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Molecular evidence of osteoblast dysfunction in elderly men with osteoporotic hip fractures ☆

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ABSTRACT

Osteoporosis is extremely frequent in post-menopausal women; nevertheless, osteoporosis in men is also a severe and frequently occurring but often underestimated disease. Increasing evidence links bone loss in male idiopathic osteoporosis and age related osteoporosis to osteoblast dysfunction rather than increased osteoclast activity as seen in postmenopausal osteoporosis. The aim of this study was to investigate gene expression of osteoblast related genes and of bone architecture in bone samples derived from elderly osteoporotic men with hip fractures (OP) in comparison to bone samples from age matched men with osteoarthritis of the hip (OA). Femoral heads and adjacent neck tissue were collected from 12 men with low-trauma hip fractures and consecutive surgical hip replacement. Bone samples of age matched patients undergoing hip replacement due to osteoarthritis served as controls. One half of the bone samples was subjected to RNA extraction, reverse transcription, and real-time polymerase chain reactions. The second half of the bone samples was analyzed by static histomorphometry. From each half samples from four different regions, the central and subcortical region of the femoral head and neck, were analyzed. OP patients displayed a significantly decreased *RUNX2*, *Osterix* and *SOST* expression compared to OA patients. Major microstructural changes in OP bone were seen in the subcortical region of the neck and were characterized by a significant decrease of bone volume, and a significant increase of trabecular separation. In conclusion, decreased local gene expression of *RUNX2* and *Osterix* in men with hip fractures strongly supports the concept of osteoblast dysfunction in male osteoporosis. Major microstructural changes in the trabecular structure associated with osteoporotic hip fractures in men are localized in the subcortical region of the femoral neck.

1. Introduction

Primary osteoporosis is an age related multifactorial disease characterized by impaired bone mass and bone microarchitecture leading to decreased bone strength and consequently to an increased risk of fragility fractures (Rachner et al., 2011). Bone loss associated with aging starts earlier and is more pronounced in women than in men (Clarke and Khosla, 2010). Nevertheless, 1 in 8 men older than 50 years will experience an osteoporotic fracture (Melton et al., 1992) and 30% of all osteoporotic hip fractures worldwide occur in men (Johnell and Kanis, 2006). With an aging population male osteoporosis will become an even increasing socioeconomic burden. Moreover, fracture-related morbidity and mortality is higher in men than in women (Khosla, 2010). In 50% of men with osteoporosis a secondary cause such as an underlying disorder or drug-induced bone loss can be identified (Gielen et al., 2011). In the absence of secondary causes primary osteoporosis is diagnosed and termed 'idiopathic osteoporosis' in young men and 'age related osteoporosis' in men older than 70 years (Gielen et al., 2011). At the tissue level, the pathophysiology of osteoporosis is characterized by loss of bone material due to a negatively balanced bone remodeling process favoring bone resorption over bone formation. Bone resorption exceeds bone formation induced either by an increased activity of osteoclasts or a decreased activity of osteoblasts. Identifying excessive bone resorption by osteoclasts as the key mechanism driving rapid postmenopausal bone loss in women, the main focus of osteoporosis research has been on osteoclast dysfunction. Hence, standard therapies available for the

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treatment of osteoporosis are antiresorptive agents targeting osteoclastogenesis and mature osteoclasts (Rachner et al., 2011). However, several studies based on histomorphometry (Ciria-Recasens et al., 2005; Fratzl-Zelman et al., 2011; Johansson et al., 1997; Kurland et al., 1997; Zerwekh et al., 1992) and studies with cell cultures derived from bone tissues from osteoporotic patients (Hu et al., 2013; Marie et al., 1991; Pernow et al., 2006; Ruiz-Gaspa et al., 2010) provide evidence for an impact of osteoblast dysfunction on the pathophysiology of male osteoporosis. In previous work we reported that, in comparison to healthy controls, local gene expression of the major osteoblast transcription factor *RUNX2*, the Wnt signaling pathway members *WNT10B* and *SOST* and the osteoclast regulating gene *RANKL* is significantly decreased in iliac crest biopsies of men with idiopathic osteoporosis (Patsch et al., 2011). This study supports the concept of decreased bone formation as the underlying pathophysiological mechanism of male osteoporosis and was the first local gene expression study in an exclusively male idiopathic study population. Previous local gene expression analyses of osteoporotic bone tissue predominantly were performed with female study populations or included both, men and women. However, as gene expression patterns are expected to be influenced by various factors including sex and age, studies with carefully selected study cohorts will help us to get a more detailed understanding on pathophysiological mechanism underlying male and female osteoporosis. The aim of the present study was to investigate local gene expression of osteoblast related genes in femoral heads of elderly men with osteoporotic hip fractures in comparison to men with osteoarthritis. Furthermore, histomorphometric characteristics of the collected bone samples were related to the local expression of the investigated genes. Extending the concept of osteoblast dysfunction to male 'age related osteoporosis', we hypothesized to find a decreased local expression of osteoblast related genes in bone samples from men with osteoporotic hip fractures.

2. Patients and methods

2.1. Human bone tissue samples

Femoral heads and the adjacent femoral neck were obtained from male patients undergoing total hip arthroplasty surgery due to fragility fractures of the hip (OP) and from male patients undergoing total hip arthroplasty surgery due to osteoarthritis of the hip (OA). Patients were recruited at the Department of Trauma Surgery, Danube Hospital, Vienna, Austria and the Department of Orthopaedics, St Vincent Hospital, Vienna, Austria respectively. 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 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 local ethic committees.

2.2. Sample preperation

To facilitate histomorphometric, as well as gene expression analysis, femoral heads and the adjacent femoral necks were cut into two halves right after hip replacement surgery. 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 histomorphometric analysis was submerged in 4% paraformaldehyde, subsequently in 70% ethanol and then stored at 4 °C. 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. Histomorphometry

Cubes (7 mm × 7 mm × 7 mm) were cut out from the above described regions of each bone sample with a bone saw and processed for histomorphometry as described previously (Patsch et al., 2011). Briefly, all bone cubes were fixed with ethanol, dehydrated in ascending ethanol series, and embedded in polymethyl methacrylate (PMMA). Histomorphometry on Goldner stained sections was performed using the semiautomatic OsteoMeasure histomorphometry system (Osteometrics Inc.; Atlanta, GE). Within the trabecular compartment of each section nine random fields of view were imaged with an ×2 objective. Histomorphometric structure parameters including bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), and bone surface density (BS/TV) were analyzed according to the standards of the American Society of Bone and Mineral Research (Dempster et al., 2013).

2.4. RNA extraction

Sample preperation for RNA extraction was performed as described previously (Patsch et al., 2011). Briefly, a small cube (approximately 5 mm × 5 mm × 5 mm) was cut out from the above described regions of each bone sample using RNAse-free instruments. The bone cube, containing bone tissue and bone marrow, was flash frozen in liquid nitrogen and together with two small steel beads placed in a grinding mill (3 min, 30 Hz) for tissue homogenization. Total RNA was extracted using TRIzol™ reagent (Invitrogen, Carlsbad, CA), chloroform extraction and isopropanol precipitation according to the manufacturer's protocol. RNA quality and quantity were checked by photometry at 260 and 280 nm.

2.5. Reverse Transcription and Quantitative PCR

cDNA was synthesized from 1 μg of total RNA using a cDNA synthesis kit (High Capacity cDNA Reverse Transcripton Kit; Applied Biosystems,

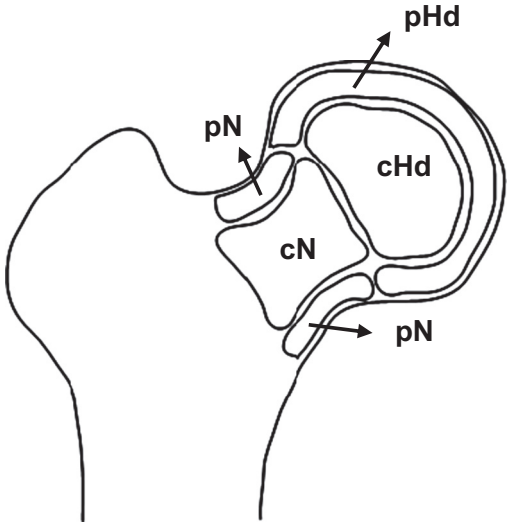

Fig. 1. Schematic diagram of the proximal femur and the sites from which samples were removed.