Is there a role for microRNAs in exercise immunology? – A synopsis of current literature and future developments

Barbara Wessner[1], Laura Gryadunov-Masutti[1,2], Harald Tschan[1], Norbert Bachl[1], Erich Roth[2]

[1] Centre for Sports Sciences and University Sports, Department of Sports and Exercise Physiology, University of Vienna, Vienna, Austria

[2] University Clinics of Surgery, Research Laboratories, Medical University of Vienna, Vienna, Austria

ABSTRACT

With the discovery of microRNAs (miRNAs), an exceptional means of regulating gene expression was introduced a few years ago. MiRNAs function to inactivate specific messenger RNA transcripts leading to depletion of the corresponding protein, whereby computational studies have shown that about one third of all animal genes might be miRNA targets. Recent publications highlight the involvement of miRNAs in regulating the immune response. The aim of this review is to provide an overview of miRNA biogenesis and function, to illustrate their impact on both the innate as well as the adaptive immune system, to show the regulation of skeletal muscle plasticity and inflammation, and finally to present their possible role within the field of exercise immunology.

Keywords: microRNA, immunology, inflammation, skeletal muscle, exercise

INTRODUCTION

In response to external stimuli the expression of genes can be regulated by different mechanisms, such as through transcription factors, alternative splicing, epigenetics and gene silencing by small RNAs including so called microRNAs (miRNAs or miRs). Since the discovery of the first miRNA *lin-4* during larval develop-

Corresponding author:
Barbara Wessner, PhD, Centre for Sports Sciences and University Sports
Department of Sports and Exercise Physiology, University of Vienna
Auf der Schmelz 6, A-1150 Vienna, Austria
Phone: +43-1-4277-28772, Fax: +43-1-4277-9287, E-mail: barbara.wessner@univie.ac.at

ment of the worm *Caenorhabditis elegans* in 1993 it became evident that this class of small endogenous RNAs control a wide range of developmental and physiological pathways, whereby the term "microRNA" was introduced as late as in 2001 (33, 36, 48). To date, more than 700 human miRNAs have been identified (and the number is still increasing), possibly regulating 30 - 60% of protein-encoding genes (24, 26, 38). MiRNAs usually are expressed in various cell types but interestingly, some miRNAs are expressed tissue-specific such as miR-1, miR-133a, and miR-206 in muscle (35). While it has been extensively shown that diseases such as cancer, muscular and cardiovascular disorders are accompanied by abnormal expression of miRNAs, recent publications highlight the involvement of miRNAs in regulating the immune response (4, 15, 21, 57). Additionally, it has been shown that miRNAs themselves are capable of directly acting antivirally (42). The aim of this review is to provide an overview of miRNA biogenesis and function, to illustrate their impact on the immune system and skeletal muscle plasticity, and finally to present their possible role within the field of exercise immunology.

Biogenesis and function of microRNAs

MiRNA biogenesis. Most of the short (20-23 nucleotide), endogenous, single-stranded miRNAs are processed through a series of post-transcriptional biogenesis steps (73, 85). MiRNAs are encoded in the genomic DNA, either in the introns of protein-coding genes, the exons of untranslated genes or apart from known genes in the intergenic region (70). In the first step of biogenesis the primary miRNA (pri-miRNA) is transcribed from the miRNA gene by RNA polymerase II or RNA polymerase III (6, 37) (Figure 1). The pri-miRNA is then cleaved to the

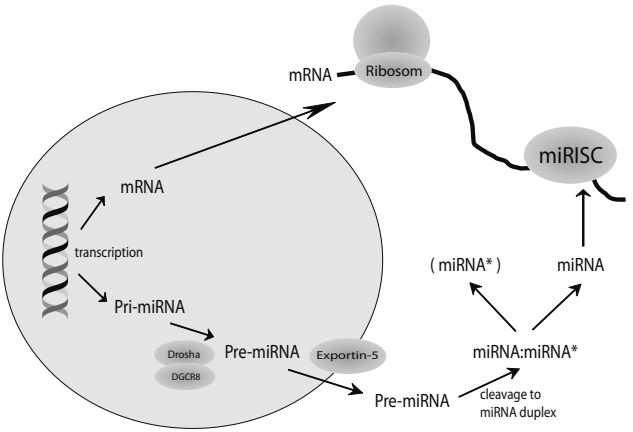


Figure 1. Biogenesis of miRNAs. Most miRNAs are transcribed from DNA as long primary transcripts (pri-miRNA) which are processed by Drosha resulting in pre-miRNA hairpins with a length of about 70 nucleotids. After being transported from nucleus to the cytosol via Exportin-5 the hairpins are cleaved to form miRNA duplexes which again are cleaved to form the mature miRNAs. This short single stranded miRNAs are incorporated into the RNA-induced silencing complex (RISC) which regulates protein expression.

precursor miRNA (pre-miRNA) by a protein complex termed Microprocessor consisting of the RNase Drosha and the protein DGCR8. The pre-miRNA is shuttled from the nucleus to the cytoplasm via the transport protein Eportin-5. The RISC loading complex (RLC) completes miRNA maturation by cleaving the pre-miRNA resulting in a miRNA duplex miRNA:miRNA*. One of the RNA strands (miRNA*) is discarded while the other mature miRNA is selected together with a member of the Argonaute family of proteins to form the core unit of the RNA-induced silencing complex (RISC) (25, 34, 45). However, recent studies reveal that this pathway is not universal to all miRNA. Many steps can be omitted or replaced suggesting multiple possibilities for post-transcriptional regulation of miRNA expression (for review see 85).

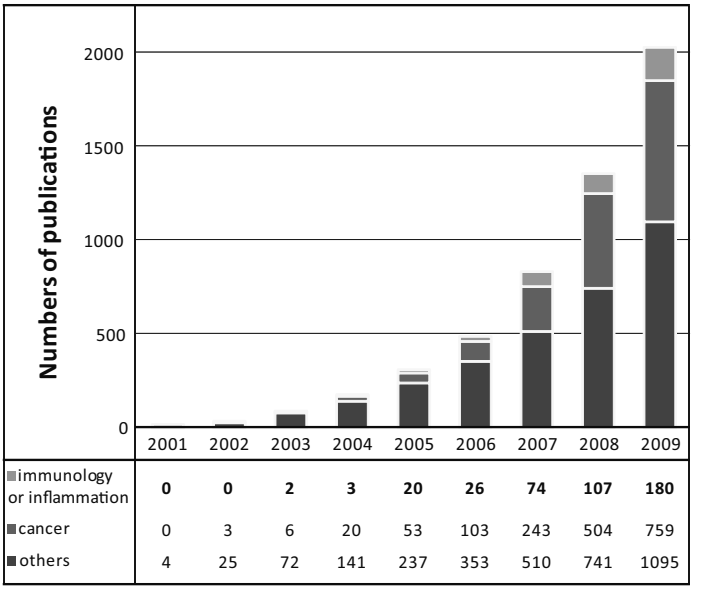
Mechanisms of miRNA function. MiRNAs control protein synthesis by sequence-specific binding to the 3' untranslated regions of mRNAs whereby one miRNA may regulate dozens of genes and each gene is likely to be regulated by several miRNAs (40, 77). Suppression of protein synthesis can either occur through inhibition of translation in the case of partial complementarity between miRNA and target mRNA or through degradation in the case of near-perfect sequence analogy (Figure 1). Interestingly, mRNAs solely suppressed by miRNAs seem to accumulate in discrete cytoplasmatic foci, so called P-bodies (62). mRNAs concentrated in these P-bodies may "wait" for a stimulation signal. Once the respective mRNA is needed again the translation process is resumed enabling a fast production of the required protein (7).

Target prediction. As miRNAs exert their functions by binding to their corresponding mRNA targets it is important to understand the miRNA-mRNA interactions. It has been shown that the ability of an miRNA to translationally repress a target mRNA is largely dictated by the free energy of binding of the first 8 nucleotides in the 5' region of the miRNA, the so called seed region (18). However, by combining computational and experimental approaches Grimson *et al.* (27) uncovered five additional features boosting site efficacy: 1) AU-rich nucleotide composition near the site, 2) proximity to sites for coexpressed miRNAs, 3) proximity to residues pairing to miRNA nucleotides 13-16, 4) positioning within the 3' untranslated region (3' UTR) at least 15 nucleotides from the stop codon, and 5) positioning away from the center of long UTRs (27). *In silico* target prediction is still challenging although several online resources are available for the non-computational biologist. The currently widely used target prediction tools for mammals are TargetScan (http://targetscan.org), miRanda (http://www.microrna.org), PicTar (http://pictar.mdc-berlin.de/), ElMMo (http://www.mirz.unibas.ch/ElMMo2), and MicroCosm Targets v5 (formerly miRBase Targets) (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5). However, although these tools improved within the last years, the obtained results need to be evaluated experimentally to exclude false positive targets (2).

Role of miRNAs in immunology and inflammation

Since the realization that miRNAs belong to a huge family with an enormous regulatory impact the number of publications has increased exponentially, especially in the field of cancer. A literature research in NCBI PubMed revealed that mi-

RNAs were mentioned together with immunology or inflammation for the first time in 2003. By the end of 2009 the proportion of publications in this field rose from 2% to nearly 10% (Figure 2). These publications provide evidence that miRNAs are involved in the innate and the adaptive immune response as well as in inflammatory disorders such as psoriasis, asthma, rheumatoid arthritis and vascular inflammation (for review of the latter see (76)).



	2001	2002	2003	2004	2005	2006	2007	2008	2009
immunology or inflammation	**0**	**0**	**2**	**3**	**20**	**26**	**74**	**107**	**180**
cancer	0	3	6	20	53	103	243	504	759
others	4	25	72	141	237	353	510	741	1095

Figure 2. Scientific articles searched by the key word microRNA which was introduced in 2001. Source: NCBI PubMed, as of December 2009.

miRNAs during activation of the innate immune response

The innate immune response comprises the first line of defense against bacteria, viruses and other pathogens and is mediated via monocytes, macrophages, dendritic cells and neutrophils. The detection of pathogens occurs via germ-line encoded receptors such as the Toll-like receptor (TLR) family with 11 different members and the Interleukin-1 (IL-1) receptor family with 10 members. Binding of the TLRs and IL-1 receptors to their ligands initiates a cascade of signaling responses finally regulating the expression of immune-responsive genes (81). To examine the potential role of miRNAs in regulating the innate immune response Taganov *et al.* (78) analyzed the expression of 200 miRNAs in human monocytic THP-1 cells after bacterial endotoxin, lipopolysaccharide (LPS), stimulation. Several of the investigated miRNAs (miR-146a/b, miR-132, and miR-155) were up-regulated after 12 hours of stimulation. By means of promoter analysis, miR-146a expression was found to be NF-κB-dependent and both miR-146a and miR-146b may function as a negative feedback regulation loop involving down-regulation of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) protein levels. Interestingly, miR-146 was only induced when

stimulated with bacterial pathogens via TLR2, TLR4 and TLR5 but not after treatment with viral stimuli via TLR3, TLR7 and TLR9 (78). After prolonged exposure to LPS a state of hyporesponsiveness to subsequent LPS challenge (LPS tolerance) can be observed. In THP-1 cells, the LPS tolerance was dependent on the LPS-priming dose and associated with miR-146a up-regulation. LPS-tolerized cells regained responsiveness in TNF-alpha production after LPS removal correlating with a decrease in miR-146a level (55). In another study A549 lung alveolar epithelial cells were stimulated with IL-1β resulting in a time- and dose-dependent up-regulation of both, miR-146a and miR146b. Examination of miRNA function by overexpression and inhibition showed that increased miRNA-146a expression negatively regulated the release of the proinflammatory chemokines IL-8 and RANTES (61). To gain insight into the human *in vivo* situation, differentially expressed miRNAs were measured in circulating leukocytes in a human model of acute inflammation triggered by *Escherichia coli* LPS infusion. Microarray data analysis of leukocyte RNA revealed that five miRNAs consistently responded to LPS-infusion, four of which were down-regulated (miR-146b, miR-150, miR-342, and let-7g) and one was up-regulated (miR-143) 4 hours after LPS-infusion. By correlating the levels of these miRNAs to LPS-induced changes of the leukocyte transcriptome it was shown that the rapid transcriptional activation of interleukin-1 receptor-associated kinase 2 (IRAK2) might be facilitated by decreased levels of LPS-responsive miRNAs. The increased level of miR-143 might be associated with the pronounced down-regulation of the B-cell CLL/lymphoma 2 (BCL2) gene expression (71). Bazzoni *et al.* (3) have shown that out of 365 tested miRNAs miR-9 has been the only one which is induced in neutrophils as well as monocytes via *ex vivo* TLR2, TLR4, TLR7/8 activation, and TNF-alpha or IFN-gamma stimulation. The expression of other miRNAs such as let-7e, miR-99b, miR125a, miR-132, miR-146a, miR-146b, miR-155 or miR-187 is enhanced solely in activated monocytes (3).

In contrast to miR-146a and miR-146b, miR-155 seems to be sensitive to bacterial and viral type stimuli and also to be inducible in a variety of different cell types (58, 78). In primary murine macrophages miR-155 was identified as being enhanced after induction by TLR2, TLR3, TLR4 and TLR9 ligands. Moreover, the pharmacological inhibition of the kinase JNK blocked induction of miR-155 in response to either polyriboinosinic:polyribocytidylic acid or TNF-α, suggesting that miR-155-inducing signals use the JNK pathway (58). Up-regulation of miR-155 upon LPS-stimulation was confirmed in mouse bone marrow-derived macrophages and Raw 264.7 macrophages (68, 80). Simultaneously, miR-125b was down-regulated in Raw 264.7 macrophages. These data were confirmed *in vivo* after injecting C57BL/6 mice with LPS and measuring miR-155 expression in splenocytes. Several proteins involved in LPS signalling such as the Fas-associated death domain protein (FADD), IkappaB kinase epsilon (IKKε), and the receptor (TNFR superfamily)-interacting serine-threonine kinase 1 (Ripk1) were reported as potential targets. As TNF-α translation was increased, the authors conclude that miR-125b down-regulation in response to LPS may be required for proper TNF-α production (80). Another animal study by Moschos *et al.* (53) observed an up-regulation of 46 different miRNAs (but not miR-146 or miR-155) in the lungs of mice inhaling aerosilised LPS. Simultaneously, the expression of TNF-α, keratinocyte-derived chemokine (KC) and macrophage inflammatory protein (MIP)-2 were down-regulated (53). MiR-155

also seems to be involved in the maturation of human monocytic-derived dendritic cells after exposure to LPS by targeting the transcription factor PU.1 and modulating pathogen binding by down-regulating DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) (47).

In summary, several miR expression levels have been shown to be altered in the acute response of myeloid cells to several pathogens and to target key players in the involved signalling pathways. As evidence is derived mainly from *in vitro* or *ex vivo* screening studies, there is a strong need to confirm these data and also to highlight the functional consequences of up- or down-regulation of a set of miRNAs in immune challenging conditions.

miRNAs and the adaptive immune responses

In addition to be involved in the first line of defense by regulating the innate immune response, miRNAs also have been shown to be important for activation and subsequent clonal expansion of antigen-specific lymphocytes. When expression profiles of hematopoetic tissue-specific miRNAs (miR-142, miR-155, miR-181 and miR-223) were compared between normal human B, T, monocytic and granulocytic lineages and malignant hematopoietic cell lines it became evident that the levels of miRNA expression among cell lines and normal cell lineages were considerably different suggesting important regulatory roles of miRNAs in human oncogenesis but also hematopoiesis (66). MiRNA profiling of the murine hematopoetic system revealed that miRNA expression patterns were very different between hematopoietic and non-hematopoietic cells, with further subtle differences observed within the hematopoietic group. Interestingly, it was suggested that in addition to regulating the process of commitment to particular cellular lineages, miRNAs might have an important general role in the mechanism of cell differentiation and maintenance of cell identity (52).

miRNAs and development B lymphocytes. The two major classes of lymphocytes (B and T cells) rely on different principles of antigen recognition, whereby B lymphocytes recognize intact forms of antigens, whereas receptors on the surface of T cells recognize antigens in the form of small peptides bound to the products of the major histocompatibility complex (MHC) (43). Development of B cells involves differentiation of common lymphocyte progenitor cells to pro-B cells, pre-B-cells and (activated) B cells (4). Several miRNAs have been shown to be differentially expressed during transition between these stages.

MiR-150 is selectively expressed in mature, resting B and T cells but not in their progenitors (52). It seems to down-regulate mRNAs that are important for pro- and pre-B cell formation. However, premature overexpression of miR-150 in hematopoetic stem cells blocked lymphopoiesis by inhibiting the pro-B to the pre-B cell stage (88). A predicted target of miR-150 is c-Myb, a transcription factor down-regulated upon maturation and again up-regulated after activation of the mature cells. It has been shown in miR-150-/- and transgenic mice that miR-150 controls c-Myb expression in vivo in a dose-dependent manner over a narrow range of miRNA and c-Myb concentrations and that this affects lymphocyte development and response (86).

MiR-155 resides within the non-coding gene known as *bic* (B-cell integration cluster), which can be regarded as its primary miRNA precursor. *Bic* as well

as miR-155 have been shown to be increased in activated B cells but also in activated T cells, macrophages and dendritic cells (20). Using a transgenic mouse model and genetic deletion it has been shown that miR-155 deletion leads to immunodeficiency and has an important role in controlling the germinal center B cell response, partly by regulating cytokine production (67, 79). B cells lacking miR-155 generated failed to produce high-affinity IgG1 antibodies, whereby the transcription factor Pu.1 has been validated as direct target of miR-155 (83).

Blocking of the whole miRNA machinery by deleting the enzyme Dicer in early B-cell progenitors leads to block at the pro- to pre-B cell transition. B cell development could be partially rescued by ablation of the pro-apoptotic molecule Bim, which seems to be a direct target of miR-17 approximately 92 cluster (miR-17~92) (31). Subsequently, miR-17~92 is regarded to be a positive regulator of B cell development as its absence leads to increased levels of Bim and inhibits the pro-B to pre-B transition (82). Overexpression on the other hand resulted in mice dying early and developing lymphoproliferative disease and autoimmunity (87). Another miRNA which can be regarded as a positive regulator during early hematopoiesis and lineage commitment is miR-181. It is preferentially expressed in the B-lymphoid cells of mouse bone marrow, and its ectopic expression in hematopoietic stem/progenitor cells led to an increased fraction of B-lineage cells in both tissue-culture differentiation assays and adult mice (12).

miRNAs and T lymphocytes. In contrast to B cells miRNAs are not required for commitment of T cell precursors to T helper cell (CD4+) or cytotoxic T cells but possibly in their proliferation as deletion of Dicer at an early stage of T cell development reduced circulating cells by 90% (14, 81). Dicer knockout at later stages of T cell development reduced the number of CD4+ T regulatory (Treg) cells and generated T cells preferentially expressing interferon-gamma, the hallmark effector cytokine of the Th1 lineage (54).

Although these studies point to an important role of miRNAs in the T cell-mediated immune response, information about the role of single miRNAs are rare. In conventional T cells miR-17~92 overexpression resulted not only in the expansion of B cells but also of CD4+ and CD8+ T Cells accompanied by suppression of the tumor suppressor PTEN and the pro-apoptotic protein Bim (87). MiR-181a, which is highly expressed in the early T cell differentiation stages, drops continuously when T cells undergo differentiation from double negative to single positive CD4 (T helper) or CD8 (cytotoxic) cells. Overexpression of miR-181a in primed T cells augmented T cell receptor signaling and activation by doubling IL-2 production (39). In contrast, reduced IL-2 production and activator-protein 1 (AP-1) activity are achieved by miR-146a overexpression acting on Fas-associated death domain and therefore as an anti-apoptotic factor (17). Finally, miR-155 has been shown to be involved in T cell commitment as mice deficient for bic/miRNA-155 show abnormal function of B and T lymphocytes. Transcriptome analysis of bic/microRNA-155-deficient CD4+ T cells identified a wide spectrum of miRNA-155-regulated genes, including cytokines, chemokines, and transcription factors (67).

Regulatory T cells (Tregs) are a subset of CD4+ T cells essential for the self-tolerance and rely on the transcription factor Foxp3 which is specifically expressed in Tregs (30). Enforced expression of Foxp3 resulted in up- regulation

of several miRNAs such as miR -146 or miR-155 (13). Elevated miR-155 expression is required for maintaining Treg cell proliferative activity whereas miR-155 deficiency resulted in increased suppressor of cytokine signaling 1 (SOCS1) expression accompanied by impaired activation of signal transducer and activator of transcription 5 (STAT5) transcription factor in response to limiting amounts of interleukin-2 (44).

Exercise-induced changes in microRNA expression

Immune-competent cells

Physical activity affects the immune system in dependency of type, duration and intensity of exercise. Moreover, subject characteristics such as gender, age, training or nutritional status but also environmental conditions such as temperature or altitude may influence the immune response. From investigating cytokine balances after strenuous endurance exercise it became evident that both pro-inflammatory and anti-inflammatory pathways are activated as plasma concentrations of pro-inflammatory cytokines (TNF-α, IL-1β), the inflammation responsive cytokine IL-6, cytokine inhibitors (IL-1ra, sTNF-r1, sTNF-r2) and the anti-inflammatory cytokine IL-10 were increased in the time course after an Marathon race (59). These changes in the cytokine balance are reflected by changes in the number of immune competent cells. However, a huge number of studies revealed that changes in leukocyte numbers vary considerably in dependency of the exercise conditions (46).

In order to understand these differences on a molecular level, microarray technology has been widely used to discover broad gene expression changes in leukocytes in response to physical activity (10, 16, 89). Although this method is hampered by a variety of influences (microarray platform, RNA and array preparation methods, sampling points and cell population) several studies revealed consistent changes in inflammatory and heat shock response genes and genes associated with cellular communication, signal transduction, cellular protection and growth and repair pathways (22). Immediate early gene expression is regarded as the first line of transcriptional response that occurs within 1h after the stimulus and mediates protein neosynthesis finally leading to adaptation of immune function. The expression of these early responsive genes is posttranscriptionally regulated either via changing the RNA-stability through interaction at recognition motives located within a mRNA or by influencing the translation process via non coding RNAs such as miRNAs (74). These processes would allow a rapid change in innate immune cellular activity which might be essential in the priming of immune cells in response to stress signals in order to act effectively against potentially invading pathogens. It has been shown that exposure to metal-rich particulate matter modifies the expression of miRNAs related with oxidative stress and inflammatory processes (miR-222, miR-21 and miR-146a) in peripheral blood leukocytes of workers of an electric-furnace steel plant (5) but up to now only one study reported changes in miRNA expression in neutrophils in response to physical activity. Radom-Aizik *et al.* (65) revealed that miRNA expression in circulating neutrophils is affected by ten 2-min bouts of cycle ergometer exercise interspersed with 1-min rest at a constant intensity equivalent to about 76% of

VO2max (65). This type of exercise increased the number of neutrophils by 56% and altered 38 miRNAs whereby 20 miRNAs were expressed at a lower and 18 at a higher level. Seven out of the 38 miRNAs were verified by quantitative RT-PCR (miR-16, miR-17, miR-18a, miR-20a, miR-20b, miR-106a, miR-223). *In silico* analysis showed that collectively 36 miRNAs potentially targeted 4,724 genes. In a previous study using a slightly different exercise protocol the authors detected 458 genes which were differentially expressed before and after the exercise (64). The miRNA-gene expression overlapping data set which comprises those genes that were both affected by exercise and potentially targeted by one or more of the 36 miRNAs consisted of 137 genes. Pathway analysis revealed three significant pathways which are known to be important in key mechanisms of inflammation: the Ubiquitin-mediated proteolysis pathway, the Jak-STAT signaling pathway and the Hedgehog signaling pathway (65). Although this study has some methodological limitations as the overlapping data set was extracted from two different subject populations one can conclude that in response to an exercise stimulus, miRNAs contribute to gene expression alterations in immune-competent cells .

MyomiRs – skeletal muscle miRNAs

(Eccentric) exercise-induced muscle damage involves a series of events starting with mechanical disruption of sarcomeres, followed by impaired excitation-contraction coupling and calcium signaling, and finally, activation of proteolytic pathways related to muscle fiber degradation and repair (63). Muscle damage coincides with ultrastructural changes in muscle architecture, loss of muscular strength and muscle soreness. In the process of assisting to remove disrupted tissue neutrophils might be recruited to the site of damage. However, in exercise and related injury models there is an ongoing debate whether these neutrophils enhance the original damage by releasing reactive oxygen and nitrogen species as well as by producing pro-inflammatory cytokines (11, 72, 75). While neutrophils are recruited to the exercised muscle within several hours and remain present up to 24 hours, macrophages are present from 24 hours up to 14 days after exercise. The inflammatory response to exercise-induced muscle damage is characterized by local production of pro-inflammatory cytokines but also by systemic release of leukocytes and cytokines. However, these inflammatory responses to muscle damage might depend on several factors such as the type of eccentric exercise, previous eccentric loading (repeated bouts), age and gender (60). Gene expression studies using skeletal muscle biopsies obtained from young, trained males prior to and at 3-h and 24-h after a 45min downhill treadmill run revealed that IL-6, IL-8, and COX2 mRNA expression were enhanced in comparison to baseline levels, whereas no significant changes for IL-12, IL-1β, TNF-α, or NFκB were noted (8). In physically active, post-menopausal women completing a series of different lower-body resistance exercises, mRNA content of TNF-α, IL-1β, SOCS2, SAA1, SAA2, IKKB, cfos, and junB were up-regulated in addition to IL-6, IL-8 and COX2 in skeletal muscle 3h post exercise, whereas IL-2, IL-5, IL-10 and IL-12 were unaffected (9). Up to now it is unproven whether miRNAs are involved in inflammatory processes within skeletal muscle tissue but a small number of striated muscle-specific miRNAs so called MyomiRs have been identified and shown to have an important role in myogenesis, embryonic muscle growth and cardiac function and hypertrophy (49). Data from animal studies show that adaptation to

functional overload (as a model for resistance training) leads to alterations in muscle-specific primary and mature miRNAs (miR-1, miR-133a, miR-206). Interestingly, pri-miRNA levels for both pri-miRNA-1-2 and pri-miRNA-133a-2 doubled, whereas pri-miRNA-206 levels were elevated even 18.3-fold. In contrast to these elevations, the expression of mature miR-1 and miR-133a were down-regulated by approximately 50% (50). An acute bout of endurance exercise results in the up-regulation of transcriptional networks that regulate mitochondrial biogenesis, glucose and fatty acid metabolism, but also skeletal muscle remodeling. When C57Bl/6J wild-type male mice are subjected to a forced-endurance exercise training, miR-181, miR-1, and miR-107 were increased by 37%, 40%, and 56%, respectively, in quadriceps femoris muscle. In contrast, miR-23 expression was decreased by 84% and miR-133 did not change 3 hours after the exercise (69). Another animal study investigated the effect of running exercise which was gradually increased over a period of 4 weeks. MiRNA microarray analysis of gastrocnemius muscle revealed that miR-696 was affected by this type of training. In parallel, protein levels of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) which plays a central role in aerobic metabolism and is a predicted target of miR-696 was increased by exercise (1). These studies show that different miRNAs are involved in skeletal muscle plasticity in a variety of conditions. Our own *in vitro* studies in a frequently used C2C12 mouse muscle cell culture system revealed that expression levels of miR-1 and miR-206 were enhanced 460-fold and 14-fold, respectively during transition of proliferating myoblasts to differentiated myotubes. At the same time mRNA level of Insulin-like growth factor-1, which is important in muscle hypertrophy, was about 35-fold higher in myotubes. Treatment of the C2C12 myotubes with TNF-α in order to mimic inflammatory conditions led to a specific decrease of mRNA levels of IGF-1 splicing variants (IGF-1Ea and MGF) to about 25% and of the muscle differentiation marker MyoD to 57% with the maximum effect at 12h but remaining low until 48h. Tests whether this down-regulation would be associated with an altered expression of muscle-specific miRNAs (miR-1, 133a, 133b, and 206) were negative at all of the tested time points (1, 3, 6, 12, 24 and 48h). However, it remains unsolved whether one or more of the other 630 currently known mouse miRNAs (miRBase sequence database version 12.0) are influenced by TNF-α or any other inflammatory mediator (28).

MiRNAs not only play an important role in muscle growth but also in skeletal muscle atrophy as a consequence of immobility or ageing. Unilateral fixation of mice hindlimb in a cast for 5 days caused an opposite response of miR-696 and its potential target PGC1α as observed after running exercise as miR-696 was enhanced whereas PGC1α was decreased (1). Comprehensive profiling of rat soleus muscle in response to hindlimb suspension revealed 151 miRs which were expressed in the soleus muscle and the expression of 18 miRs was significantly changed after 2 and/or 7 days of unloading. The expression of miR-499 and miR-208b resulted in the activation of the so called MyomiR network which regulates slow myosin expression during muscle atrophy (51). The age-induced loss of muscle mass, referred to as sarcopenia, is associated with increased falls, fractures, morbidity, and loss of independence. It has been shown that ageing alters levels of pri-miRNA-1-1, -1-2, -133a-1, and -133a-2 at baseline as well as the miRNA response to an anabolic stimulus (resistance exercise + ingestion of a 20-

g leucine-enriched essential amino acid solution), when pri-miRNA-1-2, -133a-1, -133a-2 and miR-1 were reduced after exercise only in the young men (19). Skeletal muscle loss is also seen in septic patients treated at the intensive care unit which results in the patients' protracted recovery process. Gene ontology analysis of microarray data demonstrated that losses of mitochondria and muscle mass are accompanied by sustained protein synthesis (anabolic process) while dysregulation of transcription programmes involving miR-21 appears to fail to compensate for increased damage and proteolysis (23).

Taken together, all these data suggest that miRNAs play important roles in the plasticity of skeletal muscle in response to hypertrophy but also atrophy signals, whereby local but also systemic inflammatory processes accompany the remodeling of skeletal muscle tissue. However, data especially derived form human studies are sparse, possibly due to the fact that skeletal muscle biopsies are not as easy to access and to investigate than e.g. immune cells located in the blood stream.

Future directions

The ability of miRNAs to influence the expression of multiple genes in a variety of conditions offers a new area of research but also of therapeutics and drug discovery. A disease-caused or -causing enhanced expression of a certain miRNA can be seen as a target for selective inhibition by silencing the respective miRNA (29). On the other hand it would be possible to effectively counter a loss of function by directly administering a miRNA (56). At the moment several pharmaceutical companies work on the development of miRNA-based therapeutics in areas such as oncology but also in immune or inflammatory diseases.

Moreover, many miRNAs show tissue-specific expression patterns and show a relatively high stability in plasma. These characteristics make them suitable candidates for monitoring tissue injury. In this regard liver-, muscle- and brain-specific miRNAs (miR-122, miR-133a, and miR-124) were found in samples from plasma of rats treated with liver or muscle toxicants and from a rat surgical model of stroke (32). Drug-induced liver injury is a frequent and harmful side effect of many drugs. In order to develop a diagnostic tool for the early detection of liver injury, miR-122 and miR-192 were found to be suitable candidates as their occurrence in serum parallels aminotransferase levels and the histopathology of liver degeneration, but their changes can be detected significantly earlier (84). Finally, attempts are made to use miRNA expression patterns in serum to correctly discriminate between normal and cancer patient samples (41).

Within the field of exercise immunology the investigation of miRNAs can contribute at various levels. Firstly, the puzzle about a clear mechanism of immunosuppression after intense exercise is still not resolved. MiRNAs might be involved in regulating the immune response. Secondly, muscle adaptation processes including local inflammation are regulated differently in dependency of gender, nutrition, age and the presence of wasting conditions such as systemic inflammation or cancer. MiRNAs potentially fine-tune these adaptations within the skeletal muscle. And finally, miRNAs also could be used as diagnostic tools for the detection of exercise-caused damage to skeletal muscle or the heart and guide the planning of efficient training routines.

CONCLUSIONS

In conclusion it is obvious that miRNAs play an important role in a variety of physiological and disease-associated processes including immunology and inflammation. Thereby they represent a mechanism by which the body is enabled to react very fast to various stimuli such as invading pathogens but also exercise. However, data are derived to a large extent from cell culture or animal models and need to be confirmed in human studies. Additionally, it is of upmost importance that targets which are predicted by computational tools or association studies are verified in a cause and relationship manner. Once this is implemented miRNAs might comprise interesting targets of diagnostics and therapeutics.

Disclosures
No competing interests

Abbreviations

miRNAs or miRs	microRNAs
pri-miRNA	primary miRNA
pre-miRNA	precursor miRNA
RLC	RISC loading complex
RISC	RNA-induced silencing complex
3' UTR	3' untranslated region
TLR	Toll-like receptor
IL	Interleukin
IRAK1	IL-1 receptor-associated kinase 1
TRAF6	TNF receptor-associated factor 6
FADD	Fas-associated death domain protein
IKKepsilon	IkappaB kinase epsilon
Ripk1	receptor-interacting serine-threonine kinase 1
TNF-α	tumour necrosis factor-α
KC	keratinocyte-derived chemokine
MIP-2	macrophage inflammatory protein-2
DC-SIGN	DC-specific intercellular adhesion molecule-3 grabbing non-integrin
LPS	lipopolysaccharide
bic	B-cell integration cluster
IRAK2	interleukin-1 receptor-associated kinase 2
BCL2	B-cell CLL/lymphoma 2
Treg cells	T regulatory cells
AP-1	activator- protein 1
SOCS1	suppressor of cytokine signaling 1
STAT5	signal transducer and activator of transcription 5
PGC1α	peroxisome proliferator-activated receptor gamma coactivator 1α

REFERENCES

1. Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, Yoshikawa T. The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab. 2010, 298: E799-806.
2. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009, 136: 215-233.
3. Bazzoni F, Rossato M, Fabbri M, Gaudiosi D, Mirolo M, Mori L, Tamassia N, Mantovani A, Cassatella MA, Locati M. Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. Proc Natl Acad Sci U S A. 2009, 106: 5282-5287.
4. Bi Y, Liu G, Yang R. MicroRNAs: novel regulators during the immune response. J Cell Physiol. 2009, 218: 467-472.
5. Bollati V, Marinelli B, Apostoli P, Bonzini M, Nordio F, Hoxha M, Pegoraro V, Motta V, Tarantini L, Cantone L, Schwartz J, Bertazzi PA, Baccarelli A. Exposure to Metal-rich Particulate Matter Modifies the Expression of Candidate MicroRNAs in Peripheral Blood Leukocytes. Environ Health Perspect. 2010. Epub ehead of print.
6. Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol. 2006, 13: 1097-1101.
7. Brengues M, Teixeira D, Parker R. Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science. 2005, 310: 486-489.
8. Buford TW, Cooke MB, Shelmadine BD, Hudson GM, Redd L, Willoughby DS. Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle. Appl Physiol Nutr Metab. 2009, 34: 745-753.
9. Buford TW, Cooke MB, Willoughby DS. Resistance exercise-induced changes of inflammatory gene expression within human skeletal muscle. Eur J Appl Physiol. 2009, 107: 463-471.
10. Buttner P, Mosig S, Lechtermann A, Funke H, Mooren FC. Exercise affects the gene expression profiles of human white blood cells. J Appl Physiol. 2007, 102: 26-36.
11. Cannon JG, St Pierre BA. Cytokines in exertion-induced skeletal muscle injury. Mol Cell Biochem. 1998, 179: 159-167.
12. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004, 303: 83-86.
13. Cobb BS, Hertweck A, Smith J, O'Connor E, Graf D, Cook T, Smale ST, Sakaguchi S, Livesey FJ, Fisher AG, Merkenschlager M. A role for Dicer in immune regulation. J Exp Med. 2006, 203: 2519-2527.
14. Cobb BS, Nesterova TB, Thompson E, Hertweck A, O'Connor E, Godwin J, Wilson CB, Brockdorff N, Fisher AG, Smale ST, Merkenschlager M. T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. J Exp Med. 2005, 201: 1367-1373.
15. Condorelli G, Latronico MV, Dorn GW, 2nd. microRNAs in heart disease: putative novel therapeutic targets? Eur Heart J. 2010. Epub ahead of print.
16. Connolly PH, Caiozzo VJ, Zaldivar F, Nemet D, Larson J, Hung SP, Heck JD, Hatfield GW, Cooper DM. Effects of exercise on gene expression in human peripheral blood mononuclear cells. J Appl Physiol. 2004, 97: 1461-1469.
17. Curtale G, Citarella F, Carissimi C, Goldoni M, Carucci N, Fulci V, Franceschini D, Meloni F, Barnaba V, Macino G. An emerging player in the adaptive immune

response: microRNA-146a is a modulator of IL-2 expression and activation-induced cell death in T lymphocytes. Blood. 2010, 115: 265-273.
18. Doench JG, Sharp PA. Specificity of microRNA target selection in translational repression. Genes Dev. 2004, 18: 504-511.
19. Drummond MJ, McCarthy JJ, Fry CS, Esser KA, Rasmussen BB. Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. Am J Physiol Endocrinol Metab. 2008, 295: E1333-1340.
20. Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc Natl Acad Sci U S A. 2005, 102: 3627-3632.
21. Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, Lidov HG, Kang PB, North KN, Mitrani-Rosenbaum S, Flanigan KM, Neely LA, Whitney D, Beggs AH, Kohane IS, Kunkel LM. Distinctive patterns of microRNA expression in primary muscular disorders. Proc Natl Acad Sci U S A. 2007, 104: 17016-17021.
22. Fehrenbach E. Multifarious microarray-based gene expression patterns in response to exercise. J Appl Physiol. 2007, 102: 7-8.
23. Fredriksson K, Tjader I, Keller P, Petrovic N, Ahlman B, Scheele C, Wernerman J, Timmons JA, Rooyackers O. Dysregulation of mitochondrial dynamics and the muscle transcriptome in ICU patients suffering from sepsis induced multiple organ failure. PLoS One. 2008, 3: e3686.
24. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009, 19: 92-105.
25. Gregory RI, Chendrimada TP, Shiekhattar R. MicroRNA biogenesis: isolation and characterization of the microprocessor complex. Methods Mol Biol. 2006, 342: 33-47.
26. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. miRBase: tools for microRNA genomics. Nucleic Acids Res. 2008, 36: D154-158.
27. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell. 2007, 27: 91-105.
28. Gryadunov-Masutti L, Strasser EM, Bachl N, Roth E, Wessner B. Influence of TNF-a, IL-6 and LPS on growth-regulatory pathways in C2C12 skeletal muscle cells. European Surgery. 2009, 41: S10.
29. John M, Constien R, Akinc A, Goldberg M, Moon YA, Spranger M, Hadwiger P, Soutschek J, Vornlocher HP, Manoharan M, Stoffel M, Langer R, Anderson DG, Horton JD, Koteliansky V, Bumcrot D. Effective RNAi-mediated gene silencing without interruption of the endogenous microRNA pathway. Nature. 2007, 449: 745-747.
30. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol. 2007, 8: 191-197.
31. Koralov SB, Muljo SA, Galler GR, Krek A, Chakraborty T, Kanellopoulou C, Jensen K, Cobb BS, Merkenschlager M, Rajewsky N, Rajewsky K. Dicer ablation affects antibody diversity and cell survival in the B lymphocyte lineage. Cell. 2008, 132: 860-874.
32. Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, Johnson JM, Sina JF, Fare TL, Sistare FD, Glaab WE. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem. 2009, 55: 1977-1983.

33. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science. 2001, 294: 858-862.
34. Lau PW, MacRae IJ. The molecular machines that mediate microRNA maturation. J Cell Mol Med. 2009, 13: 54-60.
35. Lee EJ, Baek M, Gusev Y, Brackett DJ, Nuovo GJ, Schmittgen TD. Systematic evaluation of microRNA processing patterns in tissues, cell lines, and tumors. Rna. 2008, 14: 35-42.
36. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993, 75: 843-854.
37. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. Embo J. 2004, 23: 4051-4060.
38. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005, 120: 15-20.
39. Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, Liu G, Braich R, Manoharan M, Soutschek J, Skare P, Klein LO, Davis MM, Chen CZ. miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell. 2007, 129: 147-161.
40. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature. 2005, 433: 769-773.
41. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One. 2009, 4: e6229.
42. Lu LF, Liston A. MicroRNA in the immune system, microRNA as an immune system. Immunology. 2009, 127: 291-298.
43. Lu LF, Rudensky A. Molecular orchestration of differentiation and function of regulatory T cells. Genes Dev. 2009, 23: 1270-1282.
44. Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, Loeb GB, Lee H, Yoshimura A, Rajewsky K, Rudensky AY. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity. 2009, 30: 80-91.
45. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004, 303: 95-98.
46. Malm C. Exercise immunology: the current state of man and mouse. Sports Med. 2004, 34: 555-566.
47. Martinez-Nunez RT, Louafi F, Friedmann PS, Sanchez-Elsner T. MicroRNA-155 modulates the pathogen binding ability of dendritic cells (DCs) by down-regulation of DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN). J Biol Chem. 2009, 284: 16334-16342.
48. Mattick JS, Makunin IV. Small regulatory RNAs in mammals. Hum Mol Genet. 2005, 14 Spec No 1: R121-132.
49. McCarthy JJ. MicroRNA-206: the skeletal muscle-specific myomiR. Biochim Biophys Acta. 2008, 1779: 682-691.
50. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. J Appl Physiol. 2007, 102: 306-313.
51. McCarthy JJ, Esser KA, Peterson CA, Dupont-Versteegden EE. Evidence of MyomiR network regulation of beta-myosin heavy chain gene expression during skeletal muscle atrophy. Physiol Genomics. 2009, 39: 219-226.

52. Monticelli S, Ansel KM, Xiao C, Socci ND, Krichevsky AM, Thai TH, Rajewsky N, Marks DS, Sander C, Rajewsky K, Rao A, Kosik KS. MicroRNA profiling of the murine hematopoietic system. Genome Biol. 2005, 6: R71.
53. Moschos SA, Williams AE, Perry MM, Birrell MA, Belvisi MG, Lindsay MA. Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. BMC Genomics. 2007, 8: 240.
54. Muljo SA, Ansel KM, Kanellopoulou C, Livingston DM, Rao A, Rajewsky K. Aberrant T cell differentiation in the absence of Dicer. J Exp Med. 2005, 202: 261-269.
55. Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced tolerance: IMPLICATION IN INNATE IMMUNITY. J Biol Chem. 2009, 284: 34590-34599.
56. Nakasa T, Ishikawa M, Shi M, Shibuya H, Adachi N, Ochi M. Acceleration of muscle regeneration by local injection of muscle-specific microRNAs in rat skeletal muscle injury model. J Cell Mol Med. 2009.
57. Negrini M, Nicoloso MS, Calin GA. MicroRNAs and cancer--new paradigms in molecular oncology. Curr Opin Cell Biol. 2009, 21: 470-479.
58. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci U S A. 2007, 104: 1604-1609.
59. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol. 1999, 515 (Pt 1): 287-291.
60. Peake J, Nosaka K, Suzuki K. Characterization of inflammatory responses to eccentric exercise in humans. Exerc Immunol Rev. 2005, 11: 64-85.
61. Perry MM, Moschos SA, Williams AE, Shepherd NJ, Larner-Svensson HM, Lindsay MA. Rapid changes in microRNA-146a expression negatively regulate the IL-1beta-induced inflammatory response in human lung alveolar epithelial cells. J Immunol. 2008, 180: 5689-5698.
62. Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol. 2007, 17: 118-126.
63. Proske U, Allen TJ. Damage to skeletal muscle from eccentric exercise. Exerc Sport Sci Rev. 2005, 33: 98-104.
64. Radom-Aizik S, Zaldivar F, Jr., Leu SY, Galassetti P, Cooper DM. Effects of 30 min of aerobic exercise on gene expression in human neutrophils. J Appl Physiol. 2008, 104: 236-243.
65. Radom-Aizik S, Zaldivar FP, Jr., Oliver SR, Galassetti PR, Cooper DM. Evidence for microRNA Involvement in Exercise-Associated Neutrophil Gene Expression Changes. J Appl Physiol. 2010.
66. Ramkissoon SH, Mainwaring LA, Ogasawara Y, Keyvanfar K, McCoy JP, Jr., Sloand EM, Kajigaya S, Young NS. Hematopoietic-specific microRNA expression in human cells. Leuk Res. 2006, 30: 643-647.
67. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. Requirement of bic/microRNA-155 for normal immune function. Science. 2007, 316: 608-611.
68. Ruggiero T, Trabucchi M, De Santa F, Zupo S, Harfe BD, McManus MT, Rosenfeld MG, Briata P, Gherzi R. LPS induces KH-type splicing regulatory protein-dependent

processing of microRNA-155 precursors in macrophages. Faseb J. 2009, 23: 2898-2908.
69. Safdar A, Abadi A, Akhtar M, Hettinga BP, Tarnopolsky MA. miRNA in the regulation of skeletal muscle adaptation to acute endurance exercise in C57Bl/6J male mice. PLoS One. 2009, 4: e5610.
70. Saini HK, Griffiths-Jones S, Enright AJ. Genomic analysis of human microRNA transcripts. Proc Natl Acad Sci U S A. 2007, 104: 17719-17724.
71. Schmidt WM, Spiel AO, Jilma B, Wolzt M, Muller M. In vivo profile of the human leukocyte microRNA response to endotoxemia. Biochem Biophys Res Commun. 2009, 380: 437-441.
72. Schneider BS, Tiidus PM. Neutrophil infiltration in exercise-injured skeletal muscle: how do we resolve the controversy? Sports Med. 2007, 37: 837-856.
73. Shomron N, Levy C. MicroRNA-biogenesis and Pre-mRNA splicing crosstalk. J Biomed Biotechnol. 2009, 2009: 594678.
74. Simon P, Fehrenbach E, Niess AM. Regulation of immediate early gene expression by exercise: short cuts for the adaptation of immune function. Exerc Immunol Rev. 2006, 12: 112-131.
75. Smith C, Kruger MJ, Smith RM, Myburgh KH. The inflammatory response to skeletal muscle injury: illuminating complexities. Sports Med. 2008, 38: 947-969.
76. Sonkoly E, Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. J Cell Mol Med. 2009, 13: 24-38.
77. Sonkoly E, Pivarcsi A. microRNAs in inflammation. Int Rev Immunol. 2009, 28: 535-561.
78. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A. 2006, 103: 12481-12486.
79. Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Frendewey D, Valenzuela D, Kutok JL, Schmidt-Supprian M, Rajewsky N, Yancopoulos G, Rao A, Rajewsky K. Regulation of the germinal center response by microRNA-155. Science. 2007, 316: 604-608.
80. Tili E, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, Fabbri M, Alder H, Liu CG, Calin GA, Croce CM. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol. 2007, 179: 5082-5089.
81. Tsitsiou E, Lindsay MA. microRNAs and the immune response. Curr Opin Pharmacol. 2009, 9: 514-520.
82. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, Newman J, Bronson RT, Crowley D, Stone JR, Jaenisch R, Sharp PA, Jacks T. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. Cell. 2008, 132: 875-886.
83. Vigorito E, Perks KL, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, Das PP, Miska EA, Rodriguez A, Bradley A, Smith KG, Rada C, Enright AJ, Toellner KM, Maclennan IC, Turner M. microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity. 2007, 27: 847-859.
84. Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, Hu Z, Hood LE, Galas DJ. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A. 2009, 106: 4402-4407.

85. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol. 2009, 11: 228-234.
86. Xiao B, Liu Z, Li BS, Tang B, Li W, Guo G, Shi Y, Wang F, Wu Y, Tong WD, Guo H, Mao XH, Zou QM. Induction of microRNA-155 during Helicobacter pylori infection and its negative regulatory role in the inflammatory response. J Infect Dis. 2009, 200: 916-925.
87. Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, Henderson JM, Kutok JL, Rajewsky K. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 2008, 9: 405-414.
88. Zhou B, Wang S, Mayr C, Bartel DP, Lodish HF. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. Proc Natl Acad Sci U S A. 2007, 104: 7080-7085.
89. Zieker D, Fehrenbach E, Dietzsch J, Fliegner J, Waidmann M, Nieselt K, Gebicke-Haerter P, Spanagel R, Simon P, Niess AM, Northoff H. cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. Physiol Genomics. 2005, 23: 287-294.