INTRODUCTION

The bone marrow stroma contains mesenchymal stem cells (MSCs), multipotent cells capable of differentiating along various mesenchymal lineages, including osteogenesis; and, in a procedure such as total hip arthroplasty (THA), MSCs are likely to be exposed to high concentrations of implant wear particles along the femoral stem of the prosthesis. We have previously reported that the osteogenic differentiation of human mesenchymal stem cells (hMSCs) is suppressed upon exposure to titanium particles accompanied by reduced bone sialoprotein (BSP) gene expression, diminished production of collagen type I and BSP, decreased cellular viability and proliferation, and inhibition of extracellular matrix mineralization. In this study, we further investigate hMSC cytotoxicity upon exposure to submicron particles of commercially pure titanium (cpTi) and zirconium oxide (ZrO2).

MATERIAL AND METHODS

Particle Preparation and Characterization: cpTi and ZrO2 particles (Sigma-Aldrich) were sterilized and resuspended in specific particle concentrations. Limulus assay of cpTi and ZrO2 particle suspensions excluded endotoxin levels exceeding 0.06 EU/ml. Laser scattering particle analysis (Horiba LA-910; EMD) revealed mean particle sizes of 0.93±0.380 and 0.87±0.540 μm for cpTi and ZrO2 respectively. MSCs Isolation and Culture: hMSCs were isolated from bone marrow aspirates of patients undergoing THA or total knee arthroplasty (TKA) for primary osteoarthritis and cultured expanded in vitro. This study was approved by the NIH Office of Human Subjects Research and the IRB of the George Washington University School of Medicine. Viability Assay: Cellular viability was determined based on exclusion of Trypan Blue staining. In Situ Cell Death Characterization: To determine if particles caused cell death by means of apoptosis or necrosis, cleavage of genomic DNA during apoptosis was assessed based on the TUNEL method. Cells were cultured with 500 particles/cell or conditioned medium (CM) for 72 h prior to determining apoptotic rate. Particle-free and serum-free medium were used as negative and positive controls respectively. Western Analysis: Rabbit IgG polyclonal antibodies (Santa Cruz) reactive to human full length p53, human p73α, and human β-actin were used. Data Analysis: All values are expressed as the mean of three separate experiments ± the standard error of the mean (SEM). Statistical significance between control values and treatment values was determined by a pairwise Student’s t-test.

RESULTS AND DISCUSSION

The purpose of this study is to test the hypothesis that exposure to wear particles compromises the viability of hMSCs through the induction of apoptosis, and that this effect may be mediated by soluble factors released by hMSCs in response to particulate stress. We demonstrate here that direct exposure to submicron cpTi and ZrO2 submicron particles adversely affects hMSC viability through the induction of apoptosis, eliciting increased expression of the tumor suppressor proteins p53 and p73 in a manner dependent on material composition, particle dosage, and exposure time. In addition, conditioned medium containing soluble factors released by hMSCs in response to cpTi particles, but not ZrO2 particles, is capable of compromising cellular viability, inducing apoptosis in a concentration-dependent manner in the absence of particle exposure. Increased expression of p53 can be induced by a variety of cellular stresses, and the ability of particle exposure and serum deprivation to induce increased p53 and p73 expression and subsequent apoptosis, suggests that these two distinct cellular insults may activate the p53 pathway through similar mechanisms. Additionally, our findings suggest that the p53-mediated apoptosis observed here may also be associated with the activation of death receptors by cytokines within the conditioned medium produced in response to particulate stress. At present, this possible mechanism of particle cytotoxicity remains unexamined and warrants further investigation. This study represents the first report to demonstrate that exposure of hMSCs to wear particles can compromise cellular viability through the direct and indirect induction of apoptosis, suggesting that long-term exposure of marrow-derived hMSCs to wear debris may reduce the population of viable osteoblastic progenitors, potentially contributing to poor periprosthetic bone quality and implant loosening.

**DIRECT AND INDIRECT INDUCTION OF APOPTOSIS IN HUMAN MESENCHYMAL STEM CELLS IN RESPONSE TO TITANIUM PARTICLES**

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