Association of presurgical circulating MicroRNAs with 1-year postsurgical pain reduction in spine facet osteoarthritis patients with lumbar spinal stenosis

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ABSTRACT

Purpose: Up to 30% of spine facet osteoarthritis patients with lumbar spinal stenosis (SF-OA + LSS) have little to no improvement in their pain after surgery. Lack of meaningful improvement in pain following surgery provides a unique opportunity to identify specific predictive biomarker signatures that might be associated with the outcomes of surgical treatment. The objective of the present study was to determine whether a microRNA (miRNA) biomarker signature could be identified in presurgical blood plasma that corresponded with levels of SF-OA + LSS patient post-surgical pain intensity one year later.

Methods: RNA was extracted from baseline plasma of SF-OA + LSS patients and prepared for miRNA sequencing. Statistical approaches were performed to identify differentially expressed miRNAs associated with reduced 1-year postsurgical pain intensity (n=56). Using an integrated computational approach, we further created predicted gene and pathway networks for each identified miRNA.

Results: We identified a panel of 4 circulating candidate miRNAs (hsa-miR-155-5p, hsa-let-7e-5p, hsa-miR-125a-5p, hsa-miR-99b-5p) with higher levels at presurgical baseline that were associated with greater changes in %NPRS20, reflecting reduced pain intensity levels at one year. Genes encoding hsa-let-7e-5p, hsa-miR-125a-5p, and hsa-miR-99b-5p are part of an evolutionarily conserved miRNA cluster. Using integrated computational analyses, we showed that mammalian target of rapamycin, transforming growth factor-β receptor, Wnt signaling, epithelial–mesenchymal transition regulators, and cholecystokinin signaling were enriched pathways of predicted gene targets.

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1. Introduction

In any given year, low back pain (LBP) affects many adults worldwide. Among patients with persistent LBP, 41% have a clinical profile and symptoms consistent with spine facet osteoarthritis-induced lumbar spinal stenosis (SF-OA) [1], the principal cause of chronic back (and leg/neurogenic claudication) pain for 1 in 5 adults over the age of 65 and is the primary reason for spinal surgery in this age group [2]. While surgery is successful for many, 30% of SF-OA + LSS patients show little to no improvement in their post-surgical pain [3] indicating ‘surgical non-response’ [4].

Currently, surgical success is gauged by patient-reported outcome measures (PROMs) [5]. Demographic, physical, and psychological factors have been extensively studied but show limited ability to explain the factors have been extensively studied but show limited ability to explain the surgical pain response.

### Table 1

| Characteristics | All SF-OA + LSS Patients | Large Reduction >70% NPRS20Δ | Mild Reduction 31–69% NPRS20Δ | Poor Reduction <30% NPRS20Δ | q-value |
|-----------------|--------------------------|-------------------------------|-------------------------------|-------------------------------|---------|
| Demographics    |                          |                               |                               |                               |         |
| Age (y)         | 69 (63–76)               | 67 (61–73)                   | 71 (60–76)                   | 71 (66–76)                   | 0.879   |
| Height (m)      | 1.7 (1.6–1.8)            | 1.7 (1.6–1.8)                | 1.7 (1.6–1.7)                | 1.7 (1.6–1.7)                | 0.852   |
| Weight (kg)     | 77.0 (70.8–92.8)         | 81.8 (74.5–92.0)             | 76.6 (68.5–92.9)             | 75.0 (69.5–89.3)             | 0.886   |
| BMI (kg/m²)     | 28 (25–32)               | 28 (25–31)                   | 27 (26–30)                   | 30 (25–33)                   | 0.969   |
| Diagnose and treatment |               |                               |                               |                               |         |
| Degenerative spondylolisthesis | 27 (48%)              | 11 (48%)                     | 9 (50%)                      | 7 (47%)                      | 0.981   |
| Multi-level SF-OA | 44 (79%)                | 18 (78%)                     | 13 (72%)                     | 13 (87%)                     | 0.852   |
| Multi-level disc degeneration | 49 (88%)                | 19 (83%)                     | 16 (89%)                     | 14 (93%)                     | 0.852   |
| Decompression   | 35 (63%)                 | 14 (61%)                     | 10 (56%)                     | 11 (73%)                     | 0.852   |
| Decompression + fusion | 21 (38%)                    | 9 (39%)                     | 8 (44%)                      | 4 (27%)                      |         |
| Back and leg pain |                         |                               |                               |                               |         |
| NPRS Back baseline | 6 (4–8)                     | 7 (5–8)                     | 6 (5–8)                      | 5 (2–7)                      | 0.590   |
| NPRS Back baseline | 7 (5–8)                     | 8 (4–8)                     | 7 (5–8)                      | 6 (4–8)                      | 0.879   |
| ODI 1-year      | 42 (32–56)               | 38 (27–44)                   | 49 (36–58)                   | 52 (32–61)                   | 0.273   |
| SF-12 PCS baseline | 53 (43–57)                | 53 (51–58)                   | 51 (44–56)                   | 48 (40–57)                   | 0.628   |
| SF-12 PCS baseline | 30 (26–38)                | 32 (27–39)                   | 26 (22–32)                   | 30 (26–38)                   | 0.312   |
| Daily medication use |                          |                               |                               |                               |         |
| OTCs            | 25 (45%)                 | 8 (35%)                      | 11 (61%)                     | 6 (40%)                      | 0.607   |
| NSAIDs          | 9 (16%)                  | 3 (13%)                      | 4 (22%)                      | 2 (13%)                      | 0.874   |
| Muscle relaxants| 2 (4%)                   | 0 (0%)                       | 1 (6%)                       | 1 (7%)                       | 0.802   |
| Antidepressants | 13 (23%)                 | 6 (26%)                      | 4 (22%)                      | 3 (20%)                      | 0.969   |
| Co-morbidities  | 17 (30%)                 | 14 (7%)                      | 7 (39%)                      | 6 (40%)                      | 0.607   |
| Lung disease    | 4 (7%)                   | 3 (13%)                      | 1 (6%)                       | 0 (0%)                       | 0.643   |
| Diabetes        | 7 (13%)                  | 1 (4%)                       | 5 (28%)                      | 1 (7%)                       | 0.273   |
| Uterus/stomach disease | 2 (4%)                  | 1 (4%)                       | 1 (6%)                       | 0 (0%)                       | 0.874   |
| Cancer          | 6 (11%)                  | 2 (9%)                       | 2 (11%)                      | 2 (13%)                      | 0.969   |
| Thyroid problems| 6 (11%)                  | 2 (9%)                       | 1 (6%)                       | 3 (20%)                      | 0.700   |
| Sleep apnea     | 9 (16%)                  | 4 (17%)                      | 2 (11%)                      | 3 (20%)                      | 0.879   |
| Heart attack    | 5 (9%)                   | 1 (4%)                       | 3 (17%)                      | 1 (7%)                       | 0.700   |
| Stroke          | 1 (2%)                   | 0 (0%)                       | 0 (0%)                       | 1 (7%)                       | 0.616   |
| High cholesterol| 19 (34%)                 | 6 (26%)                      | 9 (50%)                      | 4 (27%)                      | 0.607   |
| High blood pressure | 24 (43%)                 | 7 (30%)                      | 9 (50%)                      | 8 (53%)                      | 0.643   |
| Depression      | 7 (13%)                  | 3 (13%)                      | 3 (17%)                      | 1 (7%)                       | 0.874   |
| Rheumatoid arthritis | 5 (9%)                 | 0 (0%)                       | 2 (11%)                      | 3 (20%)                      | 0.368   |
| Chronic neck pain | 5 (9%)                  | 1 (4%)                       | 1 (6%)                       | 3 (20%)                      | 0.607   |
| Migraine        | 5 (9%)                   | 2 (9%)                       | 1 (6%)                       | 2 (13%)                      | 0.879   |
| Chronic pelvic pain | 8 (14%)                | 2 (9%)                       | 3 (17%)                      | 3 (20%)                      | 0.852   |
| Fibromyalgia    | 1 (2%)                   | 0 (0%)                       | 0 (0%)                       | 1 (7%)                       | 0.616   |

Depending on the variable, either patient number and frequency (%) or median (with interquartile range) is listed. Q-values reflect the p-value FDR adjustment (0.05, B–H method); BMI, body mass index; LSS, lumbar spinal stenosis; MCS, mental component summary score; NPRS, numeric pain rating scale; NSAIDs, Prescription Non-Steroidal Anti-Inflammatory Drugs; ODI, Oswestry Disability Index; OTC, over-the-counter medication; SF-12, 12-Item Short-Form Health Survey; SF-OA, spinal facet osteoarthritis.
variance in surgical responses. Screening for easily accessible systemic biomarkers that correlate with postsurgical pain reduction has the potential to improve the precision of surgical decision-making. Evidence suggests that both arthritis and chronic pain are regulated by epigenetic factors, like microRNAs (miRNAs) [6]. MiRNAs are short, single-stranded, non-coding RNA molecules that post-transcriptionally modulate gene expression. Several miRNAs contribute to OA pathogenesis [7] and have been found to be dysregulated in various age-associated bone diseases, which can be measured in the circulating fluids, like plasma [8].

Next generation sequencing has emerged as an important technique helping to characterize the molecular profiles of different tissues and biofluids under various conditions, driving the identification of a wide range of potential biomarkers [9]. The primary purpose of this study was to use miRNA sequencing to determine if any miRNAs in presurgical SF-OA + LSS patient plasma corresponded with changes in pain intensity 1 year after spine surgery.

2. Methods

**Study cohort** This retrospective analysis of an ongoing prospective study [Longitudinal Evaluation in the Arthritis Program; LEAP-OA; University Health Network (UHN), Toronto, Canada] was conducted under the approval of UHN’s research ethics board (REB # 16-5759-BE). Written informed consent was obtained from all SF-OA + LSS participants (n = 60) at time of surgery (2015–2018). All patients had moderate to severe degrees of LSS. None of the patients had degenerative scoliosis, hepatitis A or B, kidney or liver disease, anemia or other blood-related disease, heart failure, dementia, or a previous spine procedure. All patients had persistent LBP and/or leg pain for a minimum of 6 weeks; however due to regional surgical wait-times, median symptom duration was >1 year for 82% of the patients, with 66% patients waiting >2 years. All patients received standard post-operative observational follow-up during clinic visits at 6 weeks, 3 months, 6 months, and 1 year with a 1-year post-surgical follow-up rate of 89%. Blood collected from patients at time of surgery was handled with care to avoid excess stress and agitation, remained chilled, and was processed within 4 h of blood draw, promptly storing plasma at −80 °C 24 h before transferring to liquid nitrogen for long-term storage.

**Patient groupings and characteristic analysis** Patients reported current back and leg pain using 0–10 numeric pain rating scales (NPRS) [10], which were summed to generate a NPRS20 score, a better indicator of overall patient pain [11]. Change in overall pain from baseline to 1 year post-surgery was assessed as percentage change in NPRS20 scores (% NPRS20Δ = \( \frac{\text{NPRS20}_{\text{1 year}} - \text{NPRS20}_{\text{baseline}}}{\text{NPRS20}_{\text{baseline}}} \times 100 \)), where 100% NPRS20Δ represents full recovery 1 year after surgery and negative scores reflect worsened pain. Surgical non-response was considered for SF-OA + LSS patients with <30% NPRS20Δ as this difference has been suggested as the minimum level of important change for patients with LSS and LBP [12,13]. Changes <30% NPRS20Δ were considered poor surgical responses, 31–69% changes were mild responses, and >70% changes were large responses. Differences in patient characteristics listed in Table 1 were assessed using Wilcoxon-Kruskal-Wallis test for continuous variables and Pearson chi-square test for categorical variables. False discovery rate (FDR) adjustment of 0.05 estimated by the Benjamini-Hochberg (BH) method was used to generate corrected q-values.

**microRNA sequencing** RNA was extracted from patient baseline plasma (miRNeasy Serum/Plasma Advanced Kit, 217204, Qiagen, Germany) and sequencing libraries prepared (QIAseq miRNA Library Kit, 331505, Qiagen) following manufacturer’s adaptations for biofluid samples. Libraries were sized electrophoretically (181 bp ± 2 bp (mean ± SD); high sensitivity DNA kit, 5067-4626, Agilent, USA) and quantified (99 ± 39 nM (mean ± SD); dsDNA high sensitivity 2-point fluorometric assay, DeNovix, USA). Libraries were volumetrically pooled in two
Fig. 2. Association of SF-OA + LSS patient baseline expression levels of 4 miRNAs with postsurgical pain intensity 1 year after surgery. Postsurgical changes in pain intensity was measured as a % change of NPRS20 scores (%NPRS20Δ). A) Volcano plot of fold changes (FC) in miRNA expression and corresponding FDR-adjusted p-values (q-value) in log 2 and −log 10 scales, respectively. Dashed lines delineate cut-off values (BH-FDR, 0.05; FC, 1.5). Red dots with arrows highlight differentially expressed (DE) miRNAs. B) Heat map summarizing expression levels of the 4 DE presurgical circulating miRNAs in SF-OA + LSS patients. Individual patient 1-year postsurgical pain response relative to the %NPRS20Δ median are indicated on the left. C) t-distributed stochastic embedding plot visualizing cluster assignments of the 4 DE miRNAs in SF-OA + LSS patients grouped according to %NPRS20Δ where red dots represent patients with >70% NPRS20Δ; and blue dots, <30% NPRS20Δ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
batches, balancing for diagnosis and sex, and sequenced on a NextSeq 550 sequencing system using a 75-cycle High Output Kit v2.5 (22024906, Illumina, USA) at the Centre for Arthritis Diagnostic and Therapeutic Innovation (Schroeder Arthritis Institute, Canada). Both runs generated high-quality data (mean Q30 scores of 93.8% ± 0.7 SD) with good post-filter yield (33G ± 2 SD). 4.0 ± 1.6 SD million reads mapped to the samples.

**Data processing** Raw sequencing data were de-multiplexed and converted to FASTQ format. UMI tags were extracted (UMI-tools v0.5.5). After discarding reads that were too short (<18 bp) or too long (>30 bp), Bowtie (v1.22) was used to align remaining reads to human mature miRNA sequences (miRBase v22.1) allowing no mismatches in seed regions. Any reads that did not align in miRBase were aligned to the human reference genome (vGRCh38) selecting for those falling between chromosomal coordinates of mature miRNAs. All aligned reads were deduplicated based on extracted UMI tags (UMI-tools) and summed to generate a final raw count matrix of the 2656 miRNAs [14,15].

**Data analyses** The raw matrix was normalized by total aligned sequences (i.e., each cell is divided by its library size) before filtering to retain only miRNAs with >10 counts per million in >2 samples. Sequencing data can be found in the Gene Expression Omnibus under accession number GSE158825. Four patients were omitted from differential expression (DE) analysis as they did not provide 1-year post-surgical PROM metrics. To identify DE miRNAs (n = 56), inference was performed on the filtered miRNA counts using the % NPRS20Δ as a continuous factor in a negative binomial regression estimated by maximum likelihood with trended dispersion [16]. Multivariable models were applied, controlling for sequencing batch effects, age, sex, and body mass index (BMI). The p-values were FDR-adjusted (0.05, BH). The analysis was implemented using DESeq2 package (v1.22.2, R).

**Target predictions and pathway analysis** Putative direct targets of miRNAs associated with the level of reduced pain (assessed by % NPRS20Δ) were identified using mirDIP (microRNA Data Integration Portal v4.1.11.1; database v4.1.0.3) [17]. Only targets with a very high integrative score (i.e. top 1% score class) were selected. All the putative direct targets obtained for the 4 miRNAs were used in an extended pathway association analysis using pathDIP (v4.0.21.2; database v4.0.7.0) [18], with protein interactions set adjusted to "experimentally detected PPIs" and minimum confidence level for predicted associations set at 0.99. Pathway plots were created in matplotlib library (v 3.2.1, Python). Networks were plotted in igraph (v1.2.4.2, R).

3. Results

3.1. SF-OA + LSS patient characteristics

Patients presented with a mean facet joint grading of 2.7 ± 0.6 (1–3) and mean intervertebral disc grading of 4.0 ± 1.0 (2–5) [19]. Of the 56
Fig. 4. Interactomes of hsa-miR-155-5p (A) and hsa-let-7e-5p (B). On the left, the top 50 miRNA-associated pathways are ranked by q-values (BH, 0.05) in –log10 scale. On the right, the top 50 pathways are ranked by q-values (BH, 0.05) in –log10 scale. Both are color-coded based on frequently occurring broad pathway descriptors indicated in the legend. Many predicted targets associated with multiple pathways. In such cases, node color depicts the highest-ranking pathway. All predicted targets contributing to the top 50 pathways are presented in the network; however due to their large number, only 1 in 5 is listed to maintain legibility. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
The evidence of the role of miRNAs in drug responses is increasing [20], which meet the surgical non-response cut-off. Of note, SF-OA expression levels of the 4 DE miRNAs and the % variation of their NPRS20+ levels were positively associated with 1-year % NPRS20. BMI, and compared to 1-year % NPRS20 could in negative binomial regression analysis. As shown in Figs. 2 and 4 miRNAs were identified (hsa-miR-155-5p, hsa-let-7e-5p, hsa-miR-125a-5p, hsa-miR-99b-5p) whose reductions in this group of SF-OA patients, 15 (27%) showed a poor response to surgery and did not have any clinically meaningful improvement in pain intensity 1 year later (ΔNPRS20 > 30%) (Table 1). Forty-one patients (73%) showed meaningful improvement in pain (reduction in NPRS20Δ of >30%), where 23 patients (41%) showed large reductions in pain (>70%) and 18 patients (32%) showed mild reductions of 31–69%. Patients with large, mild, and poor reductions in pain 1 year after surgery were comparable in terms of frequency of various demographic or pre-surgical PROM measures, including diagnosis and treatment, back and leg pain, daily medication use and co-morbidities. One year after surgery, patients with poor responses reported expectedly higher 1-year NPRS scores in all domains (back, leg, or NPRS20).

3.2. Baseline circulating miRNAs associated with 1-year postsurgical pain reduction

To identify miRNAs associated with 1-year postsurgical pain, baseline miRNA circulation profiles of SF-OA + LSS patients were compared with 1-year postsurgical pain responses. A chart of the sequencing workflow is depicted in Fig. 1. The miRNA expression profiles of all 56 SF-OA + LSS patients were adjusted for sequencing run, age, sex, and BMI, and compared to 1-year % NPRS20Δ as a continuous factor in the negative binomial regression analysis. As shown in Figs. 2 and 4 miRNAs (hsa-miR-155-5p, hsa-let-7e-5p, hsa-miR-125a-5p, hsa-miR-99b-5p) were identified for which SF-OA + LSS patient presurgical plasma levels were positively associated with 1-year % NPRS20Δ. Comparison of SF-OA + LSS patients with large (>70% NPRS20Δ) and poor (<30% NPRS20Δ) pain reductions using t-SNE visualization depicted clearer cluster segregation relative to randomly selected miRNAs (Fig. 2c). Mean expression levels of the 4 DE miRNAs and the % variation of their expression levels among the SF-OA + LSS patients showing large and poor reductions in 1-year postsurgical pain are listed in Online resource 1. Of note, SF-OA + LSS patients in both the large and mild response groups showed higher levels of all 4 miRNAs than patients who did not meet the surgical non-response cut-off (ΔNPRS20Δ) (Fig. 3). Evidence of the role of miRNAs in drug responses is increasing [20], which could influence the expression of the miRNA panel identified. Thus, we examined the distribution of the 4-miRNA panel across various patient characteristics, including their medication use, and found no associations (Online Resource 2). Hemolysis has also been shown to influence miRNA profiles [21–23]. All the plasma samples used in the present study were straw in colour and none were tagged in the data repository for hemolysis. In addition, we found no differences in the expression levels of miRNAs associated with red blood cells or hemolysis upon blood draw, in vitro, or bioinformatically among patients with large, mild, and poor pain reductions (Online Resource 3) indicating that hemolysis is an unlikely contributor to the expression changes of the presurgical baseline 4-miRNA panel that corresponded with 1-year postsurgical pain reductions in this group of SF-OA + LSS patients.

Putative gene targets were identified using miRDIP [17], which were then used to identify their associated pathways using pathDIP [18].

SF-OA + LSS patients, 15 (27%) showed a poor response to surgery and did not have any clinically meaningful improvement in pain intensity 1 year later (<30% NPRS20Δ) (Table 1). Forty-one patients (73%) showed meaningful improvement in pain (reduction in NPRS20Δ of >30%). Patients with large, mild, and poor reductions in pain 1 year after surgery were comparable in terms of frequency of various demographic or pre-surgical PROM measures, including diagnosis and treatment, back and leg pain, daily medication use and co-morbidities. One year after surgery, patients with poor responses reported expectedly higher 1-year NPRS scores in all domains (back, leg, or NPRS20).
Fig. 5. Interactomes of hsa-miR-125a-5p (A) and hsa-miR-99-5p (B). The top 50 enriched pathways (left) and corresponding putative gene targets (network nodes, right) are color-coded based on a set of frequently occurring broad pathway descriptors described in the network legend. Please see Fig. 4 legend for further details. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
recently found to correlate with worse leg pain in patients with lumbar degenerative disc disease [30]. Interestingly, miR-99b, let-7e and miR-125a form an evolutionarily conserved gene cluster on chromosome 19 in humans [31]. The miR99b/let-7e/miR-125a cluster helps maintain a suppressive inflammatory state in blood-derived monocytes by stabilizing signal transducer and activator of transcription 3 activity [32]. The cluster also plays a role in promoting osteoclastogenesis in peripheral blood monocytes stimulated with receptor activator of nuclear factor kappa-B ligand and granulocyte-macrophage colony-stimulating factor [33]. While it is not known what biological role hsa-miR99b-5p, hsa-let-7e-5p, hsa-miR-125a-5p, and hsa-miR-155-5p might play in SF-OA + LSS patient post-surgical pain reduction, our results suggest that inflammatory responses modulated by these miRNAs might contribute to SF-OA + LSS patient pain recovery. This finding certainly warrants further investigation.

One limitation of our study is the possibility of additional physiological factors and comorbidities contributing to patient circulating miRNA profiles, such as the presence of medication use, or other notable factors of interest, like sex, number of affected joints, non-spinal arthritis, diabetes, or high blood pressure [34]. In the future, the effects of concurrent patient variables on the identified miRNA panel should be examined using larger sample sizes, as well as compare the expression levels of these miRNAs in surgical SF-OA patients without LSS and in people without SF-OA. Understanding how different patient factors influence the candidate biomarker signature would not only help fill an important knowledge gap in this understudied field of spine OA but also help fine-tune patient decision-making when it comes to spine surgery.

Author contributions

Mohit Kapoor, Y. Raja Rampersaud, Anthony V. Perruccio and Starlee Lively contributed to the study conception and design. Mohit Kapoor and Y. Raja Rampersaud were involved in obtaining funding for this study. Y. Raja Rampersaud performed spine surgery on lumbar spinal stenosis patients with spine facet osteoarthritis, provided tissues, and performed MRI grading. Material preparation, data collection, analysis, and interpretation were performed by Starlee Lively, Marie Miliot, Pratibha Potla, Osvaldo Espin-Garcia, Mehdi Layeghifard, Kala Sundararajan, Helal Endisha, and Akihiro Nakamura. The first draft of the manuscript was written by Starlee Lively and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the University Health Network (UHN)’s research ethics board (REB # 16-5759-BE).
Consent to publish

Patients signed informed consent regarding publishing their data.

Data and materials availability

Sequencing data sets used for analysis during the current study have been deposited in the National Center for Bioinformatics Information Gene Expression Omnibus repository under accession code GSE158825. All data generated or analyzed during this study are included in this published article and its associated supplementary files.

Declaration of competing interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eceh.2022.100283.