

## CALL FOR PAPERS | *MicroRNA and Tissue Injury*

# MicroRNAs as new maestro conducting the expanding symphony orchestra of regenerative and reparative medicine

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**Sen CK.** MicroRNAs as new maestro conducting the expanding symphony orchestra of regenerative and reparative medicine. *Physiol Genomics* 43: 517–520, 2011. First published April 5, 2011; doi:10.1152/physiolgenomics.00037.2011.—The human genome encodes 1,048 microRNAs (miRNAs). These miRNAs regulate virtually all biological processes. Leaving ignominy on the significance miRNAs behind we are approaching a new era in tissue repair where an ever expanding orchestra of events that enable tissue repair and regeneration seems to be conducted by miRNAs as the maestro. microRNAs are emerging as molecular rheostats that fine-tune and switch regulatory circuits governing tissue repair. Key elements of tissue repair such as stem cell biology, inflammation, hypoxia-response, and angiogenesis are all under the sophisticated control of a network of specific mRNAs. Embryonic stem cells lacking miRNAs lose their “stemness.” Manipulation of specific cellular miRNAs helps enhance reprogramming of somatic cells to an embryonic stem cell-like phenotype helping generate inducible pluripotent stem cells. Expression of miRNAs is subject to control by epigenetic factors. Such control influences the balance between proliferation and differentiation of stem cells. Angiomirs regulate various aspects of angiogenesis, such as proliferation, migration, and morphogenesis of endothelial cells. MiRNAs play a key role in resolution of inflammation. Hypoxia-inducible mRNAs or hypoxamirs suppress mitochondrial respiration, cause cell cycle arrest, and interfere with growth factor signaling. miRNA-210 is a good example in this category that impairs wound closure. As fine tools enabling specific and temporally controlled manipulation of cell-specific miRNAs emerge, miRNA-based therapies hold promise in facilitating tissue repair. Treatment of skin wounds has lower barriers because it lends itself to local delivery of miRNA mimics and antagonizing agents minimizing risks associated with systemic off-target toxicity.

wound; regenerative medicine; therapeutics

### MicroRNAs

Complexities underlying the biology of tissue repair are assuming an even more multidimensional configuration as new players are identified. Robust bedside solutions can only come from a team science approach that is able to successfully integrate high-resolution mechanistic science into a seamless mosaic that unlocks the yet unveiled secrets of tissue repair. Tissue repair is largely dependent on injury-inducible protein-coding genes as they serve as drivers of an inherent tissue repair program that seek to restore the injured tissue both structurally as well as functionally. There are two key steps that separate a protein coding gene from its corresponding protein. First, the DNA hosting the gene must transcribe to mRNA. Finally, the mRNA must be translated to protein.

Work emerging during the recent years demonstrates that both of these critical steps are subject to robust and redundant regulation by microRNAs (miRNAs, 19- to 22-nucleotide long), which are noncoding RNAs found in all eukaryotic cells. Work during the past decade recognizes small RNAs as a new class of regulators of eukaryotic biology. Alongside other small interfering RNAs, miRNAs execute posttranscriptional gene silencing through mRNA destabilization as well as translational repression. miRNAs form base pairs with specific sequences in protein-coding mRNAs. Near-perfect pairing induces cleavage of the target mRNA, whereas partial pairing results in translational repression and mRNA decay through deadenylation pathways (26). According to the miRbase database the human genome encodes 1,048 miRNAs. This count is rapidly growing. Based on prediction algorithms, these miRNAs may regulate more than one-third of all protein-coding genes and virtually all biological processes (22). Mammalian cells express cell type-specific miRNAs that si-

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lence unique subsets of target genes within the cell. While miRNAs are mostly known for being functional in the cytoplasm, nuclear miRNAs may also participate in gene regulation. Initially considered an oddity, miRNA-dependent control of gene expression is now accepted as being integral to the normal function of cells and organisms. This issue of *Physiological Genomics* is dedicated to understanding how miRNAs influence the ability of tissues to repair themselves after injury. Leaving ignominy on the significance miRNAs behind we are approaching a new era in tissue repair where an ever expanding orchestra of events that enable tissue repair and regeneration seem to be conducted by miRNAs as maestro.

#### STEM CELLS

Endogenous miRNA binding sites have been identified in murine embryonic stem cells (ESCs)(16). miRNAs govern ESCs function by serving as control hubs managing regulatory networks (10, 14). A central importance of such governance is highlighted by the observation that ESCs lacking microRNAs lose their “stemness.” ESCs with deficient miRNA biogenesis systems switch to a mode of ongoing cell division. They do not differentiate on demand because of failure to turn off the pluripotency regulatory program (34). miRNAs conduct the orchestra of critical gene regulatory networks controlled by pluripotency factors Sox2, Oct4, and Nanog (13, 22). Individual miRNA-dependent pathways that promote the reprogramming of somatic cells into induced pluripotent stem (iPS) cells have been now identified. Manipulation of specific cellular miRNAs helps enhance reprogramming of somatic cells to an ESC-like phenotype helping generate iPS cells (17). Expression of miRNAs is subject to control by epigenetic factors (19). Such control influences the balance between proliferation and differentiation of stem cells. In executing such control the miRNA element of epigenetics cross talks with changes in chromatin structure as well as with changes in DNA methylation (3). Collectively, this provides for a mechanism by which the tissue injury microenvironment can influence miRNA-dependent reparative and regenerative processes.

#### INFLAMMATION

Mounting of a successful inflammatory response is one of the earliest responses to injury. Blood-borne cells are transiently recruited to the wound site with the overall goal to make the injury site receptive for a repair process. In an ideal scenario, inflammation is transient and resolves facilitated by an equally sophisticated series of events. In ensuring functional tissue repair, mounting as well as resolution of inflammation are equally important. Disruption of miRNA biogenesis has a major impact on the overall immune system. Emerging studies indicate that miRNAs, especially miR-21, miR-146a/b and miR-155, play a key role in regulating several hubs that orchestrate the inflammatory process (30, 37). Pro- (miR-125b) and anti-inflammatory (miR-26a, -34a, -145, and let-7b) miRNA may also be manipulated to positively influence stroke outcomes (29). miRNAs have been directly implicated in the pathogenesis of inflammatory diseases such as osteoarthritis and rheumatoid arthritis (23). Resolvin-regulated specific miRNAs target genes involved in resolution of inflammation and establish a novel resolution circuit involving RvD1 receptor-dependent regulation of specific miRNAs (28). Furthermore, the

brain-specific miRNA-124 can tame inflammation by turning off activated microglial cells and macrophages (27). Of relevance to tissue repair is also the regulatory loop where cytokines, including those elicited following injury, are regulated by miRNAs as well as regulate miRNA expression (1, 11).

#### ANGIOGENESIS

Patent vascular supply and adequate perfusion is a key determinant of success in tissue repair especially when the injury is sizeable. In 2005–2008, the first series of observations establishing key significance of miRNAs in the regulation of mammalian vascular biology came from experimental studies involved in arresting miRNA biogenesis to deplete the miRNA pools of vascular tissues and cells (32). Dicer-dependent biogenesis of miRNA is required for blood vessel development during embryogenesis. Mice with endothelial cell-specific deletion of Dicer, a key enzyme supporting biogenesis of miRNAs, display defective postnatal angiogenesis. NADPH oxidase-derived reactive oxygen species (ROS) drive wound angiogenesis. Endothelial NADPH oxidase is subject to control by miRNAs (33). Hypoxia is widely recognized as a cue that drives angiogenesis as part of an adaptive response to vascularize the oxygen-deficient host tissue. Hypoxia-sensitive miR-200b is involved in such induction of angiogenesis via directly targeting Ets-1 (7). Various aspects of angiogenesis, such as proliferation, migration, and morphogenesis of endothelial cells, can be regulated by specific miRNAs in an endothelial-specific manner. miRNAs known to regulate angiogenesis *in vivo* are referred to as angiomiRs (36). miRNA-126 is specific to endothelial cells and regulates vascular integrity and developmental angiogenesis. Manipulating angiomiRs in the setting of tissue repair represents a new therapeutic approach that could be effective in promoting wound angiogenesis.

#### HYPOXIA RESPONSE

Tissue injury is often associated with disruption of vascular supply to the injury site. Thus, the injured tissue often suffers from insufficient oxygen supply or hypoxia. Under conditions of additional underlying ischemia, hypoxia is severe and seriously limits tissue repair (31). Hypoxia induces specific miRNAs collectively referred to as hypoxamirs (5). miRNA-210 is a classical hypoxamir. Expression of hypoxia-inducible factor 1 (HIF-1) is also controlled by specific miRNAs. In turn, HIF-1 controls the expression of hypoxamirs that are induced in the injured tissue (21). Hypoxamirs are also induced by HIF-independent pathways. Although hypoxamirs generally favor angiogenesis (7, 12), their metabolic and cell cycle arrest functions are in conflict with tissue repair especially in an ischemic setting. Silencing specific hypoxamirs may therefore represent a prudent approach to facilitate tissue repair. miRNA-210 represses mitochondrial respiration (6) and exaggerates production of undesired mitochondrial ROS (9). These outcomes are not compatible with the higher energy demands associated with tissue repair. miR-210 also silences signaling via fibroblast growth factor (35), a key contributor to wound healing. The injured tissue is highly rich in ROS (31). In addition, at the site of injury transition metal ions are released from a protein-bound state. Conditions such as these cause DNA damage that opposes tissue repair. DNA repair systems are therefore of key significance in enabling tissue repair.

miR-210 antagonizes DNA repair (8). This is another hypox-amir function that is in conflict with tissue repair. Compatible with the common observation that ischemic wounds are refractory to healing response, elevated miRNA-210 in ischemic wounds attenuated keratinocyte proliferation and impaired wound closure (2, 4).

#### miRNA-BASED THERAPEUTICS

Rapidity and reversibility of miRNA function makes them an attractive target for therapeutic manipulation. In a setting where one miRNA can regulate hundreds of genes and one gene can be regulated by a number of miRNAs, a major issue is to develop an understanding of the regulatory loops that govern miRNA-miRNA as well as miRNA-mRNA interactions (32). In addition, factors contributing to the volume control of inducible or repressible miRNA expression need to be understood. At present, major advances are being made to understand these processes. In particular, miRNA coordinated expression with other regulatory molecules, such as transcription factors, is an area under active development. miRNAs are emerging as molecular rheostats that fine-tune and switch regulatory circuits governing tissue repair. miRNA-based therapeutics now represent the most significant commercial hotspot in today's health care market space. Although there are many challenges for miRNAs as therapeutic targets such as delivery, potential off-target effects, and safety, the strategy of miRNA manipulation in vivo to regulate disease-related processes is already becoming a feasible future therapeutic approach (20). miRNA-based therapies have proven to be helpful in chronically infected chimpanzees by suppressing hepatitis C viremia and improvement of hepatitis C virus-induced liver pathology (15) and are being evaluated in the first human clinical trials of miRNA inhibition (Santaris Pharma, <http://clinicaltrials.gov/>). Miravirsin is the first miRNA-targeted drug to receive Investigational New Drug acceptance from the Food and Drug Administration, paving the way to conduct Phase 2 trials for treatment of hepatitis C in the United States. Tiny locked nucleic acid-based compounds can inhibit entire disease-associated miRNA families. This provides a potential new approach for treating a variety of diseases including cancer, viral infections, and cardiovascular and muscle diseases (25). Mirna Therapeutics, Inc. has recently published data demonstrating that miR-34 mimic developed at Mirna might be particularly effective in combating the most aggressive cancer cells in patients (18). Upon activation, miR-155 is expressed in several types of human immune cells including B cells, T cells, macrophages, and dendritic cells. Regulus Therapeutics is targeting miR-155 with anti-miRs to treat inflammatory diseases (24). As fine tools enabling precise and temporally controlled manipulation of cell-specific miRNAs emerge, miRNA-based therapies will provide lucrative returns. Treatment of skin wounds has lower barriers because it lends itself to local delivery of miRNA mimics and antagonizing agents.

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#### DISCLOSURES

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