Acta Biomaterialia xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Transcriptional profile of human macrophages stimulated by ultra-high molecular weight polyethylene particulate debris of orthopedic implants uncovers a common gene expression signature of rheumatoid arthritis

Mohamad Alaa Terkawi [a,b,*], Masanari Hamasaki [a], Daisuke Takahashi [a,*], Masahiro Ota [a], Ken Kadoya [a], Tomoyo Yutani [c], Keita Uetsuki [c], Tsuyoshi Asano [a], Tohru Irie [a], Ryuta Arai [a], Tomohiro Onodera [a,b], Masahiko Takahata [a], Norimasa Iwasaki [a,b]

[a] Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Kita-15, Nish-7, Kita-ku, Sapporo 060-8638, Japan
[b] Global Institution for Collaborative Research and Education (GI-CoRE), Frontier Research Center for Advanced Material and Life Science, Bldg No 2, Hokkaido University, Japan
[c] R&D Center, Teijin Nakashima Medical Co., Ltd., 5322, Haga, Kita-ku, Okayama 701-1221, Japan

ARTICLE INFO

Article history:
Received 16 August 2017
Received in revised form 27 October 2017
Accepted 2 November 2017
Available online xxxx

Keywords:
Osteolysis
Macrophages
Implant particles
Transcriptional profiling
Bioinformatics analyses
Bone-resorbing activity

ABSTRACT

Osteolysis is a serious postoperative complication of total joint arthroplasty that leads to aseptic loosening and surgical revision. Osteolysis is a chronic destructive process that occurs when host macrophages recognize implant particles and release inflammatory mediators that increase bone-resorbing osteoclastic activity and attenuate bone-formation osteoblastic activity. Although much progress has been made in understanding the molecular responses of macrophages to implant particles, the pathways/signals that initiate osteolysis remain poorly characterized. Transcriptomics and gene-expression profiling of these macrophages may unravel key mechanisms in the pathogenesis of osteolysis and aid the identification of molecular candidates for therapeutic intervention. To this end, we analyzed the transcriptional profiling of macrophages exposed to ultra-high molecular weight polyethylene (UHMWPE) particles, the most common components used in bearing materials of orthopedic implants. Regulated genes in stimulated macrophages were involved in cytokine, chemokine, growth factor and receptor activities. Gene enrichment analysis suggested that stimulated macrophages elicited common gene expression signatures for inflammation and rheumatoid arthritis. Among the regulated genes, tumor necrosis factor superfamily member 15 (TNFSF15) and chemokine ligand 20 (CCL20) were further characterized as molecular targets involved in the pathogenesis of osteolysis. Treatment of monocyte cultures with TNFSF15 and CCL20 resulted in an increase in osteoclastogenesis and bone-resorbing osteoclastic activity, suggesting their potential contribution to loosening between implants and bone tissues.

Statement of Significance

Implant loosening due to osteolysis is the most common mode of arthroplasty failure and represents a great challenge to orthopedic surgeons and a significant economic burden for patients and healthcare services worldwide. Bone loss secondary to a local inflammatory response initiated by particulate debris from implants is considered the principal feature of the pathogenesis of osteolysis. In the present study, we analyzed the transcriptional profiling of human macrophages exposed to UHMWPE particles and identified a large number of inflammatory genes that were not identified previously in macrophage responses to wear particles. Our data provide a new insight into the molecular pathogenesis of osteolysis and highlights a number of molecular targets with prognostic and therapeutic implications.

1. Introduction

Total joint arthroplasty is one of the most successful surgical procedures for treating severe joint diseases, restoring the mobility and improving the quality of life for millions of patients.

* Corresponding authors at: Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo, Japan.
E-mail addresses: materkawi@med.hokudai.ac.jp (M.A. Terkawi), rainbow-quest@pop02.odn.ne.jp (D. Takahashi).

https://doi.org/10.1016/j.actbio.2017.11.001
1742-7061/

Approximately 1.5 million people worldwide undergo total joint arthroplasty per year. Unfortunately, 10–15% of the patients receiving joint replacements need further surgical intervention, referred to as revision surgery, because of implant loosening [1]. Revision surgery is technically more difficult than primary arthroplasty and is often associated with a poorer prognosis and high risk of failure [1,2]. The increased numbers of these surgeries represent a great challenge for orthopedic surgeons and a significant economic burden for patients and healthcare services globally. It is estimated that the annual economic burden associated with revision surgery may exceed 3 billion dollars in the USA alone [3].

Particulate wear debris generated by motion at the bearing surfaces of implants is thought to be the main cause of this failure, which is mediated by triggering local inflammatory responses leading to osteolysis. Implant loosening because of osteolysis, referred to as aseptic loosening, is the most common mode of arthroplasty failure [4]. Osteolysis is initiated by macrophages that recognize the implant particles and release inflammatory mediators resulting in the recruitment of other types of inflammatory cells and the formation of inflamed granulomas [5–7]. Persistence of excessive inflammatory responses at the site of implants impairs local bone metabolism and promotes bone resorption resulting in a loss of implant fixation/stability and prosthesis failure [2,7]. The incidence of periprosthetic osteolysis is relatively high, ranging between 5 and 40%, and is predicted to be substantially higher in the younger population who will potentially live for decades [1,2]. Moreover, therapeutic targets that block osteoclastogenesis and osteoclastic-bone resorbing activity have failed to prevent osteolysis in clinical trials [1,8]. Thus, there is a consensus in orthopedic communities that a novel strategy for preventing periprosthetic osteolysis and improving prostheses longevity is needed [1,3,8]. This will require a better understanding of the molecular pathways and signals responsible for this serious clinical problem.

Tissue-resident macrophages are the first cells that encounter wear particulate debris and their response is driven by the size and shape of the particles. Small particles can be phagocytized by macrophages, whereas large particles induce the fusion of cells and formation of multinucleated giant cells [1,4,5]. Upon phagocytosis, macrophages produce endosomal enzymes to digest particles resulting in endosomal damage and persistent inflammasome activation. Likewise, upon binding to large particles, macrophages fuse and trigger a classical foreign body response characterized by the generation of reactive oxygen intermediates and degradative enzymes resulting in activation of the NACHT, LRR and PYD domains-containing protein 3 (NALP3) inflammasome. Consequently, activated macrophages release an array of inflammatory mediators that facilitate the recruitment of other cells such as dendritic cells and fibroblasts, osteoclastogenesis and bone resorption [5,6]. While recruited dendritic cells may play a complementary role in promoting inflammation, fibroblasts direct the formation of synovial-like pseudomembrane around prostheses. These consequent events result in chronic synovitis, joint pain, swelling, bone loss and implant loosening [1,2].

Improving the performance of implant bearing materials in younger implant recipients remains one of the major challenges in the field of orthopedics. Current bearing materials including UHMWPE, metallic alloys and ceramics have not been proven safe [4]. An approach to minimize the adverse effects of wear debris may improve the longevity of orthopedic implants in younger patients who are expected to live for decades and resume physical activities [9]. Cross-linked UHMWPE is a plastic bearing material commonly used for prosthetic components with promising clinical performance in the majority of patients [8]. However, despite the great durability of cross-linked UHMWPE materials, their wear particulates have been isolated from synovial fluid and periprosthetic tissues of loosened implants with a size ranging between 0.1 and 10 μm [10,11]. UHMWPE wear particles elicit a foreign body reaction typified by granulomatous chronic inflammation that leads to implant failure within 10 years of surgery [4]. The mechanisms/pathways by which UHMWPE particles trigger this destructive process are largely unclear and remain to be investigated [4]. Given the importance of such knowledge for the discovery of therapeutic targets, we studied the transcriptional profiling of human macrophages stimulated by UHMWPE particles using an RNA-Seq approach. We uncovered a large number of inflammatory genes that were not previously identified in macrophage responses to wear particles [12]. Our data provide a new insight into the molecular pathogenesis of osteolysis and shed light on a new molecular candidate for therapeutic intervention.

2. Materials and methods

2.1. Ethics statement

Our research protocols used in this study was approved by the Research Ethics Review Committee of Hokkaido University Hospital (approval ID: 016-0002). Informed consents for the use of samples in the research were obtained from all donors.

2.2. Preparation of polyethylene particulate debris

Particles were generated from hip-bearing materials of UHMWPE manufactured from GUR1020 powder (Celanese Japan, Tokyo, Japan) after 95 kGy irradiation and annealing at T_m (135 °C) in N_2 (Quadrant Polypenco Japan, Tokyo, Japan). The UHMWPE materials were crushed by Multi Beads Shocker (Yasui Kikai, Osaka, Japan) at 3500 rpm for 40 s to obtain particles with sizes similar to those found in patients with aseptic loosening [10,11]. Fabricated particles were resuspended in endotoxin-free-sterile water, pelleted by centrifugation at 4000 rpm for 5 min, freeze-dried and then kept for further use. Sterilization was carried out using ethylene oxide gas (EOG) sterilizer (Eogelk-SA-H160, Osaka, Japan). Distribution of particles based on their sizes (equivalent circle diameter: ECD) was then determined by a particle image analyzer Morphologi G3 (Malvern Instruments, Worcester, UK). Endotoxins in particles were determined using ToxinSensor Single Test Kit (Genscript, Piscataway, NJ, USA) and their levels were below the detection limit of kit (0.015 EU/ml).

2.3. Macrophage culture with UHMWPE particles

Human peripheral blood was collected into BD Vacutainer tubes containing ACD (Becton, Dickinson and Company, NJ, USA) from nine Asian healthy donors (ages ranged between 30 and 40 years). All donors have no history of systemic inflammatory diseases, joint disorders and total joint arthroplasty. Thereafter, mononuclear cells (PMCs) were separated from the blood samples by density gradient centrifugation (Ficoll-Paque™ PLUS: GE Healthcare, Waukesha, WI, USA) and cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 25 mg/l penicillin/streptomycin in a humidified atmosphere containing 5% CO_2 for 3 h. Adherent cells were washed twice with ice-cold phosphate-buffered saline buffer (PBS) and cultured in medium supplemented with 50 ng/ml human recombinant macrophage colony-stimulating factor (MCSF, Peprotech, Japan) for 7 days. Cultures were regularly replenished with fresh medium every 3 days. This procedure routinely resulted in >97% $CD14^+$ as verified by flow cytometric analysis (data not shown). Differentiated macrophages were detached on day 7 of culture by treatment with 1% trypsin-EDTA solution (GE Healthcare) for 5 min, washed with ice-cold PBS, counted, and then seeded onto 48-well culture plates at 2

Please cite this article in press as: M.A. Terkawi et al., Transcriptional profile of human macrophages stimulated by ultra-high molecular weight polyethylene particulate debris of orthopedic implants uncovers a common gene expression signature of rheumatoid arthritis, Acta Biomater. (2017), https://doi.org/10.1016/j.actbio.2017.11.001