REVIEW ARTICLE

Molecular Reproduction & Development (2016)

# Function and Regulation of MicroRNA-31 in Development and Disease

NADEZDA A. STEPICHEVA AND JIA L. SONG*

Department of Biological Sciences, University of Delaware, Newark, Delaware

## 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.

*Mol. Reprod. Dev. 2016.* © *2016 Wiley Periodicals, Inc.*

Received 17 April 2016; Accepted 29 June 2016

*[T]he function of miR-31 is context-dependent.*

*Corresponding author:
323 Wolf Hall
Newark, DE 19716.
E-mail: jsong@udel.edu

Grant sponsor: NSF IOS; Grant number: 1553338; Grant sponsor: NIH NIGMS; Grant number: 5P20GM103653

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/mrd.22678

## 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 divergence and miRNA expression among various species revealed that higher expression of miRNA directly

**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.

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

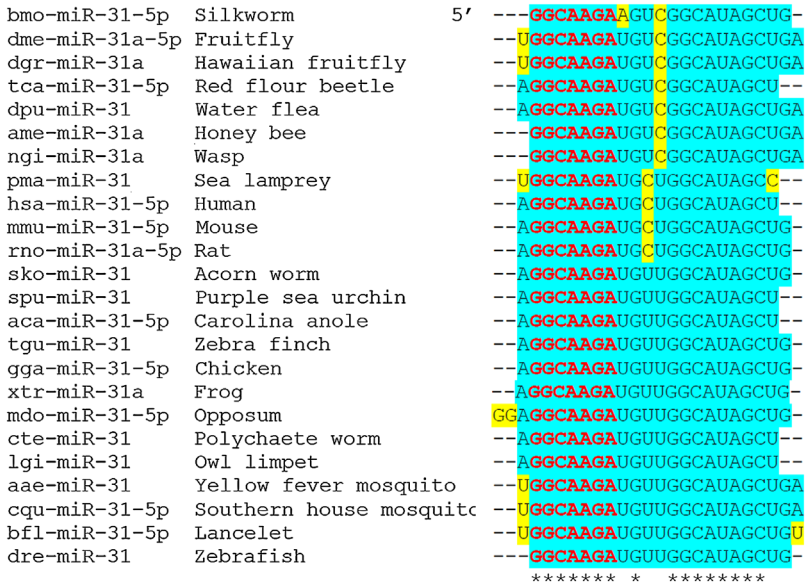


**Figure 1.** The evolutionary conservation of miR-31. Alignment of mature miR-31 sequences in metazoan species. miR-31 sequences were obtained from miRBase (Kozomara and Griffiths-Jones, 2014) and aligned using clustal multiple alignment (Sievers et al., 2011). Red nucleotides indicate miR-31 seed sequences. Blue indicates conserved nucleotides.

**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) |

(*Continued*)

**TABLE 1. (*Continued*)**

| Physiological process/disease | miR-31 expression | Direct targets | Function | Model system | Reference |
|---|---|---|---|---|---|
| | 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) |
| | 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) |

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).

**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. | |
| | Naso-pharyngeal 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. | |
| | Medullo-blastoma | *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) |
| | | *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 cholangio-carcinoma | | | Hu et al. (2013) |
| | Eosopha-geal 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).

## 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). DYSTROPHIN 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 multinucleated 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).

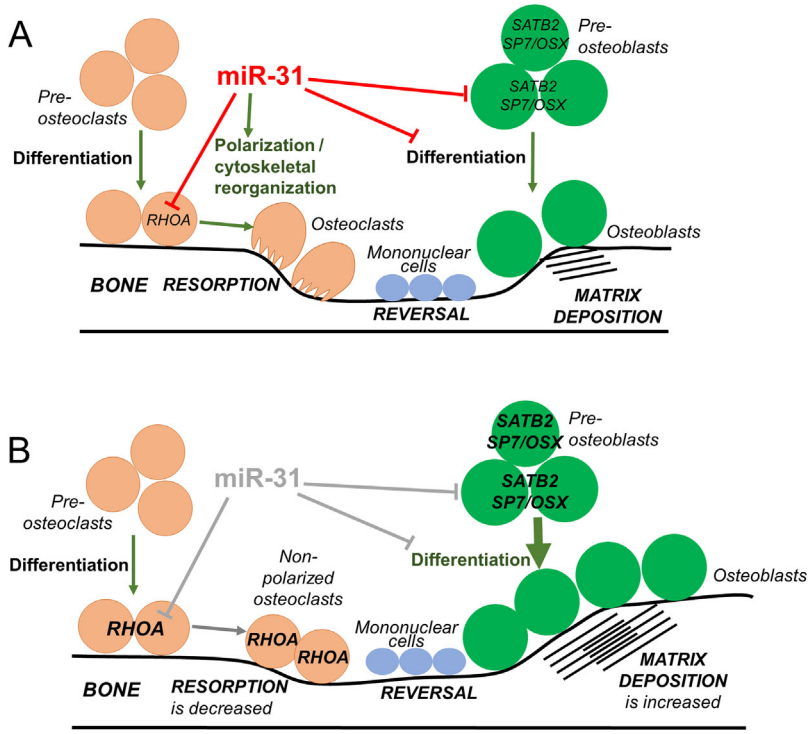


**Figure 2.** Modulation of miR-31 during bone remodeling. **A**: Bone remodeling begins with the resorption of mineralized bone by osteoclasts, which must undergo cytoskeletal reorganization, involving the formation of an actin-rich sealing zone followed by apico-basal polarization and formation of 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).

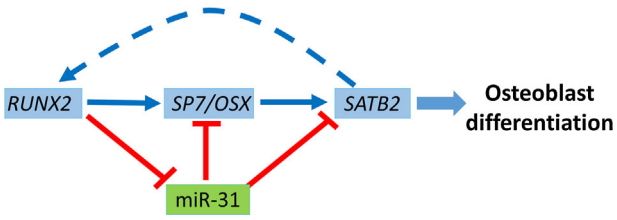


**Figure 3.** Regulatory feedback of miR-31 during osteogenic differentiation. RUNX2 transcriptionally activates (solid arrow) *SP7/OSX*, resulting in the accumulation of SATB2 in differentiating osteoblasts (Nakashima et al., 2002; Tang et al., 2011). SATB2, in turn, physically interacts (dashed arrow) 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).

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). Overexpression 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 misregulation 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 it is 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 overexpression 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

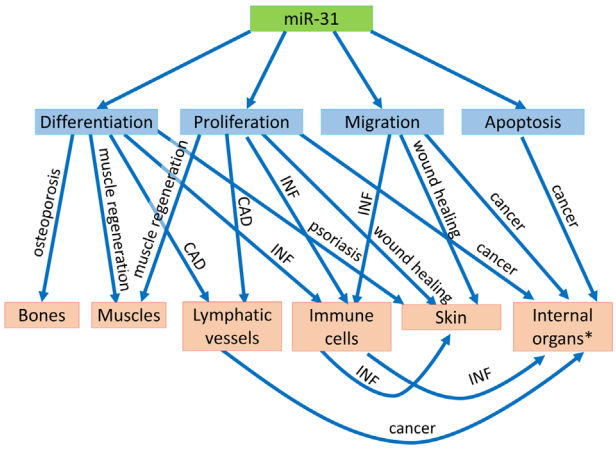


**Figure 4.** The complexity of miR-31 regulation. miR-31 regulates many biological processes—including cell differentiation, proliferation, migration, and apoptosis—in various tissues and organs. Depending on a combination of factors involved in the onset and progression of a specific disease, miR-31 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.

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.

## ACKNOWLEDGMENTS

We thank the two anonymous reviewers for their valuable feedback. We also acknowledge miRTex, where we extracted many of the miR-31 literature (Li et al., 2015b).

## REFERENCES

Aboobaker AA, Tomancak P, Patel N, Rubin GM, Lai EC. 2005. Drosophila microRNAs exhibit diverse spatial expression patterns during embryonic development. Proc Natl Acad Sci USA 102:18017–18022.

Adomako-Ankomah A, Ettensohn CA. 2013. Growth factor-mediated mesodermal cell guidance and skeletogenesis during sea urchin gastrulation. Development 140:4214–4225.

Adomako-Ankomah A, Ettensohn CA. 2014. Growth factors and early mesoderm morphogenesis: Insights from the sea urchin embryo. Genesis 52:158–172.

Alder H, Taccioli C, Chen H, Jiang Y, Smalley KJ, Fadda P, Ozer HG, Huebner K, Farber JL, Croce CM, Fong LY. 2012. Dysregulation of miR-31 and miR-21 induced by zinc deficiency promotes esophageal cancer. Carcinogenesis 33:1736–1744.

Anderson M, Creighton C, Fountain M, Yu Z, Nagaraja A, Zhu H, Khan M, Olokpa E, Gunaratne P, Matzuk M. 2010. Massively parallel sequencing identifies miR-31 as a key tumor suppressor in papillary serous ovarian cancers. Gynecol Oncol 116:S11–S11.

Asangani IA, Harms PW, Dodson L, Pandhi M, Kunju LP, Maher CA, Fullen DR, Johnson TM, Giordano TJ, Palanisamy N, Chinnaiyan AM. 2012. Genetic and epigenetic loss of microRNA-31 leads to feed-forward expression of EZH2 in melanoma. Oncotarget 3:1011–1025.

Augoff K, Das M, Bialkowska K, McCue B, Plow EF, Sossey-Alaoui K. 2011. miR-31 is a broad regulator of beta 1-integrin expression and function in cancer cells. Mol Cancer Res 9:1500–1508.

Augoff K, McCue B, Plow EF, Sossey-Alaoui K. 2012. miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. Mol Cancer 11:5–17.

Baglìo SR, Devescovi V, Granchi D, Baldini N. 2013. MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. Gene 527:321–331.

Ballarino M, Cazzella V, D'Andrea D, Grassi L, Bisceglie L, Cipriano A, Santini T, Pinnarò C, Morlando M, Tramontano A, Bozzoni I. 2015. Novel long noncoding RNAs (lncRNAs) in myogenesis: A miR-31 overlapping lncRNA transcript controls myoblast differentiation. Mol Cell Biol 35:728–736.

Bartel DP. 2009. MicroRNAs: Target recognition and regulatory functions. Cell 136:215–233.

Bleeker FE, Molenaar RJ, Leenstra S. 2012. Recent advances in the molecular understanding of glioblastoma. J Neurooncol 108:11–27.

Boyle WJ, Simonet WS, Lacey DL. 2003. Osteoclast differentiation and activation. Nature 423:337–342.

Buchan JR. 2014. mRNP granules. Assembly, function, and connections with disease. RNA Biol 11:1019–1030.

Busbee PB, Nagarkatti M, Nagarkatti PS. 2015. Natural indoles, indole-3-carbinol (I3C) and 3,3′-diindolylmethane (DIM),

attenuate staphylococcal enterotoxin B-mediated liver injury by downregulating miR-31 expression and promoting caspase-2-mediated apoptosis. PLoS One 10:e0118506.

Cacchiarelli D, Incitti T, Martone J, Cesana M, Cazzella V, Santini T, Sthandier O, Bozzoni I. 2011. miR-31 modulates dystrophin expression: New implications for Duchenne muscular dystrophy therapy. EMBO Rep 12:136–141.

Cekaite L, Rantala JK, Bruun J, Guriby M, Agesen TH, Danielsen SA, Lind GE, Nesbakken A, Kallioniemi O, Lothe RA, Skotheim RI. 2012. MiR-9,-31, and-182 deregulation promote proliferation and tumor cell survival in colon cancer. Neoplasia 14:868–879.

Chakravorty N, Hamlet S, Jaiprakash A, Crawford R, Oloyede A, Alfarsi M, Xiao Y, Ivanovski S. 2014. Pro-osteogenic topographical cues promote early activation of osteoprogenitor differentiation via enhanced TGFβ, Wnt, and Notch signaling. Clin Oral Implants Res 25:475–486.

Chen T, Yao LQ, Shi Q, Ren Z, Ye LC, Xu JM, Zhou PH, Zhong YS. 2014. MicroRNA-31 contributes to colorectal cancer development by targeting factor inhibiting HIF-1α (FIH-1). Cancer Biol Ther 15:516–523.

Cheung CC, Chung GT, Lun SW, To KF, Choy KW, Lau KM, Siu SP, Guan XY, Ngan RK, Yip TT, Busson P, Tsao SW, Lo KW. 2014. miR-31 is consistently inactivated in EBV-associated nasopharyngeal carcinoma and contributes to its tumorigenesis. Mol Cancer 13:184.

Cho JH, Dimri M, Dimri GP. 2015. MicroRNA-31 is a transcriptional target of histone deacetylase inhibitors and a regulator of cellular senescence. J Biol Chem 290:10555–10567.

Cottonham CL, Kaneko S, Xu L. 2010. miR-21 and miR-31 converge on TIAM1 to regulate migration and invasion of colon carcinoma cells. J Biol Chem 285:35293–35302.

Crist CG, Montarras D, Buckingham M. 2012. Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. Cell Stem Cell 11:118–126.

Daubas P, Crist CG, Bajard L, Relaix F, Pecnard E, Rocancourt D, Buckingham M. 2009. The regulatory mechanisms that underlie inappropriate transcription of the myogenic determination gene Myf5 in the central nervous system. Dev Biol 327:71–82.

del Peso L, González-García M, Page C, Herrera R, Nuñez G. 1997. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 278:687–689.

Deng Y, Wu S, Zhou H, Bi X, Wang Y, Hu Y, Gu P, Fan X. 2013a. Effects of a miR-31, Runx2, and Satb2 regulatory loop on the osteogenic differentiation of bone mesenchymal stem cells. Stem Cells Dev 22:2278–2286.

Deng Y, Zhou HF, Zou DH, Xie Q, Bi XP, Gu P, Fan XQ. 2013b. The role of miR-31-modified adipose tissue-derived stem cells in repairing rat critical-sized calvarial defects. Biomaterials 34:6717–6728.

Deng Y, Bi X, Zhou H, You Z, Wang Y, Gu P, Fan X. 2014a. Repair of critical-sized bone defects with anti-miR-31-expressing bone marrow stromal stem cells and poly(glycerol sebacate) scaffolds. Eur Cell Mater 27:13–24; discussion 24–15.

Deng Y, Zhou H, Gu P, Fan X. 2014b. Repair of canine medial orbital bone defects with miR-31-modified bone marrow mesenchymal stem cells. Invest Ophthalmol Vis Sci 55:6016–6023.

Destaing O, Saltel F, Gilquin B, Chabadel A, Khochbin S, Ory S, Jurdic P. 2005. A novel Rho-mDia2-HDAC6 pathway controls podosome patterning through microtubule acetylation in osteoclasts. J Cell Sci 118:2901–2911.

Dey BK, Mueller AC, Dutta A. 2014. Long non-coding RNAs as emerging regulators of differentiation, development, and disease. Transcription 5:e944014.

Dhamne C, Chung Y, Alousi AM, Cooper LJ, Tran DQ. 2013. Peripheral and thymic foxp3(+) regulatory T cells in search of origin, distinction, and function. Front Immunol 4:253.

Dobreva G, Chahrour M, Dautzenberg M, Chirivella L, Kanzler B, Fariñas I, Karsenty G, Grosschedl R. 2006. SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. Cell 125:971–986.

Dong Z, Zhong Z, Yang L, Wang S, Gong Z. 2014. MicroRNA-31 inhibits cisplatin-induced apoptosis in non-small cell lung cancer cells by regulating the drug transporter ABCB9. Cancer Lett 343:249–257.

Duloquin L, Lhomond G, Gache C. 2007. Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. Development 134:2293–2302.

Edmonds MD, Boyd KL, Moyo T, Mitra R, Duszynski R, Arrate MP, Chen X, Zhao Z, Blackwell TS, Andl T, Eischen CM. 2016. MicroRNA-31 initiates lung tumorigenesis and promotes mutant KRAS-driven lung cancer. J Clin Invest 126:349–364.

Elmore S. 2007. Apoptosis: A review of programmed cell death. Toxicol Pathol 35:495–516.

Erlebacher A. 2011. Strangers no more: Uterine NK cell recognition of the placenta in mice. Proc Natl Acad Sci USA 108:4267–4268.

Fan W, Liang D, Tang Y, Qu B, Cui H, Luo X, Huang X, Chen S, Higgs BW, Jallal B, Yao Y, Harley JB, Shen N. 2012. Identification of microRNA-31 as a novel regulator contributing to impaired interleukin-2 production in T cells from patients with systemic lupus erythematosus. Arthritis Rheum 64: 3715–3725.

Fisher S, Franz-Odendaal T. 2012. Evolution of the bone gene regulatory network. Curr Opin Genet Dev 22:390–397.

Fong LY, Taccioli C, Jing R, Smalley KJ, Alder H, Jiang Y, Fadda P, Farber JL, Croce CM. 2016. MicroRNA dysregulation and esophageal cancer development depend on the extent of zinc dietary deficiency. Oncotarget 7:10723–10738.

Ge J, Guo S, Fu Y, Zhou P, Zhang P, Du Y, Li M, Cheng J, Jiang H. 2015. Dental follicle cells participate in tooth eruption via the RUNX2-MiR-31-SATB2 loop. J Dent Res 94:936–944.

Ghorpade DS, Holla S, Kaveri SV, Bayry J, Patil SA, Balaji KN. 2013. Sonic hedgehog-dependent induction of microRNA 31 and microRNA 150 regulates Mycobacterium bovis BCG-driven toll-like receptor 2 signaling. Mol Cell Biol 33:543–556.

Greenberg E, Hershkovitz L, Itzhaki O, Hajdu S, Nemlich Y, Ortenberg R, Gefen N, Edry L, Modai S, Keisari Y, Besser MJ, Schachter J, Shomron N, Markel G. 2011. Regulation of cancer aggressive features in melanoma cells by microRNAs. PLoS One 6:e18936.

Hadjidakis DJ, Androulakis II. 2006. Bone remodeling. Ann N Y Acad Sci 1092:385–396.

Hammond SM. 2015. An overview of microRNAs. Adv Drug Deliv Rev 87:3–14.

Hanna J, Wald O, Goldman-Wohl D, Prus D, Markel G, Gazit R, Katz G, Haimov-Kochman R, Fujii N, Yagel S, Peled A, Mandelboim O. 2003. CXCL12 expression by invasive trophoblasts induces the specific migration of CD16- human natural killer cells. Blood 102:1569–1577.

Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O. 2006. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med 12:1065–1074.

Hart MW, Strathmann RR. 1994. Functional consequences of phenotypic plasticity in echinoid larvae. Biol Bull 186:291–299.

Hassan MK, Watari H, Mitamura T, Mohamed Z, El-Khamisy SF, Ohba Y, Sakuragi N. 2015. P18/Stathmin1 is regulated by miR-31 in ovarian cancer in response to taxane. Oncoscience 2:294–308.

Helms WS, Jeffrey JL, Holmes DA, Townsend MB, Clipstone NA, Su L. 2007. Modulation of NFAT-dependent gene expression by the RhoA signaling pathway in T cells. J Leukoc Biol 82:361–369.

Hill JA, Hall JA, Sun CM, Cai Q, Ghyselinck N, Chambon P, Belkaid Y, Mathis D, Benoist C. 2008. Retinoic acid enhances Foxp3 induction indirectly by relieving inhibition from CD4+CD44hi Cells. Immunity 29:758–770.

Hou C, Sun B, Jiang Y, Zheng J, Yang N, Ji C, Liang Z, Shi J, Zhang R, Liu Y, Ye C, Zuo P. 2016. MicroRNA-31 inhibits lung adenocarcinoma stem-like cells via down-regulation of MET-PI3K-Akt signaling pathway. Anticancer Agents Med Chem 16:501–518.

Hu C, Huang F, Deng G, Nie W, Huang W, Zeng X. 2013. miR-31 promotes oncogenesis in intrahepatic cholangiocarcinoma cells via the direct suppression of RASA1. Exp Ther Med 6:1265–1270.

Hua D, Ding D, Han X, Zhang W, Zhao N, Foltz G, Lan Q, Huang Q, Lin B. 2012. Human miR-31 targets radixin and inhibits migration and invasion of glioma cells. Oncol Rep 27:700–706.

Huang S, Wu B, Li D, Zhou W, Deng G, Zhang K, Li Y. 2014. Knockdown of astrocyte elevated gene-1 inhibits tumor growth and modifies microRNAs expression profiles in human colorectal cancer cells. Biochem Biophys Res Commun 444:338–345.

Ingram JL, Antao-Menezes A, Turpin EA, Wallace DG, Mangum JB, Pluta LJ, Thomas RS, Bonner JC. 2007. Genomic analysis of human lung fibroblasts exposed to vanadium pentoxide to identify candidate genes for occupational bronchitis. Respir Res 8:34.

Inui M, Martello G, Piccolo S. 2010. MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 11:252–263.

Itkin T, Ludin A, Gradus B, Gur-Cohen S, Kalinkovich A, Schajnovitz A, Ovadya Y, Kollet O, Canaani J, Shezen E, Coffin DJ, Enikolopov GN, Berg T, Piacibello W, Hornstein E, Lapidot T. 2012. FGF-2 expands murine hematopoietic stem and progenitor cells via proliferation of stromal cells, c-Kit activation, and CXCL12 down-regulation. Blood 120:1843–1855.

Ito M, Mitsuhashi K, Igarashi H, Nosho K, Naito T, Yoshii S, Takahashi H, Fujita M, Sukawa Y, Yamamoto E, Takahashi T, Adachi Y, Nojima M, Sasaki Y, Tokino T, Baba Y, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. 2014. MicroRNA-31 expression in relation to BRAF mutation, CpG island methylation and colorectal continuum in serrated lesions. Int J Cancer 135:2507–2515.

Itzstein C, Coxon FP, Rogers MJ. 2011. The regulation of osteoclast function and bone resorption by small GTPases. Small GTPases 2:117–130.

Iwama H, Masaki T, Kuriyama S. 2007. Abundance of microRNA target motifs in the 3′-UTRs of 20527 human genes. Febs Lett 581:1805–1810.

Jin Y, Xiong A, Zhang Z, Li S, Huang H, Yu TT, Cao X, Cheng SY. 2014. MicroRNA-31 suppresses medulloblastoma cell growth by inhibiting DNA replication through minichromosome maintenance 2. Oncotarget 5:4821–4833.

Kent OA, Mendell JT, Rottapel R. 2016. Transcriptional regulation of miR-31 by oncogenic KRAS mediates metastatic phenotypes by repressing RASA1. Mol Cancer Res 14:267–277.

Kiezun A, Artzi S, Modai S, Volk N, Isakov O, Shomron N. 2012. miRviewer: A multispecies microRNA homologous viewer. BMC Res Notes 5:92.

Kim SB, Zhang L, Barron S, Shay JW. 2014. Inhibition of microRNA-31-5p protects human colonic epithelial cells against ionizing radiation. Life Sci Space Res (Amst) 1:67–73.

Kim HS, Lee KS, Bae HJ, Eun JW, Shen Q, Park SJ, Shin WC, Yang HD, Park M, Park WS, Kang YK, Nam SW. 2015. MicroRNA-31 functions as a tumor suppressor by regulating cell cycle and epithelial-mesenchymal transition regulatory proteins in liver cancer. Oncotarget 6:8089–8102.

Körner C, Keklikoglou I, Bender C, Wörner A, Münstermann E, Wiemann S. 2013. MicroRNA-31 sensitizes human breast cells to apoptosis by direct targeting of protein kinase C epsilon (PKCepsilon). J Biol Chem 288:8750–8761.

Koumangoye RB, Andl T, Taubenslag KJ, Zilberman ST, Taylor CJ, Loomans HA, Andl CD. 2015. SOX4 interacts with EZH2

and HDAC3 to suppress microRNA-31 in invasive esophageal cancer cells. Mol Cancer 14:24.

Kozomara A, Griffiths-Jones S. 2014. miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 42:D68–D73.

Krawetz SA, Kruger A, Lalancette C, Tagett R, Anton E, Draghici S, Diamond MP. 2011. A survey of small RNAs in human sperm. Hum Reprod 26:3401–3412.

Kresowik JD, Devor EJ, Van Voorhis BJ, Leslie KK. 2014. MicroRNA-31 is significantly elevated in both human endometrium and serum during the window of implantation: A potential biomarker for optimum receptivity. Biol Reprod 91:17.

Kumar A, Ghosh S, Chandna S. 2015. Evidence for microRNA-31 dependent Bim-Bax interaction preceding mitochondrial Bax translocation during radiation-induced apoptosis. Sci Rep 5:15923.

Kuokkanen S, Chen B, Ojalvo L, Benard L, Santoro N, Pollard JW. 2010. Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. Biol Reprod 82:791–801.

Kurihara H, Maruyama R, Ishiguro K, Kanno S, Yamamoto I, Ishigami K, Mitsuhashi K, Igarashi H, Ito M, Tanuma T, Sukawa Y, Okita K, Hasegawa T, Imai K, Yamamoto H, Shinomura Y, Nosho K. 2016. The relationship between EZH2 expression and microRNA-31 in colorectal cancer and the role in evolution of the serrated pathway. Oncotarget 7:12704–12717.

La Rocca C, Carbone F, Longobardi S, Matarese G. 2014. The immunology of pregnancy: Regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett 162:41–48.

Laurila EM, Kallioniemi A. 2013. The diverse role of miR-31 in regulating cancer associated phenotypes. Genes Chromosomes Cancer 52:1103–1113.

Leaman D, Chen PY, Fak J, Yalcin A, Pearce M, Unnerstall U, Marks DS, Sander C, Tuschl T, Gaul U. 2005. Antisense-mediated depletion reveals essential and specific functions of microRNAs in Drosophila development. Cell 121: 1097–1108.

Lei SL, Zhao H, Yao HL, Chen Y, Lei ZD, Liu KJ, Yang Q. 2014. Regulatory roles of microRNA-708 and microRNA-31 in proliferation, apoptosis and invasion of colorectal cancer cells. Oncol Lett 8:1768–1774.

Lewis BP, Burge CB, Bartel DP. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20.

Li D, Li X, Wang A, Meisgen F, Pivarcsi A, Sonkoly E, Ståhle M, Landén NX. 2015a. MicroRNA-31 promotes skin wound healing by enhancing keratinocyte proliferation and migration. J Invest Dermatol 135:1676–1685.

Li G, Ross KE, Arighi CN, Peng Y, Wu CH, Vijay-Shanker K. 2015b. miRTex: A text mining system for miRNA-gene relation extraction. PLoS Comput Biol 11:e1004391.

Li T, Luo W, Liu K, Lv X, Xi T. 2015c. miR-31 promotes proliferation of colon cancer cells by targeting E2F2. Biotechnol Lett 37:523–532.

Li X, Zheng Y. 2015. Regulatory T cell identity: Formation and maintenance. Trends Immunol 36:344–353.

Lian JB, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, Zhang Y. 2012. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol 8:212–227.

Liang H, Li WH. 2009. Lowly expressed human microRNA genes evolve rapidly. Mol Biol Evol 26:1195–1198.

Lieberman LA, Tsokos GC. 2010. The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity. J Biomed Biotechnol 2010:740619.

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433:769–773.

Lin PC, Chiu YL, Banerjee S, Park K, Mosquera JM, Giannopoulou E, Alves P, Tewari AK, Gerstein MB, Beltran H, Melnick AM, Elemento O, Demichelis F, Rubin MA. 2013. Epigenetic repression of miR-31 disrupts androgen receptor homeostasis and contributes to prostate cancer progression. Cancer Res 73:1232–1244.

Liu X, Sempere LF, Ouyang HX, Memoli VA, Andrew AS, Luo Y, Demidenko E, Korc M, Shi W, Preis M, Dragnev KH, Li H, DiRenzo J, Bak M, Freemantle SJ, Kauppinen S, Dmitrovsky E. 2010. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. J Clin Invest 120:1298–1309.

Liu X, Cheng Y, Chen X, Yang J, Xu L, Zhang C. 2011. MicroRNA-31 regulated by the extracellular regulated kinase is involved in vascular smooth muscle cell growth via large tumor suppressor homolog 2. J Biol Chem 286:42371–42380.

Long F. 2012. Building strong bones: Molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol 13:27–38.

Lu ZD, Ye YP, Jiao DC, Qiao JH, Cui SD, Liu ZZ. 2012. miR-155 and miR-31 are differentially expressed in breast cancer patients and are correlated with the estrogen receptor and progesterone receptor status. Oncol Lett 4:1027–1032.

Lu WC, Kao SY, Yang CC, Tu HF, Wu CH, Chang KW, Lin SC. 2014. EGF up-regulates miR-31 through the C/EBPβ signal cascade in oral carcinoma. PLoS One 9:e108049.

Luo LF, Hou CC, Yang WX. 2016. Small non-coding RNAs and their associated proteins in spermatogenesis. Gene 578:141–157.

Luteijn MJ, Ketting RF. 2013. PIWI-interacting RNAs: From generation to transgenerational epigenetics. Nat Rev Genet 14:523–534.

Lynam-Lennon N, Reynolds JV, Marignol L, Sheils OM, Pidgeon GP, Maher SG. 2012. MicroRNA-31 modulates tumour sensitivity to radiation in oesophageal adenocarcinoma. J Mol Med-Jmm 90:1449–1458.

Lyons DC, Martik ML, Saunders LR, McClay DR. 2014. Specification to biomineralization: Following a single cell type as it constructs a skeleton. Integr Comp Biol 54:723–733.

Mardaryev AN, Ahmed MI, Vlahov NV, Fessing MY, Gill JH, Sharov AA, Botchkareva NV. 2010. Micro-RNA-31 controls hair cycle-associated changes in gene expression programs of the skin and hair follicle. FASEB J 24:3869–3881.

McClay DR. 2011. Evolutionary crossroads in developmental biology: Sea urchins. Development 138:2639–2648.

McClay DR. 2016. Sea Urchin Morphogenesis. Curr Top Dev Biol 117:15–29.

Meng W, Ye Z, Cui R, Perry J, Dedousi-Huebner V, Huebner A, Wang Y, Li B, Volinia S, Nakanishi H, Kim T, Suh SS, Ayers LW, Ross P, Croce CM, Chakravarti A, Jin VX, Lautenschlaeger T. 2013. MicroRNA-31 predicts the presence of lymph node metastases and survival in patients with lung adenocarcinoma. Clin Cancer Res 19:5423–5433.

Mitamura T, Watari H, Wang L, Kanno H, Hassan MK, Miyazaki M, Katoh Y, Kimura T, Tanino M, Nishihara H, Tanaka S, Sakuragi N. 2013. Downregulation of miRNA-31 induces taxane resistance in ovarian cancer cells through increase of receptor tyrosine kinase MET. Oncogenesis 2:e40.

Mizoguchi F, Murakami Y, Saito T, Miyasaka N, Kohsaka H. 2013. miR-31 controls osteoclast formation and bone resorption by targeting RhoA. Arthritis Res Ther 15:R102.

Montes M, Nielsen MM, Maglieri G, Jacobsen A, Højfeldt J, Agrawal-Singh S, Hansen K, Helin K, van de Werken HJ, Pedersen JS, Lund AH. 2015. The lncRNA MIR31HG regulates p16(INK4A) expression to modulate senescence. Nat Commun 6:6967.

Mor E, Shomron N. 2013. Species-specific microRNA regulation influences phenotypic variability: Perspectives on species-specific microRNA regulation. Bioessays 35:881–888.

Morgan JE, Partridge TA. 2003. Muscle satellite cells. Int J Biochem Cell Biol 35:1151–1156.

Morhenn VB, Nelson TE, Gruol DL. 2013. The rate of wound healing is increased in psoriasis. J Dermatol Sci 72:87–92.

Mulrane L, Gallagher WM, O'Connor DP. 2014. A novel mechanism of regulation of the anti-metastatic miR-31 by EMSY in breast cancer. Breast Cancer Res 16:467.

Muñoz X, Mata A, Bassas L, Larriba S. 2015. Altered miRNA signature of developing germ-cells in infertile patients relates to the severity of spermatogenic failure and persists in spermatozoa. Sci Rep 5:17991–18003.

Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. 2002. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29.

Nemoto E, Ebe Y, Kanaya S, Tsuchiya M, Nakamura T, Tamura M, Shimauchi H. 2012. Wnt5a signaling is a substantial constituent in bone morphogenetic protein-2-mediated osteoblastogenesis. Biochem Biophys Res Commun 422:627–632.

Nowak KJ, Davies KE. 2004. Duchenne muscular dystrophy and dystrophin: Pathogenesis and opportunities for treatment. EMBO Rep 5:872–876.

Okudela K, Suzuki T, Umeda S, Tateishi Y, Mitsui H, Miyagi Y, Ohashi K. 2014a. A comprehensive search for microRNAs with expression profiles modulated by oncogenic KRAS: Potential involvement of miR-31 in lung carcinogenesis. Oncol Rep 32:1374–1384.

Okudela K, Tateishi Y, Umeda S, Mitsui H, Suzuki T, Saito Y, Woo T, Tajiri M, Masuda M, Miyagi Y, Ohashi K. 2014b. Allelic imbalance in the miR-31 host gene locus in lung cancer-its potential role in carcinogenesis. PLoS One 9:e 100581.

Oliveri P, Davidson EH, McClay DR. 2003. Activation of pmar1 controls specification of micromeres in the sea urchin embryo. Dev Biol 258:32–43.

Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. 1999. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 401: 82–85.

Pasparakis M. 2009. Regulation of tissue homeostasis by NF-kappaB signalling: Implications for inflammatory diseases. Nat Rev Immunol 9:778–788.

Pauli A, Rinn JL, Schier AF. 2011. Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet 12:136–149.

Pedrioli DM, Karpanen T, Dabouras V, Jurisic G, van de Hoek G, Shin JW, Marino D, Kälin RE, Leidel S, Cinelli P, Schulte-Merker S, Brändli AW, Detmar M. 2010. miR-31 functions as a negative regulator of lymphatic vascular lineage-specific differentiation in vitro and vascular development in vivo. Mol Cell Biol 30:3620–3634.

Peng H, Hamanaka RB, Katsnelson J, Hao LL, Yang W, Chandel NS, Lavker RM. 2012a. MicroRNA-31 targets FIH-1 to positively regulate corneal epithelial glycogen metabolism. FASEB J 26:3140–3147.

Peng H, Kaplan N, Hamanaka RB, Katsnelson J, Blatt H, Yang W, Hao L, Bryar PJ, Johnson RS, Getsios S, Chandel NS, Lavker RM. 2012b. microRNA-31/factor-inhibiting hypoxia-inducible factor 1 nexus regulates keratinocyte differentiation. Proc Natl Acad Sci USA 109:14030–14034.

Pennington JT, Strathmann RR. 1990. Consequences of the calcite skeletons of planktonic echinoderm larvae for orientation, swimming, and shape. Biol Bull 121–133.

Piacentino ML, Ramachandran J, Bradham CA. 2015. Late Alk4/5/7 signaling is required for anterior skeletal patterning in sea urchin embryos. Development 142:943–952.

Piacentino ML, Chung O, Ramachandran J, Zuch DT, Yu J, Conaway EA, Reyna AE, Bradham CA. 2016a. Zygotic LvBMP5-8 is required for skeletal patterning and for left-right but not dorsal-ventral specification in the sea urchin embryo. Dev Biol 412:44–56.

Piacentino ML, Zuch DT, Fishman J, Rose S, Speranza EE, Li C, Yu J, Chung O, Ramachandran J, Ferrell P, Patel V, Reyna A,

Hameeduddin H, Chaves J, Hewitt FB, Bardot E, Lee D, Core AB, Hogan JD, Keenan JL, Luo L, Coulombe-Huntington J, Blute TA, Oleinik E, Ibn-Salem J, Poustka AJ, Bradham CA. 2016b. RNA-Seq identifies SPGs as a ventral skeletal patterning cue in sea urchins. Development 143:703–714.

Ponting CP, Oliver PL, Reik W. 2009. Evolution and functions of long noncoding RNAs. Cell 136:629–641.

Rafiq K, Cheers MS, Ettensohn CA. 2012. The genomic regulatory control of skeletal morphogenesis in the sea urchin. Development 139:579–590.

Raggatt LJ, Partridge NC. 2010. Cellular and molecular mechanisms of bone remodeling. J Biol Chem 285:25103–25108.

Rajbhandari R, McFarland BC, Patel A, Gerigk M, Gray GK, Fehling SC, Bredel M, Berbari NF, Kim H, Marks MP, Meares GP, Sinha T, Chuang J, Benveniste EN, Nozell SE. 2015. Loss of tumor suppressive microRNA-31 enhances TRADD/NF-κB signaling in glioblastoma. Oncotarget 6:17805–17816.

Ramirez HA, Liang L, Pastar I, Rosa AM, Stojadinovic O, Zwick TG, Kirsner RS, Maione AG, Garlick JA, Tomic-Canic M. 2015. Comparative genomic, microRNA, and tissue analyses reveal subtle differences between non-diabetic and diabetic foot skin. PLoS One 10:e0137133.

Rasheed SA, Teo CR, Beillard EJ, Voorhoeve PM, Zhou W, Ghosh S, Casey PJ. 2015. MicroRNA-31 controls G protein alpha-13 (GNA13) expression and cell invasion in breast cancer cells. Mol Cancer 14:67.

Rinn JL, Chang HY. 2012. Genome regulation by long noncoding RNAs. Annu Rev Biochem 81:145–166.

Rouas R, Fayyad-Kazan H, El Zein N, Lewalle P, Rothe F, Simion A, Akl H, Mourtada M, El Rifai M, Burny A, Romero P, Martiat P, Badran B. 2009. Human natural Treg microRNA signature: Role of microRNA-31 and microRNA-21 in FOXP3 expression. Eur J Immunol 39:1608–1618.

Russell AP, Lamon S, Boon H, Wada S, Güller I, Brown EL, Chibalin AV, Zierath JR, Snow RJ, Stepto N, Wadley GD, Akimoto T. 2013. Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. J Physiol 591:4637–4653.

Rutledge H, Baran-Gale J, de Villena FP, Chesler EJ, Churchill GA, Sethupathy P, Kelada SN. 2015. Identification of microRNAs associated with allergic airway disease using a genetically diverse mouse population. BMC Genomics 16:633.

Saunders LR, McClay DR. 2014. Sub-circuits of a gene regulatory network control a developmental epithelial-mesenchymal transition. Development 141:1503–1513.

Sebastiani G, Nigi L, Spagnuolo I, Morganti E, Fondelli C, Francesko D. 2013. MicroRNA profiling in sera of patients with type 2 diabetes mellitus reveals an upregulation of miR-31 expression in subjects with microvascular complications. J Biomed Sci Eng 6:58–64.

Sharma T, Ettensohn CA. 2010. Activation of the skeletogenic gene regulatory network in the early sea urchin embryo. Development 137:1149–1157.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539.

Song JL, Stoeckius M, Maaskola J, Friedlander M, Stepicheva N, Juliano C, Lebedeva S, Thompson W, Rajewsky N, Wessel GM. 2012. Select microRNAs are essential for early development in the sea urchin. Dev Biol 362:104–113.

Song JL, Nigam P, Tektas SS, Selva E. 2015. microRNA regulation of Wnt signaling pathways in development and disease. Cell Signal 27:1380–1391.

Sossey-Alaoui K, Downs-Kelly E, Das M, Izem L, Tubbs R, Plow EF. 2011. WAVE3, an actin remodeling protein, is regulated by the metastasis suppressor microRNA, miR-31, during the invasion-metastasis cascade. Int J Cancer 129:1331–1343.

Stepicheva NA, Song JL. 2015. microRNA-31 modulates skeletal patterning in the sea urchin embryo. Development 142:3769–3780.

Stow JL, Manderson AP, Murray RZ. 2006. SNAREing immunity: The role of SNAREs in the immune system. Nat Rev Immunol 6:919–929.

Sun D, Yu F, Ma Y, Zhao R, Chen X, Zhu J, Zhang CY, Chen J, Zhang J. 2013. MicroRNA-31 activates the RAS pathway and functions as an oncogenic MicroRNA in human colorectal cancer by repressing RAS p21 GTPase activating protein 1 (RASA1). J Biol Chem 288:9508–9518.

Taccioli C, Garofalo M, Chen H, Jiang Y, Tagliazucchi GM, Di Leva G, Alder H, Fadda P, Middleton J, Smalley KJ, Selmi T, Naidu S, Farber JL, Croce CM, Fong LY. 2015. Repression of esophageal neoplasia and inflammatory signaling by Anti-miR-31 delivery in vivo. J Natl Cancer Inst 107:1–11.

Tang MK, Zhou HY, Yam JW, Wong AS. 2010. c-Met overexpression contributes to the acquired apoptotic resistance of nonadherent ovarian cancer cells through a cross talk mediated by phosphatidylinositol 3-kinase and extracellular signal-regulated kinase 1/2. Neoplasia 12:128–138.

Tang W, Li Y, Osimiri L, Zhang C. 2011. Osteoblast-specific transcription factor Osterix (Osx) is an upstream regulator of Satb2 during bone formation. J Biol Chem 286:32995–33002.

Tateishi Y, Okudela K, Mitsui H, Umeda S, Suzuki T, Kojima Y, Watanabe K, Kawano N, Endo I, Ohashi K. 2015. The potential role of microRNA-31 expression in early colorectal cancer. Pathol Int 65:513–518.

Thompson MA, Edmonds, MD, Liang S, McClintock-Treep, Wantg, X, Li S, Eichen CM. 2016. miR-31 and miR-17-5p levels change during transformation of follicular lymphoma. Hum Pathol 50:118–126.

Tsai YH, Wu MF, Wu YH, Chang SJ, Lin SF, Sharp TV, Wang HW. 2009. The M type K15 protein of Kaposi's sarcoma-associated herpesvirus regulates microRNA expression via its SH2-binding motif to induce cell migration and invasion. J Virol 83:622–632.

Vignali DA, Collison LW, Workman CJ. 2008. How regulatory T cells work. Nat Rev Immunol 8:523–532.

Viré E, Curtis C, Davalos V, Git A, Robson S, Villanueva A, Vidal A, Barbieri I, Aparicio S, Esteller M, Caldas C, Kouzarides T. 2014. The breast cancer oncogene EMSY represses transcription of antimetastatic microRNA miR-31. Mol Cell 53:806–818.

Vrba L, Muñoz-Rodríguez JL, Stampfer MR, Futscher BW. 2013. miRNA gene promoters are frequent targets of aberrant DNA methylation in human breast cancer. PLoS One 8:e 54398.

Wang CJ, Stratmann J, Zhou ZG, Sun XF. 2010. Suppression of microRNA-31 increases sensitivity to 5-FU at an early stage, and affects cell migration and invasion in HCT-116 colon cancer cells. BMC Cancer 10:616.

Wang J, Yan CH, Li Y, Xu K, Tian XX, Peng CF, Tao J, Sun MY, Han YL. 2013. MicroRNA-31 controls phenotypic modulation of human vascular smooth muscle cells by regulating its target gene cellular repressor of E1A-stimulated genes. Exp Cell Res 319:1165–1175.

Wang N, Zhou Y, Zheng L, Li H. 2014. MiR-31 is an independent prognostic factor and functions as an oncomir in cervical cancer via targeting ARID1A. Gynecol Oncol 134:129–137.

Wang JX, Xu J, Han YF, Zhu YB, Zhang WJ. 2015a. Diagnostic values of microRNA-31 in peripheral blood mononuclear cells for pediatric pulmonary tuberculosis in Chinese patients. Genet Mol Res 14:17235–17243.

Wang Y, Men M, Yang W, Zheng H, Xue S. 2015b. MiR-31 downregulation protects against cardiac ischemia/reperfusion injury by targeting protein kinase C epsilon (PKCε) directly. Cell Physiol Biochem 36:179–190.

Weilner S, Schraml E, Wieser M, Messner P, Schneider K, Wassermann K, Micutkova L, Fortschegger K, Maier AB, Westendorp R, Resch H, Wolbank S, Redl H, Jansen-Dürr P, Pietschmann P, Grillari-Voglauer R, Grillari J. 2016. Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells. Aging Cell 15:744–754.

Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, Horvitz HR, Kauppinen S, Plasterk RH. 2005. MicroRNA expression in zebrafish embryonic development. Science 309:310–311.

Wilczynska A, Bushell M. 2015. The complexity of miRNA-mediated repression. Cell Death Differ 22:22–33.

Wong HK, Fatimy RE, Onodera C, Wei Z, Yi M, Mohan A, Gowrisankaran S, Karmali P, Marcusson E, Wakimoto H, Stephens R, Uhlmann EJ, Song JS, Tannous B, Krichevsky AM. 2015. The cancer genome atlas analysis predicts microRNA for targeting cancer growth and vascularization in glioblastoma. Mol Ther 23:1234–1247.

Wu YH, Hu TF, Chen YC, Tsai YN, Tsai YH, Cheng CC, Wang HW. 2011. The manipulation of miRNA-gene regulatory networks by KSHV induces endothelial cell motility. Blood 118:2896–2905.

Xi S, Yang M, Tao Y, Xu H, Shan J, Inchauste S, Zhang M, Mercedes L, Hong JA, Rao M, Schrump DS. 2010. Cigarette smoke induces C/EBP-β-mediated activation of miR-31 in normal human respiratory epithelia and lung cancer cells. PLoS One 5:e 13764.

Xiao GH, Jeffers M, Bellacosa A, Mitsuuchi Y, Vande Woude GF, Testa JR. 2001. Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. Proc Natl Acad Sci USA 98:247–252.

Xie Q, Wang Z, Bi X, Zhou H, Wang Y, Gu P, Fan X. 2014. Effects of miR-31 on the osteogenesis of human mesenchymal stem cells. Biochem Biophys Res Commun 446:98–104.

Xu N, Meisgen F, Butler LM, Han G, Wang XJ, Söderberg-Nauclér C, Ståhle M, Pivarcsi A, Sonkoly E. 2013a. MicroRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40. J Immunol 190: 678–688.

Xu RS, Wu XD, Zhang SQ, Li CF, Yang L, Li DD, Zhang BG, Zhang Y, Jin JP, Zhang B. 2013b. The tumor suppressor gene RhoBTB1 is a novel target of miR-31 in human colon cancer. Int J Oncol 42:676–682.

Xuan YT, Tang XL, Banerjee S, Takano H, Li RC, Han H, Qiu Y, Li JJ, Bolli R. 1999. Nuclear factor-kappaB plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. Circ Res 84:1095–1109.

Xue F, Li H, Zhang J, Lu J, Xia Y, Xia Q. 2013. miR-31 regulates interleukin 2 and kinase suppressor of ras 2 during T cell activation. Genes Immun 14:127–131.

Yadav M, Stephan S, Bluestone JA. 2013. Peripherally induced tregs—role in immune homeostasis and autoimmunity. Front Immunol 4:232.

Yamagishi M, Nakano K, Miyake A, Yamochi T, Kagami Y, Tsutsumi A, Matsuda Y, Sato-Otsubo A, Muto S, Utsunomiya A, Yamaguchi K, Uchimaru K, Ogawa S, Watanabe T. 2012. Polycomb-mediated loss of miR-31 activates NIK-dependent NF-κB pathway in adult T cell leukemia and other cancers. Cancer Cell 21:121–135.

Yan S, Xu Z, Lou F, Zhang L, Ke F, Bai J, Liu Z, Liu J, Wang H, Zhu H, Sun Y, Cai W, Gao Y, Su B, Li Q, Yang X, Yu J, Lai Y, Yu XZ, Zheng Y, Shen N, Chin YE. 2015. NF-κB-induced microRNA-31 promotes epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. Nat Commun 6:7652.

Yang MH, Yu J, Chen N, Wang XY, Liu XY, Wang S, Ding YQ. 2013. Elevated microRNA-31 expression regulates colorectal cancer progression by repressing its target gene SATB2. PLoS One 8:e85353.

Ying Z, Li J, Li M. 2011. Astrocyte elevated gene 1: biological functions and molecular mechanism in cancer and beyond. Cell Biosci 1:36.

Yu ZF, Creighton C, Fountain MD, Nagaraja AK, Zhu HF, Khan MF, Han DY, Olokpa E, Hawkins SM, Gunaratne P, Anderson ML, Matzuk MM. 2010. MiR-31 Is a tumor suppressor

microRNA that functions in ovarian cancer. Biol Reprod 83:59–59.

Yu M, Liang H, Fu Z, Wang X, Liao Z, Zhou Y, Liu Y, Wang Y, Hong Y, Zhou X, Yan X, Ma M, Zhang W, Guo B, Zhang J, Zen K, Zhang CY, Wang T, Zhang Q, Chen X. 2016. BAP1 suppresses lung cancer progression and is inhibited by miR-31. Oncotarget 7:13742–13753.

Zhang Q, Padi SK, Tindall DJ, Guo B. 2014. Polycomb protein EZH2 suppresses apoptosis by silencing the proapoptotic miR-31. Cell Death Dis 5:e 1486.

Zhang L, Ke F, Liu Z, Bai J, Liu J, Yan S, Xu Z, Lou F, Wang H, Zhu H, Sun Y, Cai W, Gao Y, Li Q, Yu XZ, Qian Y, Hua Z, Deng J, Li QJ. 2015. MicroRNA-31 negatively regulates peripherally derived regulatory T-cell generation by repressing retinoic acid-inducible protein 3. Nat Commun 6:7639.

Zhang B, Li H, Yin C, Sun X, Zheng S, Zhang C, Shi L, Liu Y, Lu S. 2016. Dock1 promotes the mesenchymal transition of glioma and is modulated by MiR-31. Neuropathol Appl Neurobiol [Epub ahead of print].

Zhao X, Xu D, Li Y, Zhang J, Liu T, Ji Y, Wang J, Zhou G, Xie X. 2014. MicroRNAs regulate bone metabolism. J Bone Miner Metab 32:221–231.

Zhou RJ, Xu XY, Liu BX, Dai WZ, Cai MQ, Bai CF, Zhang XF, Wang LM, Lin L, Jia SZ, Wang WH. 2015. Growth-inhibitory and chemosensitizing effects of microRNA-31 in human glioblastoma multiforme cells. Int J Mol Med 36:1159–1164.

Zhou M, Yu G, Yang X, Zhu C, Zhang Z, Zhan X. 2016. Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection. Mol Med Rep 13: 4620–4626.

MRD