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Molecular pathology of total knee arthroplasty instability defined by RNA-seq

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ABSTRACT

Total knee arthroplasty (TKA) is a durable and reliable procedure to alleviate pain and improve joint function. However, failures related to flexion instability sometimes occur. The goal of this study was to define biological differences between tissues from patients with and without flexion instability of the knee after TKA. Human knee joint capsule tissues were collected at the time of primary or revision TKAs and analyzed by RT-qPCR and RNA-seq, revealing novel patterns of differential gene expression between the two groups. Interestingly, genes related to collagen production and extracellular matrix (ECM) degradation were higher in samples from patients with flexion instability. Partitioned clustering analyses further emphasized differential gene expression patterns between sample types that may help guide clinical interpretations of this complication. Future efforts to disentangle the effects of physical and biological (e.g., transcriptomic modifications) risk factors will aid in further characterizing and avoiding flexion instability after TKA.

1. Introduction

Instability after total knee arthroplasty (TKA) is one of the most common modes of failure requiring revision arthroplasty [1,2]. Instability cases account for approximately 30% of revision TKAs performed in the US [3,4] and other developed countries [5]. Though improvements in implant design and surgical technique have decreased the risk of instability, the projected increase in number of primary TKAs performed will continue to burden surgeons and health care systems [6–9]. Instability can be categorized as global instability, flexion instability, extension instability, and recurvatum [1,10,11]. Flexion instability is a finite diagnosis in which there is a flexion and extension mismatch, resulting in recurrent hemarthroses and a finite constellation of signs and symptoms [10]. Patients with flexion instability after TKA present with symptoms ranging from discomfort and a subjective sense of instability to complete dislocation of their prosthesis [12–14]. Clinical examination can reveal recurrent knee effusions, anterior or medial joint line tenderness, and/or excessive laxity of the collateral ligaments [3,13,14]. Radiographic analysis can determine implant positioning and alignment in multiple planes, as well as limb alignment, tibial slope, and flexion gap to determine the severity of instability [1,10]. However, additional methods (e.g., molecular biomarkers) for characterizing the risk of instability after TKA would be beneficial for the improvement of surgical intervention strategies and long-term patient outcomes.

The failure mechanism for flexion instability after TKA is multifactorial, attributed to biomechanical insufficiencies of native tissues (e.g., ligament injury) and/or direct failure of the implant device [15]. Poor surgical techniques (e.g., undersized femoral component, internal rotation of components, excessive tibial slope, inadequate distal femoral resection, or excessive release of the medial collateral ligament) and/or the host soft-tissue status can lead to mismatched flexion/extension gaps that lead to worsening flexion instability [1,10,15,16]. The varied clinical presentations of flexion instability after TKA suggest

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pleiotropic and/or patient specific causes (and likely treatment strategies) of this orthopedic problem. However, additional insight into the molecular mechanisms that distinguish TKA instability tissues from normal tissues will yield useful insights regarding similarities among patients with similar symptoms.

Clinically, epidemiologic and retrospective studies have identified risk factors and biomechanical etiologies underlying arthroplasty failure [17], yet limited data exist regarding the relationship of biological mediators regulating this clinical scenario. Furthermore, preoperative variables and demographics alone are of limited use in predicting functional outcomes and pain scores following TKA [18]. Recognizing important biological mediators may help characterize the significance of extracellular matrix remodeling, osteolysis, the post-traumatic inflammatory cascade, and other pathogenic mechanisms involved in the development of flexion instability following TKA.

High-throughput next-generation sequencing tools can provide a basis for identifying differentially expressed genes correlated to flexion instability after TKA. For example, RNA-seq has been used to successfully identify differences in gene expression between normal tissues and tissues affected by numerous musculoskeletal diseases including osteoarthritis, and rheumatoid arthritis [19–21]. Identifying gene products that are over-expressed in patients with flexion instability after TKA will provide information regarding genes and biological pathways implicated in the condition. Dysregulation of genes involved in tissue repair and remodeling of the surrounding soft tissue enveloping the knee joint, and/or oxidative stress reactions may negatively alter postoperative healing. Furthermore, highlighting key genes involved in tissue dysfunction may help characterize novel co-morbidities of flexion instability after TKA (e.g., poor joint perfusion, smoking, diabetes) and cell signaling pathways amenable to therapeutic intervention or prophylaxis.

Matrix Metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinase (TIMPs), may be involved in the molecular pathophysiology of flexion instability after TKA. Importantly, MMPs are involved in tissue remodeling, extracellular matrix degradation, and have a prominent role in many musculoskeletal pathologies [19,22,23]. For example, a single nucleotide polymorphism (SNP) in the promoter region of *MMP1* has been associated with aseptic loosening of total hip arthroplasty when detected in peripheral blood [24]. Elevated *MMP1* expression may also induce excessive tissue degradation of the periprosthetic microenvironment [25], that results in late manifestations of prosthesis malignment. In contrast, increased *MMP3* expression may correlate with exposure of synovial tissues to particulate debris [26–28]. Nonetheless, ECM remodeling genes will be important to analyze and monitor in patients with flexion instability after TKA.

The aim of this study was to investigate differential gene expression patterns among synovial capsular tissues collected from normal adults undergoing primary TKAs and patients requiring revision procedures due to flexion instability after TKA. The pathology of flexion instability after TKA is currently defined only by clinical and radiographic parameters, so establishing a biological mechanism for this complication will not only allow refined detection strategies, but also facilitate intervention strategies to prevent and treat the condition.

2. Materials & methods

2.1. Patient enrollment & tissue handling

In accordance with our approved IRB protocol (09-000115, Mayo Clinic, Rochester, MN), all patients were identified, verbally informed, and signature-consented prior to enrollment in the study. When possible, patient clinical data were screened and matched based on age (± 5 years), gender, and body mass index (BMI) (± 5 kg/m^2). We collected a total of 32 patient samples (16 primary TKAs [control group] and 16 TKAs revised for flexion instability [experimental group]). At the time of revision surgery, suprapatellar synovial capsular tissue (either control or experimental) were carefully dissected by the operative surgeon using a scalpel. After rinsing with sterile PBS, tissues were flash-frozen in liquid nitrogen, and stored at − 80 °C for further analysis.

Table 1
Sampling scheme for qPCR and RNA-seq data collection. (a) Instability TKA (n = 16) and (b) primary TKA (n = 16). Bold text indicates samples analyzed for RNA-seq (n = 3 for each group).

A)

Primary TKA			
Sample ID	**Age (years)**	**Gender**	**BMI (kg/m^2)**
1A	68.5	F	24.5
2A	70	M	24.9
3A	68.1	F	27.8
4A	56.3	F	38.1
8A	65.7	F	41.5
9A	56.3	M	34.8
10A	56.4	F	26.9
11A	61.4	F	38.2
12A	62.7	M	27.2
14A	**70**	**F**	**28.8**
15A	70.4	F	29.2
16A	**63.5**	**M**	**38.6**
17A	65.4	M	32.2
18A	67	M	32.9
19A	**75.2**	**F**	**29.8**
20A	70.1	M	37

B)

Instability TKA			
Sample ID	**Age (years)**	**Gender**	**BMI (kg/m^2)**
1B	62.8	F	20.1
2B	**61.3**	**M**	**25.9**
3B	**70.4**	**F**	**36.6**
4B	29.8	M	35.6
5B	50.9	F	33.7
6B	59.8	M	36.2
7B	72.8	M	26.8
8B	68.8	M	25.6
9B	43.4	F	26.6
10B	52.3	M	25.9
11B	69.1	M	31
12B	69	F	27.1
13B	76.5	F	27.1
14B	72.7	F	27.1
14B	67.8	M	24.9
16B	**65.2**	**F**	**18.8**

2.2. RNA handling

Frozen tissue biopsies were ground into powder by mortar and pestle and homogenized in Qiazol reagent using the TissueLyser LT (Qiagen, Hilden, Germany). Genomic RNA extractions were performed using the miRNeasy mini kit (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol, and eluted in 50 µL total volume. A NanoDropper (Thermo Fisher Scientific, Waltham, MA) was used to measure RNA concentration and purity. The Mayo Clinic Gene Expression Core (Advanced Genomics Technology Center, Mayo Clinic, Rochester, MN) also provided an RNA quality assessment (Agilent Technologies, Santa Clara, CA) using RNA integrity number (RIN) as a tool for prioritization of samples for RNA-seq data collection.

RNA-seq data collection was performed by the Gene Expression Core, a division of the Advanced Genomics Technology Center at Mayo Clinic (Rochester, MN). Specifically, a HiSeq 2000 (Illumina, San Diego, CA) was used to run 51 bases per read, 6 samples per lane, and paired-end index reads. The RNA Sample Prep Kit v2 library preparation type was specified for use by the Gene Expression Core, to complete poly-A