Gene 599 (2017) 36–52

Contents lists available at ScienceDirect

Gene

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

Research paper

Old age and the associated impairment of bones' adaptation to loading are associated with transcriptomic changes in cellular metabolism, cell-matrix interactions and the cell cycle

Gabriel L. Galea, PhD [a,1], Lee B. Meakin, PhD [a,*,1], Marie A. Harris, PhD [b], Peter J. Delisser, BVSc(Hons) [a], Lance E. Lanyon, DSc [a], Stephen E. Harris, PhD [b], Joanna S. Price, PhD [a]

[a] School of Veterinary Sciences, University of Bristol, Bristol, UK
[b] Department of Periodontics & Cellular and Structural Biology, University of Texas Health Science Centre, San Antonio, USA

ARTICLE INFO

Article history:
Received 15 October 2016
Accepted 6 November 2016
Available online 10 November 2016

Keywords:
Ageing
Bone
Mechanical loading
Proliferation

ABSTRACT

In old animals, bone's ability to adapt its mass and architecture to functional load-bearing requirements is diminished, resulting in bone loss characteristic of osteoporosis. Here we investigate transcriptomic changes associated with this impaired adaptive response. Young adult (19-week-old) and aged (19-month-old) female mice were subjected to unilateral axial tibial loading and their cortical shells harvested for microarray analysis between 1 h and 24 h following loading (36 mice per age group, 6 mice per loading group at 6 time points). In non-loaded aged bones, down-regulated genes are enriched for MAPK, Wnt and cell cycle components, including E2F1. E2F1 is the transcription factor most closely associated with genes down-regulated by ageing and is down-regulated at the protein level in osteocytes. Genes up-regulated in aged bone are enriched for carbohydrate metabolism, TNFα and TGFβ superfamily components. Loading stimulates rapid and sustained transcriptional responses in both age groups. However, genes related to proliferation are predominantly up-regulated in the young and down-regulated in the aged following loading, whereas those implicated in bioenergetics are down-regulated in the young and up-regulated in the aged. Networks of inter-related transcription factors regulated by E2F1 are loading-responsive in both age groups. Loading regulates genes involved in similar signalling cascades in both age groups, but these responses are more sustained in the young than aged. From this we conclude that cells in aged bone retain the capability to sense and transduce loading-related stimuli, but their ability to translate acute responses into functionally relevant outcomes is diminished.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Abbreviations: MAPK, mitogen-activated protein kinase; Actn2, actinin alpha 2; AMPK, adenosine monophosphate-activated protein kinase; Atf3, activating transcription factor 3; ATP, Adenosine triphosphate; b2MG, Beta-2-microglobulin; Bcl11a, B-cell CLL/lymphoma 11A; Bcl212, Bcl-2-like protein 2; BiNGO, A Biological Network Gene Ontology tool; BMD, bone mineral density; BMP, bone morphogenetic protein; Ccna2, cyclin A2; Ccnd3, cyclin D3; Cdc20, cell division cycle 20; Cdca3, cell division cycle associated 3; Cdk6, cyclin dependent kinase 6; CO_2, Carbon dioxide; Dab2IP, disabled 2 interacting protein; DAVID, Database for Annotation, Visualization and Integrated Discovery; Des, desmin; Dkk1, dickkopf WNT signalling pathway inhibitor 1; E2F1, E2F transcription factor 1; ECM, Extracellular matrix; EGF, epidermal growth factor; EGR2, early growth response 2; Ezh2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FDR, false discovery rate; Fgf7, fibroblast growth factor 7; Fgfr1, fibroblast growth factor receptor 1; Fzd7, frizzled class receptor 7; Gnas, GNAS complex locus; Grb10, growth factor receptor bound protein 10; Gtse, G2 and S-phase expressed 1; Igf1, insulin like growth factor 1; Igf2, insulin like growth factor 2; Insig2, insulin induced gene 2; Itgb1, integrin subunit beta 1; Kcnma1, potassium calcium-activated channel subfamily M alpha 1; KEGG, Kyoto Encyclopaedia of Genes and Genomes; Mapt, microtubule associated protein tau; Mark3, microtubule affinity regulating kinase 3; MCM, Minichromosome maintenance protein complex; Mef2c, myocyte enhancer factor 2C; MEPE, matrix extracellular phosphoglycoprotein; Mki67, marker of proliferation Ki-67; MMP13, matrix metallopeptidase 13; Mmp25, matrix metallopeptidase 25; Myf6, myogenic factor 6; NADH, Nicotinamide adenine dinucleotide; Nusap1, nucleolar and spindle associated protein 1; PASTAA, Predicted Association of Transcription factors from Annotated Affinities; PBS, Phosphate buffered saline; PdgfA, platelet derived growth factor, alpha; Phex, phosphate regulating endopeptidase homolog, X-linked; Pitx2, paired-like homeodomain transcription factor 2; Pkia, protein kinase (cAMP-dependent, catalytic) inhibitor alpha; qRT-PCR, quantitative real time polymerase chain reaction; QTC, Quality Threshold Clustering; RarB, retinoic acid receptor beta; RNA, Ribonucleic acid; Scyl1, SCY1 like pseudokinase 1; Sost, sclerostin; Sox6, SRY-box 6; Sox9, SRY-box 9; Sp7, Sp7 transcription factor 7; SPEED, Signalling Pathway Enrichment using Experimental Datasets; Tcf15, transcription factor 15; TGFb, transforming growth factor beta 1; TNFa, tumor necrosis factor a; Ttn, titin; Vcam1, vascular cell adhesion molecule 1; VegfA, vascular endothelial growth factor A; Wnt16, Wnt family member 16.

* Corresponding author at: School of Veterinary Sciences, University of Bristol, Langford House, Bristol BS40 5DU, UK.
E-mail address: lee.meakin@bristol.ac.uk (L.B. Meakin).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.gene.2016.11.006
0378-1119/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

The mass and structural organisation of bone tissue necessary to sustain functional loads without damage is established and maintained throughout life by the processes of bone modelling and remodelling. Bone formation by osteoblasts is coordinated with bone resorption by osteoclasts to ensure that at each location of each bone there is sufficient tissue appropriately aligned to withstand the mechanical loads to which it is subjected during physical activity. As the skeleton ages, an imbalance between bone formation and resorption occurs resulting in net bone loss. In humans, the extent of this loss can be sufficient that fragility fractures occur with minimal trauma. This is the major characteristic of post-menopausal and age-related osteoporosis. Similar age-related deterioration in bone structure occurs in mice; 19-month-old mice have dramatic reductions in cortical and trabecular bone relative to 19-week-old mice (Meakin et al., 2014; Galea et al., 2015a).

For bone formation and resorption to maintain appropriate bone mass and architecture, osteoblasts and osteoclasts must receive a functionally-relevant stimulus that they must follow with an adequate response. It is thought that the primary local stimulus for (re)modelling originates from the response of osteoblasts and osteocytes to the mechanical strains engendered by functional loading within the bone matrix. This stimulus interacts with systemic influences to orchestrate the activity of osteoblasts and osteoclasts in order to locally maintain bone's functional integrity. Mechanisms that could contribute to the age-related imbalance between bone formation and resorption include impaired osteocyte function and/or impaired osteoblast responses to "osteogenic cues", including those resulting from mechanical simulation (Meakin et al., 2014). Mechanical loading triggers many acute responses, including the transcriptional regulation of hundreds of genes (Zaman et al., 2009). The functional outcome of any changes in gene expression depends not only upon their nature, but also the context in which they operate. For example, global depletion of the estrogen receptor ERα greatly diminishes the number of genes transcriptionally regulated following loading (Zaman et al., 2009).

It remains unclear whether the diminished adaptive response observed in old bones is a reflection of the acute responses of osteoblasts and osteocytes to loading being impaired or because the osteogenic context in which these responses occur has changed. We have recently demonstrated that several acute responses of osteoblastic lineage cells to mechanical strain are not significantly different between young and aged mice, including down-regulation of the Wnt antagonist sclerostin in osteocytes, up-regulation of the transcription factor Egr2, and entry of osteoblastic cells into the cell cycle (Meakin et al., 2014). If old age has little effect on the initial responses to strain this would suggest that age–related deterioration in the effectiveness of (re)modelling is due to reduced functional responses to the stimulus that strain engenders.

To begin to address this question, several in vitro studies have investigated replicative senescence transcriptomic changes as a model of ageing. Serial passage of human mesenchymal stem cells (osteoblast precursors) induces changes in genes related to proliferation, cell cycle progression and stress responses (Ryu et al., 2008). The bone marrow of 2-, 8- and 26-month old mice examined in vitro shows age-related changes in genes linked to differentiation, cell cycle progression and growth factors (Wilson et al., 2010). However, since ageing involves so many processes, in vitro models are severely limited. A previously-reported in vivo study examined the effect of ageing on gene expression in the bone of the cochlea and found that genes demonstrating age-related changes in expression were those involved in collagen maturation, extra-cellular matrix formation and bone mineralisation (Gong et al., 2006). A further microarray study compared gene expression patterns in transiliac bone biopsies from control and osteoporotic patients (Jemtland et al., 2011), reporting differences in the expression of the Wnt pathways antagonists (Sost, Dkk1) and bone matrix proteins (MEPE, MMP13). More recently, transcriptomic analysis of the response to loading in young versus old mice revealed blunted activation of canonical Wnt signalling, a potently osteogenic pathway, in the old following repeated bouts of loading (Holguin et al., 2016).

In this study we document age-associated differences in the basal transcriptome and in the in vivo loading-related transcriptomic responses of tibial cortical shells from young (19 week old) and aged (19 month old) female mice. Cellular processes and signalling pathways over-represented in the resulting list of differentially expressed genes were identified using a variety of bioinformatics techniques.

2. Materials and methods

2.1. Animals

19-week-old young adult and 19-month-old aged female C57BL/6 mice (36 of each age) were obtained from Charles River Inc. (Margate, UK). All mice were allowed free access to water and a maintenance diet containing 0.75% calcium (EURodent Diet 22%; PMI Nutrition International, LLC, Brentwood, MO, USA), 12-hour light/dark cycle, room temperature at 21 ± 2 °C. Cages contained wood shavings, bedding and a cardboard tube. Mice were housed in groups of up to 5 animals (Meakin et al., 2013). All procedures complied with the UK Animals (Scientific Procedures) Act 1986 and were reviewed and approved by the University of Bristol ethics committee (Bristol, UK).

2.2. In vivo external mechanical loading

Right tibias were subjected to one period of external mechanical loading under isoflurane-induced anesthesia. Left limbs were used as internal controls as previously validated (Sugiyama et al., 2010). The protocol for noninvasively loading the mouse tibia has been reported previously (Sugiyama et al., 2011,2012). The flexed knee and ankle joints are positioned in concave cups; the upper cup, containing the knee, is attached to an actuator arm of a loading device and the lower cup to a dynamic load cell. The tibia is held in place by a 0.5 N continuous static preload. Forty cycles of dynamic load are superimposed with 10 s rest intervals between each cycle. The protocol for one cycle consists of loading to the target peak load, hold for 0.05 s at the peak load, and unloading back to the 0.5 N preload. From the strain gage data previously reported (Meakin et al., 2014), different peak loads for young (15 N) and aged (12 N) mice were calculated to apply 2,500 με measured on the medial aspect of the tibia at the 37% site. Strain rate at this site was normalised to a maximum of 30,000 με s^{-1}.

2.3. RNA extraction and processing

Mice were killed by CO_2 asphyxiation and left tibiae immediately dissected from soft tissue attachments. The epiphyses were resected and marrow centrifuged out, leaving a cortical shell. Similarly-prepared samples predominantly contain osteocytes (Kelly et al., 2014). Shells were immediately snap frozen in liquid nitrogen. 2 tibiae in each group were pooled for RNA extraction giving 18 young and 18 aged bone samples. Bones in Qiazol lysis buffer were agitated with stainless steel ball bearings to disrupt the tissue according to the manufacturer's protocol (RNeasy Universal kit with TissueLyser LT, Qiagen). RNA was quantified using NanoDrop ND1000 and RNA quality assessed using a 2100 Bioanalyzer (Supplementary Fig. 1). RNA was converted to biotinylated hybridised cRNA using the Ambion Illumina TotalPrep RNA amplification kit following the manufacturer's instructions. Microarray analysis was performed at the Genomics and Microarray Core Facility at the University of Texas Southwestern Medical Centre at Dallas, USA. Microarray analysis was completed using Illumina MouseWG-6 v2.0 Expression BeadChip Kits, which probes for 45,281 probes that can measure over 21,000 coding gene expression levels in each sample. 6 chips were used for this experiment with 6 samples run on each chip.

Download English Version:

https://daneshyari.com/en/article/5589884

Download Persian Version:

https://daneshyari.com/article/5589884

Daneshyari.com