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Molecular mechanisms of osteoporotic hip fractures in elderly women☆

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ABSTRACT

A common manifestation of age-related bone loss and resultant osteoporosis are fractures of the hip. Age-related osteoporosis is thought to be determined by a number of intrinsic factors including genetics, hormonal changes, changes in levels of oxidative stress, or an inflammatory status associated with the aging process. The aim of this study was to investigate gene expression and bone architecture in bone samples derived from elderly osteoporotic women with hip fractures (OP) in comparison to bone samples from age matched women with osteoarthritis of the hip (OA). Femoral heads and adjacent neck tissue were collected from 10 women with low-trauma hip fractures (mean age 83 ± 6) and consecutive surgical hip replacement. Ten bone samples from patients undergoing hip replacement due to osteoarthritis (mean age 80 ± 5) served as controls. One half of each bone sample was subjected to gene expression analysis. The second half of each bone sample was analyzed by microcomputed tomography. From each half, samples from four different regions, the central and subcortical region of the femoral head and neck, were analyzed. We could show a significantly decreased expression of the osteoblast related genes *RUNX2*, *Osterix*, *Sclerostin*, *WNT10B*, and *Osteocalcin*, a significantly increased ratio of *RANKL* to *Osteoprotegerin*, and a significantly increased expression of the enzymes superoxide dismutase 2 (*SOD2*) and glutathione peroxidase *GPX3*, and of the inflammatory cytokine *IL6* in bone samples from hip fracture patients compared to controls. Major microstructural changes in OP bone were seen in the neck and were characterized by a significant decrease of bone volume, trabecular number, and connectivity density and a significant increase of trabecular separation. In conclusion, our data give evidence for a decreased expression of osteoblast related genes and increased expression of osteoclast related genes. Furthermore, increased expression of *SOD2* and *GPX3* suggest increased antioxidative activity in bone samples from elderly osteoporotic women with hip fractures.

1. Introduction

Age related osteoporosis is one of the most prevalent diseases in the elderly population and, with a growing population, will become an even increasing socioeconomic burden. Gradual loss of bone mass and deterioration of skeletal architecture manifesting as age related osteoporosis substantially increases the risk of fragility fractures and is a major risk factor of morbidity in elderly people. The most devastating complication of osteoporosis are fractures of the hip, as they are associated with high recurrence rates and significant morbidity and mortality (Duque et al., 2009). In women, rapid bone loss starts with the onset of menopause due to a drop in sex hormone levels and, about 8–10 years after menopause, is followed by the transition into a phase of slower bone loss which continues for the rest of life (Clarke and Khosla, 2010). Intrinsic factors thought to influence this slower age related bone loss include, besides the still prevalent sex hormone deficiency, changes in levels of oxidative stress and a proinflammatory status associated with the aging process termed "inflammaging" (Franceschi et al., 2000; Manolagas, 2010). At the cellular level, a common characteristic of osteoporosis is a net loss of bone mass following an imbalanced bone remodeling process favoring bone resorption over bone formation. Accelerated bone loss after menopause and age-related slow bone loss is driven by a high turnover status with elevated bone resorption that is

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not compensated by bone formation. Evidence for this comes from histomorphometric studies (Recker et al., 2004) and studies with biochemical markers of bone turnover (Garnero et al., 1996). In women suffering from postmenopausal osteoporosis or age related osteoporosis, bone resorption is even more increased as shown by local gene expression studies in osteoporotic bone samples compared to non-osteoporotic bone samples (Abdallah et al., 2005; D'Amelio et al., 2011; Logar et al., 2007; Zupan et al., 2012). They report on increased ratios of the expression of the pro-osteoclastogenic factor *RANKL* to the expression of the anti-osteoclastogenic factor *Osteoprotegerin* (*OPG*) in osteoporotic compared to non-osteoporotic bone samples (Abdallah et al., 2005; D'Amelio et al., 2011; Logar et al., 2007; Zupan et al., 2012). Little information is available on the local expression of markers of bone formation. The expression of markers of bone formation has been shown to be decreased in osteoporotic compared to non-osteoporotic bone samples (Dragojevic et al., 2011, 2013; Giner et al., 2013; Hopwood et al., 2007). However, the aforementioned studies were done in study populations comprising both men and women and/or comprising both middle aged and old persons. As gene expression patterns are expected to be influenced by various factors including sex and age, there is an obvious need for studies with carefully selected study cohorts, that will help us to get a more detailed understanding of differences in pathophysiological mechanisms underlying male and female osteoporosis as well as postmenopausal and age-related osteoporosis (Föger-Samwald et al., 2014). In previous work we reported on significantly decreased expression of the osteoblast transcription factors *RUNX2* and *Osterix*, but no significant differences in expression of the osteoclast related genes *RANKL* and *OPG* in bone samples from elderly men with age-related osteoporosis compared to bone samples from elderly men with osteoarthritis (Föger-Samwald et al., 2014). These data give evidence for osteoblast dysfunction rather than increased osteoclastogenesis as underlying mechanism of male age-related osteoporosis and underline the need for studies with carefully selected study cohorts. The aim of the present study was to investigate local gene expression of osteoblast and osteoclast related genes and of genes related to oxidative stress in femoral heads of elderly women with osteoporotic hip fractures in comparison to elderly women with osteoarthritis. Furthermore, microstructural characteristics of the collected bone samples were related to the local expression of the investigated genes. We hypothesized to find decreased expression of osteoblast related genes and, in contrast to the study population of elderly men investigated previously by our group, increased local expression of osteoclast related genes.

2. Patients and methods

2.1. Human bone tissue samples

Femoral heads and the adjacent femoral neck were obtained from ten female patients undergoing total hip arthroplasty surgery due to fragility fractures of the hip (OP) and from fourteen female patients undergoing total hip arthroplasty surgery due to osteoarthritis of the hip (OA). Gene expression was analyzed in bone samples from ten OP patients (mean age 83 ± 6 years) and ten OA patients (mean age 80 ± 5 years). Bone microarchitecture was analyzed in bone samples from ten OP patients (mean age 83 ± 6 years) and ten OA patients (mean age 80 ± 6 years). Some samples were excluded from gene expression and microstructural analysis due to damage of material during the process of surgical replacement or because insufficient amounts of RNA were isolated. Exclusions were randomly distributed compared to the original samples and therefore did not change the characteristics of the study population. Patients with fragility fractures were recruited at the Department of Trauma Surgery, Danube Hospital, Vienna, Austria. Patients with osteoarthritis were recruited at the Department of Orthopaedics, St. Vincent Hospital, Vienna, Austria, and the Department of Orthopaedics, Orthopaedic Hospital Gersthof, Vienna, Austria. During preoperative preparation patients were asked to participate in this study and collaborating physicians checked for predefined inclusion and exclusion criteria.

Exclusion criteria for the OP group included as described previously (Föger-Samwald et al., 2014) hip fractures caused by high energy trauma (e.g. car accidents), alcohol abuse, preoperative lab findings giving evidence for severe renal or hepatic failure, or other major chronic diseases. Moreover, patients with clinical signs or established diagnosis of liver cirrhosis, hyperthyroidism, hypogonadism, any malignancy within the last five years or other severe pathologies were excluded from enrolment. Exclusion criteria for the OA group were fragility fractures, clinical diagnosis of osteoporosis, or the previous use of specific antiosteoporotic drugs other than vitamin D and calcium supplements. Inclusion criteria for both groups, OP and OA, were a minimum age of 70 years and a signed informed consent. The study was approved by the competent local ethic committees.

2.2. Sample preparation

To facilitate microstructure as well as gene expression analysis, femoral heads and the adjacent femoral necks were cut into two halves during hip replacement surgery as described previously (Föger-Samwald et al., 2014). One half selected for subsequent gene expression analysis was submerged in RNA-Later™ (Ambion, Warrington, UK) and stored as instructed by the manufacturer. The second half selected for subsequent microstructure analysis was submerged in 70% ethanol and then stored at 4 °C. Samples obtained from the Orthopaedic Hospital Gersthof were selected either for microstructure or gene expression analysis, as for technical reasons only one half of the extracted femoral heads could be used for analyses. All analyses were performed in four different regions of the bone samples. The four regions were defined as peripheral region of the femoral head (pHd), central region of the femoral head (cHd), peripheral region of the femoral neck (pN), and central region of the femoral neck (cN) (Fig. 1). Samples from the central as well as the peripheral region included only trabecular bone.

2.3. Micro-computed tomography (μ-CT)

Cubes (appr. 10 mm × 10 mm × 10 mm) were cut out from the above described regions of each bone sample with a bone saw and microstructure was assessed with the μ-CT imaging system μCT35 (Scanco Medical AG, Brüttisellen, CH). The X-ray tube was operated at 70 kVp and 114 μA with an integration time set to 300 ms. Scans were performed at an isotropic, nominal, spatial resolution of 10 μm (high resolution mode). A cylinder shaped volume of interest (0.637 cm^3) was selected in the centre of the bone sample to eliminate possible artifacts on the surface originating from the sewing process. Morphometric

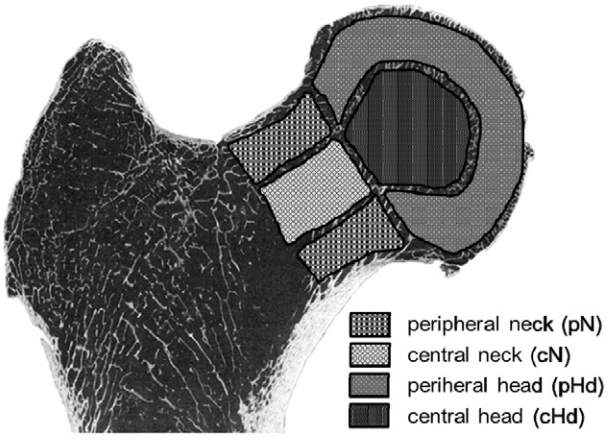

Fig. 1. Schematic diagram of the proximal femur and the sites from which samples were removed.