



microRNA Modulation

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Abstract

microRNAs are small and evolutionary conserved RNA molecules that vastly fine-tune protein expression at a posttranscriptional level. microRNA modulation has recently surfaced as powerhouse feeding the progress of novel strategies for tissue and cell engineering and regeneration. The field is growing exponentially each year and approaching clinical applications, with considerable progress in identifying biomarkers for personalized medical needs and also harnessing the therapeutic potential of these molecules to finely enhance tissue repair. This chapter aims to support beginner and expert researchers alike to delve into this emerging dynamic field. Within this chapter, we provide an overview of the biology and function of microRNAs and how they are being addressed within the tissue engineering and regenerative medicine (TERM) arena in terms of resources, applications, and development projection. Specific attention is given to the advances in the development of specialized delivery systems for microRNAs, which largely involves the application of biomaterial scaffolds, and to finalize, we review the proven therapeutic potential of microRNAs to date within the TERM space. Overall, this chapter underlines the exciting potential of microRNA modulation for cell engineering and regeneration.

1 Introduction to microRNA Biology for Cell Engineering and Regeneration

Novel approaches to engineer cells are currently gaining much traction in the field of tissue engineering and regenerative medicine (TERM) (Yau et al. 2012). These innovative strategies represent a step beyond the traditional application of cell-based therapy – with unmodified cells, biomaterial-based therapy, and the combination of both elements (Khademhosseini et al. 2009; Evans 2013). The main aim of applying cell engineering to TERM is to enhance the ability of the target cells to

regenerate tissues and re-establish a healthy function (Nerem 1991); these target cells can be part of cell therapies or be engineered directly at the host's tissue defect site. To translate the successful cell engineering methods into therapies applicable to TERM, the cross-functional collaboration of bioengineering, molecular biology, biopharmaceutics, materials science, nanotechnology, and medical/clinical experts will remain key. microRNAs represent a recent breakthrough in molecular biology, and with the understanding of how these small RNAs can influence tissue regeneration, microRNA modulation shines through as a novel promising route for cell engineering. In addition, microRNA profiling and biomaterials research has begun to merge toward the development of physiologically relevant in vitro 3D models, providing game-changing insights on the transcriptomic modulation of tissue repair (Cui et al. 2014). Key concepts of the origin and potential of microRNAs and microRNA therapeutics, including the methods and resources suitable for their investigation, will be discussed in this chapter. A specific focus will be on the state-of-the-art research on cell engineering and regeneration using biomaterials to deliver microRNA therapeutics, as well as on the hurdles for the clinical translation of these technologies.

1.1 microRNAs as Part of the Natural Cellular Mechanism of RNA Interference

microRNAs (miRNAs) have positioned themselves at the top of the natural RNA interference tools for cell engineering applications, thus challenging the 1950s dogma of hierarchical gene expression, which postulates that oligonucleotide sequences only act as units of information storage (Rinn and Chang 2012). The discovery of miRNAs led to the realization that oligonucleotides may also convey enzymatic and regulatory activities to manage the use of the very information stored within and ultimately allow the synthesis of proteins and its control (Pearson 2006). These activities entail a broad spectrum of RNA molecules such as ribosomal (r) RNAs, transfer (t)RNAs, small nuclear (sn)RNAs, small nucleolar (sno)RNAs, noncoding (nc)RNAs, or RNA interference (RNAi) (Fig. 1; Alberts et al. 2002). Ultimately, controlling the synthesis of proteins by RNAi presents an advanced and very valuable cell engineering strategy (Hackl et al. 2011).

Although miRNAs are involved in the RNA interference (RNAi) process, Fire et al. first established this process in *C. elegans* years before miRNAs were discovered (Fire et al. 1998). This process is also known as the RNA-induced silencing pathway and is initiated by long double-stranded (ds)RNA oligonucleotides. Such long dsRNA molecules can be either naturally generated or introduced exogenously as an intermediate of viral replication (Ketting et al. 1999; Mourrain et al. 2000; Li et al. 2002; Aravin et al. 2003) or via experimental gene knockdown (Fire et al. 1998). In the RNAi process, the dsRNA is processed by Dicer into smaller RNAs (Hamilton and Baulcombe 1999; Hammond et al. 2000; Knight and Bass 2001) that

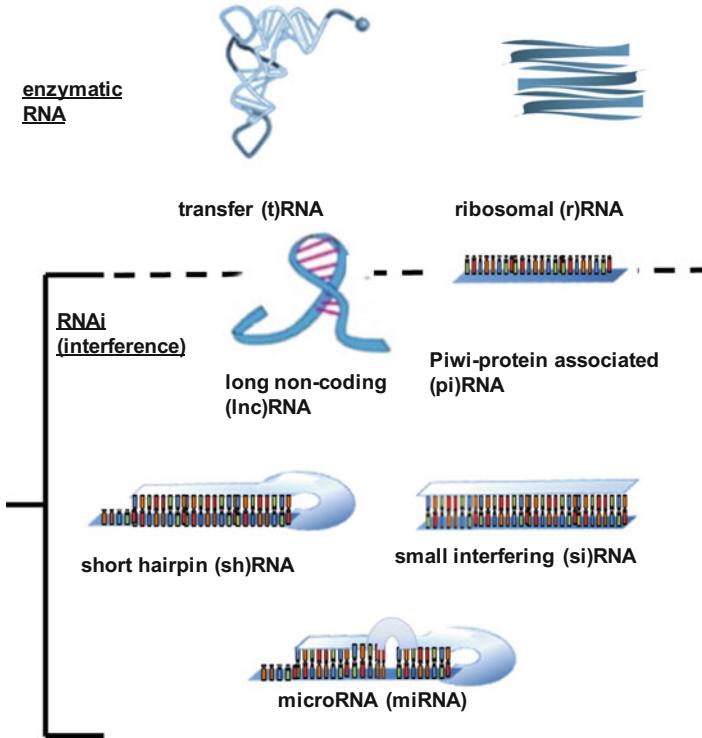


Fig. 1 Types of enzymatic RNAs that manage the expression of protein-coding genes. tRNA and rRNA cooperate in the production of protein from messenger (m)RNA as part of the translation process. This activity is subjected to the control of RNA interference (RNAi) molecules: short hairpin (sh)RNAs, small interference (si)RNAs, and microRNAs (miRNAs). Long noncoding (lnc) RNAs and Piwi-protein associated (pi)RNAs are involved in RNAi as well as in additional enzymatic functions

eventually become incorporated as single-stranded (ss)RNAs into the RNA-induced silencing complex (RISC) (Elbashir et al. 2001a; Nykanen et al. 2001; Martinez et al. 2002). The RISC is composed of the endonuclease Slicer and other structural proteins such as VIG, fragile X-related protein, and Tudor-SN (Caudy et al. 2002, 2003; Ishizuka et al. 2002). The core protein of this complex is a member of the Argonaute family with ability to bind to ssRNAs and dsRNAs through a domain denominated PAZ (Martinez et al. 2002; Hammond et al. 2001; Hutvagner and Zamore 2002; Lingel et al. 2003). Once the RISC is formed, it identifies target messenger RNAs (mRNAs) based on perfect or nearly perfect complementarity between the 3' untranslated (UTR) region of the mRNA and 5' terminal region of the RNAi, also known as the seed region (Elbashir et al. 2001b). This interaction directs the target mRNA for either cleavage or repression of its translation, thus resulting in either a decrease in target mRNA or protein levels (Bartel 2004).

1.2 The Discovery of microRNAs

To understand the potential of microRNAs as tools for cell engineering and regeneration, we need to review the origins of miRNA research as a field of study, starting with the original discovery of the *lin-4* RNA gene in *Caenorhabditis elegans* in 1993 by Victor Ambros and colleagues, Rosalind Lee and Rhonda Feinbaum (Lee et al. 1993). This team discovered that the *lin-4* gene, a known regulator of the timing in larval development, did not code for a protein but instead produced a pair of small RNAs, 61 and 22 nucleotides (nt) long, with antisense complementarity to the *lin-14* gene (Wightman et al. 1993). The breakthrough was followed by the discovery, in *C. elegans*, of a second ~22 nt regulatory RNA named *let-7* and the identification of *let-7* homologs in the genome of human, fly, and 11 other species (Reinhart et al. 2000; Slack et al. 2000; Pasquinelli et al. 2000). Within a year, the identification of additional genes for ~22 nt noncoding RNAs surpassed 100 (Lagos-Quintana et al. 2001; Lau et al. 2001; Lee and Ambros 2001); these genes with their RNA products were evolutionary conserved, but many functioned in particular cell types and were not related to embryonic development. To date, over 2500 miRNAs in humans have been identified (miRBase 2013), regulating up to one third of the protein-coding genome (Lewis et al. 2005). Increasing numbers of studies are discovering miRNAs that function to regulate key cellular events that are widely pursued in cell and tissue engineering. These include cell development, lineage commitment, differentiation, proliferation and apoptosis, immune response events and related diseases, tumor formation, and the progression of viral infections (Yau et al. 2012; Hu et al. 2010). From this evidence, miRNA-directed gene regulation has gained the attention of researchers in the tissue engineering and regenerative medicine arena seeking advanced solutions to restore and enhance normal tissue function.

1.3 The Biogenesis Process of microRNAs

Although miRNAs share cellular RNAi machinery with other types of dsRNA molecules, they must first be present in the cytoplasm to trigger their control of protein expression. This occurs following the natural biogenesis process of miRNAs (Fig. 2), which involves additional machinery and can be divided into three main phases of transcription, maturation, and RNA-induced silencing complex (RISC) assembly:

Transcription: In this step, long primary transcripts termed pri-miRNAs generate either from intronic regions of protein-coding genes or directly from intergenic or polycistronic miRNA genes containing stem-loop miRNA clusters (Lee et al. 2002). The enzyme involved in the intronic transcripts is always a type II RNA polymerase (pol) that also transcribes the corresponding protein-coding exons. However, for intergenic and polycistronic miRNA genes, both RNA pol type II

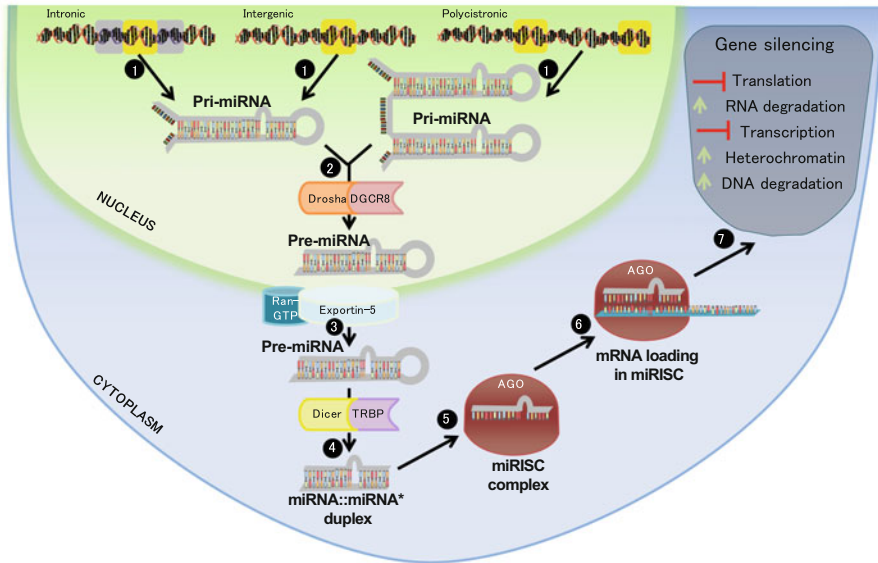


Fig. 2 miRNA biogenesis and function. (Reproduced with permission (Curtin et al. 2017). Copyright © 2017, John Wiley and Sons). (1) Transcription of miRNA genes to pri-miRNA is followed by (2) loop-processing by Drosha to generate a pre-miRNA that is then (3) transported into the cytoplasm where (4) Dicer/TRBP releases the miRNA:miRNA* duplex. (5) The mature miRNA separates to form the miRISC complex with the Argonaute (AGO) protein core, while the miRNA* strand degrades. (6) Target mRNAs interact with miRISC complexes, leading to (7) mRNA degradation or translational repression among other complex regulatory functions

and type III may carry out the transcription (Ohler et al. 2004). While RNA pol III can render efficiently processed miRNAs that function in vivo (Chen et al. 2004), RNA pol II can lead to robust expression of reporter proteins with an open reading frame downstream of intergenic miRNA genes (Johnson and Urist 1998; Johnston and Hobert 2003).

Maturation: This step is comprised of three events, two cellular locations, and several enzymes and multi-protein complexes. It starts when pri-miRNAs are cleaved in both strands leaving 60–70 nt stem-loop precursor miRNAs with 5' phosphate and ~2 nt 3' overhangs, defined as pre-miRNAs (Lee et al. 2002, 2003; Zeng and Cullen 2003; Basyuk et al. 2003): this occurs in the nucleus by the Drosha RNase III endonuclease. Following this, Ran-GTP and Exportin-5 actively transport the pre-miRNA into the cytoplasm (Yi et al. 2003; Lund et al. 2004). Finally, both strands of the pre-miRNA duplex are cleaved at the base of the stem-loop by the Dicer RNase III endonuclease. This process also creates 5' phosphate and ~2 nt 3' overhangs, generating an imperfect duplex, termed miRNA:miRNA* (Chen et al. 2004; Grishok et al. 2001; Hutvagner et al. 2001; Ketting et al. 2001). The duplex is short lived, with the two strands separating into the mature miRNA single strand and, the opposite and more unstable arm, that is, designated miRNA* or alternative strand.

RISC assembly: Finally, the single-stranded mature miRNA is selected for incorporation into the RISC, forming the miRISC complex (Mourelatos et al. 2002), while the miRNA*, detected at much lower frequencies, appears to be degraded (Aravin et al. 2003; Lim et al. 2003). Regarding the strand selection, it is hypothesized that an unknown helicase may direct the limper 5'-end strand to enter the RISC, although this must yet be fully elucidated (Khvorova et al. 2003; Schwarz et al. 2003). However, the alternative strand can be functionally active in some instances forming miRISCs, depending on the tissue, developmental, or pathophysiological state (Okamura et al. 2009). Once miRISC is formed, the interaction with the untranslated region (UTR) of the target mRNA may take place, resulting in mRNA cleavage or translational repression (Bartel 2004; Lewis et al. 2005).

The characteristic feature of miRNAs, differing from siRNAs, can be noted at the end of their biogenesis process and within a multi-protein complex. miRISC is most prone to interact with the 3'-UTR region of a target mRNA; however binding to 5'-UTR regions has also been documented (Lytle et al. 2007). Additionally, miRISC can establish both perfect and imperfect sequence complementarity binding to the mRNA targets (Guo et al. 2010). It is this partial binding that allows the multi-targeting effect of miRNAs: the seed region of a single miRNA can imperfectly bind (and thus interfere with the expression of) as many as 100 mRNAs, and the mRNA coding for a specific protein can be the subject of interaction with a collection of miRNAs (Bartel 2004; Lewis et al. 2005; Mourelatos et al. 2002; Jacobsen et al. 2013). Altogether, this overview of the known mechanism of action of miRNAs supports the increasing interest in using them as cell engineering tools to improve tissue repair.

1.4 microRNA Nomenclature

The discovery and establishment of miRNAs as a distinct class of RNA molecules necessitated the development of a uniform nomenclature system (Ambros et al. 2003; Desvignes et al. 2015; Budak et al. 2016), summarized in Table 1, to facilitate the well-ordered progress of the miRNA research field. This system is coordinated by the HUGO Gene Nomenclature Committee (HGNC) for the human miRNA genes (Gray et al. 2015) and aims to designate the different biogenic stages and variants of miRNAs as well as to keep newly discovered miRNAs generated by sequencing data and powerful bioinformatics tools in an orderly organization that would prevent overlapping in the denomination of new miRNAs (Griffiths-Jones et al. 2006). Recently, miRtrons, isomiRs, moRs, loRs, miRNA clusters, and mirror miRNAs (defined in Table 1) have been recognized as biogenic variants of miRNAs. In general, any given miRNA receives the prefix “miR” followed by a number of one to four figures and should be preceded by the three-letter code assigned to the species, i.e., *hsa*- for *Homo sapiens* (human) or *mmu*- for *Mus musculus* (mouse). The specific set of figures that identify a miRNA is set in order of discovery but also

Table 1 Nomenclature designating miRNAs and their types of interaction with target mRNAs

Term	Definition
miR-XX	miRNA-coding gene
mir-XX	Precursor hairpins (lower case)
spp – miR-XX	Mature sequence miR-XX designated for a determined species
miR-XX-1; miR-XX-2	Mature identical sequences proceeding from different genes (i.e., distinct pri-miRNA loci)
miR-XXa/b	Closely related mature sequences differing in only one or two nucleotides
miR-XX-5p/3p	Different mature miRNA sequences excised respectively from 5'- and 3'-arms of the same pre-miRNA (new nomenclature replacing miRNA and miRNA*)
miRc-XX	A miRNA cluster is a group of miRNA genes located in a small chromosomal region and subject to simultaneous regulation of their expression and thus shares a function
miRtron	Drosha-independent miR, the precursor (pre-miRNA) derives from a short intron
isomiR	A variant of posttranscriptional processing of a miRNA gene; may differ in sequence and hence in targets/functions.
moR-XX.Y	Offset RNA adjacent to the hairpin loop of the pre-miRNA, independently regulated
loR-XX	RNA single strand originating from the hairpin loop of the pre-miRNA
Mirror miR; miR-XXos	A pri-miRNA independently transcribed from both DNA strands; likely differs in seed region and hence in targets/function
miRNA::mRNA	Interaction of a miRNA and its target mRNA
6mer-1A	An exact match to positions 2–6 of the mature miRNA (the seed) followed by an adenine (“A”)
6mer	An exact match to positions 2–7 of the seed
7mer-1A	An exact match to positions 2–7 of the seed followed by an “A”
7mer-m8	An exact match to positions 2–8 of the seed + position 8
8mer-1A	An exact match to positions 2–8 of the seed + position 8 followed by an “A”

takes into account similarity or homology of the sequence with previously known miRNAs. Moreover, highly homologous miRNAs are often designated as members of “miRNA families” when they present evolutionary conserved sequences, in particular in the 5'-end region. Since the 5'-end of a miRNA contains the small nucleotide “seed region” sequence, responsible for the interaction with its mRNA targets, highly homolog seed regions between miRNAs signify that they likely share a set of mRNA targets. These miRNA families are referred to by the first discovered miRNA, i.e., the family of miR-15a/b, miR-16, miR-195, miR-424, and miR-497 is referred to as the miR-15 family. Importantly, all members of the miR-15 and miR-17 families have great impact in modulating multiple processes of interest for tissue engineering, including cell cycle, proliferation, and angiogenesis (Pekarsky et al. 2018; Porrello et al. 2011; Cimmino et al. 2005; Nunes et al. 2015; Sun et al. 2013; Caporali and Emanuelli 2011; Linsley et al. 2007), which will be further described later in this chapter. Also, specific nomenclature has been established to

designate the different types of base-pairing interaction that can take place between the seed region of a miRNA and its target mRNA.

2 Project Blueprint to Harness microRNA Research for Cell Engineering

The sequence of stages necessary to develop a successful miRNA-based cell engineering strategy can be intricate for a tissue engineering researcher approaching a miRNA research project for the first time. This is because a wide range of miRNA exploration techniques have been newly created or adapted to allow their detailed study. These include bioinformatics tools, as well as techniques ranging from miRNA isolation and identification to their detection, the elucidation of their targets and the pathways behind their regulation, and ultimately their therapeutic potential. This section provides a blueprint of the resources and techniques powering the progress in this field and the standard experimental workflows designed applying these techniques.

2.1 Bioinformatic Tools in miRNA Research

There are multiple bioinformatic tools developed to allow the *in silico* exploration of putative functions for known miRNAs, as recently reviewed by Budak et al. (2016) and also by Akhtar and colleagues (Akhtar et al. 2016). These resources are freely available online and permit a wide range of tasks such as identification of miRNAs with their regulatory, metabolic, and signaling networks. Hence, bioinformatic analysis performed ahead of experimental studies provide highly valuable data to identify mRNA targets implicated in a particular pathway, along with the type of miRNA::mRNA target interaction taking place, its homology across species, and the region occupancy by other neighboring miRNAs (Grimson et al. 2007). Although many of these platforms offer mixed services, we can generally distinguish between target prediction tools like TargetScan (TargetScan Release 6.2 2012) or microRNA.org (cBio 2010) and compiling databases such as miRTarBase (ISBLab 2013) or MirSNP (Bhattacharya et al. 2014) (Fig. 3). The target prediction tools, programmed to predict the likelihood of miRNA::mRNA interactions, often receive vast attention in screening studies. Each of these programs is based on a different and complex algorithm that computes multiple parameters of miRNA behavior in order to yield and score a list of gene target hits (Schirle et al. 2014). These parameters include, among others, the binding free energy and subsequent thermodynamic stability of the miRNA::mRNA duplex, as well as the absence of secondary structures surrounding the seed region. Therefore, results might differ widely when comparing computational predicting tools; to narrow down the chances of false-negative or false-positive predictions, it is recommended to seek overlaps within the results pooled from multiple tools.

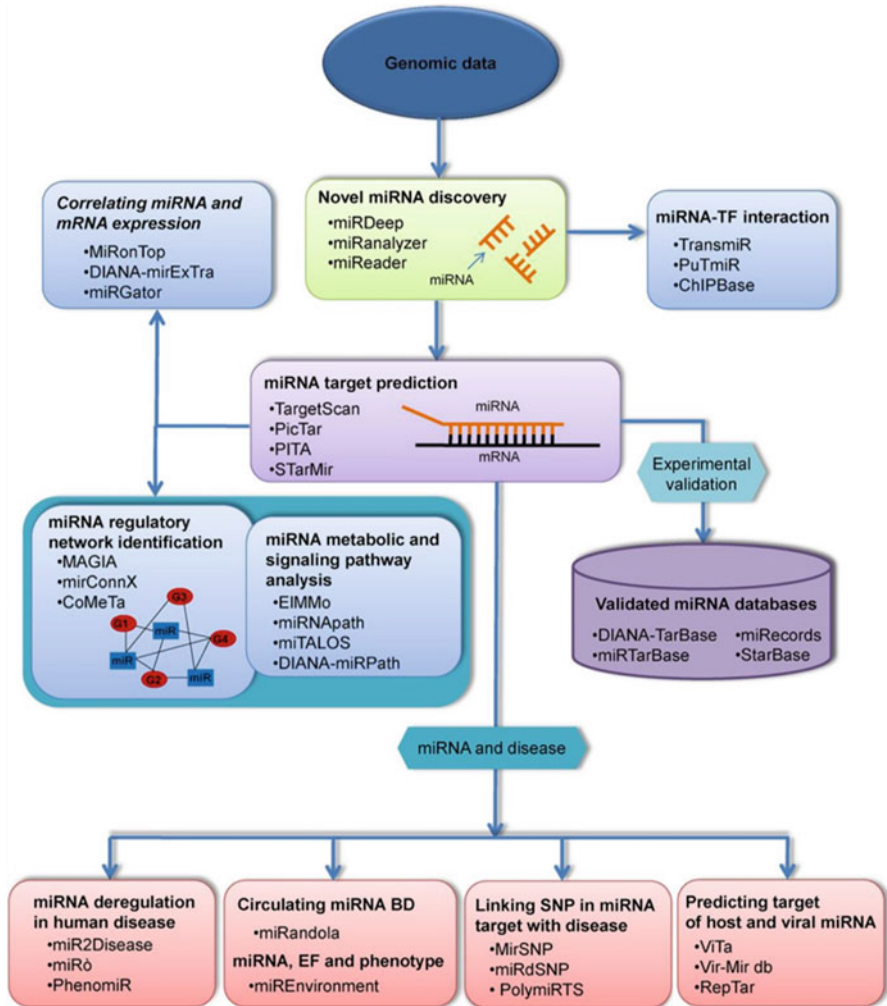


Fig. 3 Currently available bioinformatic tools classified based on principal type of data output. Illustrative examples of all categories are shown. (Reproduced with permission Akhtar et al. (2016). Copyright © 2015, the authors. Published under CC-BY 4.0 license)

Ultimately, experimental verification is an indispensable step to fully establish the miRNA::mRNA interaction encountered by computational methods. Regarding the database sites, miRBase currently serves as the reference for the miRNAs indexed in a number of species. miRBase encompasses a vast record of mature miRNA sequences identified in mouse and human and a much lower count in most other species including zebrafish, the increasingly used model for developmental research. Although this disparity may relate to lower genome content in the less documented species, it must also be interpreted as an indicator of differences in

gene annotation stringency (Desvignes et al. 2015) and sequencing efforts in the research carried out to date.

2.2 microRNA Isolation and Quality Check

miRNA isolation, also referred to as “extraction,” is commonly the initial experimental step when studying expression level of any miRNA(s) of interest. Previously, the separation of nucleic acids from other tissue components was developed to yield RNA strands longer than 200 nt from any given sample (Lin et al. 2010). This separation could be obtained using one of the following techniques: guanidine isothiocyanate in β-mercaptoethanol followed by silica binding or phenol-chloroform phase exchange. To allow for the isolation of smaller RNA strands, including miRNAs, the combination of phenol-chloroform phase exchange and purification by silica binding in high polarity and the presence of nuclease-inhibiting guanidinium salts was introduced (Reddy and Gilman 2001; Farrell 2006). This method is currently supplied by several companies in the form of kits (Table 2) and yields total RNA above 10 nt with a high purity and integrity, including ribosomal RNAs as well as miRNAs and short house-keeping RNAs, i.e., the snoRNA family. With this recommended optimization, a subsequent enrichment procedure for small RNAs is generally not necessary, and the starting quantity of sample needed for a satisfactory yield remains small (<1 × 10⁵⁻⁶ cells). Ultimately, the effectiveness of the miRNA isolation process varies depending on the kind of sample and how it was collected and stored until

Table 2 Main commercially available platforms for miRNA research, with their respective suppliers

miRNA isolation (phenol + kit)	Array systems	PCR systems	Modulation	Reporter (target validation)
PureZOL + Aurum (Bio-Rad)	GeneChip miRNA (4.0) array (Thermo Fisher)	IScript + MystiCq (Bio-Rad)	miRCURY LNA (Qiagen)	LightSwitch™ 3' UTR (SwitchGear Genomics)
QIAzol + miRNeasy (Qiagen)	miScript array (Qiagen)	miScript + SYBR Green (Qiagen)	MiRIDIAN/shMIMIC lentiv. (Dharmacon/GE Healthcare LifeSciences)	pMIR-GLO™ (Promega)
TRIzol + PureLink (Thermo Fisher)	TaqMan A +B cards (Applied Biosystems) TrueQuant SmallRNA Seq kit (GenXPro)	TaqMan assays and TaqMan advanced assays (Applied Biosystems/Thermo Fisher)	miRVana Ambion (Thermo Fisher)	pMIR-REPORT™ (Thermo Fisher)

the time of extraction; addition of a stabilizing agent like RNAlater[®] and performing mechanical digestion of the sample are technical steps that the researchers can consider to optimize this process in their specific work.

Once the miRNA isolation is completed, it is important to verify the quantity and quality of product obtained; spectrophotometry via NanoDrop is routinely used to do so. This technique uses just 1–2 μ l of sample and performs an absorbance sweep between 200 and 300 nm; from the readout it is important to note the absorbance ratios at 260/280 nm and 260/230 nm, which should lay \sim 2.0 to indicate good RNA quality (Bernardo et al. 2012). The NanoDrop measurement can also inform genomic contamination when blanked with the same diluent (RNAse-free water) used for the isolation and observing the result of the DNA reading setting. Moreover, modern tools can also provide an RNA integrity number, with 8–10 indicating high RNA quality. However, more advance microfluidics platforms have also been developed – like the Agilent 2100 Bioanalyzer – to generate electropherograms of the peaks corresponding to the ribosomal RNA levels (5S, 18S, and 28S; Fleige et al. 2006). These systems perform an automated electrophoretic sequencing and render the ratios of those ribosomal species and their predicted bands upon northern blotting. This level of detail is of critical importance to proceed further with those samples onto microarray or deep sequencing experiments.

2.3 microRNA Profiling and Detection

Precision is the key for the utility of any miRNA profiling/detection technique, where identifying a particular miRNA and reporting its presence hold multiple challenges (Ozsolak and Milos 2011). These include the distinction between the precursors (pri-/pre-miRs) and mature forms of the given miRNA or the distinction among miRNA family members that present just one different nucleotide. The currently available techniques to achieve this task can be divided in high-throughput, i.e., microarray and deep sequencing, or low-throughput, like northern blotting, quantitative real-time polymerase chain reaction and in situ hybridization (Git et al. 2010).

High-throughput: These techniques are carried out in the early stages of exploratory miRNA research and provide large and highly valuable amounts of information that can serve as the foundation for multiple individual projects to follow (Kozarewa et al. 2009). *Microarrays* display probes matching a set of previously identified species-specific target miRNAs, thus serving to simultaneously test the level of expression of numerous miRNAs. This technique is performed seeking relative changes between conditions, such as healthy in comparison to diseased and treated compared with untreated. The limitation is the need for known sequence information to predesign the probes, although some companies have developed probes based on proprietary algorithms to potentially match unknown miRNAs (Bernardo et al. 2012). In contrast, *deep RNA sequencing* (RNAseq) does not rely on the input of publicly available sequence information for the predesign of the system. Instead, the sequencing platforms support the discovery of novel small RNA molecules, not limited to miRNAs, as well as longer RNA species like mRNA or

lncRNA. Additionally, this technique can also render information on the absolute expression level, or absolute abundance, eliminating the need to compare with a set reference. Although the capacity of the first platforms was focused on the identification of long DNA and RNA sequences, platforms such as the *Roche 454*, *Illumina*, and *SoLiD* currently have a much improved power to detect shorter strands. To date, the main factors limiting the use of RNAseq are the cost involved and the development of better computational tools to facilitate the storage, analysis, and interpretation of the complex and large datasets that this technique generates. Further developments in the systems biology area will permit the visualization of distinct datasets and facilitate the identification of novel interactions; this progress will be the key to enhancing uptake of high-throughput methods in tissue engineering. With new benchtop tools like *Ion Torrent* and *Miseq* beginning to emerge, together with the online software packages *Partek-RNAseq* (commercial), *miRanalyzer*, and *mirTools* (free), the accessibility of RNAseq seems likely to extend to many more TE researchers in the coming years.

Once the findings from the high-throughput techniques are obtained, the next step in the workflow is to verify these using some of the low-throughput options which retain better power to elude both false-positive and false-negative results (van Rooij 2011).

Low-throughput: Northern blotting is a classical semiquantitative technique based on electrophoretic properties and applicable to miRNA research, which uses polyacrylamide gels and nitrocellulose membranes. Although it requires big quantities of starting sample and arduous work, its advantages include robustness and resolution capacity to distinguish between mature miRNAs and their precursors. The sensitivity of this technique can be enhanced incorporating LNA technology and/or radioactive labeling in the design of the oligonucleotide probes (Várallyay et al. 2008). A more widely employed method to validate high-throughput results is *quantitative real-time PCR (qRT-PCR)*, with two variants in the reverse transcription (RT) reaction: universal or single detection of specific miRNAs with stem-loop primers (Varkonyi-Gasic and Hellens 2011). At the qPCR stage, the universal approach uses SYBR Green dye and both a linear miRNA-specific primer and a universal qPCR primer (Benes and Castoldi 2010). On the contrary, the single detection system is based on TaqMan[®] assays and, in addition to the dye label, always applies miRNA-specific primers (forward and reverse). Both options hold their pros and cons: single assays have constant pre-validated reaction efficiencies and sufficient specificity to differentiate mature from precursor miRNAs, but the separate RT reactions required make it costly and time-consuming; the universal method results in much more time and cost-effectiveness to study broader miRNA sets using substantially less starting amount of sample, but in exchange their discrimination between precursor and mature miRNAs is limited. There are three main considerations to ensure quality of the miR-PCR analysis: First, efficiency rate needs to be kept at 90–110% by adjusting a standard curve of miRNA, for example, with tenfold dilutions, to one-log amplifications every 3–3.5 cycles, especially in SYBR Green-based assays. Next, a melting curve must be added to ensure that the amplicon generated is of the right length. Finally, the selection of the reference gene for normalization must be standardized for each experimental model to ensure its sufficient and unaltered expression across the tested conditions. Frequently a

snoRNA or the mean of several snoRNAs can serve as the reference value to calculate the relative miRNA expression; however other housekeeping RNAs like the ribosomal 18S may also offer a stern reference. Another technique known as *in situ hybridization (ISH)* has been developed in support of the robust quantitative miR qRT-PCR results, which visually informs regarding the localization and spatiotemporal expression patterns of miRNAs (Obernosterer et al. 2007). ISH is recommended for the detection of miRNAs known to be highly expressed but can be troublesome when targeting rare miRNAs. As previously seen with northern blotting, LNA-detection probes appear to be a promising option to improve miRNA ISH (Silahtaroglu et al. 2007). This technique can furnish our understanding of many miRNA functions and can be optimized for both frozen- and paraffin-embedded tissues, by tuning the fixation procedures and introducing amplifier chemicals for the tyramide signals, as seen in the work by Silahtaroglu et al. (2007) and Pena et al. (2009).

2.4 Studies of Target Validation and Biological Effects

After a microRNA and its expression profile in a given physiological or pathological state has been elucidated, the next target typically focusses on its biological impact, which starts by identifying the direct target of such miRNA (Ørom and Lund 2010). Bioinformatic prediction algorithms discussed earlier in this chapter are very useful drivers at this stage of the process, but the immediate following step is to experimentally validate the existence of such predicted interactions (Bernardo et al. 2012). In order to do so, the most widely extended approach is to carry out luciferase reporter assays. In these assays, the 3'-UTR region of the putative mRNA target of the miRNA of interest is cloned downstream of a luciferase reporter plasmid vector (Fig. 4 and Table 2). Upon confirming the successful construction and expression of this reporter vector, the cells of interest are exposed to the 3'-UTR reporter plasmid using a conventional transfection method. Additional groups will receive a co-transfection with a synthetic miRNA mimic/inhibitor; see Sect. 3.1 for details on how these work. Further controls including the vector with a mutation in the 3'-UTR region and a non-targeting miRNA (or *scrambled*) shall be included in the experiment (Fig. 4). Following a short incubation time, samples are to be collected and analyzed for luciferase activity. Substantial changes in luciferase activity across the groups will indicate that a direct interaction exists with the miRNA mimic or inhibitor, which is capable of altering the expression of a functional protein.

Typically, a positive result would show that, compared with the cells that only received the luciferase reporter vector, the cells co-treated with the miR-mimic present lower luciferase activity, whereas the cells that co-treated with the miR-inhibitor present higher luciferase activity. The scrambled treatment should not alter the reporter luciferase activity vs. the vector only cells and neither should the miR-mimic when co-transfected with the mutated 3'-UTR reporter.

An alternative to these reporter assays is the comparative transfection with miR-mimics and inhibitors followed by the assessment of putative targets at the

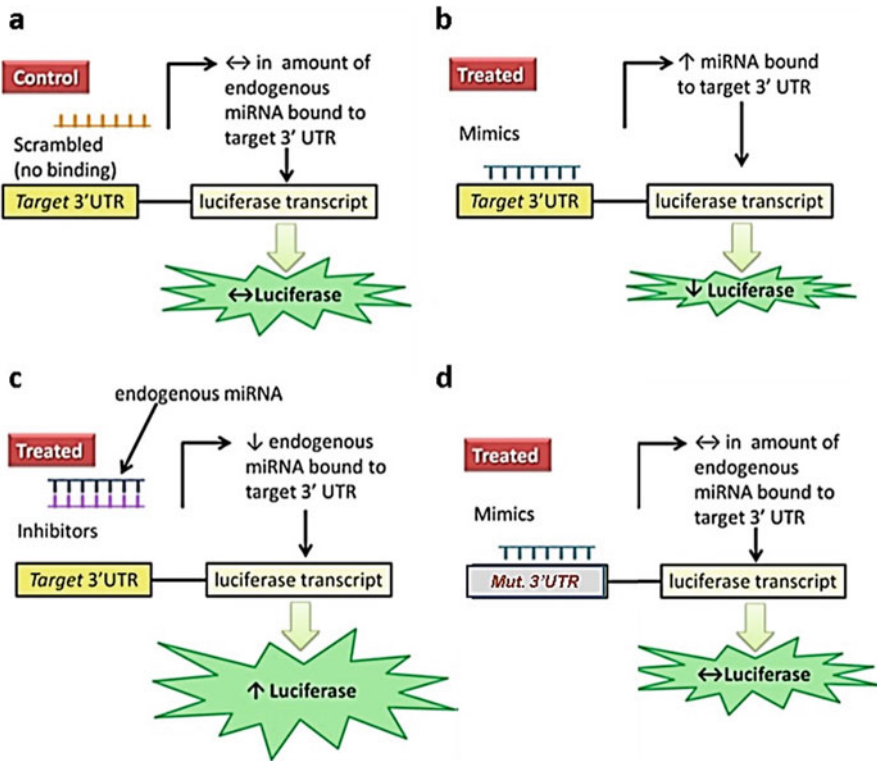


Fig. 4 Diagram of luciferase reporter assays for miRNA:mRNA interaction validation. (Adapted with permission Bernardo et al. (2012); Copyright © 2012; Elsevier B.V. All rights reserved). Cells transfected with (a) scrambled control, (b–c) the miR-mimic or the inhibitor, in addition of the reporter construct or the (d) mutated reporter construct, will result in different levels of luciferase expression, whose activity is readily quantified with commercially available kits

mRNA level – by qPCR – and/or at the protein level, via western blotting or immunostaining. However, detecting changes in the levels of the putative target following this approach would not rule out the possibility of an indirect interaction with the miRNA of interest. Furthermore, the effect of successfully transfected miR-mimics and inhibitors on the expression of its direct mRNA target tends to be of moderate amplitude, i.e., 0.5-fold reduction or 1.5-fold increase. This complicates the clear detection of the sought interaction using said PCR and immunostaining techniques, sometimes prompting researchers to explore methodologies like pull-down assays, proteomics, and even transcriptomics. For further details on the molecular techniques fit to evaluate the presence and levels of the putative target, which are not specific to miRNA research but general to protein studies, we direct the reader to other available reviews (van Rooij 2011; Ørom and Lund 2010). Ultimately, a confirmation of the validated direct interaction between a particular miRNA and its mRNA target of interest for a specific application is essential to

continue the evaluation of the biological implications of that given miRNA. It is important to note that abundant information on validated miRNA::mRNA interactions can be found from the literature or compiled in bioinformatic databases. Thus, a project pursuing the therapeutic application of a certain miRNA can frequently be designed based on such externally reported information; this is indeed an extended practice in the application of miRNA therapeutics to tissue engineering. However, consideration must be given that validation of a miRNA::mRNA interaction in a particular cell type, animal model, or species does not ensure consistency of that interaction in a different setting. It is recommended hence to perform preliminary studies of species homology at the bioinformatics level and also to determine the basal expression of the miRNA and its target experimentally, before aiming to assess a potential therapeutic effect.

Focusing on the exploration of the biological or therapeutic effects of miRNAs, multiple options are worthy of consideration, ranging from *in vitro* tests to preclinical evaluation using animal models. As a common denominator of this phase of the miRNA research, the effect of miRNA overexpression or inhibition is evaluated for phenotypic – and sometimes also genotypic – changes. The key elements of resolution when planning *in vitro* or *in vivo* miRNA experiments should be:

1. Determination of the timing and dose of mimic/inhibitor that is effective and nontoxic – for cells and tissues – delivered to the specific model
2. Establishment of the time-course of treatment and analysis points, testing guided by literature reports
3. Comparative evaluation of the effect, i.e., across two cell types or culture conditions, to obtain a broader insight in the amplitude and impact of the biological response
4. Confirmation of efficient target silencing to ensure system reproducibility
5. Assessment of downstream targets and markers standardized for the application of interest
6. Evaluation of biodistribution and adverse effects of the optimized treatment to main organs like the liver and kidney (Bernardo et al. 2012)

The next sections of this chapter will provide a detailed review of the current knowledge regarding the role of miRNAs in tissue repair and the routes by which those roles have been harnessed to date to provide improved therapeutic options in the various subfields of tissue engineering and regenerative medicine.

3 miRNA Modulation as a Therapeutic for Cell and Tissue Engineering

The emerging understanding of miRNA function and regulation has sparked their exploration in the development of more advanced therapies for tissue engineering applications (Hu et al. 2010; Jensen et al. 2010; Haussecker 2014). The competitive advantage of miRNAs over other gene therapy variants is multifold, but

perhaps the main point resides in the ability of a single miRNA to imperfectly bind to a multitude of targets, resulting in a multi-targeting effect capable of modulating complex signaling pathways (van Rooij 2011; van Rooij et al. 2012; Bader et al. 2010). Such an effect potentially incurs a robust and enhanced biological response (Beavers et al. 2014). Other additional benefits of miRNAs are their small molecular size and cytoplasmic activity, which can simplify delivery methods as they do not depend on access to the cell nucleus or transcription machinery. Furthermore, the possibility of inhibiting or blocking the function of miRNAs to achieve the upregulation of their direct target provides a tool for the bidirectional control of gene and protein expression. Consequently, the landscape of miRNA-related patents denotes fast-paced progress with positive projections for commercialization, with over 500 filed US patents related to miRNA-based therapies as of 2015 (Fig. 5; Christopher et al. 2016). This includes inventions in areas of wound healing, bone, muscular, ocular, respiratory, and cardiovascular tissues, as well as inflammatory diseases, approximately accounting for nearly one fifth (20%) of the total filings, which collectively underlines the high impact of miRNA therapies in tissue engineering (Monaghan and Pandit 2011; Chew 2015). Taken together with its speedy progression to enter clinical trials, this suggests that miRNA therapy holds great promise as an effective protein modulating alternative to the delivery of high doses of protein (Takeda 2009; Vo et al. 2012).

The first candidates entering clinical trials were the liver-targeted miravirsin and MRX34 (Janssen et al. 2013; ClinicalTrials.gov 2013), because this organ retains the highest concentrations of unmodified miRNAs upon systemic administration. Further progress currently includes Phase I/IIa trials for nonalcoholic fatty liver disease and type 2 diabetes sponsored by Regulus Therapeutics or Phase I trials for mesothelioma and scleroderma. Moreover, many miRNAs are in the R&D pipeline as tissue repair treatments for diseases of the circulatory system including miR-92a, miR-33, miR-15, and miR-208 or diseases caused by uncontrolled fibrosis or inflammation such as miR-21 and miR-155.

3.1 Types of miRNA Therapeutics

As outlined earlier in this chapter, synthetic molecules exist to modulate – i.e., mimic or inhibitor – the natural activity of miRNAs in a cell type of interest and thus render a therapeutically engineered cell phenotype with a physiological miRNA profile (van Rooij et al. 2012; Fig. 6). The design of synthetic miRNA variants includes sequence editing and chemical modifications to tailor the affinity of binding to the target or provide increased cellular uptake and stability against degrading enzymes. These include the locked nucleic acid (LNA) technology developed by Exiqon (Braasch and Corey 2001; Petersen and Wengel 2003) or the phosphate backbone functionalization which creates hybrid peptide nucleic acids (PNA) (Fabani and Gait 2008; Table 3). These modifications can be applied simultaneously within a single nt but not in every unit of the oligo chains; therefore perfecting the balance between unmodified and multi-modified units is a task

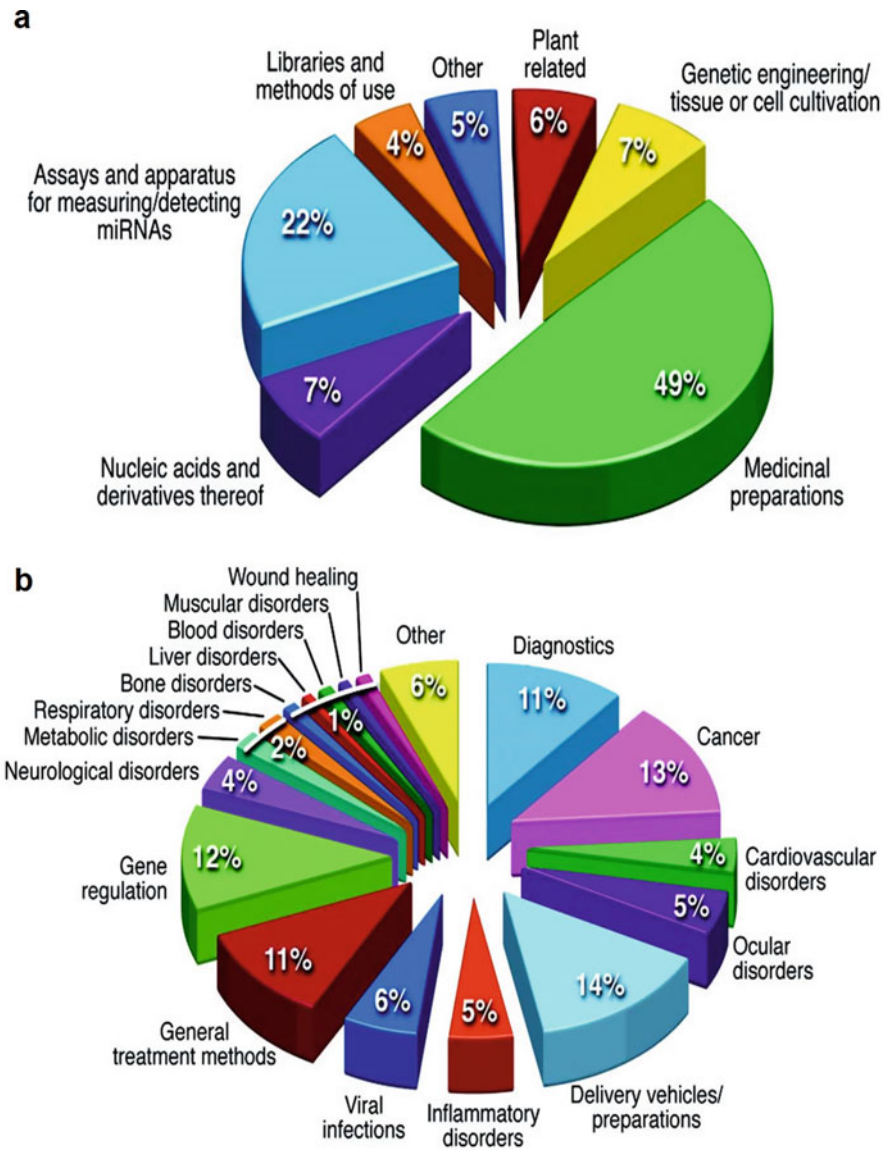


Fig. 5 miRNA-related distribution of US patents. (Reproduced with permission van Rooij et al. (2012). Copyright © 2012, American Heart Association, Inc). **(a)** The distribution of different technological fields that incorporate applications of miRNA, as determined by International Patent Classification codes. **(b)** Distribution of filed patents related to miRNA-based medicinal preparations divided by particular indications

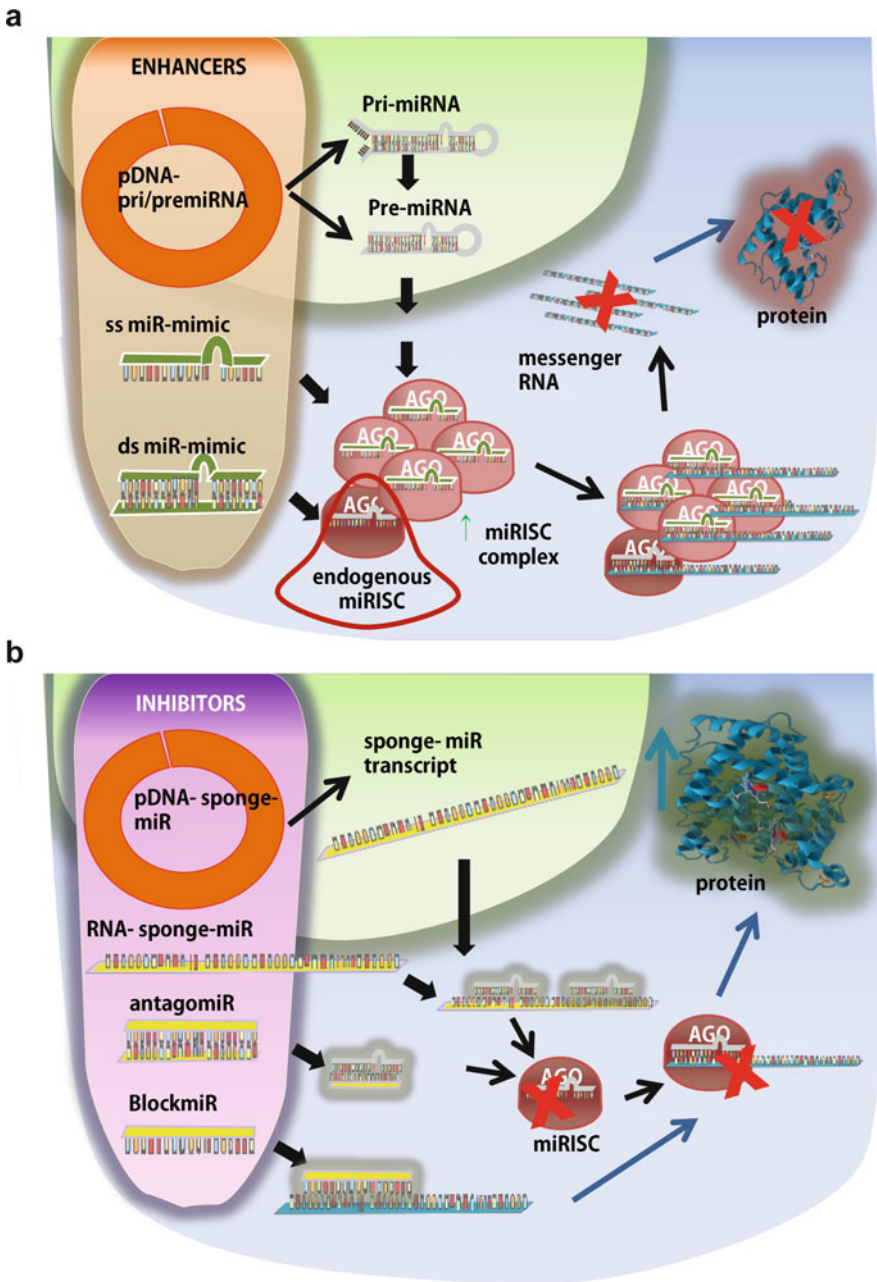


Fig. 6 Types of miRNA modulators. (Reproduced with permission from (Curtin et al. 2017). Copyright © 2017, John Wiley and Sons). (a) miR-mimicking by pDNA encoding for miRNA precursors, single- or double-stranded oligonucleotides serve to enhance the formation of miRISC

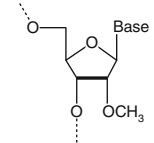
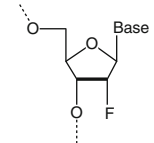
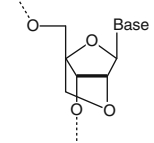
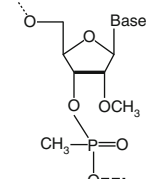
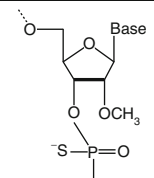
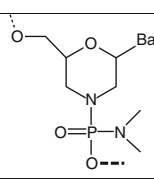
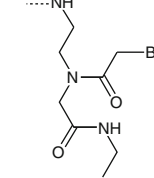
which garners intensive work. This is especially so with single-stranded miR-mimics because their guide strand must be recognized as identical to the miRNA of interest to form the miRISC complex (van Rooij and Kauppinen 2014). However, double-stranded miR-mimics can also be used; these can feature a more modified chemistry in the passenger strand, rendering potency improvements of above 1000-fold in comparison to the single-stranded molecules. An alternative strategy to enhance the level or activity of endogenous miRNAs is the application of plasmid (p)DNA vectors coding for precursors of the miRNA, instead of direct delivery of the mature miRNA form. This strategy remains cost-effective as bacterial cultures allow continuous propagation of the vector; however, it presents higher risks of overloading the enzymatic activity of endogenous miRNA biogenesis (Bonadio et al. 1999).

Focusing on the options for miRNA inhibition, we can distinguish three categories which are antagomiRs, miR-sponges, and small chemical inhibitors (Fig. 6b). *AntagomiRs*, also named “antimiRs” or “AMOs,” are short antisense oligonucleotides which have high binding affinity for their target miRNA, owing to their modified chemistry; this pairing impedes the interaction with the mRNA, whose levels and translation into protein are effectively increased (Bernardo et al. 2012). Hence, antagomiRs have the capacity to modulate the various mRNA targets of their inhibited miRNA and are the first effective miRNA inhibitors preclinically that have progressed to clinical trials (Kruzfeldt et al. 2005). *BlockmiRs* are a subtype of short antisense inhibitors able to selectively engage with a single target mRNA in the region that would bind the desired miRNA but that support protein translation instead of entry in the miRISC. *miR-sponges* are long and single strands that function via competitive substitution of the mRNA in its interaction with the corresponding miRNA, ultimately confiscating away multiple miRNA copies at a time and thus preventing their activity (Ebert et al. 2007; Ebert and Sharp 2010). miR-sponges are usually constructs of pDNA vectors, like miRNA eraser, miRNA mower, tough decoy (TuD), and LidNA, and are preferred as a stable genetic modification to treat chronic diseases. Finally, *small chemical inhibitors* like dihydropteridinone ATP analogues, diazobenzenes, and helix-threading peptides act by impeding miRNA biogenesis unspecifically (Sakurai et al. 2008). Although they are valuable in developmental studies and for their good pharmacokinetics, they present an enhanced risk of side effects in comparison to the other types of inhibitors (Deiters 2010; Zhang et al. 2010).



Fig. 6 (continued) complex alongside endogenous miRISC, ultimately leading to decreased levels of messenger (m)RNA or protein of the target present in the cell. **(b)** miRNA inhibition by pDNA/RNA sponges or antagomiRs (antimiRs) impedes the entry of the mature miRNA to form miRISC and abrogates downstream interaction with the target mRNA. “BlockmiRs” conceal the binding sites of mRNA directly preventing its entry in miRISC. The three approaches act to ultimately enhance mRNA and protein levels of the target in the cell

Table 3 Effects of chemical design of miRNA therapeutics. (Reproduced with permission from Curtin et al. (2017). Copyright © 2017, John Wiley and Sons)

Types of modification	Chemical structure	Function	Properties conferred
<u>Ribose methylation: 2'O-Me</u>		<ul style="list-style-type: none"> Enhances hydrophobicity Increases duplex melting temperature 	<ul style="list-style-type: none"> ↑ Cellular uptake
<u>Ribose fluoridation: 2'F</u>		<ul style="list-style-type: none"> Enhances hydrophobicity Increases duplex melting temperature 	<ul style="list-style-type: none"> ↑ Cellular uptake ↑ Stability
<u>Conformational ribose locking: LNA</u>		<ul style="list-style-type: none"> Ribose ring structurally locked in C3'-endo conformation Enhances hydrophobicity Improved target-binding affinity 	<ul style="list-style-type: none"> ↑ Cellular uptake ↑ Bioactivity
<u>Phosphate backbone methylation: P-Me</u>		<ul style="list-style-type: none"> Improved enzymatic resistance Enhances hydrophobicity Increases duplex melting temperature 	<ul style="list-style-type: none"> ↑ Cellular uptake
<u>Phosphate backbone thiolation: PS</u>		<ul style="list-style-type: none"> Introduces phosphorothioate linkages Improved enzymatic resistance Enhanced binding to plasma proteins Improved pharmacokinetics 	<ul style="list-style-type: none"> ↑ Stability ↑ Bio-distribution time, slowed excretion
<u>Morpholino-ribose substitution: PMO</u>		<ul style="list-style-type: none"> Introduces phosphorodiamidate linkages Removes negative charge Improved target-binding affinity 	<ul style="list-style-type: none"> ↑ Cellular uptake ↑ Bioactivity ↑ Stability
<u>Peptide-phosphate backbone substitution: PNA</u>		<ul style="list-style-type: none"> N-(2-aminoethyl)-glycine units substitute the phosphate backbone Removes negative charge Improved enzymatic resistance 	<ul style="list-style-type: none"> ↑ Cellular uptake ↑ Stability

3.2 miRNA Delivery Methods

The delivery of miRNAs presents multiple technical challenges due to their negative charge, rigidity, and low charge density (Pan et al. 2012a; Lee et al. 2014), as well as their susceptibility to enzymatic degradation and poor intracellular cytosolic delivery, owing to the lysosomal digestion process. These difficulties explain the limited success obtained to date with the delivery of naked miRNAs and the need for delivery methods that can function safely and efficiently. As such, the field of miRNA delivery accounts for nearly a third of the total share of the miRNA-related US patents, representing a bigger portion than the ~20% of the combined tissue engineering and regenerative medicine (TERM) areas mentioned at the beginning of this Sect. 3 (Monaghan and Pandit 2011; Chew 2015). miRNA delivery systems specifically developed for tissue engineering purposes often combine vectors and biomaterial scaffold platforms to spatiotemporally sustain modulation of miRNA and target. This subsection describes the main properties and highlights applications of both vectors and scaffolds, which are roughly transferable across systems developed to deliver pDNA, siRNA, or miRNA. A series of prerequisites are generally asked of a successful delivery system – summarized in Table 4. The continuous efforts in this area no longer aim to develop a single ideal system fitting all applications; instead, the goal is to satisfy as many of these prerequisites as possible while using fine-tuned platforms for each particular application.

3.2.1 miRNA Delivery Vectors

The principal role of the delivery vectors is to improve cellular uptake and prevent lysosomal degradation, and they are broadly divided into viral and non-viral methods (Ilina et al. 2012; Midoux et al. 2009). Generally, *non-viral vectors* present adequate properties with regard to fabrication, safety, and stability but have modest functionality (Santos et al. 2011). Non-viral vectors are associated with temporal and variable transfection efficiencies per cell type (Midoux et al. 2009; Santos et al. 2011; Pack et al. 2005). However, the temporal effect is beneficial in tissue

Table 4 Prerequisites of miRNA delivery vectors to progress toward clinical applications

Fabrication	Safety	Stability	Functionality
Ease of fabrication	Safe administration route	Robustness/stability (chemical)	Internalization
Inexpensive	Nonpathogenic	In physiologic fluids, including serum	Endolysosomal escape
Facile purification	Nontoxic	For storage and administration	Entry in nondividing cells
Targetability (to specific cells/tissue)	Non-immunogenic	Protection of cargo	Nuclear transport (generally not RNAi)
		Packaging sufficient amount of cargo	Efficient unpackaging/packaging

engineering to allow the termination of tissue repair without undesired tissue overgrowth. Although non-viral physical transfection methods like electroporation or microinjection offer consistently high efficiencies, they are relatively invasive techniques and complicated to adapt to off-the-shelf tissue engineering therapeutics. Meanwhile, the opposite occurs with chemical vectors: a plethora of highly adaptable materials and nano-sized systems that allow noninvasive miRNA delivery by forming complexes via electrostatic interaction, charge adsorption, or physical encapsulation. These include natural and synthetic polymers, generally with cationic properties. Among the cationic polymers, polyamines are common structural features that provide an efficient path to escape lysosomal degradation. This path, known as the “proton sponge” effect, attracts anions to disrupt the endolysosomal compartment before the degradation can start in the mature lysosome, thereby allowing the miRNA cargo to be released into the cytoplasm (Behr 1997). To optimize this effect for each cell type, the ideal proportion of polyamines (“N”) to phosphate groups (“P”) present in the cargo, i.e., the N/P ratio, must be determined. The principal synthetic cationic polymers explored for miRNA delivery are derivatives of polyethyleneimine (PEI) (Zhang et al. 2013; McKiernan et al. 2013; Schade et al. 2013, 2014), poly(β -amino esters) (P β AE), methacrylates, dendrimers (Raftery et al. 2016), cyclodextrins (Fitzgerald et al. 2015, 2016), and cell-penetrating peptides (CPP) (Guo et al. 2016; Sathy et al. 2017); examples and their main limitations are described below.

Beyond commercially available transfection agents like ExGen500 and jetPEI™, PEI derivatives have shown high miRNA transfection efficiencies both within poly(lactic-co-glycolic acid) (PLGA) microspheres embedded in poly(L-lactic acid) (PLLA) scaffolds (Zhang et al. 2016a) and alginate-based hydrogels (Liu et al. 2016). The main drawback of PEI derivatives is their tendency for exacerbated proton sponge effects that generate notable cell damage and thus compromise the clinical applicability (Ghosh et al. 2012). Contrastingly, P β AEs display low cytotoxicity while keeping high transfection efficiencies (60–70%) in various cell types including hMSCs and human adipose-derived stem cells (ADSCs) (Vuorimaa et al. 2011; Yang et al. 2009; Guk et al. 2013; Yin et al. 2011; Jere et al. 2008; Remaut et al. 2010) and also allow kinetically controlled biodegradation for temporal delivery of their cargo (Gupta et al. 2015). Recent innovations in methacrylate-based miRNA/siRNA delivery systems, dendrimers, cyclodextrins, and CPPs offer the main overall advantage of chemical versatility. These provide precise physicochemical tuning and thus control of the pharmacokinetic profile, hence holding great potential for incorporation into scaffold-based platforms as miRNA delivery vectors. In particular, a dimethylaminoethyl methacrylate copolymer formulated as a dendrimer with polylactic acid (PLA) (Wang et al. 2017a; Nelson et al. 2014) demonstrated cargo protection, adequate cytosolic release, and significant gene knockdown when delivering miR-21 as a treatment for glioma (Qian et al. 2014). Additionally, advanced polyamidoamine (PAMAM) dendrimers have also demonstrated highly efficient macropinocytosis-mediated delivery of a miR-205 mimic or an antagonomiR-221 within a hydrogel to treat breast cancer (Conde et al. 2016; Haensler and Szoka 1993).

Interestingly, the non-viral vectors above described remain underutilized compared to the extensively researched *lipid-based cationic vectors*. FuGENE, GenePORTER, TransFast, DOTAP, Lipofectamine[®] 2000, and Lipofectamine RNAiMAX are but a few examples of widely commercialized lipid transfection agents (Wu et al. 2013; Eskildsen et al. 2011; Wang et al. 2015; Hoseinzadeh et al. 2016; Chen et al. 2011a; Li et al. 2013). These lipid vectors are often presented as liposomes, spherical bilayers of polyamines, and cholesterol that easily fuse with cell membranes to aid the uptake of their miRNA cargo. Typically, the cationic lipids interact electrostatically with the miRNAs resulting in a net positive charge, while core encapsulation of the cargo occurs more frequently with liposomes of neutral charge. Liposomes offer a vast versatility, a distinctively high loading capacity, and a renowned high efficiency, but their main clinical drawback is their immunogenicity and toxic-detergent effect on cell membranes, generating intracellular vacuoles (Li et al. 2014). With intensive efforts on developing vectors that lack these cytotoxicity issues, materials of a natural base like chitosan and inorganic nanoparticles have garnered the excitement of tissue engineering researchers (Ghosh et al. 2012). *Chitosan* is a crustacean-derived polysaccharide with commendable biodegradability and biocompatibility and was introduced as a gene delivery vector approximately two decades ago (MacLaughlin et al. 1998). Altering its polymerization, acetylation degree, and/or chain architecture allows the optimization for miRNA delivery (McKiernan et al. 2013; Malmo et al. 2012), with highly depolymerized chitosan showing optimized miR-145 delivery in adenocarcinoma studies with approximately 50% target silencing (Santos-Carballeda et al. 2015).

Nanoparticles (NP) assembled from inorganic materials like gold (Au), silver (Ag), iron (Fe), and calcium phosphates (CaP) also hold interesting properties. *Au-based NPs* are amphiphilic vectors with demonstrated ability to deliver miRNA reporters in cancer applications like ovarian cancer or neuroblastoma (Ghosh et al. 2012). *Silver- and iron-based systems* include additional physical triggers such as light or magnetism to switch on and off the miRNA delivery; this offers on-demand spatiotemporal control over miRNA delivery with high precision in surface body areas. Relevantly, an 80% uptake efficiency range was reported for photocleavable Ag NPs delivering miR-148b mimic to hADSCs (Qureshi et al. 2013) and paramagnetic Fe₃O₄ NPs delivering miR-335 to hMSCs to treat cardiovascular diseases (Schade et al. 2013). Finally in this group are the *CaPs*, biodegradable and highly biocompatible ceramics naturally found in bone (Pedraza et al. 2008; Cunniffe et al. 2016) which can be fabricated easily and cost-effectively (Bose and Tarafder 2012; Wu et al. 2008). CaP-aided transfection was first reported in 1973 (Graham and van der Eb 1973), and it facilitates cellular uptake by dynamin/clathrin-endocytosis after precipitating at the cell surface (Ilina et al. 2012). CaPs feature low pDNA delivery efficiency and instability in solution (Li et al. 2010) but have been surface-modified with PEG, chitosan, hyaluronic acid, and 3,4-hydroxyphenylalanine-dopa for improved efficiency, specificity, and loading capacity (Lee et al. 2013, 2014; Wu et al. 2008; Zhang et al. 2009; Giger et al. 2013). Non-aggregating CaPs with hydroxyapatite stoichiometry (HA; Cunniffe et al. 2010) proved superior for hMSC transfection when compared to a commercial CaP kit (Curtin et al. 2012),

with 90% functional efficiency of reporter miR-mimics and antagomiRs (Mencia Castano et al. 2015); a scaffold system incorporating these has demonstrated potential as a new bone repair therapeutic (Mencia Castano et al. 2016, 2018).

To conclude this section on miRNA delivery, *viral vectors* are worthy of discussion, as they lead gene therapy development with the recently approved Zalmoxis and Strimvelis, by MolMed and GlaxoSmithKline, respectively ((Agency), E.E.M 2016a, b). As nature's transfection agents, viruses have characteristically high transfection efficiencies with little variability. Viral miRNA therapy for tissue engineering might be of interest to permanently correct endogenous levels of a miRNA in chronic degenerative diseases like OA. It is worth highlighting that viral miRNA delivery has proven reduced dose-dependent toxicities versus shRNA and siRNA, with improved silencing efficiency (Boudreau et al. 2009). The principal drawback of viral miRNA delivery is insertional mutagenesis, although the risks of fatal effects are much reduced in lentiviruses versus retroviruses (Laufs et al. 2006; Montini et al. 2009). This has possibly swayed the greater use of lentiviral-based systems combined with biomaterial scaffolds (Deng et al. 2013a, 2014a; Xie et al. 2016), although adenoviruses and adeno-associated viruses are widely used in in vitro miRNA transfection studies (Mowa et al. 2010) which may be due to their reduced size and non-pathogenicity for humans (Liu and Berkhout 1809). More recently, vast attention has been directed to baculoviruses, which also lack pathogenicity in humans and present reduced risks by not replicating or integrating in mammalian cells (Chen et al. 2011b; Liao et al. 2014a). Clinical translation of viral miRNA approaches remains hindered also by difficulties in up-scaling the complex production and the high costs associated with this (Gordeladze et al. 2010). Viruslike particles, consisting of noninfectious recombinant variants of virus capsids that maintain the original cell tropism and trafficking capacities (Pan et al. 2012a, b), may offer a valuable tool to accelerate clinical progress.

3.2.2 Application of Biomaterial Scaffolds to miRNA Delivery

The concept of using biomaterial scaffolds, originally designed to support tissue growth, as therapeutic delivery devices is now widely established among tissue engineering researchers (Lee et al. 2011). Evolving from the “gene-activated matrix” idea which surfaced at the end of the 1990s (Bonadio et al. 1999; Fang et al. 1996), scaffold-based miRNA delivery is now growing (Chew 2015). This approach offers physical shielding from degrading agents and delays the clearance of miRNAs from the target site, enhancing the time frame of therapeutic effects within a localized area (Bonadio et al. 1999; O'Brien et al. 2007; Sriram et al. 2015; Nguyen et al. 2014). In this way, scaffold-based miRNA delivery may minimize the biodistribution to off-target tissues, representing an important advantage in terms of safety (Chew 2015). Importantly, the 3D microenvironment has been shown to alter the transfection efficiency of the vectors employed, in comparison to monolayer settings, which has been associated with changes in treatment exposure within the scaffold matrix. This highlights the need to perform extensive in vitro testing of the scaffolds once activated with the miRNA therapeutic. In this section we review the main properties required to successfully adapt the different materials and existing scaffold types as miRNA delivery platforms, while their therapeutic application is summarized in Table 5 and later detailed in Sect. 5

Table 5 Summary of miRNA therapeutic potential reports in tissue engineering using scaffold-based delivery. (Reproduced with permission Curtin et al. 2017. Copyright © 2017, John Wiley and Sons)

Scaffold type	Vector type	Therapeutic	Endpoint analysis result	In vivo model/cell type	Ref.	Appl.
Gelatin-coated PLGA scaffold disc	Baculovirus	ASCs, BMP2+ miR-148b mimic transduced	12 weeks: complete bridging: bone area = 94%, BV = 89%, BD = 95%	4 mm critical-sized calvarial defect in nude mice	Liao et al. (2014a)	Bone
Gelatin-coated PLGA scaffold disc	Baculovirus	ASCs, BMP2+ miR-148b mimic transduced	12 weeks: filling 83% defect area, 75% BV, bone density 89% of original	4 mm critical-sized calvarial bone defects in nude mice	Sung et al. (2013) and Mariner et al. (2012)	
Gelatin-spongostan scaffold	Baculovirus	OVX-BM-MSCs transduced with miR-214 or miR-140 sponges	Bone healing, bone density, trab. no, trab. thickness, and trab. space: further synergy by BMP2 co-expression	Osteoporotic rat femoral metaphysis defect model	Li et al. (2016a)	
Poly(glycerol sebacate) porous scaffold discs	Lentivirus	BM-MSCs, miR-31 inhibitor transduced	8 weeks postoperatively: significant improvement in healing vs. control groups (near full bridging)	8 mm calvarial rat defect (12-w.o. male Fischer 344)	Deng et al. (2014a)	
β -TCP scaffold	Lentivirus	ASCs or BM-MSCs, miR-31 inhibitor transduced	Bone regeneration in 8 weeks in rat model and in 16 weeks in canine, Runx2-Satb2 loop mechanism	5 mm rat calvarial defect, 10 mm canine orbital bone defect	Deng et al. (2013a, 2014b)	
Poly(sebacoyl diglyceride) (PSED) scaffold	Lentivirus	BM-MSCs, miR-135 mimic or inhibitor transduced	8 weeks: respectively enhanced or decreased newly formed bone and trabecular number	8 mm critical-sized rat cranial defects	Xie et al. (2016)	
PSED scaffold	Lentivirus	BM-MSCs transduced with miR-125b inhibitor	8 weeks: enhanced SMAD4, Runx2 and Osterix expression in newly formed bone	8 mm critical-sized rat cranial defects	Xie et al. (2017)	

Demineralized bone matrix	Lentivirus	BM-MSCs transduced with miR125b inhibitor	51% increased BMD, 79% increase in Tb.N; histologically enhanced new bone generation and bone maturity	Bilateral segmental femoral defects in athymic mice	Wang et al. (2017b)
Nanofibrous PLLA scaffold	HP-PEI-PEG	26a mimic (cell-free in vivo)	Bone regeneration in 8 weeks in both healthy and osteoporotic mice	5 mm critical-sized mice calvarial bone defect	Zhang et al. (2016a)
HyStem-HP™ and Hya/gelatin/PEG hydrogels	siPORT NeoFx™	hBM-MSCs, miR-26a mimic transduced	Histomorphometry at 8 weeks (ectopic model): increased bone formation vs. control; micro-CT analysis at 12W (calvarial defect model) full bridging	3 mm and 5 mm mice calvarial defect (nude C57BL6J), mouse (m) or hBM-MSCs	Li et al. (2013)
HA/TCP scaffold disc	Lipofectamine® 2000	Rat ASCs, miR-26a mimic transduced	Histomorphometry at 12 weeks: increased new bone forming area vs. control groups	3.5 mm diameter rat cortical tibial defect	Wang et al. (2015)
Matrigel™ or PCL scaffold	PC-SilverNP	Pre-miR-148b-transduced ASCs	4 and 12 weeks: healing improved 32.53 ± 8.3%	4 mm critical-sized mouse calvarial defect	Qureshi et al. (2015)
Porous collagen-nHA scaffold	nHA particles	GAPDH targeting miR-mimic, antiagomiR-16	Silencing functionality of ~20% and 88.4%, respectively	hMSC, 2D and 3D delivery profile characterization	Mencia Castano et al. (2015)
Porous collagen-nHA scaffold	nHA particles	antiagomiR-133a-3p	Enhanced Runx2 and osteocalcin expression, increased alkaline phosphatase activity and Ca ²⁺ deposition	hMSC, 2D and 3D assessment of therapeutic osteogenesis	Mencia Castano et al. (2016)
EXg 3D scaffold	Naked	miR-2861	miR-2861 and RunX2 overexpression	Human periodontal ligament (hPDL) SCs	Diomede et al. (2016)

(continued)

Bone

Table 5 (continued)

Scaffold type	Vector type	Therapeutic	Endpoint analysis result	In vivo model/cell type	Ref.	Appl.
Atelocollagen	Naked	miR-222 inhibitor	8 weeks: positive radiographic, μ CT and histological evaluation	Rat refractory fracture model	Yoshizuka et al. (2016)	
PEG-norbormene hydrogel	Naked-nucleofection	miR-148b mimic + miR-489 inhibitor	66% increase alkaline phosphatase activity and fourfold increase in Ca^{2+} deposition (day 10) over untreated control	hBM-MSC, 2D and 3D assessment of therapeutic osteogenesis	Mariner et al. (2011)	
Alginate hydrogel +osteochondral biopsy	RNAiMAX	BM-MSCs transduced 2X with miR-221 inhibitor	12 weeks: cartilage repair with absence of coil X	Subcutaneously implant in nude mice	Lolli et al. (2016)	Cartilage
Fibrin hydrogel	siPORT NeoFX™	antagomiR-133a and antagomiR-696	Enhanced expression of PGC-1 α and contractile force	2 weeks myotubes culture	Cheng et al. (2016)	Muscle
PEG-PCL-gelatin electrospun fibers	REDV peptide-trimethyl chitosan-PEG	miR-126	8 weeks: in vitro downregulation of SPRED-1, improved endothelialization in vivo	Rabbit 1 cm left common carotid artery resection	Zhou et al. (2016)	Cardiovascular
Fibrinogen, Matrigel™ and thrombin hydrogel	DharmaFECT	miR-1 + miR-133+ miR-208+ miR-499	MMP-dependent mechanism of successful reprogramming	Direct reprogramming of fibroblasts into cardiomyocytes	Li et al. (2016b)	

4S-StarPEG collagen scaffold (gel)	Naked	miR-29b	4 weeks: reduced wound contraction, improved collagen/1 ratio, higher MMP-8; TIMP-1 ratio 2 and 5 weeks following MI: myocardial function maintenance at	Full-thickness rat skin excisional wounds	Monaghan et al. (2014, 2018)
Glycosan HyStem hydrogel				Mice ischemia reperfusion model	
Pluronic F127 gel + vein segments	Lentivirus	miR-26a mimics	4 weeks: reduced hyperplasia and inhibited SMCs proliferation	In rat vein neointimal hyperplasia model	Tan et al. (2017)
Electrospun PCL fiber scaffold	TransIT-TKO	miR-219 and miR-338		OPC remyelination	Diao et al. (2015a)
Sed scaffold	n/a	miR-20	Unraveling miR signature	NSCs self-renewal and differentiation control	Cui et al. (2014)
Aminopropyl-silica nanofibers	n/a	Let-7			Mercado et al. (2016)
					Neurological

In addition to the general principles for apt biomaterials such as ease of fabrication, biodegradability, and biocompatibility (O'Brien et al. 2007), ease of sterilization and long-term stability are key limiting requisites from the angle of clinical translation (Buchholz 2002). However, the most specific requisite for any miRNA delivery scaffold is the structural ability to retain the miRNAs and sufficiently expose them to the infiltrating cells, thereby mediating the localized miRNA release while not impeding cellular uptake. Thus, a balanced interaction strength must be met between scaffold and miRNA complexes (Zhang and Webster 2009). For this reason, highly porous scaffold architectures with nano-topographic features are generally well suited to permit miRNA delivery while also being favorable for tissue ingrowth and repair (Stevens and George 2005). The amount of miRNA complexes loaded on the scaffold is also important, so that the addition of miRNA complexes to the scaffold does not have a deleterious effect on mechanical integrity or reduce its cellular attachment/retention properties (Hedberg et al. 2005). Although the low miRNA dose generally applied is unlikely to cause major disruptions, the varied vector types forming the miRNA complexes can significantly impact the viscosity of the starting mixtures for scaffold manufacture. Depending on the target application, the presence of the miRNA complexes may require the corroboration of different structural features. These include mechanical strength of orthopedic graft substitutes (Cui et al. 2014; Zhou et al. 2016; Diao et al. 2015a; Mercado et al. 2016; Wu et al. 2013; Deng et al. 2014b; Sung et al. 2013; Mariner et al. 2012; Diomedea et al. 2016; Lolli et al. 2016; Li et al. 2016a) or rheology, viscoelasticity, and physical stimuli responsiveness in the case of hydrogels (Monaghan et al. 2014, 2018; Li et al. 2016b; Cheng et al. 2016; Tan et al. 2017).

Two primary types of scaffold-based miRNA delivery have typically been used: in situ “cell-free” or ex situ “cell-mediated” delivery of miRNAs. Based on the nature of their components and their fabrication method, some materials may be better suited for either ex situ or in situ incorporation of miRNA complexes. In the case of *cell-mediated* miRNA delivery, a defined cell population undergoes in vitro miRNA transfection prior to being seeded onto the scaffold of choice. This approach thus necessitates a preliminary in vitro incubation time to obtain the miRNA-modified cells that will become the therapeutic agent delivered by the scaffold. In this way, when the scaffold is implanted in a tissue void, it supports the activity of the miR-modified cells but does not administer more miRNA to any infiltrating cells. In contrast with *cell-mediated* miRNA delivery, the less reported *cell-free* approach does not rely on a preliminary in vitro incubation, denoting an increased “off-the-shelf” potential which might simplify the path to clinical translation (Nguyen et al. 2014; Mariner et al. 2011). In this approach the scaffolds are prepared as depots of miRNA complexes before the cells are seeded or come into contact with the scaffold. These scaffolds can thus be implanted without adding in vitro cultured cells. This way they provide the environment for the infiltrating cells to become transfected with the miRNAs contained in the scaffold and repair the tissue defect (Pelled et al. 2010; Kimelman-Bleich et al. 2011).

A variety of natural or synthetic *hydrogels* have shown ability to deliver miRNA therapeutics using both ex situ and in situ approaches, being the first platforms to

deliver naked miRNA therapeutics (i.e., not complexed with a delivery vector; Monaghan et al. 2014, 2018; Mariner et al. 2011). This simplified concept is likely to provide a quick bolus-like miRNA delivery due to quick diffusion from the biomaterial without further protection from degradation, which would limit treatment efficiency. Natural polymer hydrogels that have proven currently successful for miRNA delivery include the commercial Matrigel™ and formulations of thrombin, silk fibrin, fibrinogen, and alginate (Lolli et al. 2016; Li et al. 2016b; Cheng et al. 2016; Qureshi et al. 2015). Synthetic hydrogels used for miRNA delivery reported to date include an ex situ PEG-norbornene system encapsulating miR-148b mimic + miR-489 inhibitor-modified human MSCs (Mariner et al. 2011), as well as both in situ PEG systems of HyStem-HP™-based delivery of miR-26a mimics (Li et al. 2013) and “on-demand” miR-20a delivery triggered by UV light (Huynh et al. 2016; Nguyen et al. 2014). Another commercial synthetic gel, an amphiphilic triblock copolymer, *Pluronic F127*, was recently employed to mediate the in situ delivery of miR-26a mimic lentiviral complexes (Tan et al. 2017). These are among the biodegradable synthetic materials approved for TERM applications by the US Food and Drug Administration (FDA); however the acidic by-products of their degradation can inhibit tissue repair (Dawes and Rushton 1994). To reduce the detrimental effects of these synthetic polymers, they are often mixed with hyaluronic acid (HyA) among other natural polymers, as found in commercial systems like Glycosan HyStem™.

Interestingly, *electrospun materials* gather most of the miRNA delivery work carried out with non-viral and non-lipid vectors such as the PEI-PEG polyplexes (Zhang et al. 2016a), peptide-chitosan-PEG complexes (Zhou et al. 2016), or silver NPs (Qureshi et al. 2015). Materials like gelatin, or the synthetic PLLA, PCL, and PEG-PCL polymers, are commonly preferred to produce electrospun nanofibrous scaffolds compatible with miRNA delivery (Zhang et al. 2016a; Zhou et al. 2016; Diao et al. 2015a; Qureshi et al. 2015; James et al. 2014). The versatility of the electrospinning methodology explains that these scaffolds have generally been developed as in situ miRNA delivery platforms with great potential as off-the-shelf products. Among the non-electrospun porous spongelike scaffolds, very diverse composition mixtures have been adapted to miRNA delivery, with nearly half of the reports relying on lipid (Eskildsen et al. 2011; Wang et al. 2015; Hoseinzadeh et al. 2016; Vimalraj et al. 2016) and viral delivery (Deng et al. 2013a, b). This collection of work is firmly based on the ex situ methodology of pre-transfecting a target cell population in advance of seeding the scaffolds, thus negating a role for the scaffolds to deliver the miRNA cargo. In clear distinction with this research climate, composites of collagen/nHA developed in the Tissue Engineering Research Group (TERG, Dublin) have been successfully applied to the strategy of cell-free in situ delivery of both miR-mimics and antagomiRs (Mencia Castano et al. 2015, 2016). This work reassures the potential of porous scaffolds to serve as clinically translatable off-the-shelf miRNA delivery platforms. In summary, most existing fabrication processes and biomaterial types appear tunable as vehicles for improved miRNA delivery in combination with either viral or non-viral delivery vectors. Further exploration of the vast biomaterial types available to deliver

miRNAs will provide the next generation of advanced tissue engineering therapeutics; the next section illustrates reported therapeutic potential of some of these strategies.

4 The Role of miRNAs as Modulators of Tissue Repair

miRNAs have a pivotal role in regulating embryonic development (Bartel 2004), and their capacity to induce and modulate the differentiation of pluripotent, multipotent, and progenitor cells, in addition to promoting their accelerated differentiation, holds particular interest for tissue engineering researchers (Levy et al. 2010). Hence, much investigation has concentrated on how specific miRNAs affect different cell types to dictate their function in building and repairing the many tissue types of the body (Weilner et al. 2015, 2016; Feichtinger et al. 2018), in instances with one miRNA influencing several tissue types differently. This knowledge has set the foundation for the later pursuit of miRNA-based tissue engineering approaches (Hackl et al. 2011). This section provides an overview of the known roles of miRNAs by tissue type, while Sect. 5 will discuss how some of these miRNAs have been combined with a wide range of biomaterials, thus beginning to show promise as therapeutics in tissue engineering approaches.

4.1 Bone

The bone is a highly dynamic tissue, with remarkable remodeling and repair capabilities, but unable to heal completely in cases of critical-sized non-union fractures (Martini et al. 2006). The bone repair capability resides in mesenchymal stem cells (MSC), and osteoprogenitors are found in the bone marrow (approx. 1% of its cell population), which replenish the population of osteoblasts and osteocytes via osteogenic differentiation (Aubin 1999). The activation of the osteogenesis process in the bone precursors is thus key to direct bone repair. This can be triggered by many anabolic factors, such as bone morphogenetic proteins (BMPs), which have been pursued as therapeutics with this aim. A selection of over 40 miRNAs have been identified to modulate osteogenesis either positively or negatively (Fig. 7; Hu et al. 2010; Jensen et al. 2010; Li et al. 2008, 2009a, b; Luzi et al. 2008; Mizuno et al. 2008, 2009; Huang et al. 2010; Baglio et al. 2013; Kim et al. 2009; Kapinas et al. 2009; Schoolmeesters et al. 2009; Clark et al. 2014; Grunhagen and Ott 2013; Eguchi et al. 2013), and miRNA modulation is explored to treat bone defects and diseases such as osteoporosis and osteoarthritis (Hu et al. 2010; Jensen et al. 2010). Many miRNAs of this panel have direct targets that only play a secondary role in the osteogenesis pathway (Kim and Lim 2014), like Crim1/BAMBI, Dickkopf-related proteins (DKK) 1–2, connexin 43, or the focal adhesion kinase (FAK). On the contrary, another set of miRNAs is known to target the main driver of osteogenesis, transcription factor Runx2: miR-133a-3p, miR-204, miR-211, miR-335, and miR-3077-5p (Li et al. 2008; Liao et al.

Generally, the therapeutic levels of osteogenesis achieved with these candidates is reported as enhanced mRNA levels of Runx2 and OCN to about threefold the expression of the control group (Li et al. 2009a, b; Kim et al. 2009; Hassan et al. 2012) and modal increases of twofold over untreated cells in alkaline phosphatase (ALP) activity (Schoolmeesters et al. 2009). In terms of calcium deposition, the end-stage marker of functional osteogenesis, non-viral delivery approaches using, for example, miR-148b mimic, incurred ~2-fold increase after 14 days of osteogenic culture (Qureshi et al. 2013), while baculovirus-based delivery has demonstrated 3- to 7-fold increases over the control group (Liao et al. 2014a). Of note, results surpassing all these parameters listed above have recently been reported by the inhibition of both miR-133a-3p and miR-16 using a non-viral delivery system based on nanohydroxyapatite particles (Mencia Castano et al. 2016; Mencia Castano et al. 2018), which denotes exciting possibilities in the realization of miRNA therapeutics for bone repair.

4.2 Cartilage

Cartilage tissue is subclassified histologically into hyaline, elastic, or fibrocartilage. Hyaline cartilage, often referred to as articular cartilage, is found on the articulating surface of joints and is extremely difficult to repair using current tissue engineering methods. This is because articular cartilage is generated and maintained by a population of cells called articular chondrocytes (ACs), which rarely proliferate in adults, functioning primarily to produce extracellular matrix (ECM) components such as glycosaminoglycans (Ulrich-Vinther et al. 2003). Also, the intricate balance of cartilage catabolism and anabolism rates can be disrupted leading to progressive, inflammatory, degenerative lesions of articular cartilage; such is the case of osteoarthritis (OA), a chronic disease ultimately affecting the entire joint. Early treatment of articular cartilage lesions may abrogate disease progression; however suitable treatments with long-term efficacy remain elusive (Huey et al. 2012; Niemeyer et al. 2008; Schnabel et al. 2002; Stenberg et al. 2014). Tissue-engineered alternatives stimulate MSCs to differentiate toward chondrocytes *in vitro* by modulating several signaling molecules. These include transforming growth factor- β superfamily cytokines (TGF β , BMPs, GDF5; Johnstone et al. 1998; Legendre et al. 2017; Hatakeyama et al. 2004), the Sox trio – particularly Sox-9 – (Hattori et al. 2010; Lefebvre et al. 2007; Liu and Lefebvre 2015), Wnt/ β -catenin signaling (Yuan et al. 2016), as well as histone deacetylase (HDAC) 4, which inhibits chondrocyte hypertrophy, thereby stabilizing the AC population (Huh et al. 2007). Several miRNAs which target the aforementioned factors have also been investigated for their role in promoting chondrogenesis or inhibiting endochondral ossification or inflammation (Fig. 8). In a microarray screening, several miRNAs, including miR-23b, miR-140, and miR-210, were associated with successful chondrogenic differentiation of MSCs, while miR-221, miR-31, and miR-100 were downregulated during chondrogenic differentiation (Gabler et al. 2015). miR-140 is involved in Sox-9-mediated chondrogenesis and is a direct regulator of aggrecanase-2 (Karlsen et al.

miRNAs are consistently upregulated in OA patients including miR-27b and miR-22 which inhibit matrix metalloproteinase-13 (MMP-13) production (Akhtar et al. 2010; Iliopoulos et al. 2008) and miR-146a which modulates inflammation by inhibiting NF- κ B (Zhao et al. 2011).

4.3 Angiogenesis

Angiogenesis is the process that generates functional vascular networks from already existing vasculature by sprouting or intussusceptive budding, while vasculogenesis generates de novo vasculature within avascular tissues (Balaji et al. 2013). In both processes, endothelial cell differentiation takes place and cells become arranged in tubules with an internal luminal space stabilized by supporting cells like pericytes (Caplan 2008). Both processes are crucial in tissue repair, especially in cases of large or extensive damage, and remain a limiting obstacle for the realization of full-size tissue-engineered grafts of clinical use (Jaklenec et al. 2012). Approaches to develop tissue-engineered vascular networks can focus on either endothelial cell progenitors or supporting cells such as MSCs (Clark et al. 2014; Clarkin and Gerstenfeld 2013). Additionally, natural activators of angiogenesis and vasculogenesis are harnessed as stimuli to further promote the process: low oxygen tissue levels, i.e., hypoxia, which trigger the secretion of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), angiopoietin (Ang)-1/2, and chemokines like stromal cell-derived factor 1-alpha (SDF1 α ; Martini et al. 2006; Tayalia and Mooney 2009). All these factors act in an endocrine and paracrine manner to guide the growth, remodeling, and maturation of the newly formed capillary vessels (des Rieux et al. 2011) and can be secreted by both endothelial cells and MSCs. An extended set of miRNAs is currently known to modulate angiogenesis, vasculogenesis, and the pro-angiogenic capacity of MSCs either with a positive or a negative effect (Fig. 9; Li et al. 2013; Suarez and Sessa 2009; Polisenio et al. 2006; Suarez et al. 2007; Fish et al. 2008; Wang et al. 2008; Harris et al. 2008; Fasanaro et al. 2008). Hence, miRNA modulation strategies have arisen to treat angiogenesis-related pathologies such as cardiovascular diseases (van Rooij 2012), diabetes and diabetes-induced retinopathy (Nunes et al. 2015; McArthur et al. 2011; Feng et al. 2011), as well as exacerbated vascularization associated with several cancer types (Ma et al. 2010). Of particular interest for tissue engineering, pro-angiogenic miRNAs that may simultaneously enhance other tissue repair processes, such as promoting the proliferation of progenitor cells, can present additional benefit clinically (Wang and Olson 2009). Some outstanding pro-angiogenic miRNAs considered as tissue engineering therapeutics involve miR-mimics of miR-26a, miR-126, and miR-210 as well as inhibitors of miR-15/16, miR-24, and miR-200b. In particular, a critical role has been shown for miR-126 in developmental studies (Fish et al. 2008) and in coronary ligation infarction models (Wang et al. 2008), while intra-articular injections of atelocollagen containing naked miR-210 mimic accelerated angiogenesis in anterior cruciate ligament (ACL) injuries in vivo (Shoji et al. 2012). Additionally, the systemic delivery of antagomiR-24 and antagomiR-100 improved

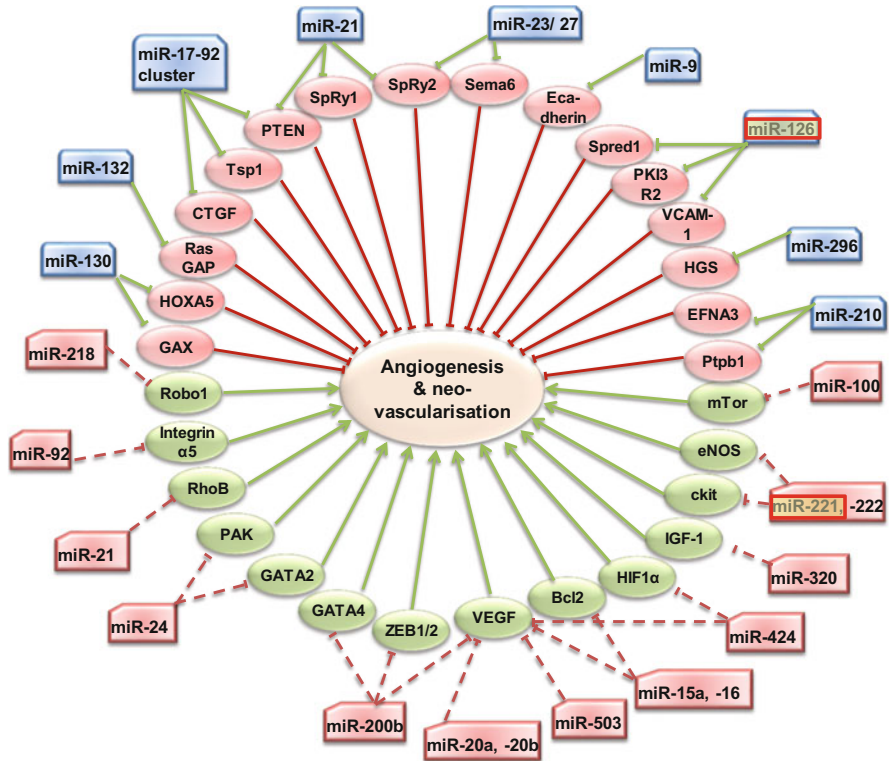


Fig. 9 miRNAs involved in angiogenesis and neovascularization and their validated targets. Inner circle: red ovals linked to red brake symbols mark proteins that inhibit angiogenesis, green ovals linked to green arrows mark proteins that trigger angiogenesis. Outer circle: miRNAs represented in blue or red are positive or negative regulators of the process, respectively (Suarez and Sessa 2009; Poliseno et al. 2006; Suarez et al. 2007; Fish et al. 2008; Wang et al. 2008; Harris et al. 2008; Fasanaro et al. 2008). Highlighting boxes with red border and yellow filling indicate that the miRNA has been assessed as a therapeutic candidate

myocardial angiogenesis in mice myocardial ischemia studies (Grundmann et al. 2011). Contrastingly, therapeutic inhibition of angiogenesis has been shown through the fine-tuning role of miR-200b on multiple growth factors and in epithelial-mesenchymal transition (EMT) in multiple types of cancer, as extensively reviewed by Sinha et al. (Sinha et al. 2015). In a similar line of work, hMSCs were used to deliver miR-16 mimics to prostate cancer xenografts established in mice, and the anti-angiogenic effect of this treatment significantly slowed down tumor growth (Jones et al. 2017). Additionally, subcutaneous implantation – in mice – of microvessels engineered by culturing miR-16-transfected endothelial cells into collagen-fibrinogen gels demonstrated effective modulation of angiogenesis in vivo (Chamorro-Jorganes et al. 2011). This heterogeneous body of work highlights the ample possibilities of angiogenesis-modulating miRNAs in tissue engineering applications, including its recently increasing interplay with cancer research, where

anti-angiogenic treatments have begun to show promise in tumor size reduction (Yihai 2003; Fang et al. 2012; Dai and Tan 2015).

4.4 Skin

The skin is a tri-layered tissue forming the largest organ of the body (Tumbar et al. 2004). It has excellent self-repair capacity in injuries limited to the superficial or dermal layer; however deep damage to all three layers, i.e., full-thickness wounds, requires further treatment to heal: excessive scarring must be prevented, and often skin grafts or bioengineered skin equivalents (BSE) are needed (Leblebici et al. 2006). Although currently multiple BSEs are commercially available, the regeneration of fully functional skin is yet to be achieved (Shevchenko et al. 2010). miRNA modulation might offer the necessary control over cell behavior to meet this goal (Miller et al. 2015). A number of cell types are involved in the wound repair process, including epithelial cells or keratinocytes, melanocytes, Langerhans cells, Merkel cells, fibroblasts, basal stem cells, and bulge stem cells – from hair follicles and sebaceous glands (Watt and Jensen 2009). The adequate proliferation of these cells, followed by their deposition of ECM components, must also be accompanied by the re-establishment of a vascular network, mainly within the dermal layer (Blanpain and Fuchs 2009). Following the findings that follicular and epithelial morphogenesis defects occur in mice with globally impaired miRNA biogenesis (i.e., Dicer or Ago KO mice Wang et al. 2012; Andl et al. 2006), many miRNA modulators of skin development and repair have been identified (Fig. 10). These miRNAs can regulate (i) the proliferation and cell cycle of epithelial stem cells and fibroblasts (Yi et al. 2008; Suh et al. 2012), (ii) the production of melanin (Kim et al. 2014), (iii) the follicular development and sebogenesis (Schneider et al. 2013), and very notably (iv) the migration of keratinocytes (Yi et al. 2006; Pastar et al. 2012) and (v) the deposition of ECM by fibroblasts (Madhyastha et al. 2012). In many cases one single miRNA affects different cell types, and can have opposing effects depending on the cell type, as seen with miR-21, miR-24, miR-31, miR-125b, miR-200b, miR-203, or the miR-29 family (Peng et al. 2012). In the case of epithelial stem cells, the relevant miRNAs tend to silence the transcription factor p63 or the fibroblast growth factor receptor 2 (FGFR2) to maintain stemness and proliferation, preventing differentiation (Lena et al. 2008). The control of miRNAs over the function of keratinocytes and fibroblasts relates to fine-tuning of the TGF β signaling pathway and the control of collagenous ECM deposition (Sundaram et al. 2013; Sun et al. 2008). Despite this extensive knowledge, miRNA therapeutic strategies pursued to date in wound healing have predominantly focused on pro-angiogenic miRNAs, described in the section above. Interesting work focused on miR-7 inhibition to rescue the responsiveness of aging fibroblasts to epidermal growth factor (EGF) and hyaluronan (Midgley et al. 2014); this lack of responsiveness leads to chronic non-healing of wounds among the elderly. The anti-miR-7 treatment demonstrated restored differentiation of fibroblasts to myofibroblasts by enhanced formation of α -SMA fibrils and ~4-fold increased cell mobility in photobleaching studies, overall showing

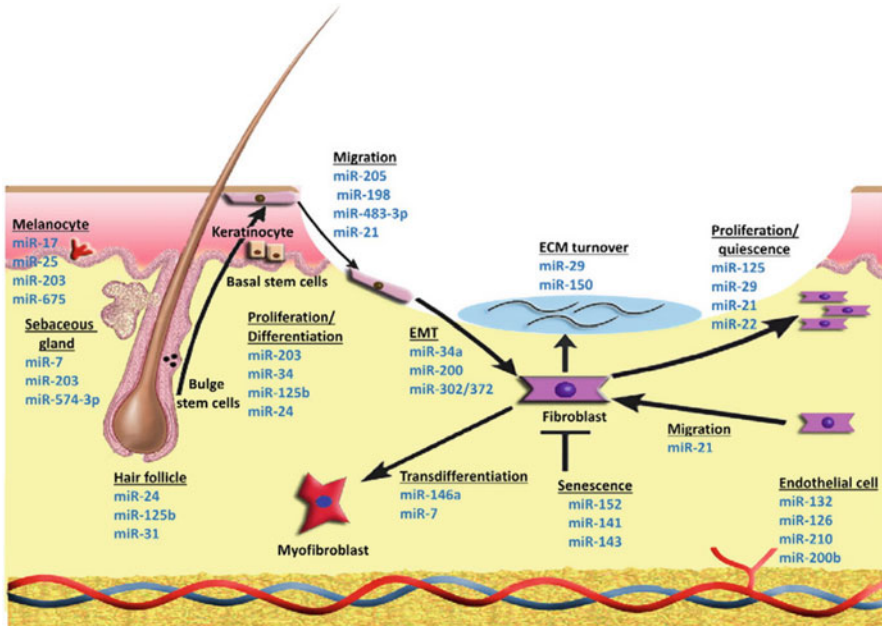


Fig. 10 miRNA modulation of cell behavior in the different skin cell types. (Reproduced with permission Miller et al. (2015); Copyright © 2015 Elsevier B.V. All rights reserved)

promise to prevent chronic wounds. Considering the sound work establishing the role of all these miRNAs in the different cell types found in the skin, we can speculate that the combination of miRNA therapeutics with biomaterials to provide highly functional BSEs is yet to be exploited.

4.5 Muscle

The body is composed of different muscle types, first classified into smooth or striated muscle and then subclassified within striated muscle into the pharyngeal, skeletal, and cardiac muscle types (Cezar and Mooney 2015). Damage or injury to striated muscles includes ischemic heart disease, chronic genetic myopathies, and volumetric muscle loss (VML) injuries, for which muscle-regenerating treatments remain an unmet clinical need (Pascual-Gil et al. 2015). The repair capacity of skeletal muscle is limited by the contribution of resident mononuclear stem cells (or satellite cells) (Lepper et al. 2011), while the limiting factor for cardiac muscle repair is the slow proliferation of adult cardiomyocytes (van Amerongen and Engel 2008). Unsurprisingly, miRNAs have been found to modulate differentiation of satellite cells and myoblasts, with miR-134, miR-296, and miR-470 demonstrating myogenic differentiation of ESCs matching the efficiency previously reported by siRNA silencing of Nanog, Oct4, and Sox-2 (Tay et al. 2008a, b). Hence, the

application of miRNA therapeutics for muscle repair has begun to generate interest, with mimics of miR-26a and miR-206, as well as miR-682 inhibition showing ability to enhance myogenesis after injury (Chen et al. 2011a; McCarthy 2008; Dey et al. 2012). Interestingly, miR-206 can silence multiple myogenesis inhibitors, such as Pax7, Notch3, Igfbp5, connexin 43 (CX43), and histone deacetylase 4 (HDAC4) (McCarthy 2008), denoting a potentially high therapeutic benefit of this miRNA. In line with this, delivery of naked miR-206 mimics along with miR-1 or miR-133 mimics in an atelocollagen mix significantly increased the regeneration of centronucleated myofibers by ~2.4-fold in a rat model of laceration of the tibialis anterior muscle (Nakasa et al. 2010). In addition to myogenesis modulation, miRNA signatures have also been associated with chronic muscle maladies, including Duchenne myodystrophy, sarcopenia, aging-related degeneration, or muscular atrophy (Arashiro et al. 2009; Dimmeler and Nicotera 2013; Wada et al. 2011). For example, aged muscles present decreased levels of miR-181a, while the presence of the miR-15 family members is increased (Mercken et al. 2013). The identification of these expression profiles in patients could serve as a cue to apply personalized miRNA therapeutics to reinstate the balance and normal function of the affected muscles (Feichtinger et al. 2018).

Distinct miRNAs have shown specific potential to improve cardiomyocyte function (van Amerongen and Engel 2008); this includes miR-590 and miR-199a, which halved the infarct size by promoting cardiomyocyte proliferation in a mouse myocardial infarction model (Eulalio et al. 2012). Additionally, miR-302 mimics were able to reduce over 60% cardiac fibrosis size in a period of 50 days (Tian et al. 2015). Meanwhile, inhibiting miR-34a (Boon et al. 2013) or members of the miR-15 family (Porrello et al. 2011), all of which act through arresting the cell cycle of cardiomyocytes, can also result in enhanced cardiomyocyte proliferation and up to 75% less cell death after infarction. Moreover, miRNA modulation can aid cardiac stem cell (CSC) differentiation, as shown by the local injection of miR-499 mimic modified CSCs post-infarction in a mouse model (Wilson et al. 2010; Sluijter et al. 2010). This work demonstrated myocyte mass increase to double that in the control, and improved left ventricular function in the modified CSCs vs. control CSCs, which occurred through targeting Sox-6 and Rod1. In summary, the collective work reported on miRNA-mediated modulation of striated muscle repair suggests an attractive potential to be harnessed in tissue engineering; as seen before, this research area will also rely on the availability of adequately safe and efficient delivery systems to be fully exploited in the near future.

4.6 Neural Tissue

Neural tissue is formed by a number of cell types and has a limited capacity to regenerate after injury, more so in the central nervous system (CNS) than in the peripheral nervous system (PNS) (Schmidt and Leach 2003). Following CNS injury, glial scars are formed which inhibit axonal regeneration and neurogenesis

(GrandPré et al. 2000). Additionally, neurite outgrowth is also inhibited by molecules contained in the CNS myelin (McKerracher et al. 1994). Thus, to recapitulate an environment favorable to neural growth after injury, tissue engineering approaches must promote neurite outgrowth and remyelination while preventing excessive inflammatory reactions and glial scar formation. To achieve successful tissue repair, two divergent avenues are possible: cell replacement therapies and treatments for the local cell population. Because several miRNAs have different effects on various cell types of the neural tissue (Nguyen et al. 2015), treating the local cell population requires neuron or Schwann cell-specific miRNA delivery systems that remain to be developed. Thus, initial efforts have been placed on cell replacement therapies by miRNA-engineering the cells in vitro. A panel of miRNAs modulate dendritogenesis, miR-134 (Christensen et al. 2010); neurogenesis, let 7b/d, miR-34a, miR-125b, miR-137, miR-184, and miR-195 (Nguyen et al. 2015; Stappert et al. 2015; Perruisseau-Carrier et al. 2011); and oligodendrocyte maturation, miR-219 and miR-338 (Svaren 2014). Hence, it is envisaged that miRNA modulation can improve the neuronal cell reprogramming yields that limit this cell replacement strategy. The reprogramming can be targeted to different types of adult stem cells or to trans-differentiate fibroblasts. For example, successful trans-differentiation of mouse embryonic fibroblasts (MEFs) to neuronal-like cells has been shown following lentiviral delivery of miR-9/9* and miR-124 mimics (Yoo et al. 2011; Xue et al. 2013). While both miRNAs can silence the *a* subunit of the BAF chromatin-remodeling complex (BAF53a), they also possess additional targets involved in neurogenesis: miR-9 targets the Hes family members (Bonev et al. 2012), in addition to TLX, a nuclear receptor directing neural stem cell self-renewal (Zhao et al. 2009). Meanwhile, miR-124 is known to also silence a repressor of neuron-specific splicing, that is, polypyrimidine tract binding protein 1 (PTBP1) (Makeyev et al. 2007), and to target multiple elements of Notch signaling (Cheng et al. 2009). This body of work highlights the beneficial effect achieved by the multi-targeting potential of these miRNAs. Other interesting work on miRNAs for neural tissue engineering has shown enhanced neurogenesis with mimics of miR-124a (Hwang et al. 2011), the miR-106b~25 cluster (Brett et al. 2011), or screening of miR-153, miR-181a/a*, miR-324/* (Stappert et al. 2013), and miR-146b, miR-23b, and miR-99a (Stevanato and Sinden 2014). These reports have generally targeted neural stem/progenitor cells (NSPCs) using retrovirus (Brett et al. 2011) or commercial lipid vectors to deliver the miRNAs (Stappert et al. 2013; Stevanato and Sinden 2014), achieving ~2.6-fold increases in the key neuronal marker Tuj1. However, a miR-124 delivery system (Hwang et al. 2011) employed a rabies virus glycoprotein (RVG)-enabled carrier with ability to specifically target nicotinic acetylcholine receptors on neuronal cells, showing significant promise for in vivo miRNA delivery. This delivery system showed 90% reporter silencing efficiency in Neuro2a cells, and combined with mannitol for in vivo administration, it accumulated in the brain after successfully passing the blood-brain barrier. This development thus showed promise as a treatment for the localized cell population and alternative to cell replacement approaches.

5 The Proven Therapeutic Potential of miRNAs to Date

The progress in miRNA therapeutics research for TERM is increasingly proving to have potential for applications in angiogenesis and vasculogenesis, the skin, the cardiovascular system, and orthopedics. From the summary presented in Table 5, it is clear that most work has been done in the area of bone repair; however, positive preclinical results have been reported in viral miRNA delivery for muscle repair (Cheng et al. 2016) and enhanced neurogenesis (Brett et al. 2011). In this highly dynamic field, miRNAs are also being combined with other therapeutics like small chemicals or separate pDNA vectors (Liao et al. 2014a; Shi et al. 2014).

5.1 Bone Healing

The area of bone healing has been the most prolific to date in demonstrating the therapeutic potential of miRNA-based treatments, with a number of reports focusing on the use of miR-26a mimics. These employed varied delivery platforms like Lipofectamine[®] 2000 on porous HA scaffolds (Wang et al. 2015), PEI polyplexes on nanofibrous PLLA scaffolds (Zhang et al. 2016a), or the siPORT NeoF_x[™] on HyStem-HP hydrogels (Li et al. 2013). The interest on miR-26a began by investigating a pro-angiogenic effect seen through BMP2/Drosophila mothers against decapentaplegic (SMAD) signaling (Icli et al. 2013) and the p38-MAPK/VEGF pathway (Zuo et al. 2015). However, a direct effect of miR-26a on modulating osteoblast activity has more recently been proven through targeting glycogen synthase kinase-3beta (GSK-3beta; Zhang et al. 2016a). The current therapeutic effects derived from the miR-26a mimic treatments include the repair of bone defects 12 weeks after implantation (Li et al. 2013; Wang et al. 2015). Additionally the cell-free system by Zhang et al., encompassing the intermediate polyplex encapsulation in scaffold-immobilized PLGA microspheres, increased bone healing in both healthy and osteoporotic mice just 8 weeks after implantation (Zhang et al. 2016a).

miR-148b mimics consistently demonstrated increased ALP activity and were used on PEG-norbornene scaffolds (Mariner et al. 2012) and cell-seeded baculovirus-/gelatin-coated PLGA (Liao et al. 2014b) and Ag NP/PCL (Qureshi et al. 2015) scaffolds, before the BMP2 inhibitor *NOG* was identified as its direct target (Li et al. 2016). In the first two of these studies, miR-148b was co-delivered with miR-489 inhibitors or BMP2 pDNA, respectively. The baculovirus systems enhanced healing by ~80% 12 weeks post-implantation (Liao et al. 2014b; Li et al. 2016). Comparatively, the non-viral Ag NP/PCL system reported ~32% increased healing, although this platform was successfully controlled by photoactivation, an elegant development to confer temporal control and improved safety to this proposed bone graft substitute (Qureshi et al. 2015). Non-viral delivery of miR-15b or miR-20a mimics to MSCs has also shown significant potential to enhance therapeutic osteogenesis in vitro (Huynh et al. 2016; Nguyen et al. 2014; Vimalraj et al. 2016). Specifically, miR-15b modulation induced paracrine osteogenesis on osteoblasts using conditioned medium from mMSCs that were transfected using the

X-tremeGENE agent, with a greater effect when MSCs were cultured on the carboxymethyl cellulose-nHA scaffolds (Vimalraj et al. 2016). The work performed with miR-20a mimics first used PEI polyplexes and in situ gelling PEG hydrogels co-delivering Noggin siRNA (Nguyen et al. 2014), to later incorporate on-demand release using photodegradation triggered by UV light (Huynh et al. 2016).

Lentivirus systems pre-modifying ASCs with miR-135 mimics (Xie et al. 2016) or inhibitors of miR-146a (Xie et al. 2017), and miR-31 (Deng et al. 2013b, 2014a, b) have demonstrated relevant potential to modulate osteogenesis and bone healing as cell-based approaches. In the lentivirus systems applied to poly(sebacoyl diglyceride) (PSeD) scaffolds, the treatments significantly enhanced ASC-directed bone regeneration, while counter-treatments with miR-135 inhibitors or miR-146a mimics reverted the therapeutic effect (Xie et al. 2016, 2017). Works on the lentiviral miR-31 inhibitory strategy have shown significant bone repair in both rat calvarial defects (Deng et al. 2013b, 2014a) and canine orbital defects (Deng et al. 2014b). This work underlined a feedback mechanism activated by BMPs and leading to miR-31 suppression. Finally, demineralized bone matrices (DBMs) were combined with MSCs pre-modified by lentiviral miR-125b inhibition targeting the BMP type-1b receptor (BMPRIb), leading to ~50% higher bone mineral density and maturity (Wang et al. 2017b). Multiple non-viral approaches to miRNA inhibitor delivery have also shown bone repair to date. The ADSC-based application of antagomiR-221 complexes with the commercial agent Lipofectamine[®] 2000 on nHA/PCL scaffolds proved successfully increased osteogenesis while highlighting differences between the monolayer and scaffold settings (Hoseinzadeh et al. 2016). Meanwhile, the commercial TKO transfection agent was applied to cell-free antagomiR-29a delivery within gelatin nanofibers to target several collagens as well as osteonectin, significantly increasing the synthesis of ECM proteins, although mineralization data were not provided (James et al. 2014).

A growing body of work using cell-free non-viral miRNA delivery platforms has focused on nHA particles as delivery vectors added to porous collagen-nHA scaffolds first designed for bone repair applications (Mencia Castano et al. 2015, 2016). The scaffold system has shown promising functionality at ~20% silencing with miR-mimic reporters and 88.4% inhibition with antagomiR reporters (Mencia Castano et al. 2015); these scaffolds delivering antagomiR-16 and antagomiR-133a demonstrated increased osteogenesis by hMSCs to levels seen only with viral systems (Mencia Castano et al. 2018; Li et al. 2008) and are currently being pursued in preclinical studies. Among preclinical reports, gelatin/spongostan scaffolds carrying naked miR-214 inhibiting sponges pre-transfected in ovariectomized (OVX) MSCs increased osteoporotic bone healing, especially when co-delivered with plasmid BMP2 (Li et al. 2016a), while a naked antagomiR-222 in an atelocollagen mix lead to successful refractory fracture healing after 8 weeks (Yoshizuka et al. 2016).

5.2 Cartilage Healing

In comparison with the increasing understanding of how miRNA modulation can enhance in vitro chondrogenesis, realization of miRNA therapeutics for enhanced

cartilage healing is noticeably delayed. This is partly because single or focal defects present difficulties of low cell density and avascularity and partly because degenerative cartilage damage resulting from osteoarthritis (OA) or advanced tear of the anterior cruciate ligament (ACL) is greatly affected by impaired functions of the immune system. Recently reported *in vitro* studies of 3D pellet cultures without the support of a biomaterial scaffold have demonstrated the fine-tuning and space-dependent effect of different miRNA therapeutic candidates. This work entails the inhibition of miR-374, miR-379, or miR-503 with Lipofectamine® 2000 complexes in rat primary chondrocytes (Jee et al. 2018), as well as the lentiviral delivery of miR-140 mimics administered in combination with TGFβ3 supplements to human-induced pluripotent stem cells (Mahboudi et al. 2018). The thorough zonal study of growth plate chondrocytes presented by Jee and colleagues (2018) underscored a parallel between the concentration of parathyroid hormone-related protein (PTHrP) and the three miRNAs: miR-374, miR-379, and miR-503. High levels of all PTHrP and three miRNAs enhanced chondrocyte proliferation while inhibiting hypertrophy, but low levels of these directed hypertrophic differentiation. Mahboudi et al. showed enhanced aggrecan and collagen type II expression in the pellets after 21 days, validating the pro-chondrogenic role of miR-140 (Mahboudi et al. 2018); however, collagen type X expression was also enhanced with the miRNA treatment, indicative of the undesired hypertrophy that leads to dysfunctional calcification of cartilage.

Based on these reports, miRNA-based approaches for cartilage tissue engineering are still envisaged to involve the delivery of miRNA-modified cells within biomaterials. There has only been one successful *in vivo* report on cartilage regeneration, which demonstrated >95% successful inhibition of miR-221 in pellet culture and later incorporated the miR-221 inhibited cells in a hydrogel suspension of alginate injected within osteochondral biopsies (Lolli et al. 2016). This miR-221 silencing system used Lipofectamine RNAiMAX to transfect hMSCs and was introduced by Lolli et al. in their previous *in vitro* work showing the increase of chondrogenic markers with this therapeutic in the absence of TGFβ (Lolli et al. 2014). These complex constructs were then inserted into subcutaneous pockets in the back of immunosuppressed mice and lead to enhanced deposition of cartilaginous matrix within the constructs at a 12-week time-point. Moreover, the study assessed the hallmark indicator of hypertrophy, collagen type X, and did not find evidence of expression in the miR-221 silenced group. These results point to an exciting direction to provide alternatives to the limitedly successful options of chondroplasty/mosaicplasty, microfracture, or articular replacement.

5.3 Myogenesis and Myocardium Repair

Similarly to the landscape of cartilage repair, the pursuit of miRNA therapeutics in the area of myocyte functional regeneration remains in its infancy. However, progress in this arena appears valuable not only to address diseased or injured muscles but also as *in vitro* models for drug testing in the effort to reduce animal testing. To date, miRNA modulation has successfully augmented contractile force of human

skeletal myoblasts grown on fibrin-based hydrogels via delivery of miR-133a and miR-696 inhibitors with the commercial agent siPORT NeoF_xTM (Cheng et al. 2016). This recent work by Cheng et al. demonstrated that inhibition of both miRs could enhance the peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) among other markers, indicating increased myogenesis of the myobundles after 2 weeks in static 3D culture. Cardiomyocyte function modulation has also been reported with miRNA treatments attempting to restore cardiovascular function after myocardium damage. With the premise of recovering cardiomyocyte function and enhancing their proliferation rate, the complications generated by insufficient angiogenesis could be somewhat prevented (van Amerongen and Engel 2008). This was shown using a mouse ischemia/reperfusion model where the animals received myocardial injections of a Glycosan HyStem/hyaluronan hydrogel carrying naked miR-29b mimics (Monaghan et al. 2018). The treatment enhanced the de novo deposition of collagen fibers bordering the infarct zone and improved scar remodeling and vascularity, ultimately preserving the myocardial function and increasing the cardiac ejection fraction by \sim 10%. In another elegant approach to therapeutically enhance the functional population of cardiomyocytes, neonatal cardiac fibroblasts were successfully reprogrammed with a multi-miRNA mimic treatment of miR-1, miR-133, miR-208, and miR-499, delivered using the commercial agent DharmaFECT and fibrinogen-based hydrogels as the support biomaterial (Li et al. 2016b). The combination of the treatment with the 3D hydrogel culture improved – over the 2D culture results – the yield of successfully reprogrammed cardiomyocytes through a MMP-dependent mechanism. The realization of injectable biomaterials adapted for successful delivery of miRNA therapeutics, such as these hydrogels, can provide an attractive route for noninvasive regenerative treatment of myocardial dysfunctions and ischemic diseases.

5.4 Angiogenesis, Vasculogenesis, and Wound Healing

Angiogenesis is a crucial process not only for the homeostasis and repair of the circulatory system but also for the successful repair of most tissue types, with the exception of cartilage healing. A recent application of miRNA therapeutics for vascular tissue engineering was introduced by delivering miR-126 mimics to vascular endothelial cells using multi-modified peptide/chitosan/PEG NPs (Zhou et al. 2016). The miR-chitosan complexes were embedded in the innermost layer of a fibrous electrospun composite based on PEG, PLL, PCL, and gelatin (PELCL) and, through silencing SPRED-1, effectively enhanced cell proliferation and in vivo endothelialization. Importantly, exacerbated angiogenesis is a leading cause of tumor growth, and numerous anti-angiogenic treatments have been proposed to inhibit tumor growth. With the use of 3D biomaterials emerging in cancer research, a number of reports have already explored miRNAs in tissue-engineered approaches to control vascular hyperplasia. To this end, fragments of jugular vein enhanced with a lentiviral miR-26a mimic and Pluronic F127 hydrogel mix reduced venous neointimal hyperplasia of vascular smooth muscle cells in a rat model (Tan et al. 2017).

This treatment worked by inhibiting cell proliferation through silencing the target MAPK6, and also reducing the intima-to-media ratio, reducing the neointimal hyperplasia by ~30%. Similarly, the Pluronic F127 hydrogel carrying adenoviral miR-221 inhibiting sponge mixtures also hindered cell proliferation and neointimal formation in the rat vein grafting model, with reduced neointima/media ratio, neointimal area, and thickness (Wang et al. 2016). Additionally, this study also determined improved hemodynamics and related these effects with the targeting of Kip1. Taking this body of work together, miRNA modulation appears as a significant promise to bypass failure in vein grafting procedures.

Delivery of naked miR-29b mimics from a collagen-based scaffold has been used in the area of wound healing, with the aim of directing beneficial remodeling of the ECM while preventing the formation of scar tissue (Monaghan et al. 2014). The scaffolds were first tested *in vitro* culturing primary fibroblasts, and the miR-29b mimics released from this platform showed beneficial ~40% and ~15% reductions of collagen type I and type III levels at the 2-week time-point. Following these observations, the scaffolds were implanted in full-thickness skin excisional wounds in rats and achieved dose-dependent improvements in the ratios of collagen type III/I and matrix metalloproteinase (MMP)-8/tissue inhibitor of metalloproteinase (TIMP)-1, as well as reducing the contraction of the wound.

5.5 Neurogenesis and Neural Tissue Healing

With the limited clinical options currently available to treat neurological pathologies and improve nerve healing, miRNA therapeutics might have significant potential for neural tissue engineering. Of note, various 3D scaffold systems have been used to better elucidate the miRNA mechanisms regulating the self-renewal vs. differentiation fate of neural stem cells, in comparison with the traditional monolayer culture. To do so, Cui et al. employed spongy collagen-based scaffolds together with PA-1 cells and neural stem cells (NSCs), reporting differential miRNA profiles in 3D culture and decreased NSC differentiation (Cui et al. 2014). This effect was associated with the reduced expression of a panel of miRNAs with validated targets involved in NSC fate decision, including miR-20. Additionally, NSCs differentiated on nanofibrous scaffolds of aminopropyl-silica have been reported to enhance the levels of let-7 miRNAs (Mercado et al. 2016), whose decisive control of NSC differentiation had previously been reported (Brett et al. 2011). Building on the physiologically relevant data obtained from these studies of 3D-cultured neural progenitors, three different miRNAs have been assessed as therapeutic candidates in this nascent field: miR-222 mimics and miR-219 and miR-338 inhibitors. The therapeutic potential of miR-219 and miR-338 inhibitors, complexed with TRANSIT-TKO, to enhance the development of oligodendroglial precursor cells (OPC) was assessed *in vitro* in topographically controlled electrospun PCL scaffolds (Diao et al. 2015a, b). Both antagomiR treatments significantly enhanced OPC maturation and remyelination, and this was favored by the topography (Diao et al. 2015b). More recently, micellar NPs delivering miR-222 mimics within an aligned nanofibrous

hydrogel based on collagen, PCL, and *co*-ethylene phosphate have shown therapeutic promise in a rat spinal cord injury model (Nguyen et al. 2017). This work demonstrated axonal regeneration with adequate alignment just 1 week after injury induction while finding minimal scar tissue formation and inflammation and represents an attractive development for advanced neural tissue repair.

6 Future Outlook

The discovery of miRNAs and the recent explorations of their therapeutic potential have widely impacted the landscape of gene therapy. This impact has been more prominent in the areas of genetic disorders (i.e., single gene mutations) and oncology, with research only recently beginning to focus on the field of TERM and concentrating on establishing the role of miRNAs in tissue development and repair. The fruition of this knowledge now opens up vast and novel therapeutic opportunities, propelling a research area that has grown exponentially in the last 5–8 years. Perhaps the most immediate opportunity exists on the application of miRNAs in the areas of biomarkers and personalized medicine (Hackl et al. 2016); miRNA profiles of individual patients may serve both to prevent and diagnose diseases and to monitor the patient response to the assigned treatments. The use of miRNAs as biomarkers accounts for the highest number of clinical trials currently ongoing (Ginn 2018). Nonetheless, the application of miRNAs as therapeutics remains very appealing, as their chemical manipulation and incorporation into efficient delivery systems can be facilitated by their low molecular weight and small size, in comparison with plasmid DNA or proteins. More importantly, miRNAs have the potential to interact with multiple targets and thus regulate biological processes at several points. Despite limitations with clinical translation related to issues such as pharmacokinetic and intracellular delivery obstacles, commercialization of miRNA products is increasing all the time, and many companies have taken stakes in the clinical application of miRNAs. For example, Santaris Pharma, Regulus, miRagen, InteRNA Tech., Mirna Therapeutics, and EnGeneIC are currently competing with the giant of RNA therapeutics, Moderna, and have led a number of miRNA therapeutics to enter clinical trials, progressing up to Phase II. This progress strengthens the idea that miRNA treatments bear a bright future and the capacity to make an impact in the generation of the next-generation TERM therapeutics.

7 Conclusions

This chapter has reviewed the wide range of techniques and research tools currently available for miRNA exploration. While these currently allow the investigation of miRNAs from the discovery of novel units to the understanding of their physiopathological roles, research efforts need to make more accessible the use of high-throughput RNAseq. Specific focus must be placed in developing new benchtop equipment, as well as better computational and systems biology tools, to simplify the storage of the

complex and large datasets that this technique generates, permit their quick visualization, and facilitate the identification of novel interactions. In addition, genetically modified animal models that suppress the expression of a particular miRNA are currently underdeveloped in comparison with the tools to generate models over-expressing the miRNA of interest. The application of CRISPR technology to generate miR-specific and tissue- and cell-specific KO animal models will allow an improved understanding of the importance of anomaly low levels of a miRNA of interest for the normal function of a given tissue type or organ. This enhanced knowledge will serve to test better and more accurately the novel miRNA modulation strategies and TE systems surfacing in the field. Ultimately the biggest research need for the use of miRNA therapeutics in TERM is invariably the development, availability, and standardization of safe, noninvasive, and marketable biomaterial-based delivery systems of high quality. These systems must serve the main goal of enhancing the miRNA bioavailability in a spatiotemporally controlled manner and reducing the undesired uptake by nontarget tissues, which would occur when employing systemic routes of administration. Some of the key factors which might support the potential of miRNA applications in tissue engineering and regenerative medicine include accumulating translatable knowledge gained from siRNA and anti-tumor miRNA-based therapies, where translation toward the clinic is at a more advanced stage of development, as well as exploiting the highly innovative systems for biomaterial-based miRNA delivery. If these developments are accompanied by extensive advances at the regulatory level, the field of biomaterial-based miRNA delivery will likely begin the transition from bench to bedside within the next decade.

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References

- (Agency), E.E.M (2016a) Strimvelis autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence. EMA/CHMP/249031/2016: European Medicines Agency
- (Agency), E.E.M (2016b) Zalmoxis allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (Δ LNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2). EMA/454627/2016: European Medicines Agency
- Akhtar N, Rasheed Z, Ramamurthy S, Anbazhagan AN, Voss FR, Haqqi TM (2010) MicroRNA-27b regulates the expression of matrix metalloproteinase 13 in human osteoarthritis chondrocytes. *Arthritis Rheum* 62:1361
- Akhtar MM, Micolucci L, Islam MS, Olivieri F, Procopio AD (2016) Bioinformatic tools for microRNA dissection. *Nucleic Acids Res* 44:24
- Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) *Molecular biology of the cell*, 4th edn. Garland Science, New York
- Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, and Tuschl T (2003) A uniform system for microRNA annotation. *RNA* 9:277

- Andl T, Murchison EP, Liu F, Zhang Y, Yunta-Gonzalez M, Tobias JW, Andl CD, Seykora JT, Hannon GJ, Millar SE (2006) The miRNA-processing enzyme dicer is essential for the morphogenesis and maintenance of hair follicles. *Curr Biol* 16:1041
- Arashiro P, Eisenberg I, Kho AT, Cerqueira AM, Canovas M, Silva HC, Pavanello RC, Verjovski-Almeida S, Kunkel LM, Zatz M (2009) Transcriptional regulation differs in affected facioscapulohumeral muscular dystrophy patients compared to asymptomatic related carriers. *Proc Natl Acad Sci U S A* 106:6220
- Aravin AA, Lagos-Quintana M, Yalcin A, Zavolan M, Marks D, Snyder B, Gaasterland T, Meyer J, Tuschl T (2003) The small RNA profile during *Drosophila melanogaster* development. *Dev Cell* 5:337
- Aubin JE (1999) Osteoprogenitor cell frequency in rat bone marrow stromal populations: role for heterotypic cell-cell interactions in osteoblast differentiation. *J Cell Biochem* 72:396
- Bader AG, Brown D, Winkler M (2010) The promise of microRNA replacement therapy. *Cancer Res* 70:7027
- Baglio 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
- Balaji S, King A, Crombleholme TM, Keswani SG (2013) The role of endothelial progenitor cells in postnatal vasculogenesis: implications for therapeutic neovascularization and wound healing. *Adv Wound Care (New Rochelle)* 2:283
- Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281
- Barter MJ, Tselepi M, Gómez R, Woods S, Hui W, Smith GR, Shanley DP, Clark IM, Young DA (2015) Genome-wide MicroRNA and gene analysis of mesenchymal stem cell chondrogenesis identifies an essential role and multiple targets for miR-140-5p. *Stem Cells (Dayton, Ohio)* 33:3266
- Basyuk E, Suavet F, Doglio A, Bordonne R, Bertrand E (2003) Human let-7 stem-loop precursors harbor features of RNase III cleavage products. *Nucleic Acids Res* 31:6593
- Beavers KR, Nelson CE, Duvall CL (2014) MiRNA inhibition in tissue engineering and regenerative medicine. *Adv Drug Deliv Rev*
- Behr J-P (1997) The proton sponge: a trick to enter cells the viruses did not exploit. *CHIMIA Int J Chem* 51:34
- Benes V, Castoldi M (2010) Expression profiling of microRNA using real-time quantitative PCR, how to use it and what is available. *Methods* 50:244
- Bernardo BC, Charchar FJ, Lin RCY, McMullen JR (2012) A MicroRNA guide for clinicians and basic scientists: background and experimental techniques. *Heart, Lung Circ* 21:131
- Bhattacharya A, Ziebarth JD, Cui Y (2014) PolymiRTS database 3.0: linking polymorphisms in microRNAs and their target sites with human diseases and biological pathways. *Nucleic Acids Res* 42:D86
- Blanpain C, Fuchs E (2009) Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol* 10:207
- Bonadio J, Smiley E, Patil P, Goldstein S (1999) Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration. *Nat Med* 5:753
- Bonev B, Stanley P, Papalopulu N (2012) MicroRNA-9 modulates Hes1 ultradian oscillations by forming a double-negative feedback loop. *Cell Rep* 2:10
- Boon RA, Iekushi K, Lechner S, Seeger T, Fischer A, Heydt S, Kaluza D, Tréguer K, Carmona G, Bonauer A, Horrevoets AJ, Didier N, Girmatsion Z, Biliczki P, Ehrlich JR, Katus HA, Müller OJ, Potente M, Zeiher AM, Hermeking H, Dimmeler S (2013) MicroRNA-34a regulates cardiac ageing and function. *Nature* 495:107
- Bose S, Tarafder S (2012) Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. *Acta Biomater* 8:1401
- Boudreau RL, Martins I, Fau-Davidson BL, Davidson BL (2009) Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in vitro and in vivo. *Mol Ther* 17:169
- Braasch DA, Corey DR (2001) Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem Biol* 8:1

- Brett JO, Renault VM, Rafalski VA, Webb AE, Brunet A (2011) The microRNA cluster miR-106b-25 regulates adult neural stem/progenitor cell proliferation and neuronal differentiation. *Aging (Albany NY)* 3:108
- Bucholz RW (2002) Nonallograft osteoconductive bone graft substitutes. *Clin Orthop Relat Res* 395:44
- Budak H, Bulut R, Kantar M, Alptekin B (2016) MicroRNA nomenclature and the need for a revised naming prescription. *Brief funct genomics*. The Author 2015. Published by Oxford University Press For permissions, please email: journals.permissions@oup.com, England, p 65
- Caplan AI (2008) All MSCs are pericytes? *Cell Stem Cell* 3:229
- Caporali A, Emanuelli C (2011) MicroRNA-503 and the extended microRNA-16 family in angiogenesis. *Trends Cardiovasc Med* 21:162
- Caudy AA, Myers M, Hannon GJ, Hammond SM (2002) Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev* 16:2491
- Caudy AA, Ketting RF, Hammond SM, Denli AM, Bathoorn AM, Tops BB, Silva JM, Myers MM, Hannon GJ, Plasterk RH (2003) A micrococcal nuclease homologue in RNAi effector complexes. *Nature* 425:411
- cBio (2010) MicroRNA.org. In: M.S.-K.C.C.M (ed) Computational biology center. Memorial Sloan-Kettering Cancer Center™, New York
- Cezar CA, Mooney DJ (2015) Biomaterial-based delivery for skeletal muscle repair. *Adv Drug Deliv Rev* 84:188
- Chamorro-Jorganes A, Araldi E, Penalva LOF, Sandhu D, Fernandez-Hernando C, Suarez Y (2011) MicroRNA-16 and microRNA-424 regulate cell-autonomous angiogenic functions in endothelial cells via targeting vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1. *Arterioscler Thromb Vasc Biol* 31:2595
- Chen CZ, Li L, Lodish HF, Bartel DP (2004) MicroRNAs modulate hematopoietic lineage differentiation. *Science* 303:83
- Chen Y, Gelfond J, McManus LM, Shireman PK (2011a) Temporal microRNA expression during in vitro myogenic progenitor cell proliferation and differentiation: regulation of proliferation by miR-682. *Physiol Genomics* 43:621
- Chen CL, Luo WY, Lo WH, Lin KJ, Sung LY, Shih YS, Chang YH, Hu YC (2011b) Development of hybrid baculovirus vectors for artificial MicroRNA delivery and prolonged gene suppression. *Biotechnol Bioeng* 108:2958
- Cheng LC, Pastrana E, Tavazoie M, Doetsch F (2009) miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat Neurosci* 12:399
- Cheng CS, Ran L, Bursac N, Kraus WE, Truskey GA (2016) Cell density and joint microRNA-133a and microRNA-696 inhibition enhance differentiation and contractile function of engineered human skeletal muscle tissues. *Tissue Eng Part A* 22:573
- Chew SY (2015) MicroRNAs in tissue engineering & regenerative medicine. *Adv Drug Deliv Rev* 88:1
- Christensen M, Larsen LA, Kauppinen S, Schratt G (2010) Recombinant adeno-associated virus-mediated microRNA delivery into the postnatal mouse brain reveals a role for miR-134 in dendritogenesis in vivo. *Front Neural Circ* 3:16
- Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V, Bansal P (2016) MicroRNA therapeutics: Discovering novel targets and developing specific therapy. *Perspect Clin Res* 7:68
- Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 102:13944
- Clark EA, Kalomoiris S, Nolte JA, Fierro FA (2014) Concise review: microRNA function in multipotent mesenchymal stromal cells. *Stem Cells* 32:1074
- Clarkin CE, Gerstenfeld LC (2013) VEGF and bone cell signalling: an essential vessel for communication? *Cell Biochem Funct* 31:1

- ClinicalTrials.gov (2013) Multicenter phase I study of MRX34, MicroRNA miR-RX34 Liposome Injectable Suspension. October 28, 2013 ed: U.S. National Institutes of Health
- Conde J, Oliva N, Atilano M, Song HS, Artzi N (2016) Self-assembled RNA-triple-helix hydrogel scaffold for microRNA modulation in the tumour microenvironment. *Nat Mater* 15:353
- Cui Y, Xiao Z, Chen T, Wei J, Chen L, Liu L, Chen B, Wang X, Li X, Dai J (2014) The miR-7 identified from collagen biomaterial-based three-dimensional cultured cells regulates neural stem cell differentiation. *Stem Cells Dev* 23:393
- Cunniffe GM, O'Brien FJ, Partap S, Levingstone TJ, Stanton KT, Dickson GR (2010) The synthesis and characterization of nanophase hydroxyapatite using a novel dispersant-aided precipitation method. *J Biomed Mater Res A* 95:1142
- Cunniffe GM, Curtin CM, Thompson EM, Dickson GR, O'Brien FJ (2016) Content-dependent osteogenic response of nanohydroxyapatite: an in vitro and in vivo assessment within collagen-based scaffolds. *ACS Appl Mater Interfaces* 8:23477
- Curtin CM, Cunniffe GM, Lyons FG, Bessho K, Dickson GR, Duffy GP, O'Brien FJ (2012) Innovative collagen nano-hydroxyapatite scaffolds offer a highly efficient non-viral gene delivery platform for stem cell-mediated bone formation. *Adv Mater* 24:749
- Curtin CM, Mencía Castaño I, O'Brien FJ (2017) Scaffold-based microRNA therapies in regenerative medicine and cancer. *Adv Healthc Mater* 7:1700695
- Dai X, Tan C (2015) Combination of microRNA therapeutics with small-molecule anticancer drugs: mechanism of action and co-delivery nanocarriers. *Adv Drug Deliv Rev* 81:184
- Dawes E, Rushton N (1994) The effects of lactic acid on PGE2 production by macrophages and human synovial fibroblasts: a possible explanation for problems associated with the degradation of poly(lactide) implants? *Clin Mater* 17:157
- Deiters A (2010) Small molecule modifiers of the microRNA and RNA interference pathway. *AAPS J* 12:51
- Deng Y, Zhou H, Zou D, Xie Q, Bi X, Gu P, Fan X (2013a) The role of miR-31-modified adipose tissue-derived stem cells in repairing rat critical-sized calvarial defects. *Biomaterials* 34:6717
- Deng Y, Wu S, Zhou H, Bi X, Wang Y, Hu Y, Gu P, Fan X (2013b) Effects of a miR-31, Runx2, and Satb2 regulatory loop on the osteogenic differentiation of bone mesenchymal stem cells. *Stem Cells Dev* 22:2278
- 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
- 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
- des Rieux A, Ucakar B, Mupendwa BP, Colau D, Feron O, Carmeliet P, Preat V (2011) 3D systems delivering VEGF to promote angiogenesis for tissue engineering. *J Control Release* 150:272
- Desvignes T, Batzel P, Berezikov E, Eilbeck K, Eppig JT, McAndrews MS, Singer A, Postlethwait JH (2015) microRNA nomenclature: a view incorporating genetic origins, biosynthetic pathways, and sequence variants. *Trends Genet*: TIG 31:613
- Dey BK, Gagan J, Yan Z, Dutta A (2012) miR-26a is required for skeletal muscle differentiation and regeneration in mice. *Genes Dev* 26:2180
- Diao HJ, Low WC, Lu QR, Chew SY (2015a) Topographical effects on fiber-mediated microRNA delivery to control oligodendroglial precursor cells development. *Biomaterials* 70:105
- Diao HJ, Low WC, Milbreta U, Lu QR, Chew SY (2015b) Nanofiber-mediated microRNA delivery to enhance differentiation and maturation of oligodendroglial precursor cells. *J Control Release* 208:85
- Dimmeler S, Nicotera P (2013) MicroRNAs in age-related diseases. *EMBO Mol Med* 5:180
- Diomedea F, Merciaro I, Martinotti S, Cavalcanti MF, Caputi S, Mazzon E, Trubiani O (2016) miR-2861 is involved in osteogenic commitment of human periodontal ligament stem cells grown onto 3D scaffold. *J Biol Regul Homeost Agents* 30:1009
- Ebert MS, Sharp PA (2010) MicroRNA sponges: progress and possibilities. *RNA* 16:2043

- Ebert MS, Neilson JR, Sharp PA (2007) MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* 4:721
- Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR (2018) Gene therapy clinical trials worldwide to 2017: An update. *J Gene Med* 20:e3015
- Eguchi T, Watanabe K, Hara ES, Ono M, Kuboki T, Calderwood SK (2013) OsteMiR: a novel panel of microRNA biomarkers in osteoblastic and osteocytic differentiation from mesenchymal stem cells. *PLoS One* 8:e58796
- Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, and Tuschl T (2001a) Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J* 20:6877
- Elbashir SM, Lendeckel W, Tuschl T (2001b) RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev* 15:188
- Eskildsen T, Taipaleenmaki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M (2011) MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. *Proc Natl Acad Sci U S A* 108:6139
- Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M (2012) Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* 492:376
- Evans CH (2013) Advances in regenerative orthopedics. *Mayo Clin Proc* 88:1323
- Fabani MM, Gait MJ (2008) miR-122 targeting with LNA/2'-O-methyl oligonucleotide mixmers, peptide nucleic acids (PNA), and PNA-peptide conjugates. *RNA* 14:336
- Fang J, Zhu YY, Smiley E, Bonadio J, Rouleau JP, Goldstein SA, McCauley LK, Davidson BL, Roessler BJ (1996) Stimulation of new bone formation by direct transfer of osteogenic plasmid genes. *Proc Natl Acad Sci U S A* 93:5753
- Fang L, Du WW, Yang W, Rutnam ZJ, Peng C, Li H, O'Malley YQ, Askeland RW, Sugg S, Liu M, Mehta T, Deng Z, Yang BB (2012) MiR-93 enhances angiogenesis and metastasis by targeting LATS2. *Cell Cycle* 11:4352–4365
- Farrell RE (2006) RNA methodologies. *Rev Cell Biol Mol Med*. Wiley-VCH Verlag GmbH & Co. KGaA
- Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, Capogrossi MC, Martelli F (2008) MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. *J Biol Chem* 283:15878
- Feichtinger X, Muschitz C, Heimel P, Baierl A, Fahrleitner-Pammer A, Redl H, Resch H, Geiger E, Skalicky S, Dormann R, Plachel F, Pietschmann P, Grillari J, Hackl M, Kocjan R (2018) Bone-related circulating MicroRNAs miR-29b-3p, miR-550a-3p, and miR-324-3p and their association to bone microstructure and histomorphometry. *Sci Rep* 8:4867
- Feng B, Chen S, McArthur K, Wu Y, Sen S, Ding Q, Feldman RD, Chakrabarti S (2011) miR-146a-mediated extracellular matrix protein production in chronic diabetes complications. *Diabetes* 60:2975
- Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806
- Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, Ivey KN, Bruneau BG, Stainier DY, Srivastava D (2008) miR-126 regulates angiogenic signaling and vascular integrity. *Dev Cell* 15:272
- Fitzgerald KA, Malhotra M, Curtin CM, O'Brien FJ, O'Driscoll CM (2015) Life in 3D is never flat: 3D models to optimise drug delivery. *J Control Release* 215:39–54
- Fitzgerald KA, Guo J, Raftery RM, Castano IM, Curtin CM, Gooding M, Darcy R, O'Brien FJ, O'Driscoll CM (2016) Nanoparticle-mediated siRNA delivery assessed in a 3D co-culture model simulating prostate cancer bone metastasis. *Int J Pharm* 511:1058
- Fleige S, Walf V, Huch S, Prgomet C, Sehm J, Pfaffl MW (2006) Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. *Biotechnol Lett* 28:1601
- Gabler J, Ruetze M, Kynast KL, Grossner T, Diederichs S, Richter W (2015) Stage-specific miRs in chondrocyte maturation: differentiation-dependent and hypertrophy-related miR clusters and the miR-181 family. *Tissue Eng A* 21:2840

- Ghosh R, Singh LC, Shohet JM, Gunaratne PH (2012) A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells. *Biomaterials* 34:807
- Giger EV, Castagner B, Raikkonen J, Monkkonen J, Leroux JC (2013) siRNA transfection with calcium phosphate nanoparticles stabilized with PEGylated chelators. *Adv Healthc Mater* 2:134
- Git A, Dvinge H, Salmon-Divon M, Osborne M, Kutter C, Hadfield J, Bertone P, Caldas C (2010) Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. *RNA* 16:991
- Gordeladze JO, Reseland JE, Duroux-Richard I, Apparailly F, Jorgensen C (2010) From stem cells to bone: phenotype acquisition, stabilization, and tissue engineering in animal models. *ILAR J* 51:42
- Graham FL, van der Eb AJ (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456
- GrandPré T, Nakamura F, Vartanian T, Strittmatter SM (2000) Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature* 403:439
- Gray KA, Yates B, Seal RL, Wright MW, Bruford EA (2015) Genenames.org: the HGNC resources in 2015. *Nucleic Acids Res* 43:D1079
- Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ (2006) miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 34:D140
- Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP (2007) MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell* 27:91
- Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC (2001) Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. *Cell* 106:23
- Grundmann S, Hans FP, Kinniry S, Heinke J, Helbing T, Bluhm F, Sluijter JP, Hofer I, Pasterkamp G, Bode C, Moser M (2011) MicroRNA-100 regulates neovascularization by suppression of mammalian target of rapamycin in endothelial and vascular smooth muscle cells. *Circulation* 123:999
- Grunhagen J, Ott CE (2013) On microRNA-214 suppressing osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. *Bone* 57:325
- Guk K, Lim H, Kim B, Hong M, Khang G, Lee D (2013) Acid-cleavable ketal containing poly(beta-amino ester) for enhanced siRNA delivery. *Int J Pharm* 453:541
- Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 466:835
- Guo Z, Peng H, Kang J, Sun D (2016) Cell-penetrating peptides: possible transduction mechanisms and therapeutic applications. *Biomed Rep* 4:528
- Gupta P, Authimoolam SP, Hilt JZ, Dziubla TD (2015) Quercetin conjugated poly(beta-amino esters) nanogels for the treatment of cellular oxidative stress. *Acta Biomater* 27:194
- Hackl M, Jakobi T, Blom J, Doppmeier D, Brinkrolf K, Szczepanowski R, Bernhart SH, Honer Zu Siederdisen C, Bort JA, Wieser M, Kunert R, Jeffs S, Hofacker IL, Goesmann A, Puhler A, Borth N, Grillari J (2011) Next-generation sequencing of the Chinese hamster ovary microRNA transcriptome: identification, annotation and profiling of microRNAs as targets for cellular engineering. *J Biotechnol* 153:62
- Hackl M, Heilmeyer U, Weilner S, Grillari J (2016) Circulating microRNAs as novel biomarkers for bone diseases – complex signatures for multifactorial diseases? *Mol Cell Endocrinol*. 2015 The Authors, Ireland. Published by Elsevier Ireland Ltd. p 83
- Haensler J, Szoka FC Jr (1993) Synthesis and characterization of a trigalactosylated bisacridine compound to target DNA to hepatocytes. *Bioconjug Chem* 4:85
- Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286:950
- Hammond SM, Bernstein E, Beach D, Hannon GJ (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 404:293
- Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ (2001) Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* 293:1146
- Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ (2008) MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. *Proc Natl Acad Sci U S A* 105:1516

- Hassan MQ, Maeda Y, Taipaleenmaki H, Zhang W, Jafferji M, Gordon JA, Li Z, Croce CM, Van Wijnen AJ, Stein JL, Stein GS, Lian JB (2012) miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells. *J Biol Chem* 287:42084–42092
- Hatakeyama Y, Tuan RS, Shum L (2004) Distinct functions of BMP4 and GDF5 in the regulation of chondrogenesis. *J Cell Biochem* 91:1204
- Hattori T, Müller C, Gebhard S, Bauer E, Pausch F, Schlund B, Bösl MR, Hess A, Surmann-Schmitt C, von der Mark H, de Crombrughe B, von der Mark K (2010) SOX9 is a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. *Development* 137:901
- Haussecker D (2014) Current issues of RNAi therapeutics delivery and development. *J Control Release* 195:49
- Hedberg EL, Shih CK, Lemoine JJ, Timmer MD, Liebschner MA, Jansen JA, Mikos AG (2005) In vitro degradation of porous poly(propylene fumarate)/poly(DL-lactic-co-glycolic acid) composite scaffolds. *Biomaterials* 26:3215
- Hoseinzadeh S, Atashi A, Soleimani M, Alizadeh E, Zarghami N (2016) MiR-221-inhibited adipose tissue-derived mesenchymal stem cells bioengineered in a nano-hydroxy apatite scaffold. *In Vitro Cell Dev Biol Anim* 52:479
- Hu R, Li H, Liu W, Yang L, Tan Y, Luo X (2010) Targeting miRNAs in osteoblast differentiation and bone formation. *Expert Opin Ther Targets* 14:1109
- Huang J, Zhao L, Xing L, Chen D (2010) MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation. *Stem Cells* 28:357
- Huey DJ, Hu JC, Athanasiou KA (2012) Unlike bone, cartilage regeneration remains elusive. *Science (New York, NY)* 338:917
- Huh YH, Ryu J-H, Chun J-S (2007) Regulation of type II collagen expression by histone deacetylase in articular chondrocytes. *J Biol Chem* 282:17123
- Hutvagner G, Zamore PDA (2002) microRNA in a multiple-turnover RNAi enzyme complex. *Science* 297:2056
- Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 293:834
- Huynh CT, Nguyen MK, Naris M, Tonga GY, Rotello VM, Alsberg E (2016) Light-triggered RNA release and induction of hMSC osteogenesis via photodegradable, dual-crosslinked hydrogels. *Nanomedicine (Lond)* 11:1535
- Hwang DW, Son S, Jang J, Youn H, Lee S, Lee D, Lee YS, Jeong JM, Kim WJ, Lee DS (2011) A brain-targeted rabies virus glycoprotein-disulfide linked PEI nanocarrier for delivery of neurogenic microRNA. *Biomaterials* 32:4968
- Icli B, Wara AK, Moslehi J, Sun X, Plovie E, Cahill M, Marchini JF, Schissler A, Padera RF, Shi J, Cheng HW, Raghuram S, Arany Z, Liao R, Croce K, MacRae C, Feinberg MW (2013) MicroRNA-26a regulates pathological and physiological angiogenesis by targeting BMP/SMAD1 signaling. *Circ Res* 113:1231
- Iilina P, Hyvonen Z, Saura M, Sandvig K, Yliperttula M, Ruponen M (2012) Genetic blockage of endocytic pathways reveals differences in the intracellular processing of non-viral gene delivery systems. *J Control Release* 163:385
- Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A (2008) Integrative MicroRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One* 3:e3740
- ISBLab (2013) miRTarBase: the experimentally validated microRNA-target interactions database. In: National Chiao Tung University (ed) Department of Biological Science and Technology I.o. B.a.S.B., miRTarBase Release 45. Taiwan © ISBLab, Hsinchu
- Ishizuka A, Siomi MC, Siomi HA (2002) Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev* 16:2497

- Itoh T, Nozawa Y, Akao Y (2009) MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5. *J Biol Chem* 284:19272
- Jacobsen A, Silber J, Harinath G, Huse JT, Schultz N, Sander C (2013) Analysis of microRNA-target interactions across diverse cancer types. *Nat Struct Mol Biol* 20:1325
- Jaklenec A, Stamp A, Deweerd E, Sherwin A, Langer R (2012) Progress in the tissue engineering and stem cell industry "are we there yet?". *Tissue Eng B-Rev* 18:155
- James EN, Delany AM, Nair LS (2014) Post-transcriptional regulation in osteoblasts using localized delivery of miR-29a inhibitor from nanofibers to enhance extracellular matrix deposition. *Acta Biomater* 10:3571
- Janssen HL, Kauppinen S, Hodges MR (2013) HCV infection and miravirsin. *N Engl J Med* 369:878
- Jee YH, Wang J, Yue S, Jennings M, Clokie SJ, Nilsson O, Lui JC, Baron J (2018) mir-374-5p, mir-379-5p, and mir-503-5p regulate proliferation and hypertrophic differentiation of growth plate chondrocytes in male rats. *Endocrinology* 159:1469
- Jensen ED, Gopalakrishnan R, Westendorf JJ (2010) Regulation of gene expression in osteoblasts. *Biofactors* 36:25
- Jere D, Xu CX, Arote R, Yun CH, Cho MH, Cho CS (2008) Poly(beta-amino ester) as a carrier for si/shRNA delivery in lung cancer cells. *Biomaterials* 29:2535
- Johnson EE, Urist MR (1998) One-stage lengthening of femoral nonunion augmented with human bone morphogenetic protein. *Clin Orthop Relat Res*, 347:105
- Johnston RJ, Hobert O (2003) A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans*. *Nature* 426:845
- Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU (1998) In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* 238:265
- Jones E, Mazirka P, McNurlan MA, Brink P, Caso G (2017) Inhibition of human prostate cancer growth by mesenchymal stem cells delivering MiR-16. *FASEB J* 31:lb177
- Kapinas K, Kessler CB, Delany AM (2009) miR-29 suppression of osteonectin in osteoblasts: regulation during differentiation and by canonical Wnt signaling. *J Cell Biochem* 108:216
- Karlsen TA, Jakobsen RB, Mikkelsen TS, Brinchmann JE (2013) microRNA-140 targets RALA and regulates chondrogenic differentiation of human mesenchymal stem cells by translational enhancement of SOX9 and ACAN. *Stem Cells Dev* 23:290
- Karlsen TA, de Souza GA, Ødegaard B, Engebretsen L, Brinchmann JE (2016) microRNA-140 inhibits inflammation and stimulates chondrogenesis in a model of interleukin 1 β -induced osteoarthritis. *Mol Ther Nucleic Acids* 5:e373
- Ketting RF, Haverkamp TH, van Luenen HG, Plasterk RH (1999) Mut-7 of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD. *Cell* 99:133
- Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH (2001) Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. *Genes Dev* 15:2654
- Khademhosseini A, Vacanti JP, Langer R (2009) Progress in tissue engineering. *Sci Am* 300:64
- Khvorova A, Reynolds A, Jayasena SD (2003) Functional siRNAs and miRNAs exhibit strand bias. *Cell* 115:209
- Kim KM, Lim S-K (2014) Role of miRNAs in bone and their potential as therapeutic targets. *Curr Opin Pharmacol* 16:133
- Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS (2009) miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. *J Bone Miner Res Off J Am Soc Bone Miner Res* 24:816
- Kim NH, Choi SH, Kim CH, Lee CH, Lee TR, Lee AY (2014) Reduced MiR-675 in exosome in H19 RNA-related melanogenesis via MITF as a direct target. *J Invest Dermatol* 134:1075

- Kimelman-Bleich N, Pelled G, Zilberman Y, Kallai I, Mizrahi O, Tawackoli W, Gazit Z, Gazit D (2011) Targeted gene-and-host progenitor cell therapy for nonunion bone fracture repair. *Mol Ther: J Am Soc Gene Ther* 19:53
- Knight SW, Bass BL (2001) A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. *Science* 293:2269
- Kozarewa I, Ning Z, Quail MA, Sanders MJ, Berriman M, Turner DJ (2009) Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of (G+C)-biased genomes. *Nat Methods* 6:291
- Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M (2005) Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 438:685
- Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. *Science* 294:853
- Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294:858
- Laufs S, Guenechea G, Gonzalez-Murillo A, Zsuzsanna Nagy K, Luz Lozano M, del Val C, Jonnakuty S, Hotz-Wagenblatt A, Jens Zeller W, Bueren JA, Fruehauf S (2006) Lentiviral vector integration sites in human NOD/SCID repopulating cells. *J Gene Med* 8:1197
- Leblebici B, Adam M, Bagis S, Tarim AM, Noyan T, Akman MN, Haberal MA (2006) Quality of life after burn injury: the impact of joint contracture. *J Burn Care Res Engl* 27:864
- Lee RC, Ambros V (2001) An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* 294:862
- Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75:843
- Lee Y, Jeon K, Lee JT, Kim S, Kim VN (2002) MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J* 21:4663
- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN (2003) The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425:415
- Lee K, Silva EA, Mooney DJ (2011) Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface* 8:153
- Lee K, Oh MH, Lee MS, Nam YS, Park TG, Jeong JH (2013) Stabilized calcium phosphate nano-aggregates using a dopa-chitosan conjugate for gene delivery. *Int J Pharm* 445:196
- Lee MS, Lee JE, Byun E, Kim NW, Lee K, Lee H, Sim SJ, Lee DS, Jeong JH (2014) Target-specific delivery of siRNA by stabilized calcium phosphate nanoparticles using dopa-hyaluronic acid conjugate. *J Control Release* 192:122–130
- Lefebvre V, Dumitriu B, Penzo-Méndez A, Han Y, Pallavi B (2007) Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. *Int J Biochem Cell Biol* 39:2195
- Legendre F, Ollitrault D, Gomez-Leduc T, Bouyoucef M, Hervieu M, Gruchy N, Mallein-Gerin F, Leclercq S, Demoor M, Galéra P (2017) Enhanced chondrogenesis of bone marrow-derived stem cells by using a combinatory cell therapy strategy with BMP-2/TGF- β 1, hypoxia, and COL1A1/HtrA1 siRNAs. *Sci Rep* 7:3406
- Lena AM, Shalom-Feuerstein R, Rivetti di Val Cervo P, Aberdam D, Knight RA, Melino G, Candi E (2008) miR-203 represses 'stemness' by repressing DeltaNp63. *Cell Death Differ* 15:1187
- Lepper C, Partridge TA, Fan CM (2011) An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development* 138:3639
- Levy O, Ruvinov E, Reem T, Granot Y, Cohen S (2010) Highly efficient osteogenic differentiation of human mesenchymal stem cells by eradication of STAT3 signaling. *Int J Biochem Cell Biol* 42:1823
- 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
- Li H, Li WX, Ding SW (2002) Induction and suppression of RNA silencing by an animal virus. *Science* 296:1319

- Li Z, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, Lian JB, Stein GS (2008) A microRNA signature for a BMP2-induced osteoblast lineage commitment program. *Proc Natl Acad Sci U S A* 105:13906
- Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, van Wijnen AJ, Stein JL, Stein GS, Lian JB (2009a) Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J Biol Chem* 284:15676
- Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, Luo XH (2009b) A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest* 119:3666
- Li J, Chen YC, Tseng YC, Huang L (2010) Biodegradable calcium phosphate nanoparticle with lipid coating for systemic siRNA delivery. *J Control Release* 142:416
- Li Y, Fan L, Liu S, Liu W, Zhang H, Zhou T, Wu D, Yang P, Shen L, Chen J, Jin Y (2013) The promotion of bone regeneration through positive regulation of angiogenic-osteogenic coupling using microRNA-26a. *Biomaterials* 34:5048
- Li J, Yu X, Wang Y, Yuan Y, Xiao H, Cheng D, Shuai X (2014) A reduction and pH dual-sensitive polymeric vector for long-circulating and tumor-targeted siRNA delivery. *Adv Mater* 26:8217
- Li KC, Lo SC, Sung LY, Liao YH, Chang YH, Hu YC (2016) Improved calvarial bone repair by hASCs engineered with Cre/loxP-based baculovirus conferring prolonged BMP-2 and MiR-148b co-expression. *J Tissue Eng Regen Med* 11:3068
- Li KC, Chang YH, Yeh CL, Hu YC (2016a) Healing of osteoporotic bone defects by baculovirus-engineered bone marrow-derived MSCs expressing MicroRNA sponges. *Biomaterials* 74:155
- Li Y, Dal-Pra S, Mirotso M, Jayawardena TM, Hodgkinson CP, Bursac N, Dzau VJ (2016b) Tissue-engineered 3-dimensional (3D) microenvironment enhances the direct reprogramming of fibroblasts into cardiomyocytes by microRNAs. *Sci Rep* 6:38815
- Liao XB, Zhang ZY, Yuan K, Liu Y, Feng X, Cui RR, Hu YR, Yuan ZS, Gu L, Li SJ, Mao DA, Lu Q, Zhou XM, de Jesus Perez VA, Yuan LQ (2013a) MiR-133a modulates osteogenic differentiation of vascular smooth muscle cells. *Endocrinology* 154:3344
- Liao L, Yang X, Su X, Hu C, Zhu X, Yang N, Chen X, Shi S, Shi S, Jin Y (2013b) Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. *Cell Death Dis* 4:e600
- Liao Y-H, Chang Y-H, Sung L-Y, Li K-C, Yeh C-L, Yen T-C, Hwang S-M, Lin K-J, Hua Y-C (2014a) Osteogenic differentiation of adipose-derived stem cells and calvarial defect repair using baculovirus-mediated co-expression of BMP-2 and miR-148b. *Biomaterials* 35:4901
- Liao YH, Chang YH, Sung LY, Li KC, Yeh CL, Yen TC, Hwang SM, Lin KJ, Hu YC (2014b) Osteogenic differentiation of adipose-derived stem cells and calvarial defect repair using baculovirus-mediated co-expression of BMP-2 and miR-148b. *Biomaterials* 35:4901
- Lim LP, Lau NC, Weinstein EG, Abdelhakim A, Yekta S, Rhoades MW, Burge CB, Bartel DP (2003) The microRNAs of *Caenorhabditis elegans*. *Genes Dev* 17:991
- Lin RC, Weeks KL, Gao XM, Williams RB, Bernardo BC, Kiriazis H, Matthews VB, Woodcock EA, Bouwman RD, Mollica JP, Speirs HJ, Dawes IW, Daly RJ, Shioi T, Izumo S, Febbraio MA, Du XJ, McMullen JR (2010) PI3K (p110 alpha) protects against myocardial infarction-induced heart failure: identification of PI3K-regulated miRNA and mRNA. *Arterioscler Thromb Vasc Biol* 30:724
- Lingel A, Simon B, Izaurralde E, Sattler M (2003) Structure and nucleic-acid binding of the Drosophila Argonaute 2 PAZ domain. *Nature* 426:465
- Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M, Lim L (2007) Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. *Mol Cell Biol* 27:2240
- Liu YP, Berkhout B (1809) miRNA cassettes in viral vectors: problems and solutions. *Biochim Biophys Acta (BBA) – Gene Regul Mech* 732:2011
- Liu C-F, Lefebvre V (2015) The transcription factors SOX9 and SOX5/SOX6 cooperate genome-wide through super-enhancers to drive chondrogenesis. *Nucleic Acids Res* 43:8183

- Liu L, Shu S, Cheung GS, Wei X (2016) Effect of miR-146a/bFGF/PEG-PEI nanoparticles on inflammation response and tissue regeneration of human dental pulp cells. *Biomed Res Int* 2016:3892685
- Lolli A, Lambertini E, Penolazzi L, Angelozzi M, Morganti C, Franceschetti T, Pelucchi S, Gambari R, Piva R (2014) Pro-chondrogenic effect of miR-221 and slug depletion in human MSCs. *Stem Cell Rev* 10:841
- Lolli A, Narcisi R, Lambertini E, Penolazzi L, Angelozzi M, Kops N, Gasparini S, van Osch GJ, Piva R (2016) Silencing of Antichondrogenic MicroRNA-221 in human mesenchymal stem cells promotes cartilage repair in vivo. *Stem Cells* 34:1801
- Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U (2004) Nuclear export of microRNA precursors. *Science* 303:95
- Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML (2008) Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. *J Bone Miner Res Off J Am Soc Bone Miner Res* 23:287
- Lytle JR, Yario TA, Steitz JA (2007) Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5' UTR as in the 3' UTR. *Proc Natl Acad Sci* 104:9667
- Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, Teruya-Feldstein J, Reinhardt F, Onder TT, Valastyan S, Westermann F, Speleman F, Vandesompele J, Weinberg RA (2010) miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* 12:247
- MacLaughlin FC, Mumper RJ, Wang J, Tagliaferri JM, Gill I, Hinchcliffe M, Rolland AP (1998) Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. *J Control Release* 56:259
- Madhyastha R, Madhyastha H, Nakajima Y, Omura S, Maruyama M (2012) MicroRNA signature in diabetic wound healing: promotive role of miR-21 in fibroblast migration. *Int Wound J* 9:355
- Mahboudi H, Soleimani M, Enderami SE, Kehtari M, Ardeshtyrlajimi A, Eftekhary M, Kazemi B (2018) Enhanced chondrogenesis differentiation of human induced pluripotent stem cells by MicroRNA-140 and transforming growth factor beta 3 (TGFβ3). *Biologicals* 52:30–36
- Makeyev EV, Zhang J, Carrasco MA, Maniatis T (2007) The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol Cell* 27:435
- Malmö J, Sorgard H, Varum KM, Strand SP (2012) siRNA delivery with chitosan nanoparticles: molecular properties favoring efficient gene silencing. *J Control Release* 158:261
- Mariner PD, Johannesen E, Anseth KS (2011) Manipulation of miRNA activity accelerates osteogenic differentiation of hMSCs in engineered 3D scaffolds. *J Tissue Eng Regen Med* 6:314
- Mariner PD, Johannesen E, Anseth KS (2012) Manipulation of miRNA activity accelerates osteogenic differentiation of hMSCs in engineered 3D scaffolds. *J Tissue Eng Regen Med* 6:314
- Martinez J, Patkaniowska A, Urlaub H, Luhrmann R, and Tuschl T (2002) Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell* 110:563
- Martini FH, Nath JL, Bartholomew EF (2006) *Fundamentals of anatomy and physiology*, 9th edn. P.E.I. Editorial, San Francisco
- McArthur K, Feng B, Wu Y, Chen S, Chakrabarti S (2011) MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. *Diabetes* 60:1314
- McCarthy JJ (2008) MicroRNA-206: the skeletal muscle-specific myomiR. *Biochim Biophys Acta* 1779:682
- McKerracher L, David S, Jackson DL, Kottis V, Dunn RJ, Braun PE (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron* 13:805
- McKiernan P, Cunningham O, Greene C, Cryan S-A (2013) Targeting miRNA-based medicines to cystic fibrosis airway epithelial cells using nanotechnology. *Int J Nanomedicine* 8:3907
- Mencia Castano I, Curtin CM, Shaw G, Murphy JM, Duffy GP, O'Brien FJ (2015) A novel collagen-nanohydroxyapatite microRNA-activated scaffold for tissue engineering applications

- capable of efficient delivery of both miR-mimics and antagomiRs to human mesenchymal stem cells. *J Control Release* 200:42
- Mencia Castano I, Curtin CM, Duffy GP, O'Brien FJ (2016) Next generation bone tissue engineering: non-viral miR-133a inhibition using collagen-nanohydroxyapatite scaffolds rapidly enhances osteogenesis. *Sci Rep* 6:27941
- Mencia Castano I, Curtin CM, Duffy GP, O'Brien FJ (2018) Harnessing a novel role of miR-16 in osteogenesis for scaffold-based bone tissue engineering. *Tissue Eng A* 25:24
- Mercado AT, Yeh JM, Chin TY, Chen WS, Chen-Yang YW, Chen CY (2016) The effect of chemically modified electrospun silica nanofiber on the mRNA and miRNA expression profile of neural stem cell differentiation. *J Biomed Mater Res A* 104:2730
- Mercken EM, Majounie E, Ding J, Guo R, Kim J, Bernier M, Mattison J, Cookson MR, Gorospe M, de Cabo R, Abdelmohsen K (2013) Age-associated miRNA alterations in skeletal muscle from rhesus monkeys reversed by caloric restriction. *Aging (Albany NY)* 5:692
- Midgley AC, Bowen T, Phillips AO, Steadman R (2014) MicroRNA-7 inhibition rescues age-associated loss of epidermal growth factor receptor and hyaluronan-dependent differentiation in fibroblasts. *Aging Cell* 13:235
- Midoux P, Pichon C, Yaouanc JJ, Jaffres PA (2009) Chemical vectors for gene delivery: a current review on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carrier. *Br J Pharmacol* 157:166
- Miller KJ, Brown DA, Ibrahim MM, Ramchal TD, Levinson H (2015) MicroRNAs in skin tissue engineering. *Adv Drug Deliv Rev* 88:16
- miRBase (2013) ed: Faculty of Life Sciences University of Manchester
- Mizuno Y, Yagi K, Tokuzawa Y, Kanesaki-Yatsuka Y, Suda T, Katagiri T, Fukuda T, Maruyama M, Okuda A, Amemiya T, Kondoh Y, Tashiro H, Okazaki Y (2008) miR-125b inhibits osteoblastic differentiation by down-regulation of cell proliferation. *Biochem Biophys Res Commun* 368:267
- Mizuno Y, Tokuzawa Y, Ninomiya Y, Yagi K, Yatsuka-Kanesaki Y, Suda T, Fukuda T, Katagiri T, Kondoh Y, Amemiya T, Tashiro H, Okazaki Y (2009) miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b. *FEBS Lett* 583:2263
- Monaghan M, Pandit A (2011) RNA interference therapy via functionalized scaffolds. *Adv Drug Deliv Rev* 63:197
- Monaghan M, Browne S, Schenke-Layland K, Pandit A (2014) A collagen-based scaffold delivering exogenous microrna-29B to modulate extracellular matrix remodeling. *Mol Ther: J Am Soc Gene Ther* 22:786
- Monaghan MG, Holeiter M, Brauchle E, Layland SL, Lu Y, Deb A, Pandit A, Nsair A, Schenke-Layland K (2018) Exogenous miR-29B delivery through a hyaluronan-based injectable system yields functional maintenance of the infarcted myocardium. *Tissue Eng Part A* 24:57
- Montini E, Cesana D, Schmidt M, Sanvito F, Bartholomae CC, Ranzani M, Benedicenti F, Sergi LS, Ambrosi A, Ponzoni M, Doglioni C, Di Serio C, von Kalle C, Naldini L (2009) The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. *J Clin Invest* 119:964
- Mourelatos Z, Dostie J, Paushkin S, Sharma A, Charroux B, Abel L, Rappsilber J, Mann M, Dreyfuss G (2002) miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. *Genes Dev* 16:720
- Mourrain P, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Jouette D, Lacombe AM, Nikic S, Picault N, Remoue K, Sanial M, Vo TA, Vaucheret H (2000) Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* 101:533
- Mowa MB, Crowther C, Arbuthnot P (2010) Therapeutic potential of adenoviral vectors for delivery of expressed RNAi activators. *Expert Opin Drug Deliv* 7:1373
- Nakasa T, Ishikawa M, Shi M, Shibuya H, Adachi N, Ochi M (2010) Acceleration of muscle regeneration by local injection of muscle-specific microRNAs in rat skeletal muscle injury model. *J Cell Mol Med* 14:2495

- Nelson CE, Kim AJ, Adolph EJ, Gupta MK, Yu F, Hocking KM, Davidson JM, Guelcher SA, Duvall CL (2014) Tunable delivery of siRNA from a biodegradable scaffold to promote angiogenesis in vivo. *Adv Mater* 26:607
- Nerem RM (1991) Cellular engineering. *Ann Biomed Eng* 19:529
- Nguyen MK, Jeon O, Krebs MD, Schapira D, Alsborg E (2014) Sustained localized presentation of RNA interfering molecules from in situ forming hydrogels to guide stem cell osteogenic differentiation. *Biomaterials* 35:6278
- Nguyen LH, Diao HJ, Chew SY (2015) MicroRNAs and their potential therapeutic applications in neural tissue engineering. *Adv Drug Deliv Rev* 88:53
- Nguyen LH, Gao M, Lin J, Wu W, Wang J, Chew SY (2017) Three-dimensional aligned nanofibers-hydrogel scaffold for controlled non-viral drug/gene delivery to direct axon regeneration in spinal cord injury treatment. *Sci Rep* 7:42212
- Niemeyer P, Pestka JM, Kreuz PC, Erggelet C, Schmal H, Suedkamp NP, Steinwachs M (2008) Characteristic complications after autologous chondrocyte implantation for cartilage defects of the knee joint. *Am J Sports Med* 36:2091
- Nunes DN, Dias-Neto E, Cardo-Vila M, Edwards JK, Dobroff AS, Giordano RJ, Mandelin J, Brentani HP, Hasselgren C, Yao VJ, Marchio S, Pereira CA, Passetti F, Calin GA, Sidman RL, Arap W, Pasqualini R (2015) Synchronous down-modulation of miR-17 family members is an early causative event in the retinal angiogenic switch. *Proc Natl Acad Sci U S A* 112:3770
- Nykanen A, Haley B, Zamore PD (2001) ATP requirements and small interfering RNA structure in the RNA interference pathway. *Cell* 107:309
- Obermosterer G, Martinez J, Alenius M (2007) Locked nucleic acid-based in situ detection of microRNAs in mouse tissue sections. *Nat Protoc* 2:1508
- O'Brien FJ, Harley BA, Waller MA, Yannas IV, Gibson LJ, Prendergast PJ (2007) The effect of pore size on permeability and cell attachment in collagen scaffolds for tissue engineering. *Technol Health Care* 15:3
- Ohler U, Yekta S, Lim LP, Bartel DP, Burge CB (2004) Patterns of flanking sequence conservation and a characteristic upstream motif for microRNA gene identification. *RNA* 10:1309
- Okamura K, Liu N, Lai EC (2009) Distinct mechanisms for microRNA strand selection by *Drosophila* Argonautes. *Mol Cell* 36:431
- Ørom UA, Lund AH (2010) Experimental identification of microRNA targets. *Gene* 451:1
- Ozsolak F, Milos PM (2011) RNA sequencing: advances, challenges and opportunities. *Nat Rev Genet* 12:87
- Pack DW, Hoffman AS, Pun S, Stayton PS (2005) Design and development of polymers for gene delivery. *Nat Rev Drug Discov* 4:581
- Pan Y, Zhang Y, Jia T, Zhang K, Li J, Wang L (2012a) Development of a microRNA delivery system based on bacteriophage MS2 virus-like particles. *FEBS J* 279:1198
- Pan Y, Jia T, Zhang Y, Zhang K, Zhang R, Li J, Wang L (2012b) MS2 VLP-based delivery of microRNA-146a inhibits autoantibody production in lupus-prone mice. *Int J Nanomedicine* 7:5957
- Park K, Lee K-M, Yoon D, Park K, Choi W, Lee J, Kim S-H (2016) Inhibition of microRNA-449a prevents IL-1 β -induced cartilage destruction via SIRT1. *Osteoarthr Cartil* 24:2153
- Pascual-Gil S, Garbayo E, Díaz-Herráez P, Prosper F, Blanco-Prieto MJ (2015) Heart regeneration after myocardial infarction using synthetic biomaterials. *J Control Release* 203:23
- Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 408:86
- Pastar I, Khan AA, Stojadinovic O, Lebrun EA, Medina MC, Brem H, Kirsner RS, Jimenez JJ, Leslie C, Tomic-Canic M (2012) Induction of specific microRNAs inhibits cutaneous wound healing. *J Biol Chem* 287:29324
- Pearson H (2006) Genetics: what is a gene? *Nature* 441:398

- Pedraza CE, Bassett DC, McKee MD, Nelea V, Gbureck U, Barralet JE (2008) The importance of particle size and DNA condensation salt for calcium phosphate nanoparticle transfection. *Biomaterials* 29:3384
- Pekarsky Y, Balatti V, Croce CM (2018) BCL2 and miR-15/16: from gene discovery to treatment. *Cell Death Differ* 25:21
- Pelled G, Ben-Arav A, Hock C, Reynolds DG, Yazici C, Zilberman Y, Gazit Z, Awad H, Gazit D, Schwarz EM (2010) Direct gene therapy for bone regeneration: gene delivery, animal models, and outcome measures. *Tissue Eng Part B Rev* 16:13
- Pena JT, Sohn-Lee C, Rouhanifard SH, Ludwig J, Hafner M, Mihailovic A, Lim C, Holloch D, Berninger P, Zavolan M, Tuschl T (2009) miRNA in situ hybridization in formaldehyde and EDC-fixed tissues. *Nat Methods* 6:139
- Peng WJ, Tao JH, Mei B, Chen B, Li BZ, Yang GJ, Zhang Q, Yao H, Wang BX, He Q, Wang J (2012) MicroRNA-29: a potential therapeutic target for systemic sclerosis. *Expert Opin Ther Targets* 16:875
- Perruisseau-Carrier C, Jurga M, Forraz N, McGuckin CP (2011) miRNAs stem cell reprogramming for neuronal induction and differentiation. *Mol Neurobiol* 43:215
- Petersen M, Wengel J (2003) LNA: a versatile tool for therapeutics and genomics. *Trends Biotechnol* 21:74
- Poliseno L, Tuccoli A, Mariani L, Evangelista M, Citti L, Woods K, Mercatanti A, Hammond S, Rainaldi G (2006) MicroRNAs modulate the angiogenic properties of HUVECs. *Blood* 108:3068
- Porrello ER, Johnson BA, Aurora AB, Simpson E, Nam YJ, Matkovich SJ, Dorn GW, van Rooij E, Olson EN (2011) MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes. *Circ Res* 109:670
- Qian X, Long L, Shi Z, Liu C, Qiu M, Sheng J, Pu P, Yuan X, Ren Y, Kang C (2014) Star-branched amphiphilic PLA-b-PDMAEMA copolymers for co-delivery of miR-21 inhibitor and doxorubicin to treat glioma. *Biomaterials* 35:2322
- Qureshi AT, Monroe WT, Dasa V, Gimble JM, Hayes DJ (2013) miR-148b eNanoparticle conjugates for light mediated osteogenesis of human adipose stromal/stem cells. *Biomaterials* 34:7799
- Qureshi AT, Doyle A, Chen C, Coulon D, Dasa V, Del Piero F, Levi B, Monroe WT, Gimble JM, Hayes DJ (2015) Photoactivated miR-148b-nanoparticle conjugates improve closure of critical size mouse calvarial defects. *Acta Biomater* 12:166
- Raftery RM, Walsh DP, Mencía Castaño I, Heise A, Duffy GP, Cryan S-A, O'Brien FJ (2016) Delivering nucleic-acid based nanomedicines on biomaterial scaffolds for orthopedic tissue repair: challenges, progress and future perspectives. *Adv Mater* n/a
- Reddy KJ, Gilman M (2001) Preparation of bacterial RNA. *Curr Protoc Mol Biol*. Wiley
- Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 403:901
- Remaut K, Symens N, Lucas B, Demeester J, De Smedt SC (2010) Efficient delivery of intact phosphodiester oligonucleotides by poly-beta-amino esters. *J Control Release* 144:65
- Rinn JL, Chang HY (2012) Genome regulation by long noncoding RNAs. *Annu Rev Biochem* 81:145
- Sakurai H, Kawabata K, Sakurai F, Nakagawa S, Mizuguchi H (2008) Innate immune response induced by gene delivery vectors. *Int J Pharm* 354:9
- Santos JL, Pandita D, Rodrigues J, Pego AP, Granja PL, Tomas H (2011) Non-viral gene delivery to mesenchymal stem cells: methods, strategies and application in bone tissue engineering and regeneration. *Curr Gene Ther* 11:46
- Santos-Carballal B, Aaldering LJ, Ritzeveld M, Pereira S, Sewald N, Moerschbacher BM, Gotte M, Goycoolea FM (2015) Physicochemical and biological characterization of chitosan-microRNA nanocomplexes for gene delivery to MCF-7 breast cancer cells. *Sci Rep* 5:13567

- Sathy BN, Olivera D, Gonzalez-Fernandez T, Cunniffe GM, Pentlavalli S, Chambers P, Jeon O, Alsberg E, McCarthy HO, Dunne N, Haut Donahue TL, Kelly DJ (2017) RALA complexed α -TCP nanoparticle delivery to mesenchymal stem cells induces bone formation in tissue engineered constructs in vitro and in vivo. *J Mater Chem B* 5:1753
- Schade A, Delyagina E, Scharfenberg D, Skorska A, Lux C, David R, Steinhoff G (2013) Innovative strategy for microRNA delivery in human mesenchymal stem cells via magnetic nanoparticles. *Int J Mol Sci* 14:10710
- Schade A, Muller P, Delyagina E, Voronina N, Skorska A, Lux C, Steinhoff G, David R (2014) Magnetic nanoparticle based nonviral microRNA delivery into freshly isolated CD105(+) hMSCs. *Stem Cells Int* 2014:197154
- Schirle NT, Sheu-Gruttadauria J, MacRae IJ (2014) Structural basis for microRNA targeting. *Science* 346:608
- Schmidt CE, Leach JB (2003) Neural tissue engineering: strategies for repair and regeneration. *Annu Rev Biomed Eng* 5:293
- Schnabel M, Marlovits S, Eckhoff G, Fichtel I, Gotzen L, Vecsei V, Schlegel J (2002) Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. *Osteoarthr Cartil* 10:62
- Schneider MR, Samborski A, Bauersachs S, Zouboulis CC (2013) Differentially regulated microRNAs during human sebaceous lipogenesis. *J Dermatol Sci* 70:88
- Schoolmeesters A, Eklund T, Leake D, Vermeulen A, Smith Q, Force Aldred S, Fedorov Y (2009) Functional profiling reveals critical role for miRNA in differentiation of human mesenchymal stem cells. *PLoS One* 4:e5605
- Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD (2003) Asymmetry in the assembly of the RNAi enzyme complex. *Cell* 115:199
- Shevchenko RV, James SL, James SE (2010) A review of tissue-engineered skin bioconstructs available for skin reconstruction. *J R Soc Interface* 7:229
- Shi S, Han L, Deng L, Zhang Y, Shen H, Gong T, Zhang Z, Sun X (2014) Dual drugs (microRNA-34a and paclitaxel)-loaded functional solid lipid nanoparticles for synergistic cancer cell suppression. *J Control Release* 194:228
- Shoji T, Nakasa T, Yamasaki K, Kodama A, Miyaki S, Niimoto T, Okuhara A, Kamei N, Adachi N, Ochi M (2012) The effect of intra-articular injection of MicroRNA-210 on ligament healing in a rat model. *Am J Sports Med* 40:2470
- Silahtaroglu AN, Nolting D, Dyrskjøt L, Berezikov E, Møller M, Tommerup N, Kauppinen S (2007) Detection of microRNAs in frozen tissue sections by fluorescence in situ hybridization using locked nucleic acid probes and tyramide signal amplification. *Nat Protoc* 2:2520
- Sinha M, Ghatak S, Roy S, Sen CK (2015) microRNA-200b as a switch for inducible adult angiogenesis. *Antioxid Redox Signal* 22:1257
- Slack FJ, Basson M, Liu Z, Ambros V, Horvitz HR, Ruvkun G (2000) The lin-41 RBCC gene acts in the *C. elegans* heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. *Mol Cell* 5:659
- Sluijter JP, van Mil A, van Vliet P, Metz CH, Liu J, Doevendans PA, Goumans MJ (2010) MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler Thromb Vasc Biol* 30:859
- Sriram M, Sainitya R, Kalyanaraman V, Dhivya S, Selvamurugan N (2015) Biomaterials mediated microRNA delivery for bone tissue engineering. *Int J Biol Macromol* 74:404
- Stappert L, Borghese L, Roese-Koerner B, Weinhold S, Koch P, Terstegge S, Uhrberg M, Wernet P, Brustle O (2013) MicroRNA-based promotion of human neuronal differentiation and subtype specification. *PLoS One* 8:e59011
- Stappert L, Roese-Koerner B, Brustle O (2015) The role of microRNAs in human neural stem cells, neuronal differentiation and subtype specification. *Cell Tissue Res* 359:47
- Stenberg J, de Windt TS, Synnergren J, Hynsjö L, van der Lee J, Saris DB, Brittberg M, Peterson L, Lindahl A (2014) Clinical outcome 3 years after autologous chondrocyte implantation does not correlate with the expression of a predefined gene marker set in chondrocytes prior to

- implantation but is associated with critical signaling pathways. *Orthop J Sports Med* 2:2325967114550781
- Stevanato L, Sinden JD (2014) The effects of microRNAs on human neural stem cell differentiation in two- and three-dimensional cultures. *Stem Cell Res Ther* 5:49
- Stevens MM, George JH (2005) Exploring and engineering the cell surface interface. *Science* 310:1135
- Suarez Y, Sessa WC (2009) MicroRNAs as novel regulators of angiogenesis. *Circ Res* 104:442
- Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC (2007) Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ Res* 100:1164
- Suh EJ, Remillard MY, Legesse-Miller A, Johnson EL, Lemons JM, Chapman TR, Forman JJ, Kojima M, Silberman ES, Collier HA (2012) A microRNA network regulates proliferative timing and extracellular matrix synthesis during cellular quiescence in fibroblasts. *Genome Biol* 13:R121
- Sun Q, Zhang Y, Yang G, Chen X, Cao G, Wang J, Sun Y, Zhang P, Fan M, Shao N, Yang X (2008) Transforming growth factor-beta-regulated miR-24 promotes skeletal muscle differentiation. *Nucleic Acids Res* 36:2690
- Sun Q, Mao S, Li H, Zen K, Zhang CY, Li L (2013) Role of miR-17 family in the negative feedback loop of bone morphogenetic protein signaling in neuron. *PLoS One* 8:e83067
- Sundaram GM, Common JE, Gopal FE, Srikanta S, Lakshman K, Lunny DP, Lim TC, Tanavde V, Lane EB, Sampath P (2013) 'See-saw' expression of microRNA-198 and FSTL1 from a single transcript in wound healing. *Nature* 495:103
- Sung LY, Chen CL, Lin SY, Hwang SM, Lu CH, Li KC, Lan AS, Hu YC (2013) Enhanced and prolonged baculovirus-mediated expression by incorporating recombinase system and in cis elements: a comparative study. *Nucleic Acids Res* 41:e139
- Svaren J (2014) MicroRNA and transcriptional crosstalk in myelinating glia. *Neurochem Int* 77:50
- Takeda K (2009) Delivery of magic bullets: on the still rocky road to gene therapy. *Br J Pharmacol* 157:151
- Tan J, Yang L, Liu C, Yan Z (2017) MicroRNA-26a targets MAPK6 to inhibit smooth muscle cell proliferation and vein graft neointimal hyperplasia. *Sci Rep* 7:46602
- TargetScan Release 6.2 (2012) In: Computing B.a. R., ed. © 2006–2012 Whitehead Institute for Biomedical Research, Cambridge, MA
- Tay YM, Tam WL, Ang YS, Gauthwin PM, Yang H, Wang W, Liu R, George J, Ng HH, Perera RJ, Lufkin T, Rigoutsos I, Thomson AM, Lim B (2008a) MicroRNA-134 modulates the differentiation of mouse embryonic stem cells, where it causes post-transcriptional attenuation of Nanog and LRH1. *Stem Cells* 26:17
- Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I (2008b) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature* 455:1124
- Tayalia P, Mooney DJ (2009) Controlled growth factor delivery for tissue engineering. *Adv Mater* 21:3269
- Tian Y, Liu Y, Wang T, Zhou N, Kong J, Chen L, Snitow M, Morley M, Li D, Petrenko N, Zhou S, Lu M, Gao E, Koch WJ, Stewart KM, Morrisey EE (2015) A microRNA-hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice. *Sci Transl Med* 7:279ra38
- Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E (2004) Defining the epithelial stem cell niche in skin. *Science* 303:359
- Ulrich-Vinther M, Maloney MD, Schwarz EM, Rosier R, O'keefe RJ (2003) Articular cartilage biology. *J Am Acad Orthop Surg* 11:421
- van Amerongen MJ, Engel FB (2008) Features of cardiomyocyte proliferation and its potential for cardiac regeneration. *J Cell Mol Med* 12:2233
- van Rooij E (2011) The art of microRNA research. *Circ Res* 108:219
- van Rooij E (2012) Introduction to the series on microRNAs in the cardiovascular system. *Circ Res* 110:481
- van Rooij E, Kauppinen S (2014) Development of microRNA therapeutics is coming of age. *EMBO Mol Med* 6:851

- van Rooij E, Purcell AL, Levin AA (2012) Developing microRNA therapeutics. *Circ Res* 110:496
- Várallyay E, Burgyán J, Havelda Z (2008) MicroRNA detection by northern blotting using locked nucleic acid probes. *Nat Protoc* 3:190
- Varkonyi-Gasic E, Hellens RP (2011) Quantitative stem-loop RT-PCR for detection of microRNAs. *Methods Mol Biol* 744:145
- Vimalraj S, Saravanan S, Vairamani M, Gopalakrishnan C, Sastry TP, Selvamurugan N (2016) A combinatorial effect of carboxymethyl cellulose based scaffold and microRNA-15b on osteoblast differentiation. *Int J Biol Macromol* 93:1457
- Vo TN, Kasper FK, Mikos AG (2012) Strategies for controlled delivery of growth factors and cells for bone regeneration. *Adv Drug Deliv Rev* 64:1292
- Vuorimaa E, Ketola TM, Green JJ, Hanzlikova M, Lemmetyinen H, Langer R, Anderson DG, Urtti A, Yliperttula M (2011) Poly(beta-amino ester)-DNA complexes: time-resolved fluorescence and cellular transfection studies. *J Control Release* 154:171
- Wada S, Kato Y, Okutsu M, Miyaki S, Suzuki K, Yan Z, Schiaffino S, Asahara H, Ushida T, Akimoto T (2011) Translational suppression of atrophic regulators by microRNA-23a integrates resistance to skeletal muscle atrophy. *J Biol Chem* 286:38456
- Wang S, Olson EN (2009) AngiomiRs – key regulators of angiogenesis. *Curr Opin Genet Dev* 19:205
- Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R, Olson EN (2008) The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Dev Cell* 15:261
- Wang D, Zhang Z, O’Loughlin E, Lee T, Houel S, O’Carroll D, Tarakhovskiy A, Ahn NG, Yi R (2012) Quantitative functions of Argonaute proteins in mammalian development. *Genes Dev* 26:693
- Wang Z, Zhang D, Hu Z, Cheng J, Zhuo C, Fang X, Xing Y (2015) MicroRNA-26a-modified adipose-derived stem cells incorporated with a porous hydroxyapatite scaffold improve the repair of bone defects. *Mol Med Rep* 12:3345
- Wang XW, He XJ, Lee KC, Huang C, Hu JB, Zhou R, Xiang XY, Feng B, Lu ZQ (2016) MicroRNA-221 sponge therapy attenuates neointimal hyperplasia and improves blood flows in vein grafts. *Int J Cardiol* 208:79
- Wang Y, Malcolm DW, Benoit DSW (2017a) Controlled and sustained delivery of siRNA/NPs from hydrogels expedites bone fracture healing. *Biomaterials* 139:127
- Wang H, Xie Z, Hou T, Li Z, Huang K, Gong J, Zhou W, Tang K, Xu J, Dong S (2017b) MiR-125b regulates the osteogenic differentiation of human mesenchymal stem cells by targeting BMPRIb. *Cell Physiol Biochem* 41:530
- Watt FM, Jensen KB (2009) Epidermal stem cell diversity and quiescence. *EMBO Mol Med* 1:260
- Weilner S, Skalicky S, Salzer B, Keider V, Wagner M, Hildner F, Gabriel C, Dovjak P, Pietschmann P, Grillari-Voglauer R, Grillari J, Hackl M (2015) Differentially circulating miRNAs after recent osteoporotic fractures can influence osteogenic differentiation. *Bone* 79:43
- 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-Durr P, Pietschmann P, Grillari-Voglauer R, Grillari J (2016) Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells. *Aging Cell* 15:744
- Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75:855
- Wilson KD, Hu S, Venkatasubrahmanyam S, Fu JD, Sun N, Abilez OJ, Baugh JJ, Jia F, Ghosh Z, Li RA, Butte AJ, Wu JC (2010) Dynamic microRNA expression programs during cardiac differentiation of human embryonic stem cells: role for miR-499. *Circ Cardiovasc Genet* 3:426
- Wu Q, Chen D, Zuscik MJ, O’Keefe RJ, Rosier RN (2008) Overexpression of Smurf2 stimulates endochondral ossification through upregulation of beta-catenin. *J Bone Miner Res* 23:552
- Wu K, Song W, Zhao L, Liu M, Yan J, Andersen MO, Kjems J, Gao S, Zhang Y (2013) MicroRNA functionalized microporous titanium oxide surface by lyophilization with enhanced osteogenic activity. *ACS Appl Mater Interfaces* 5:2733

- Xie Q, Wang Z, Zhou H, Yu Z, Huang Y, Sun H, Bi X, Wang Y, Shi W, Gu P, Fan X (2016) The role of miR-135-modified adipose-derived mesenchymal stem cells in bone regeneration. *Biomaterials* 75:279
- Xie Q, Wei W, Ruan J, Ding Y, Zhuang A, Bi X, Sun H, Gu P, Wang Z, Fan X (2017) Effects of miR-146a on the osteogenesis of adipose-derived mesenchymal stem cells and bone regeneration. *Sci Rep* 7:42840
- Xue Y, Ouyang K, Huang J, Zhou Y, Ouyang H, Li H, Wang G, Wu Q, Wei C, Bi Y, Jiang L, Cai Z, Sun H, Zhang K, Zhang Y, Chen J, Fu XD (2013) Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. *Cell* 152:82
- Yang F, Green JJ, Dinio T, Keung L, Cho SW, Park H, Langer R, Anderson DG (2009) Gene delivery to human adult and embryonic cell-derived stem cells using biodegradable nanoparticulate polymeric vectors. *Gene Ther* 16:533
- Yau WW, Rujitanaroj PO, Lam L, Chew SY (2012) Directing stem cell fate by controlled RNA interference. *Biomaterials* 33:2608
- Yi R, Qin Y, Macara IG, Cullen BR (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 17:3011
- Yi R, O'Carroll D, Pasolli HA, Zhang Z, Dietrich FS, Tarakhovskiy A, Fuchs E (2006) Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. *Nat Genet* 38:356
- Yi R, Poy MN, Stoffel M, Fuchs E (2008) A skin microRNA promotes differentiation by repressing 'stemness'. *Nature* 452:225
- Yihai C (2003) Angiogenesis inhibitors and their therapeutic potentials. *Adv Exp Med Biol* 532:109
- Yin Q, Gao Y, Zhang Z, Zhang P, Li Y (2011) Bioreducible poly (beta-amino esters)/shRNA complex nanoparticles for efficient RNA delivery. *J Control Release* 151:35
- Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR (2011) MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature* 476:228
- Yoshizuka M, Nakasa T, Kawanishi Y, Hachisuka S, Furuta T, Miyaki S, Adachi N, Ochi M (2016) Inhibition of microRNA-222 expression accelerates bone healing with enhancement of osteogenesis, chondrogenesis, and angiogenesis in a rat refractory fracture model. *J Orthop Sci: Off J Jpn Orthop Assoc* 21:852
- Yuan X, Liu H, Huang H, Liu H, Li L, Yang J, Shi W, Liu W, Wu L (2016) The key role of canonical Wnt/ β -catenin signaling in cartilage chondrocytes. *Curr Drug Targets* 17:475
- Zeng Y, Cullen BR (2003) Sequence requirements for micro RNA processing and function in human cells. *RNA* 9:112
- Zhang L, Webster TJ (2009) Nanotechnology and nanomaterials: promises for improved tissue regeneration. *Nano Today* 4:66
- Zhang M, Ishii A, Nishiyama N, Matsumoto S, Ishii T, Yamasaki Y, Kataoka K (2009) PEGylated calcium phosphate nanocomposites as smart environment-sensitive carriers for siRNA delivery. *Adv Mater* 21:3520
- Zhang S, Chen L, Jung EJ, Calin GA (2010) Targeting microRNAs with small molecules: from dream to reality. *Clin Pharmacol Ther* 87:754
- Zhang Y, Wang Z, Gemeinhart RA (2013) Progress in microRNA delivery. *J Control Release* 172:962
- Zhang Z, Hou C, Meng F, Zhao X, Zhang Z, Huang G, Chen W, Fu M, Liao W (2015) MiR-455-3p regulates early chondrogenic differentiation via inhibiting Runx2. *FEBS Lett* 589:3671
- Zhang X, Li Y, Chen YE, Chen J, Ma PX (2016a) Cell-free 3D scaffold with two-stage delivery of miRNA-26a to regenerate critical-sized bone defects. *Nat Commun* 7:10376
- Zhang Z, Chen W, Zhang Z, Kang Y, Kang Y, Liao W (2016b) MIR-455-3P and HDACs co-regulate chondrogenesis. *Osteoarthr Cartil* 24:S224
- Zhao C, Sun G, Li S, Shi Y (2009) A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nat Struct Mol Biol* 16:365

- Zhao JL, Rao DS, Boldin MP, Taganov KD, O'Connell RM, Baltimore D (2011) NF- κ B dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. *Proc Natl Acad Sci U S A* 108:9184
- Zhou F, Jia X, Yang Y, Yang Q, Gao C, Hu S, Zhao Y, Fan Y, Yuan X (2016) Nanofiber-mediated microRNA-126 delivery to vascular endothelial cells for blood vessel regeneration. *Acta Biomater* 43:303
- Zuo K, Zhi K, Zhang X, Lu C, Wang S, Li M, He B (2015) A dysregulated microRNA-26a/EphA2 axis impairs endothelial progenitor cell function via the p38 MAPK/VEGF pathway. *Cell Physiol Biochem* 35:477