cancer	*GNA13*	GNA-13 promotes cell invasion mainly through activation of RHOA.	Rasheed et al. (2015)
	Liver cancer	*HDAC2, CDK2*	HDAC2 and CDK2 are cell cycle regulators (acceleration of cell cycle if overexpressed).	Kim et al. (2015)
		CDH1 & 2, VMN, FN1	CDH1/2, VMN, and FN1 are regulators of the epithelial-to-mesenchymal transition.	
	Ovarian cancer	*STMN1*	STMN1 destabilized microtubules.	Hassan et al. (2015)
		MET	MET is a membrane receptor that mediates apoptotic resistance to therapeutic drugs if overexpressed	Mitamura et al. (2013)
	Brain Tumors	*DOCK1*	Dock1 promotes epithelial-to-mesenchymal transition through NF-kB/SNAIL signaling.	Zhang et al. (2016)
		TRADD	TRADD is an upstream activator of NF-κB.	Rajbhandari et al. (2015)
		FIH1	FIH1 inhibits HIF1α and NOTCH. Down- regulation of FIH1 promotes angiogenesis.	Wong et al. (2015)
	Follicular lymphoma	*E2F2*	E2F2 regulates pro-proliferation genes.	Thompson et al. (2016)
		PIK3C2A	PIK3C2A is an oncogene, and its suppression results in apoptosis.	
	Nasopharyngeal carcinoma	*FIH1*	FIH1 inhibits HIF1α, which is a master regulator of oxygen homeostasis.	Cheung et al. (2014)
		MCM2	MCM2 is important in the initiation of DNA replication.	
	Medulloblastoma	*MCM2*	MCM2 is important in the initiation of DNA replication.	Jin et al. (2014)
OncomiR (up-regulated)	Lung cancer	*BAP1*	BAP1 is a tumor suppressor in lung cancer (nuclear-localized deubiquitinating enzyme).	Yu et al. (2016)
		MET	MET is a proto-oncogene and a hepatocyte growth factor receptor.	Hou et al. (2016)
		ABCB9	ABCB9 is a transporter involved in cellular trafficking and chemotherapy-related multidrug resistance.	Dong et al. (2014)
		RASA1, SPRED1 & 2, SPRY1, 3, & 4	RASA1, SPRED1, SPRED2, SPRY1, SPRY3, and SPRY4 are negative regulators of RAS/ MAPK signaling.	Edmonds et al. (2016)
	Cervical cancer	*ARID1A*	ARID1A is a tumor suppressor that remodels chromatin to regulate cell cycle progression.	Wang et al. (2014)
	Colon/ colorectal cancer	*E2F2*	E2F2 acts as a tumor suppressor in colon cancer by inhibiting cell cycle.	Li et al. (2015c)

	Cancer type	Validated target	Function of the target	References
		SATB2	SATB2 is a tumor suppressor; its down-regulation is associated with metastasis.	Yang et al. (2013)
		FIH1	FIH1 inhibits HIF1α, which is a master regulator of oxygen homeostasis. Down-regulation of FIH1 promotes tumor angiogenesis, cell proliferation and cell invasion.	Chen et al. (2014)
		CDKN2B	CDKN2B is a cell growth regulator that controls cell cycle G1 progression.	Lei et al. (2014)
	Pancreatic cancer	*RASA1*	RASA1 is a suppressor of RAS function. Down- regulation of RASA1 increases cell proliferation.	Kent et al. (2016)
	Intra-hepatic cholangiocarcinoma			Hu et al. (2013)
	Eosophageal neoplasia	*STK40*	STK40 is a negative regulator of NF-κB- mediated transcription.	Taccioli et al. (2015)
		CPM	CPM is a cancer biomarker, but the mechanism for its carcinogenesis not known.	

ABCB9, ATP-Binding Cassette, sub-family B (MDR/TAP), member 9; *ARID1A*, AT-rich Interactive Domain 1A (SWI-like); *BAP1*, BRCA1-associated Protein-1; *CDH1*, E-Cadherin; *CDH2*, N-Cadherin; *CDK2*, Cyclin-dependent Kinase 2; *CDKN2B*, Cyclin-dependent Kinase Inhibitor 2B; *CPM*, Carboxypeptidase M; *DOCK1*, Dedicator of Cytokinesis 1; *E2F2*, E2F Transcription Factor 2; *FIH1*, Factor Inhibiting Hypoxia-inducible Transcription Factor 1 alpha; *FN1*, Fibronectin; *GNA13*, G Protein Alpha-13; *HDAC2*, Histone Deacetylase 2; *MCM2*, Minichromosome Maintenance Complex Component 2; *MET*, receptor tyrosine kinase; *PIK3C2A*, Phosphatidylinositol-4-Phosphate 3 Kinase, catalytic subunit type 2 alpha; *RASA1*, RAS P21 Protein Activator 1; *SATB2*, Special AT-rich Sequence-binding Protein 2; *SPRED1/ 2*, Sprouty-related, EVH1 domain containing 1/2; *SPRY1/3/4*, Sprouty RTK Signaling Antagonist 1/3/4; *STK40*, Serine/Threonine Kinase 40; *STMN1*, Stathmin 1; *TRADD*, TNF Receptor-associated Death Domain; *VMN*, Vimentin.

*This table does not include references in the review by Laurila and Kallioniemi (2013).