Involvement of the Osteoblast in Paget's Disease of Bone

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ABSTRACT

Paget's disease is characterised by focal regions of accelerated bone turnover. The aetiology is unknown, but genetic and environmental factors have been implicated. Pagetic lesions contain increased numbers of osteoclasts with abnormal morphology, so an osteoclast defect has been considered central to the pathogenesis. However, given osteoblasts regulate osteoclast differentiation and activity; osteoblast abnormalities may be important in the disease. This study aimed to identify features of pagetic osteoblasts that could clarify their role in Paget's disease.

Gene expression in osteoblasts and bone marrow cultured from pagetic lesions of 23 patients was compared to cells from unaffected tissue using both microarrays and real time RT-PCR. The results indicated global changes in gene expression in pagetic osteoblasts. A number of genes that can stimulate osteoclastogenesis, including interleukins 6 and 1β, and monocyte chemotactic factor 1 were up-regulated, but the *RANKL/OPG* ratio tended to be decreased. Genes involved in osteoblast differentiation were down-regulated, including the transcription factors *RUNX2*, *DLX5* and *SATB2*, the osteogenic factor *BMP2*, and the matrix proteins osteocalcin and bone sialoprotein. Markers of less mature osteoblastic cells, alkaline phosphatase and matrix gla protein were up-regulated. The intermediate filament, keratin 18, was very significantly up-regulated in pagetic cells. Over-expression of this protein in osteoblasts using an adenoviral vector produced some changes in gene expression, but did not produce an overtly pagetic phenotype. Over-expression of *SQSTM1* mutants found in some patients with Paget's disease also produced only minor changes in osteoblast phenotype. The RNA from the primary cell cultures was also used to investigate the presence of measles virus and somatic mutations in *SQSTM1* in the disease, but neither were identified in any of the patients.

These results suggest that there are important changes in pagetic osteoblasts that are maintained when the cells are removed from the affected bone microenvironment. These include enhanced production of factors to stimulate osteoclastogenesis, while osteoblast differentiation and activity may be impaired. We were unable to identify genetic or environmental factors that could trigger these changes. The pagetic osteoblast is distinct from control cells, and is likely to contribute to the development of Paget's disease.

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ABBREVIATIONS

$1,25(OH)_2D_3$	1,25 dihydroxyvitamin D_3
A2P	L-ascorbic acid 2-phosphate
AAA	ATPases associated with a variety of activities
ALP	alkaline phosphatase
aPKC	atypical protein kinase C
ATF	activating transcription factor
αMEM	minimum essential medium, alpha
BM	bone marrow
BMD	bone mineral density
BMP	bone morphogenetic protein
BMU	basic multicellular unit
bp	base pair
BSA	bovine serum albumin
BSP	bone sialoprotein
C/EBP	CAAT/enhancer-binding protein
cAMP	cyclic adenosine monophosphate
CAR	Coxsackie-adenovirus receptor
Cath K	cathepsin K
CBP	CREB binding protein
CCL2	monocyte chemotactic protein 1
CFU-GM	granulocyte macrophage colony forming unit
CHI3L1	chitinase 3-like 1
Col-I/II/X	type I/II/X collagen
CREB	cAMP response element binding
CTx	C-telopeptide of collagen crosslinks
DCt	delta Ct
DKK1	dickkopf 1
DMEM	Dulbecco's modified Eagle's medium
DMP1	dentin matrix protein 1
Dpd	deoxypyridolines
Dsh	dishevelled
ER	oestrogen receptor

ERK	extracellular signal-regulated kinase
FBS	foetal bovine serum
FEO	familial expansile osteolysis
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FPPS	farnesyl pyrophosphate synthase
Fra	fos-related antigen
GATA6	GATA binding protein 6
GCOS	GeneChip operating software
GFP	green fluorescent protein
gla	γ-carboxylic acid
GM-CSF	granulocyte macrophage colony stimulating factor
GO	gene ontology
GSK	glycogen synthase kinase
HOB	human osteoblasts
hr	hour
IBMPFD	inclusion body myopathy, Paget's disease and frontotemporal dementia
IGF	insulin-like growth factor
Ihh	Indian hedgehog
IKK	IκB kinase
IL	interleukin
IL-1R	Interleukin 1 receptor
I-Smad	inhibitory Smad
IκB	inhibitor of NFκB
JAK	Janus kinase
JNK	jun N-terminal kinase
KEGG	Kyoto encyclopaedia for genes and genomes
LRP	low-density lipoprotein receptor-related protein
M gene	matrix gene
MAPK	mitogen activated protein kinase
M-CSF	macrophage colony stimulating factor
MEF2	myocyte-enhancer factor 2
MEM	minimum essential medium
MEPE	matrix extracellular phosphoglycoprotein

MGB	minor groove binder
MGP	matrix gla protein
min	minute
MMP	matrix metalloproteinase
MOI	multiplicity of infection
MRFs	myogenic regulatory factors
MV	measles virus
N gene	nucleocapsid gene
NCoR	nuclear receptor corepressor
NFAT	nuclear factor of activated T cells
NFκB	nuclear factor kappa B
NGFR	nerve growth factor receptor
NIBSC	National Institute of Biological Standards and Control
NTC	no template control
NTx	N-telopeptide of collagen crosslinks
OB	osteoblast
OC	osteocalcin
ONPG	o-nitrophenyl-β-D-galactopyranoside
OPG	osteoprotegerin
Osx	osterix
P1CP	carboxy-terminal propeptide of type I collagen
P1NP	amino-terminal propeptide of type I collagen
p62	sequestosome 1
PB1	phox and bem1p-1
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCOT2	principal coordinates and Hotelling's T squared
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
pfu	plaque-forming units
PGD_2	prostaglandin D_2
PGE_2	prostaglandin E_2
PI3K	phosphatidylinositol-3 kinase
PPARγ	peroxisome proliferator-activated receptor γ

PTGDS	prostaglandin D2 synthase, brain isoform
PTH	parathyroid hormone
PTHrP	parathyroid hormone related protein
Pyd	pyridinolines
RANK	receptor activator of nuclear factor κB
RANKL	RANK ligand
RGS	regulator of G-protein signalling
RIN	RNA integrity number
RIP	receptor interacting protein
RMA	robust multichip analysis
R-Smad	receptor-activated Smad
RT-PCR	reverse transcription polymerase chain reaction
s	second
SAFE	significance analysis of function and expression
SERM	selective oestrogen receptor modulators
sFRP	secreted frizzled-related protein
SGK1	serum/glucocorticoid regulated kinase 1
SIBLING	small integrin-binding ligand, N-glycosylated proteins
SMRT	silencing mediator of retinoid and thyroid receptors
SOST	sclerostin
SQSTM1	sequestosome 1
SSPE	subacute sclerosing panencephalitis
STAT	signal transducers and activators of transcription
TBS	tris-buffered saline
TBS-T	tris-buffered saline with Tween20
TFGβ	transforming growth factor β
TNF	tumour necrosis factor
TNFR1	TNFα receptor
TRADD	TNFR1-associated via death domain
TRAF6	TNF receptor-associated factor 6
TRAP	tartrate resistant acid phosphatase
UBA	ubiquitin associated
VCP	valosin-containing protein
VDR	vitamin D receptor

WIF	Wnt inhibitory factor
WISP	WNT1 inducible signalling pathway protein

Selected amino acid abbreviations

D	aspartic acid
G	glycine
K	lysine
L	leucine
P	proline
R	arginine
X	truncation mutation/stop codon