Transcriptomic Activities of Human Hosts in Response to Periprosthetic Joint Infection

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Introduction

Periprosthetic joint infection (PJI) is a devastating complication of total joint arthroplasty (TJA) as it substantially affects morbidity, impairs quality of life, and is often associated with significantly high clinical cost. Although PJI occurs at a relatively low rate with <3% of total hip or knee arthroplasties affected, given a steadily increasing volume of TJAs over time with 4 million total joint replacements estimated by 2030 in the US alone, the number of PJIs and their impact are significant.

PJI is difficult and challenging to treat because it is often associated with bacterial biofilms that provide a protective layer from host defense system or antimicrobial drugs. PJI can be caused by a variety of microorganisms, with *Staphylococcus epidermidis* and *Staphylococcal aureus* being the most common agents, accounting for 35 and 21% of monomicrobial PJI cases, respectively.

PJI diagnosis can be challenging and relies on a large combination of clinical findings. No single universal diagnostic test exists to provide accurate and timely diagnosis. Here we applied transcriptomic sequencing to investigate host response with aim of identifying unique molecular signatures for PJI.

Specific Aims

Multiomic analysis of biomaterials dislodged from surfaces of resected prosthetic hip or knee hardware (known as sonicate fluid):

1. Identify host genes exhibiting highly differential expression in PJI compared to aseptic cohort
2. Identify molecular signatures of staphylococcal compared to non-staphylococcal PJIs
3. Identify molecular signatures of *Staphylococcus aureus* compared to *Staphylococcus epidermidis* PJI

Methods

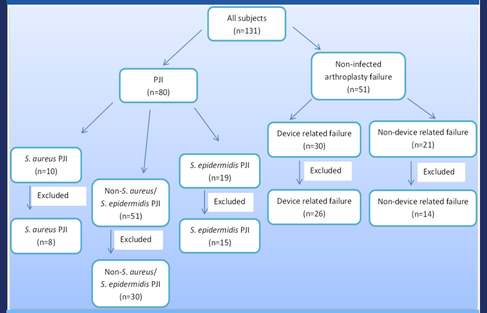

Figure 1: Study design

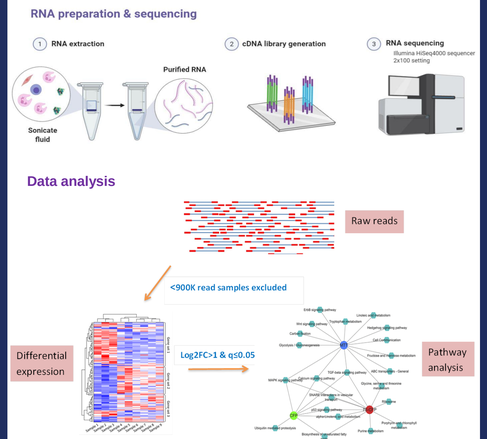

Figure 2: Sample processing and analysis workflows

Results

Transcriptomic profiles of PJI *versus* aseptic failure

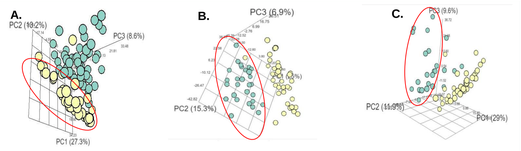

Figure 3: Principle component analysis of all PJI group (A), staphylococcal PJI (B), and non-staphylococcal PJI (C) *versus* aseptic failure. Green circles, PJI cases; yellow circles, aseptic failure cases.

Expression of proposed PJI-related genes

57 PJI-associated genes defined in prior studies were assessed for expression in PJI *versus* aseptic cohort:

- 28 genes increased expression in PJI groups
  - PI3: most expressed gene across all PJI groups
  - MMP3 and IL17D: 2-3 fold higher expression in staphylococcal *versus* non-staphylococcal PJI
  - IL8 and IL1B: 3-4 fold higher expression in *S. aureus* PJI *versus* *S. epidermidis* and non-staphylococcal PJI subgroups
- 2 genes paradoxically downregulated (A2M and LDHD)
- 27 no significant change in expression

Table 1: Twenty-eight proposed PJI related genes with increased expression

Gene	All PJI	Staphylococcal PJI	Non-staphylococcal PJI	*S. aureus* PJI	*S. epidermidis* PJI
PI3	910.3*	918.8*	1179*	2603*	799.6*
IL8	30.9*	30.3*	32.6*	86.8*	26.0*
IL5RA	29.8*	27.0*	33.6*	26.9*	27.6*
IL1B	22.7*	20.7*	26.2*	67.5*	15.2*
IL1A	19.8*	17.2*	27.2*	35.6*	14.5*
CSF3	10.7*	10.3*	13.4*	7.4*	4.9*
IL6	7.5*	8.3*	7.1*	13.0*	8.6*
FCGR1B	5.6*	5.7*	5.9*	8.1*	6.1*
BPI	5.3*	4.1*	6.3*	8.6*	2.6
MMP3	4.8*	6.9*	2.7*	15.4*	4.9*
IL17D	4.7*	6.7*	3.5*	7.1*	8.6*
CCL4	4.5*	4.1*	5.8*	8.1*	3.3*
LBP	4.2*	5.4*	3.4*	9.1*	4.8*
IL13RA2	3.9*	4.7*	3.3*	7.2*	n/r
CSF2RB	3.5*	3.5*	3.8*	5.0*	2.8*
LDHC	3.4*	4.4*	2.5*	n/r	n/r
DEFA4	3.3*	2.7*	3.2*	4.3	1.6
LCN2	3.0*	2.4	3.3*	5.2*	1.5
CAMP	2.9*	2.3*	3.2*	3.9*	1.6
LTF	2.6*	2.1	3.0*	3.4	1.3
VEGFA	2.6*	2.5*	2.8*	3.4*	2.2*
CXCL2	2.5*	2.4*	2.8*	3.2*	2.1*
CCL3	2.4*	2.1*	3.1*	3.7*	2
IL1RN	2.4*	2.5*	2.6*	5.1*	1.9
IFNG	2.2*	1.6	3.3*	n/r	n/r
IL2RA	2.2*	2.3*	2.3*	3.2*	2.3*
ICAM1	2.1*	2.2*	2.0*	3.1*	2.1*
IL2RG	2.0*	2.0*	2.3*	2.8*	1.7

Expression analysis revealed novel markers for PJI

Table 2: Top 50 genes with highest expression in PJI cohorts

Staphylococcus/Non-*Staphylococcus* PJI

Both PJI groups	Staphylococcal PJI	Non-staphylococcal PJI
Immune function		
F7 FCRL4	PI3 UMOD	CCL20
Cell metabolism /proliferation/ regulation function		
ARSI ATOH7 CCNI2 CELA1 DLX2 DMRTA2 EFNA2 EN1 GABRG3 GDF2 GPM6A HDGFL1 HOXC13 LYPD4 NDST4	NEUROD1 NEUROD2 NKX2-5 NKX3-1 OR52E8 PAN3 PRSS56 S1PR2 SALL3 SLITRK1 SLITRK2 SPATA22 USP26 VAX1 WT1	SEPTIN3 CAMK2N2 FOXE3 HMX3 NKX1-2 ONECUT1 ONECUT3 TAC TGM3	CDH10 CRNDE FOXI3 GPR123 KRT20 MSI1 PHOX2B SCN2A SOX11
Unknown function		
BPESC1 DCAF4L2 LDLRAD1	MGC2889 NOL4 VSIG10		

S. aureus/*S. epidermidis* PJI

Both PJI groups	*S. aureus* PJI	*S. epidermidis* PJI
Immune function		
F7 FCRL4	PI3 UMOD	CCL20 IL8 PF4V1 IL1B	
Cell metabolism /proliferation/ regulation function		
ARSI ATOH7 CAMK2N2 CCNI2 CELA1 DLX2 DMRTA2 EFNA2 EN1 FOXE3 FOXI3 GABRG3 GDF2 GPM6A HDGFL1 LYPD4	NEUROD1 NEUROD2 NKX2-5 NKX3-1 ONECUT3 OR52E8 PAN3 PRSS56 S1PR2 SALL3 SLITRK1 SLITRK2 SPATA22 TGM3 VAX1 WT1	CRNDE CYP26C1 HOXC13 NKX1-2	SEPTIN3 C1QL1 ENPP7 HMX3 KCNG3 ONECUT1 PHOX2B THEG
Unknown function		
BPESC1 DCAF4L2 LDLRAD1	MGC2889 PPY2 VSIG10		

Analysis of top expressed genes:

- F7, PI3, FCRL4, and UMOD: highly expressed in all PJI groups
- IL8, IL1B, and PF4V1: uniquely highly expressed in *S. aureus* PJI

Pathway analysis

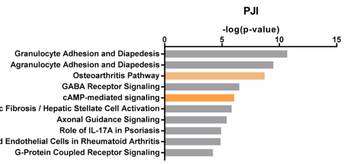

Figure 4: Top 10 enriched pathways in PJI cohort

Top enriched pathways involved:

- Host immune response (IL-17A pathway, granulocyte and agranulocyte adhesion)
- Cellular development and repair functions (osteoarthritis, cAMP, axonal guidance)

Conclusions

1. PJI cohorts display uniquely distinct expression profiles from aseptic failure.
2. Multiomic analysis identified potential PJI molecular signatures:

- ❑ Of previously-investigated PJI-related genes
  - ✓ PI3: most differentiating PJI from aseptic failure
  - ✓ MMP3 and IL17D: distinguish staphylococcal from non-staphylococcal PJI
  - ✓ IL8 and IL1B: potential signatures for *S. aureus* PJI
- ❑ Top expressed genes,
  - ✓ F7, FCRL4 and UMOD: most expressed in PJI
  - ✓ IL8, IL1B and PF4V1: uniquely expressed in *S. aureus* PJI

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