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Preoperative serum circulating microRNAs as potential biomarkers for chronic postoperative pain after total knee replacement

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Abstract

Background: Chronic postoperative pain affects approximately 20% of patients with knee osteoarthritis after total knee replacement. Circulating microRNAs can be found in serum and might act as biomarkers in a variety of diseases. The current study aimed to investigate the preoperative expression of circulating microRNAs as potential predictive biomarkers for the development of chronic postoperative pain in the year following total knee replacement.

Methods: Serum samples, collected preoperatively from 136 knee osteoarthritis patients, were analyzed for 21 circulatory microRNAs. Pain intensity was assessed using a visual analog scale before and one year after total knee replacement. Patients were divided into a low-pain relief group (pain relief percentage $<30\%$) and a high-pain relief group (pain relief percentage $>30\%$) based on their pain relief one year after total knee replacement, and differences in microRNAs expression were analyzed between the two groups.

Results: We found that three microRNAs were preoperatively dysregulated in serum in the low-pain relief group compared with the high-pain relief group. MicroRNAs hsa-miR-146a-5p, -145-5p, and -130 b-3p exhibited fold changes of 1.50, 1.55, and 1.61, respectively, between the groups (all P values < 0.05). Hsa-miR-146a-5p and preoperative pain intensity correlated positively with postoperative pain relief (respectively, $R = 0.300$, $P = 0.006$; $R = 0.500$, $P < 0.001$).

Discussion: This study showed that patients with a low postoperative pain relief present a dysregulation of circulating microRNAs. Altered circulatory microRNAs expression correlated with postoperative pain relief, indicating that microRNAs can serve as predictive biomarkers of pain outcome after surgery and hence may foster new strategies for preventing chronic postoperative pain after total knee replacement (TKR).

Keywords

Knee osteoarthritis, circulating microRNA, pain, serum biomarker

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Introduction

Osteoarthritis (OA) is the most frequent painful musculoskeletal diagnosis in the elderly population and the most prominent cause of disability.[1] Total knee

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replacement (TKR) is the end-stage treatment of knee OA and provides pain relief for the majority of patients with severe OA. However, around 20% of knee OA patients will experience chronic postoperative pain after TKR surgery.[2,3] Several studies have found that high preoperative pain intensities[4,5] and sensitization of central pain pathways[6] act as predictors for chronic postoperative pain following TKR.

Recently, an exploratory study found that certain preoperative pro-inflammatory cytokines were associated with the development of chronic postoperative pain following TKR.[7] There is evidence that pro-inflammatory and anti-inflammatory cytokines are involved in pain.[8] Consequently, there is increasing interest in the evaluation of small non-coding RNAs, defined by Sommer et al. as "master switches," acting in the development and maintenance of inflammation and pain.[9] The action of circulating non-coding RNAs, already verified in pathologies such as cancer and autoimmune disease, is receiving increasing attention in the pain field.[10–12] In this context, studies have shown that small non-coding RNAs are directly involved in the production of cytokines.[13,14]

This indicates that the preoperative assessment of non-coding RNAs could be a potential prognostic biomarker venue in terms of assessing the risk for chronic postoperative pain following TKR and may represent a way to explore new therapeutic opportunities.

MicroRNA (miRNA) is a group of small non-coding RNAs (~20–25 nucleotides)[15] involved in post-transcriptional gene expression regulation, with more than 7000 miRNAs recognized in human between precursor and mature structure.[16] Unlike the majority of miRNAs, which are usually detectable intracellularly, *circulating* or *extracellular* miRNAs have also been found in the interstitial environment, cell culture media, and in different biological fluids such as serum or plasma.[17–20] Moreover, circulating miRNAs are known to be protected from the action of degradation enzymes, due to their inclusion in extracellular microvesicles[18] or formation of protein-miRNA complexes.[19] This makes the circulating miRNAs highly stable and their expression can be quantified in body fluids, making them suitable to act as potential biomarkers.[21] Circulating miRNAs have been found to be dysregulated in patients with various chronic pain conditions including complex regional pain syndrome,[22] migraine,[23] peripheral neuropathy,[24] and fibromyalgia when compared to healthy controls.[24] Recently, a study highlighted the different functions as well as potential diagnostic and predictive value of miRNAs in various pain states,[25] but no study thus far has assessed the potential use of circulating miRNA as predictive biomarkers for chronic postoperative pain. The current study aimed to investigate the preoperative expression of circulating miRNAs validated or predicted in previous study to be associated with OA pain and inflammation, as potential predictive biomarkers for the development of chronic postoperative pain one year after TKR.

Material and methods

Patients

One hundred thirty-six patients with a knee osteoarthritis (KOA) scheduled for TKR were recruited consecutively from the outpatient clinic at Hospital Vendsyssel, Frederikshavn, Denmark, and tested for preoperative miRNAs expression in serum. Patients with other diagnosed pain conditions (e.g., hip OA, rheumatoid arthritis, fibromyalgia, and neuropathic pain), sensory dysfunction, or mental impairment were excluded from the study. Radiological KOA progression was evaluated using the Kellgren and Lawrence (KL) score.[26] The KL score is a radiological assessment score of knee OA. The score ranges from 0 (no OA) to 4 (severe OA).[26]

The patients were asked not to take any analgesic medication, such as non-steroidal anti-inflammatory drugs or paracetamol, 24 h before the pain scoring examination. The study was approved by The North Denmark Region Committee on Health Research Ethics (N-20120015) and conducted in accordance with the Helsinki Declaration. All patients read and signed an informed consent form prior to enrollment.

Pain assessment

Before surgery and one year after, the peak pain intensity within the last 24 h was collected (using the visual analog scale, hereafter VAS). The patients were asked to rate their pain intensity on the VAS scale from "0–10" where "0" represents "no pain" and "10" represents "worst pain imaginable." The patients were divided into two groups based on the percentage of postoperative pain relief (the difference between pre- and postoperative VAS scores divided by preoperative VAS score). Patients achieving >30% pain relief after TKR were assigned to the "high-pain relief" group, whereas patients who achieved <30% pain relief were assigned to the "low-pain relief" group. This classification for the patients was based on the minimum clinical relevance observed in previous study.[27]

Blood withdrawal and microRNA isolation

Venous blood was collected following standard procedures from patients before they underwent the surgery, between 07:30 and 09:00 in the morning. For the real-time PCR (qRT-PCR), 9 ml of whole blood was withdrawn in an untreated tube. After collection, the whole blood was left at room temperature for 15 min and

allowed to clot; the serum was then separated from clotted cells by low-speed centrifugation (3000 rpm) for 15 min to allow the serum separation. The serum obtained was stored at −80°C until used. After thawing on ice, circulating miRNAs were isolated from 200 µl of serum using miRNeasy Serum/Plasma Advanced Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. As a positive control, 3.5 µl (1.6×10^8 copies/µl) of Serum/Plasma Spike-in Control cel-miR-39-3p (QIAGEN, Hilden, Germany) was added to each reaction during the extraction protocol.

MicroRNA array expression

Isolated miRNAs were retrotranscribed to cDNA, using miScript II RT Kit (QIAGEN, Hilden, Germany) in a thermocycler (Applied Biosystems, Foster City, CA, USA) at 37°C for 1 h with heat inactivation of the retro-transcriptase at 95°C for 5 min. To perform qRT-PCR, miScript SYBR® Green qPCR Kit (QIAGEN, Germany) was added to each sample. Furthermore, this mixture was aliquoted into the wells of Custom miScript miRNA PCR Array (QIAGEN, Germany). The cDNA for every patient was analyzed for 21 putative miRNAs candidates known to be associated with inflammation, pain, and cartilage degeneration. Plates were pre-manufactured by QIAGEN and contained specific primers for miRNAs of interest based on previous evidence of their detection in human body fluids, and their involvement in pathological OA pathways, inflammation process, and findings of direct connections with pain sensation (see Table 1). Thermal cycling conditions consisted of a hot start at 95°C for 15 min followed by 40 cycles of each qPCR step: (denaturation) 94°C for 15 s, (annealing) 55°C for 30 s and (extension) 70°C for 30 s (AriaMx Agilent Technologies, Santa Clara, CA, USA). A melting curve analysis was carried out to ensure the specificity of the corresponding qRT-PCR reactions. The Cycle quantification (Cq) data obtained setting a single threshold of 12 between the assays using Agilent Aria software 5.1 (Agilent Technologies, Santa Clara, CA, USA), exported to a Microsoft Excel file (Microsoft 2016) and subsequently uploaded in Statistical Package for Social Sciences (SPSS, v. 25, IBM).

Statistical analysis

Before the statistical analysis, the raw Cq values were normalized using the Global Mean normalization method on the expression of all miRNA assays.[51] Initially, patient demographics were calculated for the two groups with chi-square tests for categorical data and *t*-tests for continuous data. The qRT-PCR data were transformed into the fold change domain, defined as measure describing how much a quantity changes between an original and a subsequent measurement. Unpaired Student's *t*-tests were used to compare the normalized Cq values for each miRNA between the two groups of patients. The data have been visualized in a

Table 1. MicroRNA candidates list and list of microRNA sequences contained in the RT-qPCR customized array.

miRNA	miRBase#	miRNA sequence	References
hsa-miR-146a-5p	MIMAT0000449	ugagaacugaauuccauggguu	28,29
hsa-miR-29a-3p	MIMAT0000086	uagcaccaucugaaaucgguua	30,31
hsa-miR-29b-3p	MIMAT0000100	uagcaccauuugaaaucaguguu	31
hsa-miR-183-5p	MIMAT0000261	uauggcacugguagaauucacu	32
hsa-miR-149-5p	MIMAT0000450	ucuggcuccgugucuucacuccc	33
hsa-miR-145-5p	MIMAT0000437	guccaguuuucccaggaaucccu	34
hsa-miR-16-5p	MIMAT0000069	uagcagcacguaaauauuggcg	35
hsa-miR-103a-3p	MIMAT0000101	agcagcauuguacagggcuauga	36
hsa-miR-320a	MIMAT0000510	aaaagcuggguugagagggcga	36,37
hsa-miR-374b-5p	MIMAT0004955	auauaauacaaccugcuaagug	36
hsa-miR-93-5p	MIMAT0000093	caaagugcuguucgugcagguag	38
hsa-miR-19a-3p	MIMAT0000073	ugugcaaaucuaugcaaaacuga	39
hsa-miR-19b-3p	MIMAT0000074	ugugcaaauccaugcaaaacuga	39,40
hsa-miR-195-5p	MIMAT0000461	uagcagcacagaaauauuggc	41,42
hsa-miR-92a-3p	MIMAT0000092	uauugcacuugucccggccugu	39,40
hsa-miR-130a-3p	MIMAT0000425	cagugcaauguuaaaagggcau	43
hsa-miR-130b-3p	MIMAT0000691	cagugcaaugaugaaagggcau	44,45
hsa-miR-17-5p	MIMAT0000070	caaagugcuuacagugcagguag	39
hsa-miR-155-5p	MIMAT0000646	uuaaugcuaaucgugauagggguu	29,46,47
hsa-miR-106b-5p	MIMAT0000680	uaaagugcugacagugcagau	48
hsa-miR-34a-5p	MIMAT0000255	uggcagugucuuagcugguugu	49,50

miRNA: microRNA.

volcano plot, combining P values (y-axis) with the fold changes (x-axis), thus highlighting miRNAs with higher or lower levels of expression. Correlations were conducted using Pearson's correlation coefficients. Linear regression models were constructed to predict the pain relief following TKR, using the preoperative pain intensity and the miRNAs, which were significantly different between the two groups. Furthermore, linear regressions were utilized to identify independent preoperative predictive factors based on the miRNAs. Data analysis was performed in SPSS software V.25 (IBM, Armonk, NY, USA). $P < 0.05$ was considered significant.

In silico target prediction

A gene target analysis was conducted for the differentially expressed miRNAs to identify genes that represent putative targets. In silico prediction analyses were performed using DIANA-Tool TarBase v.8,[52] choosing the predicted targeted mRNA genes by their prediction score with a value between 0.5 and 1.0. To determine the biological relevance of the predicted mRNA genes targeted by the miRNAs, gene ontology (GO) analysis was subsequently explored using PANTHER classification system[53] and Reactome (www.reactome.org). Manual curation of targets associated with the immune system process, biological regulation, cellular process, and response to stimuli was performed.

Results

Demographics

Of 136 patients with KOA (82 female), 114 patients (84%) had pain relief percentage >30% (high-pain relief group) and 22 patients (16%) had pain relief percentage <30% (low-pain relief group). No significant differences were found between the two groups regarding preoperative patient demographics or KL (Table 2). Moreover, no statistical difference was found for preoperative pain intensity in patients when subdivided for gender ($P = 0.944$). From the 82 females enrolled in the study, 82% were in the high-pain relief group and 18% in the low-pain relief group. From the 54 men enrolled in the study, 87% were in the high-pain relief group and 13% in the low-pain relief group.

MiRNA serum assays quality control

MiRNAs amplification cut-off was set at 35 cycles (Figure S1). Moreover, to avoid "false positive" results, a melt curve analysis was carried out to ensure the specificity of the corresponding RT-qPCR reactions. Figure S2 shows that the assessed miRNAs peak at the same time as the positive control cel-miR-39-3p (both peak at temperatures of 76.5°C). Conversely, the PPC (internal PCR positive control) peaked at higher temperatures (81.5°C). The single aligned peak for assessed miRNA and cel-miR-39-3p suggests that no unspecific amplicons were produced.

Differential expression of miRNAs in patients with low-pain relief following TKR

Three miRNAs were differentially expressed between low-pain relief and high-pain relief group (a volcano plot is displayed in Figure 1). Hsa-miR-146a-5p (fold change $= 1.50 \pm 0.86$ SD, P value $= 0.021$), hsa-miR-145-5p (fold change $= 1.55 \pm 1.24$ SD, P value $= 0.037$), and hsa-miR-130b-3p (fold change $= 1.61 \pm 1.4$ SD, P value $= 0.039$) had a significantly higher expression in the low-pain relief group relative to the high-pain group.

Prediction of postoperative pain relief

Pooling all patient data showed significant Pearson correlations between pain relief and preoperative pain intensity ($R = 0.500$, $P < 0.001$) and hsa-miR-146a-5p ($R = 0.300$, $P = 0.006$). Furthermore, linear regression models were established to investigate the predictive value using the significant miRNAs hsa-miR-146a-5p, hsa-miR-130b-3p, hsa-miR-145-5p, and preoperative pain intensity. Model 1 consisted of all the parameters with a predictive value (R^2) of 30% and identified preoperative pain intensity ($P < 0.001$) as significant factors (Table 3). Model 2 was constructed using a backward selection of the parameters included in model 1 and identified preoperative pain intensity ($P < 0.001$) as a significantly independent parameter for postoperative pain

Table 2. Demographic characteristics of sub grouped patients with severe knee osteoarthritis before total knee replacement.

	High pain relief	Low pain relief	P value
Number of patients, n	114	22	
Sex, female, %	58.7	68.1	
Preoperative pain VAS score, cm, mean ± SEM	6.56 ± 1.8	6.32 ± 1.8	0.58
BMI, kg/m2, mean ± SEM	28.8 ± 4.6	30.78 ± 4.6	0.08
Age, y, mean ± SEM	69.03 ± 8.7	68.00 ± 10.1	0.62
KL, mean (range)	3.77 (2–4)	3.68 (2–4)	0.43

BMI: body mass index; KL: Kellgren and Lawrence radiological scores; SEM: standard error of the mean; VAS: visual analog scale (0–10).

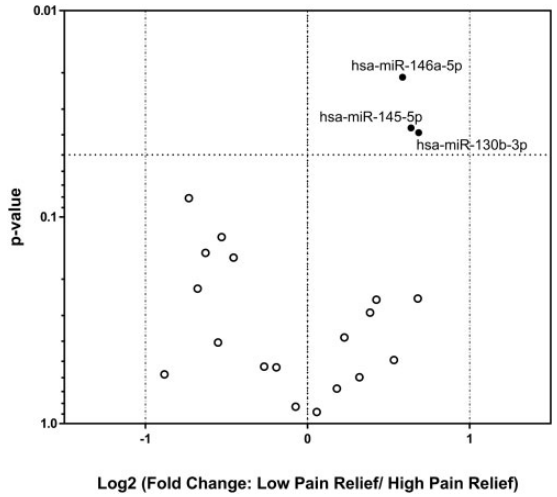

Figure 1. Volcano plot of the differential serum miRNAs expression. Statistical significance versus fold change was showed on the y- and x-axes, respectively. Volcano plot of data shows the fold change of the 21 assessed miRNAs in patients with less than 30% pain relief (low-pain relief group) compared with patients with more than 30% pain relief (high-pain relief group) following total knee replacement. Three miRNAs exhibited higher significantly (black dots) expression ($P < 0.05$) between the two groups.

Table 3. Linear regression models of preoperative pain intensity, hsa-miR-146a-5p, hsa-miR-130b-3p, and hsa-miR-145-5p aiming to predict postoperative pain relief in patients with knee osteoarthritis following total knee replacement.

Model	Variable	Standardized coefficient	P value	R_2
1				0.29
	Preoperative pain intensity	0.522	<0.001	
	miRNA-146a-5p	0.167	0.096	
	miRNA-145-5p	−0.013	0.889	
	miRNA-130b-3p	0.91	0.359	
2				0.30
	Preoperative pain intensity	0.504	<0.001	
	miRNA-146a-5p	0.183	0.063	

Note: Model 1 consists of all the parameters and model 2 is constructed using backward selection. R^2 indicates the combined predictive value. miRNA: microRNA.

relief prediction with a value of R^2 of 30% and showed a trend of hsa-miR-146a-5p ($P = 0.06$) (Table 3, model 2).

Potential target mRNA genes of differentially expressed circulating miRNAs

In silico target prediction analyses, through DIANA-TarBase v.8, were performed to highlight the potential mRNA targets of the dysregulated miRNAs. An mRNA was considered a potential target if it has been validated with *high/low throughput experiment* and if its *prediction score* was between 0.5 and 1.0. Using these criteria, the potential number of mRNA targets for the hsa-miR-146a-5p was 103, for hsa-miR-145-5p was 35, and for hsa-miR-130b-3p was 337. All the targeted genes were analyzed for the GO analysis of the biological processes (Figure 2). The majority of the identified gene targets were involved in various cellular and metabolic processes. Our interest in qualitative exploration were genes related to sensory transduction, inflammatory response, and neuronal sensitization. The interleukin-1 receptor-associated kinase 1 (IRAK1) gene was found to be targeted by hsa-miR-146a-5p and is involved broadly in cytokine-mediated signaling as well as the toll-like receptor signaling pathways.[54,55] Moreover, the hsa-miR-145-5p, which was expressed at higher levels in the low-pain relief group, had transcription-factor JunB (JUNB) as a target. This transcription factor is part of the family of the Jun-protein normally identified as a transcriptional repressor; however, there is evidence that shows its involvement in the positive transcription regulation of IL-2, IL-4, IL-6, and TNF-α[56–58] and its activation by external or endogenous stimuli leads to a cellular control, regulating pathway of differentiation or cell death (osteoclast and osteoblast genesis and proliferation).[59–61]

Discussion

The current study is the first to assess preoperative circulating miRNAs as serological preoperative predictors for postoperative pain relief one year after TKR in painful knee OA patients. Higher levels of hsa-miR-146a-5p, hsa-miR-145-5p and hsa-miR-130b-3p were demonstrated in patients with low-pain relief as compared to patients with high postoperative pain relief. Furthermore, preoperative pain intensity was found to be an independent predictor of postoperative pain relief with a trend for hsa-miR-146a-5p.

The current analysis highlights that hsa-miR-146a-5p, hsa-miR-145-5p, and hsa-miR-130b-3p showed higher preoperatively levels in serum of patients with low postoperative pain relief one year after TKR. All of these miRNAs have been assessed in previous study, where their action to different pathological conditions, for example, cancer, has been proved through high-throughput analysis.[62–64] As previously stated, these miRNAs are involved in numerous processes that can include metabolic, cellular, or pathological processes. In this study, their involvement in inflammatory processes regulation seemed to be of considerable interest since inflammation is one of the main actors for the sensitization of peripheral nerve endings that leads to pain.[8]

Hsa-miR-146a-5p is codified by a gene located on chromosome 5 and its mature product differs only by 2–6 nucleotides in the 3′ region from the other products of miRNA-146's family.[65] It has been shown that

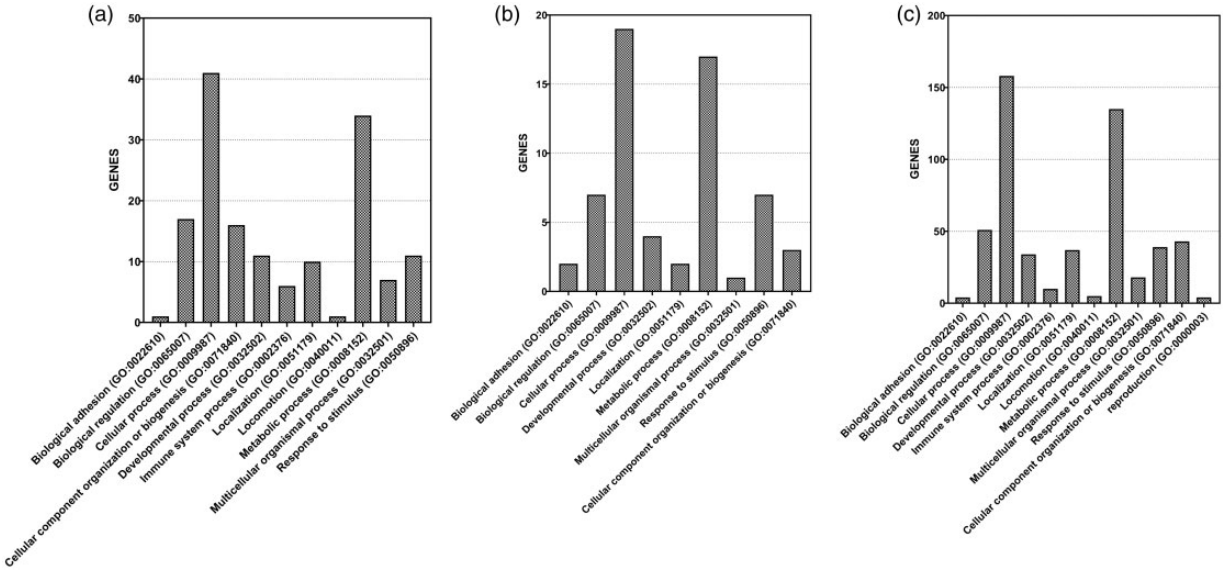

Figure 2. Gene ontology analysis. (a) Bar chart of biological process for genes regulated by hsa-miR-146a-5p. (b) Bar chart of biological process for genes regulated by hsa-miR-145-5p. (c) Bar chart of biological process for genes regulated by hsa-miR-130b-3p.

hsa-miR-146a-5p expression is regulated by nuclear factor kappa B (NFκB), induced by the action of proinflammatory mediators and in response to the activation of the innate immune response in monocytes and macrophages.[66]Hsa-miR-146a-5p has been shown to be upregulated in synovial tissues of patients with rheumatoid arthritis when compared to healthy controls, and its level of expression is stimulated by inflammatory cytokines such as tumor necrosis factor α (TNF-α) and Interleukin-1β (IL-1β).[67] A previous study has suggested that suggest that hsa-miR-146a-5p is expressed in OA cartilage at higher levels than in normal cartilage.[68] In addition, a study found higher levels of miR-146-5p in peripheral human blood mononuclear cells in early-stage OA patients when compared with healthy controls, indicating that the dysregulation of miR-146-5p is important for the initial stage of OA.[69] In a mouse model, Lu et al. showed higher levels of miR-146-5p may partially attenuate neuropathic pain conditions in rats by decreasing the expression of TNF receptor-associated factor 6 (TRAF6).[70]Hsa-miR-146a-5p also governs feedback of cytokine expression, and consequently the production of cartilage–degrading enzymes, such as matrix metalloproteinase (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS).[28] The current study shows that hsa-miR-146a-5p is present at higher levels circulating in serum of patients with low chronic postoperative pain relief and shows a trend for this miRNA as an independent predictor of postoperative pain relief.

Recent studies have highlighted that high levels of TNF-α and IL-1β induce the expression of hsa-miR-145-5p, a pathway involved in the pathogenesis of OA.[34,71] Previously, hsa-miR-145-5p has also been identified as a regulator of chondrogenic differentiation, and miRNA microarray data show that hsa-miR-145-5p is significantly upregulated in human chondrocytes isolated from KOA patients at the last stage (K&L scale III and IV) of the pathology.[71] The expression of hsa-miR-145-5p is sensitive to the level of TNF-α and is implicated in modulating the expression of TNF-α-induced enzymes such as MMPs and ADAMTS, which are involved in the cartilage disruption and OA progression.[34] Furthermore, the hsa-miR-145-5p expression is significantly upregulated in OA chondrocytes in response to IL-1β stimulation, which is important for cartilage degradation.[71–73]Has-miR-145-5p have also been found to be involved in another painful condition wherein cerebrospinal fluid of patients with fibromyalgia was strongly downregulated compared with healthy controls, but higher levels of it were found to correlate to higher levels of pain and fatigue.[74] The present results show preoperative higher expression levels of hsa-miR-145-5p in the serum of patients with post-operative low-pain relief, suggesting that hsa-miR-145-5p might increase and sustain the inflammation in low postoperative pain relief patients causing their painful condition. However, further studies to confirm this hypothesis are needed.

Hsa-miR-130b-3p is a well-known marker, studied in cellular and animal models, involved at different level in the progression and maintenance of different kinds of cancer,[75,76] but it has also been shown to be involved in chondrogenesis and osteogenesis,[77] associated with obesity, insulin resistance, and other pathophysiological processes.[45,78] A previous study has shown how

inflammatory signals, such as TNFα and IL-1β, induce its action in adipose inflamed tissue.[44] So far, the presented results show a higher expression of hsa-miR-130b-3p in patients with low chronic postoperative pain relief. Future studies are needed to demonstrate whether the involvement of this miRNA can be directly related to the postoperative pain condition in patients with KOA and then serve as a new biomarker for it.

In this study, before the qRT-PCR, a per sample quantification of the extracted total RNA was not performed; however, by employing a standardized kit and several internal control assays (PPC, miRTC, cel-miR-39-3p), the quality of the qRT-PCR was validated. Data normalization, a considerable challenge for circulating miRNA data analysis given the lack of robust house-keeping genes,[79,80] was performed using the common global mean normalization approach.[51] Moreover, adjustments for multiple comparisons using the Benjamini–Hochberg method (false discovery rate, FDR) was not performed due to the exploratory nature of this study. Many cell types are capable of secreting and receiving circulating free and exosomal miRNAs,[18,19,21] which makes it impossible to determine the tissue origin as well as the recipient cells of the presently observed dysregulated miRNAs. Future research on the transporting mechanisms of circulating miRNAs is needed. With this in mind, in silico target predictions should be interpreted with caution until luciferase assays have been conducted on suspected miRNA-mRNA interactions of interest. In addition, interactions between miRNA and circulating metabolites released in the blood stream by any pharmaceutical treatment was not evaluated.

The current study utilized a 30% decrease in pain after surgery as a cut-off for chronic postoperative pain. This yielded 16% of patients with chronic postoperative pain, which is in line with the overall risk for chronic postoperative pain following TKR,[3] but it is important to note that different cut-offs have been utilized previously.[4,5]

Results obtained from this study and the biological pathways potentially involved in the action of miRNAs highlighted need to be better investigated and validated in further studies, and a second independent cohort of patients. Females were frequent in the current study and further research is encouraged to address gender differences in expressions of miRNA in relation to chronic postoperative pain.

In conclusion, this is the first study to show that postoperative pain is associated with specific preoperative serum circulating miRNA signatures. In the subset of patients who developed chronic pain one year after TKR, hsa-miR-146a-5p, hsa-miR-145-5p, and hsa-miR-130b-3p showed higher levels of expression. Furthermore, a prediction model shows that preoperative pain intensity is an independent predictive factor for postoperative pain relief and highlights a trend for hsa-miR-146a-5p, although further validation analysis is needed in this regard. This exploratory study gives the first insight into preoperative circulating miRNAs dysregulation and how they can serve as potential biomarkers for postoperative pain condition.

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Declaration of Conflicting Interests

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Supplemental material

Supplemental material for this article is available online.

References

1. Peat G, McCarney R, Croft P. Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care. *Ann Rheum Dis* 2001; 60: 91–97.
2. Petersen KK, Arendt-Nielsen L. Chronic postoperative pain after joint replacement. *Pain* 2016; 24: 1–6.
3. Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P. What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. *BMJ Open* 2012; 2: e000435.
4. Petersen KK, Arendt-Nielsen L, Simonsen O, Wilder-Smith O, Laursen MB. Presurgical assessment of temporal summation of pain predicts the development of chronic postoperative pain 12 months after total knee replacement. *Pain* 2015; 156: 55–61.

5. Petersen KK, Graven-Nielsen T, Simonsen O, Laursen MB, Arendt-Nielsen L. Preoperative pain mechanisms assessed by cuff algometry are associated with chronic postoperative pain relief after total knee replacement. *Pain* 2016; 157: 1400–1406.
6. Arendt-Nielsen L. Pain sensitisation in osteoarthritis. *Clin Exp Rheumatol* 2017; 35: S68–74.
7. Gandhi R, Santone D, Takahashi M, Dessouki O, Mahomed NN. Inflammatory predictors of ongoing pain 2 years following knee replacement surgery. *Knee* 2013; 20: 316–318.
8. Calvo M, Dawes JM, Bennett DL. The role of the immune system in the generation of neuropathic pain. *Lancet Neurol* 2012; 11: 629–642.
9. Sommer C, Leinders M, Üçeyler N. Inflammation in the pathophysiology of neuropathic pain. *Pain* 2018; 159: 595–602.
10. Furer V, Greenberg JD, Attur M, Abramson SB, Pillinger MH. The role of microRNA in rheumatoid arthritis and other autoimmune diseases. *Clin Immunol* 2010; 136: 1–15.
11. Henriksen M, Johnsen KB, Andersen HH, Pilgaard L, Duroux M. MicroRNA expression signatures determine prognosis and survival in glioblastoma multiforme—a systematic overview. *Mol Neurobiol* 2014; 50: 896–913.
12. Kress M, Hüttenhofer A, Landry M, Kuner R, Favereaux A, Greenberg D, Bednarik J, Heppenstall P, Kronenberg F, Malcangio M, Rittner H, Üçeyler N, Trajanoski Z, Mouritzen P, Birklein F, Sommer C, Soreq H. microRNAs in nociceptive circuits as predictors of future clinical applications. *Front Mol Neurosci. Frontiers* 2013; 6: 33.
13. Zhao J, Lee M-C, Momin A, Cendan C-M, Shepherd ST, Baker MD, Asante C, Bee L, Bethry A, Perkins JR, Nassar MA, Abrahamsen B, Dickenson A, Cobb BS, Merkenschlager M, Wood JN. Small RNAs control sodium channel expression, nociceptor excitability, and pain thresholds. *J Neurosci* 2010; 30: 10860–10871.
14. Bali KK, Kuner R. Noncoding RNAs: key molecules in understanding and treating pain. *Trends Mol Med* 2014; 20: 437–448.
15. Hammond SM. An overview of microRNAs [Internet]. *Adv Drug Deliv Rev* 2015; 87: 3–14.
16. Londin E, Loher P, Telonis AG, Quann K, Clark P, Jing Y, Hatzimichael E, Kirino Y, Honda S, Lally M, Ramratnam B, Comstock CES, Knudsen KE, Gomella L, Spaeth GL, Hark L, Katz LJ, Witkiewicz A, Rostami A, Jimenez SA, Hollingsworth MA, Yeh JJ, Shaw CA, McKenzie SE, Bray P, Nelson PT, Zupo S, Van Roosbroeck K, Keating MJ, Calin GA, Yeo C, Jimbo M, Cozzitorto J, Brody JR, Delgrosso K, Mattick JS, Fortina P, Rigoutsos I. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. *Proc Natl Acad Sci USA* 2015; 112: E1106–E1115.
17. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee MLT, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. Lo YMD, editor. *PLoS One* 2008; 3: e3694.
18. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007; 9: 654–659.
19. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci USA* 2011; 108: 5003–5008.
20. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang C-Y. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; 18: 997–1006.
21. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics [Internet]. *J Cell Physiol* 2016; 231: 25–30.
22. Orlova IA, Alexander GM, Qureshi RA, Sacan A, Graziano A, Barrett JE, Schwartzman RJ, Ajit SK. MicroRNA modulation in complex regional pain syndrome. *J Transl Med* 2011; 9: 195.
23. Andersen HH, Duroux M, Gazerani P. Serum MicroRNA signatures in migraineurs during attacks and in pain-free periods. *Mol Neurobiol* 2016; 53: 1494–1500.
24. Leinders M, Doppler K, Klein T, Deckart M, Rittner H, Sommer C, Üçeyler N. Increased cutaneous miR-let-7d expression correlates with small nerve fiber pathology in patients with fibromyalgia syndrome. *Pain* 2016; 157: 2493–2503.
25. Andersen HH, Duroux M, Gazerani P. MicroRNAs as modulators and biomarkers of inflammatory and neuropathic pain conditions. *Neurobiol Dis* 2014; 71: 159–168.
26. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis* 1957; 16: 494–502.
27. Arendt-Nielsen L, Egsgaard LL, Petersen KK. Evidence for a central mode of action for etoricoxib (COX-2 inhibitor) in patients with painful knee osteoarthritis. *Pain* 2016; 157: 1634–1644.
28. Li X, Gibson G, Kim J-S, Kroin J, Xu S, van Wijnen AJ, Im H-J. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. *Gene* 2011; 480: 34–41.
29. Soyocak A, Kurt H, Ozgen M, Turgut Cosan D, Colak E, Gunes HV. miRNA-146a, miRNA-155 and JNK expression levels in peripheral blood mononuclear cells according to grade of knee osteoarthritis. *Gene* 2017; 627: 207–211.
30. Ko JY, Lee MS, Lian WS, Weng WT, Sun YC, Chen YS, Wang FS. MicroRNA-29a counteracts synovitis in knee osteoarthritis pathogenesis by targeting VEGF. *Sci Rep* 2017; 7: 3584.
31. Le LTT, Swingler TE, Crowe N, Vincent TL, Barter MJ, Donell ST, Delany AM, Dalmay T, Young DA, Clark IM. The microRNA-29 family in cartilage homeostasis and osteoarthritis. *J Mol Med* 2016; 94: 583–596.
32. Li X, Kroin JS, Kc R, Gibson G, Chen D, Corbett GT, Pahan K, Fayyaz S, Kim JS, Van Wijnen AJ, Suh J, Kim

SG, Im HJ. Altered spinal MicroRNA-146a and the microRNA-183 cluster contribute to osteoarthritic pain in knee joints. *J Bone Miner Res* 2013; 28: 2512–2522.
33. Santini P, Politi L, Vedova PD, Scandurra R, Scotto d'Abusco A. The inflammatory circuitry of miR-149 as a pathological mechanism in osteoarthritis. *Rheumatol Int* 2014; 34: 711–716.
34. Hu G, Zhao X, Wang CC, Geng Y, Zhao J, Xu J, Zuo B, Zhao C, Wang CC, Zhang X. MicroRNA-145 attenuates TNF-α-driven cartilage matrix degradation in osteoarthritis via direct suppression of MKK4. *Cell Death Dis* 2017; 8: e3140.
35. Yu X-M, Meng H-Y, Yuan X-L, Wang Y, Guo Q-Y, Peng J, Wang A-Y, Lu S-B. MicroRNAs' involvement in osteoarthritis and the prospects for treatments. *Evid Based Complement Altern Med* 2015; 2015: 1–13.
36. Bjersing JL, Bokarewa MI, Mannerkorpi K. Profile of circulating microRNAs in fibromyalgia and their relation to symptom severity: an exploratory study. *Rheumatol Int* 2015; 35: 635–642.
37. Jin Y, Chen X, Gao ZY, Liu K, Hou Y, Zheng J. The role of mir-320a and il-1β in human chondrocyte degradation. *Bone Joint Res* 2017; 6: 196–203.
38. Borgonio Cuadra VM, González-Huerta NC, Romero-Córdoba S, Hidalgo-Miranda A, Miranda-Duarte A. Altered expression of circulating microRNA in plasma of patients with primary osteoarthritis and in silico analysis of their pathways. *PLoS One* 2014; 9: e97690.
39. Sakai A, Saitow F, Maruyama M, Miyake N, Miyake K, Shimada T, Okada T, Suzuki H. MicroRNA cluster miR-17-92 regulates multiple functionally related voltage-gated potassium channels in chronic neuropathic pain. *Nat Commun* 2017; 8: 16079.
40. Kong R, Gao J, Si Y, Zhao D. Combination of circulating miR-19b-3p, miR-122-5p and miR-486-5p expressions correlates with risk and disease severity of knee osteoarthritis. *Am J Transl Res* 2017; 9: 2852–2864.
41. Bras JP, Silva AM, Calin GA, Barbosa MA, Santos SG, Almeida MI. miR-195 inhibits macrophages pro-inflammatory profile and impacts the crosstalk with smooth muscle cells. *PLoS One* 2017; 12: e0188530.
42. Bai R, Zhao AQ, Zhao ZQ, Liu WL, Jiang DM. MicroRNA-195 induced apoptosis in hypoxic chondrocytes by targeting hypoxia-inducible factor 1 alpha. *Eur Rev Med Pharmacol Sci* 2015; 19: 545–551.
43. Li Z-C, Han N, Li X, Li G, Liu Y-Z, Sun G-X, Wang Y, Chen G-T, Li G-F. Decreased expression of microRNA-130a correlates with TNF-α in the development of osteoarthritis. *Int J Clin Exp Pathol* 2015; 8: 2555–2564.
44. Kim C, Lee H, Cho YM, Kwon OJ, Kim W, Lee EK. TNFα-induced miR-130 resulted in adipocyte dysfunction during obesity-related inflammation. *FEBS Lett* 2013; 587: 3853–3858.
45. Lv C, Zhou Y, Hong Wu C, Shao Y, Lu C, Lu, Wang QY. The changes in miR-130b levels in human serum and the correlation with the severity of diabetic nephropathy. *Diabetes Metab Res Rev* 2015; 31: 717–724.
46. Ceppi M, Pereira PM, Dunand-Sauthier I, Barras E, Reith W, Santos MA, Pierre P. 155 Modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. *Proc Natl Acad Sci USA* 2009; 106: 2735–2740.
47. Tan Y, Yang J, Xiang K, Tan Q, Guo Q. Suppression of MicroRNA-155 attenuates neuropathic pain by regulating SOCS1 signalling pathway. *Neurochem Res* 2015; 40: 550–560.
48. Tao Y, Wang Z, Wang L, Shi J, Guo X, Zhou W, Wu X, Liu Y, Zhang W, Yang H, Shi Q, Xu Y, Geng D. Downregulation of miR-106b attenuates inflammatory responses and joint damage in collagen-induced arthritis. *Rheumatology (Oxford)* 2017; 56: 1804–1813.
49. Abouheif MM, Nakasa T, Shibuya H, Niimoto T, Kongcharoensombat W, Ochi M. Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro. *Rheumatology (Oxford)* 2010; 49: 2054–2060.
50. Yan S, Wang M, Zhao J, Zhang H, Zhou C, Jin L, Zhang Y, Qiu X, Ma B, Fan Q. MicroRNA-34a affects chondrocyte apoptosis and proliferation by targeting the SIRT1/p53 signaling pathway during the pathogenesis of osteoarthritis. *Int J Mol Med* 2016; 38: 201–209.
51. D'haene B, Mestdagh P, Hellemans J, Vandesompele J. miRNA expression profiling: from reference genes to global mean normalization. In: *Next-generation MicroRNA expression profiling technology*. Totowa: Humana Press, 2012, pp. 261–272.
52. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, Papadimitriou D, Kavakiotis I, Maniou S, Skoufos G, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. *Nucleic Acids Res* 2018; 46: D239–D245.
53. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function analysis with the panther classification system. *Nat Protoc* 2013; 8: 1551–1566.
54. Hou J, Wang P, Lin L, Liu X, Ma F, An H, Wang Z, Cao X. MicroRNA-146a feedback inhibits RIG-I-dependent type I IFN production in macrophages by targeting TRAF6, IRAK1, and IRAK2. *J Immunol* 2009; 183: 2150–2158.
55. Ye E-A, Steinle JJ. miR-146a attenuates inflammatory pathways mediated by TLR4/NF-κB and TNFα to protect primary human retinal microvascular endothelial cells grown in high glucose. *Mediators Inflamm* 2016; 2016: 1–9.
56. Gomard T, Michaud H-A, Tempé D, Thiolon K, Pelegrin M, Piechaczyk M. An NF-κB–dependent role for JunB in the induction of proinflammatory cytokines in LPS-activated bone marrow–derived dendritic cells. *PLoS One* 2010; 5: e9585.
57. Garaude J, Farrás R, Bossis G, Charni S, Piechaczyk M, Hipskind RA, Villalba M. SUMOylation regulates the transcriptional activity of JunB in T lymphocytes. *J Immunol* 2008; 180: 5983–5990.
58. Li B, Tournier C, Davis RJ, Flavell RA. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *Embo J* 1999; 18: 420–432.

59. Chalaux E, Lopez-Rovira T, Rosa JL, Bartrons R, Ventura F. JunB is involved in the inhibition of myogenic differentiation by bone morphogenetic protein-2. *J Biol Chem* 1998; 273: 537–543.
60. Wang N, Liu W, Tan T, Dong C-Q, Lin D-Y, Zhao J, Yu C, Luo X-J. Notch signaling negatively regulates BMP9-induced osteogenic differentiation of mesenchymal progenitor cells by inhibiting JunB expression. *Oncotarget* 2017; 8: 109661–109674.
61. Piechaczyk M, Farràs R. Regulation and function of JunB in cell proliferation. *Biochem Soc Trans* 2008; 36: 864–867.
62. Hu X-Y, Li L, Wu H-T, Liu Y, Wang B-D, Tang Y. Serum miR-130b level, an ideal marker for monitoring the recurrence and prognosis of primary hepatocellular carcinoma after radiofrequency ablation treatment. *Pathol – Res Pract* 2018; 214: 1655–1660.
63. Luo J, Si Z-Z, Li T, Li J-Q, Zhang Z-Q, Chen G-S, Qi H-Z, Yao H-L. MicroRNA-146a-5p enhances radiosensitivity in hepatocellular carcinoma through replication protein A3 induced activation of the DNA repair pathway. *Am J Physiol Physiol* 2019; 316: C299–C311.
64. Li B, Ding C, Li Y, Peng J, Geng N, Qin W. MicroRNA-145 inhibits migration and induces apoptosis in human non-small cell lung cancer cells through regulation of the EGFR/PI3K/AKT signaling pathway. *Oncol Rep* 2018; 40: 2944–2954.
65. Griffiths-Jones S. The microRNA registry. *Nucleic Acids Res* 2004; 32: 109D–1011.
66. Taganov KD, Boldin MP, Chang K-J, Baltimore D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* 2006; 103: 12481–12486.
67. Nakasa T, Miyaki S, Okubo A, Hashimoto M, Nishida K, Ochi M, Asahara H. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum* 2008; 58: 1284–1292.
68. Yamasaki K, Nakasa T, Miyaki S, Ishikawa M, Deie M, Adachi N, Yasunaga Y, Asahara H, Ochi M. Expression of microRNA-146a in osteoarthritis cartilage. *Arthritis Rheum* 2009; 60: 1035–1041.
69. Okuhara A, Nakasa T, Shibuya H, Niimoto T, Adachi N, Deie M, Ochi M. Changes in microRNA expression in peripheral mononuclear cells according to the progression of osteoarthritis. *Mod Rheumatol* 2012; 22: 446–457.
70. Lu Y, Cao D-LL, Jiang B-CC, Yang T, Gao Y-JJ. MicroRNA-146a-5p attenuates neuropathic pain via suppressing TRAF6 signaling in the spinal cord. *Brain Behav Immun* 2015; 49: 119–129.
71. Yang B, Kang X, Xing Y, Dou C, Kang F, Li J, Quan Y, Dong S. Effect of microRNA-145 on IL-1β-induced cartilage degradation in human chondrocytes. *FEBS Lett* 2014; 588: 2344–2352.
72. Goldring MB. Articular cartilage degradation in osteoarthritis. *HSS J* 2012; 8: 7–9.
73. Hashimoto M, Nakasa T, Hikata T, Asahara H. Molecular network of cartilage homeostasis and osteoarthritis [Internet]. *Med Res Rev* 2008; 28: 464–481.
74. Bjersing JL, Lundborg C, Bokarewa MI, Mannerkorpi K. Profile of cerebrospinal microRNAs in fibromyalgia. *PLoS One* 2013; 8: e78762.
75. Zhang Q, Zhang B, Sun L, Yan Q, Zhang Y, Zhang Z, Su Y, Wang C. MicroRNA-130b targets PTEN to induce resistance to cisplatin in lung cancer cells by activating wnt/β-catenin pathway. *Cell Biochem Funct* 2018; 36: 194–202.
76. Gu J-J, Fan K-C, Zhang J-H, Chen H-J, Wang S-S. Suppression of microRNA-130b inhibits glioma cell proliferation and invasion, and induces apoptosis by PTEN/AKT signaling. *Int J Mol Med* 2018; 41: 284–292.
77. Han J, Yang T, Gao J, Wu J, Qiu X, Fan Q, Ma B. Specific microRNA expression during chondrogenesis of human mesenchymal stem cells. *Int J Mol Med* 2010; 25: 377–384.
78. Ma Y, Shi J, Wang F, Li S, Wang J, Zhu C, Li L, Lu H, Li C, Yan J, Zhang X, Jiang H. MiR-130b increases fibrosis of HMC cells by regulating the TGF-β1 pathway in diabetic nephropathy. *J Cell Biochem* 2019; 120: 4044–4013.
79. Hardikar AA, Farr RJ, Joglekar MV. Circulating microRNAs: understanding the limits for quantitative measurement by real-time PCR. *J Am Heart Assoc* 2014; 3: e000792.
80. Rice J, Roberts H, Rai SN, Galandiuk S. Housekeeping genes for studies of plasma microRNA: a need for more precise standardization. *Surg (United States)* 2015; 158: 1345–1351.