Table 2. Main characteristics of studies in cell cultures investigating the role of statins in bone metabolism

Author	Method	Statin	Outcome	Comments
Hughes A et al 2007[52]	Mouse macrophage like cells, osteoclast like cells from rabbit bone marrow	Rosuvastatin, pravastatin, simvastatin, cerivastatin	i) Order of potency for inhibition of bone resorption CER>SIM>RSV>PRA ii) PRA inhibited resorption at concentrations >50μM iii) Single injections of RSV and CER sufficient to prenylate bone marrow cells	i) Hydrophobic and hydrophylic statins can inhibit osteoclast function in vitro ii) > doses of statins can inhibit protein prenylation in osteoclasts in vivo iii) > doses of CER or RSV mildly prevent the bone loss
Ruis-Gaspa et al 2007[53]	Cultures of bone specimens of 3 post menopausal women and MG3 osteosarcoma like cells.Cells incubated with the presence of statin	Simvastatin or atorvastatin fro 10^{-6}M -10^{-9}M	COLLIA1, OC, BMP2 gene expression significant ▲, similar effects to MG3 cells	SIM and atorvastatin stimulatory effects in COLLIA1, OC, BMP2 genes, (+) effect in osteoporosis
Ahn KS et al 2008[54]	Mouse macrophage cells, human breast adenocarcinoma and multiple myeloma cells exposed to statins and RANKL	Simvastatin	i) Osteoclast ▼ with▲ concentrations of simvastatin, ii) the inhibitory effect▼ in time-dependent manner, iii)inhibited osteoclastogenesis induced by tumor cells	Simvastatin inhibits the RANKL-induced NF-kappaB activation, suppresses osteoclastogenesis, therapeutic potential in osteoporosis and in cancer-related bone loss.
Yamashita et al 2008[55]	Cultures of mouse myoblast cell line C2C12 treated with BMP-2, TNF-a, SIM	Simvastatin	SIM no effects on Runx2 and ALP activity, SIM reversed TNF-a inhibition of BMP-induced Smad 1, 5, 8 phosphorylation, SIM ▲ expression of Smad in C2C12 cells exposed to TNF-a, SIM suppressed TNF-a phosphorylation of ERK1/2 and SARK/JNK, FPP and GGPP reversed the SIM effects on TNF-a induced activation of Ras/Rho/MARK pathway	SIM supports BMP-induced osteoblasts differentiation through antagonizing TNF-a-to-Ras/Rho/MARK pathway and augmenting BMP-Smad signaling suggesting potential usage of SIM to inflammatory bone damage
Monjo et al 2010[56]	Cultures of mouse osteoblastic cell line MC3T3-E1	Different concentrations of rosuvastatin (0.001-10μ M)	< concentrations of RSV were protective against cell death and > showed cytotoxicity	RSV promotes osteoblast differentiation and regulates the expression of Slco1a1 which may constitute the transport system for RSV across the cell membrane in mature osteoblasts
Yamashita et al 2010[57]	Mouse osteoclast line cell MLC-6 from mouse bone marrow co-cultured with mouse chondrocytes	Simvastatin	SIM suppressed osteoclastic activity and▲ RANK, TRAP and cathepsin K expression, SIM activated ERK, SARK/JNK, AKT pathways and inactivated Ras, Src phosphorylation suppressed by SIM	SIM inhibits osteoclastic differentiation through inhibiting Src and enhancing MARK/AKT pathways
Chen et al 2010[58]	Cell cultures of mice osteoblast like cells after 3 days examined the mitochondria osteoblastic activity with various concentrations of SIM	Simvastatin	With 10^{-6}M SIM ALP enhanced and BMP-2, ALP, sialoprotein, type I collagen up-regulated, RasGRF1 and phosphoRasGRF1 ▼	i) SIM can promote osteoblast viability and differentiation via Smad/Erk/BMP-2 pathway, ii) statins stimulate osteoblast differentiation in vitro

Table 2. (continued)

Author	Method	Statin	Outcome	Comments
Zhou et al 2010[59]	An ITB composed of hADSCs and hPRP was preliminarily constructed, but its osteogenic capability needs improving	Simvastatin	0.01 microm, 0.1 microm, and 1 microm SIM induce hADSCs' osteoblastic differentiation in vitro accompanied with non-inhibition on cell proliferation, > ALP activity, more mineralization deposition and more expression of osteoblast-related genes such as OC, Cbfa1 , BMP-2,VEGF, and basic FGF	1) Simvastatin at 1 mIcrom seemed the most optimal concentration due to its high osteocalcin secretion in media, 2) simvastatin at optimal concentrations can be used to promote this ITB's osteogenesis
Pagkalos et al 2010[60]	ESCs, derived from the inner cell mass of the blastocyst	Simvastatin	1) Simvastatin induces murine ESC differentiation toward the osteogenic lineage in the absence of osteoinductive supplements, 2) simvastatin concentration in the micromolar range and > was toxic to the cells and that an effective concentration for osteoinduction is 0.1 nM	Lipophilic simvastatin may provide a novel pharmacologic agent for bone tissue engineering applications

CER: cerivastatin; SIM: simvastatin; PRA: pravastatin; RSV: rosuvastatin, COLLIA1: collagen type I a 1; BMP-2: bone morphogenetic protein-2; OC: osteocalcin; RANKL: receptor activator of nuclear factor kappa-b ligand; TNF-a: tumor necrosis factor alpha, FPP: farnesyl pyrophosphate, GGPP: geranylgeranyl pyrophosphate, TRAP: tartrate resistant acid phosphatase, ALP: alkaline phosphatase, ITB: injectable tissue engineered bone, hADSCs: human adipose-derived cells; h PRP: human platelet rich plasma; Cbfa1: core binding factor alpha 1; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; ESCs: embryonic stem cells; >: higher; <: lower, (+): positive; ▲: increased; ▼: decreased.