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Original Article

Transcriptome-Wide High-Density Microarray Analysis Reveals Differential Gene Transcription in Periprosthetic Tissue From Hips With Chronic Periprosthetic Joint Infection vs Aseptic Loosening

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ABSTRACT

Background: Differentiating between periprosthetic hip infection and aseptic hip prosthesis loosening can be challenging, especially in patients with chronic infections. This study used whole-genome microarray analysis to investigate the transcriptomes of periprosthetic hip tissues to identify genes that are differentially transcripted between chronic periprosthetic hip infection and aseptic hip prosthesis loosening.

Methods: In this pilot study, a total of 24 patients with either chronic periprosthetic hip infection (n = 12) or aseptic hip prosthesis loosening (n = 12) were analyzed. Periprosthetic hip infection was diagnosed based on modified criteria of the Musculoskeletal Infection Society. To evaluate differences in gene transcription, whole-genome microarray analysis was performed on the mRNA of periprosthetic tissue.

Results: Microarray analysis revealed differential gene transcription in periprosthetic hip tissue affected by chronic hip infection vs aseptic hip prosthesis loosening. A total of 39 genes had area under the curve values greater than 0.9 for diagnosing chronic periprosthetic hip infection; 5 genes had annotations relevant to infection and metabolism. The 39 genes also included 7 genes that were differentially transcribed but that have no apparent connection to immune response processes plus 27 genes with unknown function.

Conclusion: Differences in gene transcription profiles might represent novel diagnostic targets that can be used to differentiate between chronic periprosthetic hip infections and aseptic hip prosthesis loosening. Secondary metabolites of differentially transcripted genes might serve as easily accessible markers for detecting chronic periprosthetic joint infection in future.

Although joint arthroplasty is a useful treatment that improves patient mobility and alleviates pain, periprosthetic joint infections (PJIs) remain a serious concern and result in considerable morbidity, mortality, and increased hospital stays, thus contributing to a high economic burden [1,2]. A variety of strategies are used to help prevent infection after joint arthroplasty [3]. In particular, early diagnosis plays a pivotal role in the appropriate treatment of PJIs [4]. While acute infections caused by highly virulent pathogens are generally easy to detect, it can be difficult to differentiate between chronic infections and aseptic prosthesis loosening [5]. Since the treatment for these 2 entities is fundamentally different, an accurate diagnosis is important [6]. To improve diagnostic accuracy, the Musculoskeletal Infection Society (MSIS) developed a consensus definition for PJI that defines infection based on a combination of clinical, microbiological, histological, serum, and synovial fluid characteristics [7,8]. This diagnostic procedure is relatively complex, and therefore, intensive research has focused on identifying new biomarkers for these conditions [9-14].

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Since serum markers can be influenced by other inflammatory conditions, researchers have aimed to identify and measure biomarkers at the infection site itself, that is, in synovial fluid. A couple of markers have proven promising for distinguishing between infection and aseptic loosening [9-14]. To identify new biomarkers, it is essential to determine gene transcription patterns in the surrounding tissue. There is evidence for differential gene transcription in the periprosthetic membranes of aseptically loosened prostheses compared to infected periprosthetic tissues [15-24]. Although these results are relevant, the practical implications are limited because only selected candidate genes were analyzed in a limited number of subjects.

The purpose of this study was to investigate gene transcription levels in periprosthetic tissues from patients with chronic periprosthetic hip infection and in patients with aseptic hip loosening using transcriptome-wide mRNA analysis. We hypothesized that this approach would identify differences in gene transcription profiles between chronic periprosthetic hip infection and aseptic hip prosthesis loosening that could identify new diagnostic markers for PJIs.

Materials and Methods

Approval for this study was obtained from our institution's ethics committee (registration number 5544). Informed consent was obtained from all of the patients who participated in this study.

Patients and Sampling

Consecutive patients that underwent revision hip arthroplasty at least 4 weeks after the index surgery due to aseptic prosthesis loosening or chronic periprosthetic hip infection were investigated over a 2-year period. The patients' demographic, clinical, and radiographic data were analyzed. Synovial fluid specimens were obtained preoperatively for culture and for determination of the white blood cell count and the percentage of polymorphonuclear cells. Blood samples were taken to measure C-reactive protein levels and peripheral blood leukocyte counts. During surgery, synovial fluid was aspirated for fluid culture, and tissue samples were collected for high-density microarray analysis.

The diagnosis of chronic periprosthetic hip infection was performed postoperatively based on modified criteria of the MSIS [7,8]. Briefly, a diagnosis of infection included the following: (1) a sinus tract or an open wound in communication with the prosthesis, or (2) a pathogen isolated from 2 separate tissues or cultures, or (3) 4 of the following 5 minor criteria: (a) serum C-reactive protein concentration >10 mg/L, (b) white blood cell >3000 cells/μL, (c) %polymorphonuclearcells >80%, (d) the presence of purulence in the affected joint, (e) isolation of a microorganism in one culture of periprosthetic tissues or fluid. In contrast to the original MSIS criteria, we did not include the minor criteria elevated erythrocyte sedimentation rate and elevated neutrophil count in histologic analysis. Septic subjects with both fixed and loosened prostheses were included in the infected cohort.

Aseptic hip prosthesis loosening was deemed in subjects that did not fulfill the above mentioned criteria for infection and had clinical and radiographic signs for loosening.

Specimens from patients who received antibiotics before the diagnostic work-up were not analyzed further. A total of 24 samples were processed for microarray analysis that fulfilled the criteria of either aseptic loosening (n = 12) or chronic infection (n = 12).

Total mRNA Extraction From Periprosthetic Tissues and mRNA Quality Control

Periprosthetic tissues were frozen at −80°C immediately after surgical removal. Total RNA was extracted from the tissue using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Briefly, the tissue samples were disrupted in lysis buffer and homogenized using a mortar and pestle. The samples were then frozen immediately in liquid nitrogen and ground to a fine powder. The suspension was transferred into a liquid nitrogen-cooled tube for the nitrogen to evaporate. The lysis buffer was added and the samples were homogenized using the QIAshredder (Qiagen). The lysate was loaded onto the QIAshredder spin column in a 2-mL collection tube, spinned for 2 minutes at maximum speed in a microfuge, and the homogenized lysate was collected for total RNA quality analysis.

Microarray Experiments

The Whole Human Genome Oligo Microarray Kit 8 × 60K v2 (G4851B, design ID 039494, 50.599 genes and RNA, Agilent Technologies, Santa Clara, CA) was utilized in this study. Total RNA was used to prepare Cy3-labeled cRNA (Amino Allyl MessageAmp II Kit; Ambion, Life Technologies, Carlsbad, CA) as directed by the instructions. Prior to the reverse transcription reaction, 1 μL of a 1:25,000 dilution of Agilent's One-Color spike-in Kit (#5188-5282, Agilent Technologies) was added to 200 ng of total RNA from each analyzed sample. cRNA fragmentation and the hybridization and washing steps were carried out according to Agilent's One-Color Microarray-Based Gene Transcription Analysis Protocol V5.7 except that 300 ng of each labeled cRNA sample were used for hybridization. Slides were scanned on the Agilent Microarray Scanner G2565 CA (pixel resolution 5 μm, bit depth 20). Data extraction was performed with the "Feature Extraction Software V10.7.3.1" using the recommended default extraction protocol file (GE1_107_Sep09.xml). The processed intensity values of the green channel ("gProcessedSignal" or "gPS") were normalized globally by a linear scaling approach in which all of the gPS values of one sample were multiplied by an array-specific scaling factor. This scaling factor was calculated by dividing a "reference 75th percentile value" (set as 1500 for the whole series) by the 75th percentile value of the particular microarray ("Array i" in the formula shown below). Accordingly, normalized gPS values for all samples (microarray data sets) were calculated using the following formula:

$$normalized\ gPS_{Array\ i} = gPS_{Array\ i} \times \left(1500 \Big/ 75^{th}\ Percentile_{Array\ i}\right)$$

A lower intensity threshold was defined as 1% of the reference 75th percentile value (=15). All of the normalized gPS values that fell below this intensity threshold were substituted by the respective surrogate value of 15.

Data Analysis

All patient data were analyzed using the SPSS 22.0 software package. Demographic and laboratory data were compared between the aseptic loosening and chronic periprosthetic infection groups using a *t* test. Gene transcription data were normalized within arrays using GeneSpring GX software. The processed signal and the background median signal were entered into the Bioconductor program, which normalized the processed signal between arrays using the quantile method.

The area under the curve (AUC) value was calculated for each gene and RNA to measure how well it could detect chronic