Oncostatin M (OSM) and leukemia inhibitory factor (LIF) are IL-6 family members with a wide range of biological functions. Human OSM (hOSM) and murine LIF (mLIF) act in mouse cells via a LIF receptor (LIFR)-glycoprotein 130 (gp130) heterodimer. In contrast, murine OSM (mOSM) signals mainly via an OSM receptor (OSMR)-gp130 heterodimer and binds with only very low affinity to mLIFR. hOSM and mLIF stimulate bone remodeling by both reducing osteoclastic resorption and up-regulating the pro-osteoclastic factor receptor activator of NF-κB ligand (RANKL) in osteoblasts. In the absence of OSMR, mOSM still strongly suppressed sclerostin and stimulated bone formation but did not induce RANKL, suggesting that intracellular signaling activated by the low affinity interaction of mOSM with mLIFR is different from the downstream effects when mLIF or hOSM interacts with the same receptor. Both STAT1 and STAT3 were activated by mOSM in wild type cells or by mLIF/hOSM signals by binding first to the ubiquitously expressed glycoprotein 130 co-receptor (hLIFR) or the human leukemia inhibitory factor receptor (hOSMR) or the human leukemia inhibitory factor receptor (hOSM). The authors declare that they have no conflicts of interest with the contents of this article.

Oncostatin M (OSM) \(^2\) is a member of the IL-6 cytokine superfamily with a diverse range of activities \((1)\). It was first identified as a secreted product of macrophage-like cells that inhibited proliferation of melanoma-, neuroblastoma-, and lung cancer-derived cell lines \((2)\), and although it suppresses breast cancer cell proliferation \((3)\), it has been suggested to support metastasis of breast cancer in the skeleton due to pro-osteoclastic activities \((4)\) and to stimulate Kaposi’s sarcoma \((5)\). Studies in knock-out mice have shown that OSM supports hematopoiesis \((6)\) and bone formation at the expense of adipogenesis \((7)\), but it has also been implicated in pulmonary tissue fibrosis \((8)\), cardiac disease and repair \((9)\), prostate cancer \((10)\), osteogenesis \((7, 22)\) and stimulates bone formation \((7)\) at least in vivo.

In the skeleton, OSM is a paracrine factor expressed by osteoblasts \((7)\), osteocytes \((7)\), and macrophages \((16–19)\). OSM stimulates the formation of bone-resorbing osteoclasts indirectly by acting on osteoblasts \((20)\) to promote their expression of receptor activator of NF-κB ligand (RANKL) \((21)\). OSM also promotes osteoblast differentiation at the expense of adipogenesis \((7, 22)\) and stimulates bone formation formation \((7)\) at least in vivo.

\(^{2}\) The abbreviations used are: OSM, oncostatin M; LIF, leukemia inhibitory factor; hOSM, human OSM; mLIF, murine LIF; LIFR, LIF receptor; gp130, glycoprotein 130; mOSM, murine OSM; OSMR, OSM receptor; RANKL, receptor activator of NF-κB ligand; hOSMR, hOSM receptor; hLIFR, human leukemia inhibitory factor receptor; mOSMR, murine OSMR; mLIFR, murine LIFR; qPCR, quantitative real time PCR; logFC, fold change; LA, LIFR antagonist; mmp13, murine gp130; nM, minimum Eagle’s medium; pSTAT, phospho-STAT; AF, Alexa Fluor; CT, computed tomography; MFI, mean fluorescence intensity.
part by acting on osteocytes (23) (matrix-embedded osteoblasts) and inhibiting their production of the Wnt antagonist sclerostin (7, 23). These actions mediated by OSMR are required for normal levels of bone formation and resorption in juvenile and adult skeletons (7) and for normal skeletal response to parathyroid hormone (24), a therapeutic agent used to reduce fracture risk in osteoporosis. Surprisingly, in the absence of OSMR, mOSM can signal through LIFR to suppress sclerostin expression and stimulate bone formation (7). Unlike LIF and hOSM, when mOSM acted through mLIFR, it did not significantly stimulate RANKL production (7). This suggested a novel pathway that could increase bone mass by stimulating bone formation without promoting bone resorption.

The mechanism by which mOSM-mLIFR activates downstream signals different from those activated by canonical murine LIF (mLIF)-mLIFR signaling is unknown. This study sought to (a) identify unique downstream signaling pathways of mOSM-mLIFR compared with hOSM-mLIFR and mLIF-mLIFR, (b) determine mechanisms by which such distinct signaling pathways could be activated, and (c) ascertain the benefit of promoting the mOSM-mLIFR-specific pathway in low bone mass.

Results

Identification of Downstream Gene Signatures by Microarray—Microarray analysis indicated that the expression changes caused by mLIF and hOSM in OsMr−/− primary calvarial osteoblasts (defined as mLIF-mLIFR effects and hOSM-mLIFR effects, respectively) were very highly correlated (Fig. 1A), supporting previous observations that hOSM acts entirely through LIFR in murine cells (25). A similar correlation between mLIF and hOSM responses was observed in WT (data not shown).

FIGURE 1. Identification of the distinct mOSM-LIFR gene signature. A, scatterplot of log₂-fold changes of gene expression in 17-day-differentiated OsMr−/− primary calvarial osteoblasts treated with mLIF or hOSM for 6 h. The genes biological correlation between the log₂-fold change profiles is 99.7%.

Values are means from three independent cultures, each performed in triplicate; error bars represent S.E.

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Mt2 confirmed strong enrichment of STAT3 target genes in those retained the five most strongly regulated probes, positive targets of canonical LIFR signaling with a strong genes regulated by mOSM-mLIFR), we first determined the subset of all those regulated by the canonical pathway ((mLIF/mLIFR)-LIFR targets; the slash represents a possible substitution either hOSM or mLIF). To identify the distinctive subset (only nine genes were identified at 6 h).

For this reason, mLIF-mLIFR and hOSM-mLIFR responses were averaged for all subsequent analyses.

No unique set of genes was differentially expressed in OsMr−/− cells treated with mOSM (i.e. mOSM-mLIFR targets) compared with mLIF- and hOSM-treated WT or OsMr−/− cells. Instead, mOSM-mLIFR-regulated target genes formed a subset of all those regulated by the canonical pathway (mLIF/ hOSM)-LIFR targets; the slash represents a possible substitution (either hOSM or mLIF). To identify the distinctive subset of gene activation retained by mOSM in OsMr−/− cells (i.e. the genes regulated by mOSM-mLIFR), we first determined the positive targets of canonical LIFR signaling with a strong mOSM response by selecting genes up-regulated at least 2-fold by hOSM/mLIF in OsMr−/− cells with an mOSM -fold change above the slope of the line of regression between the differential expression of hOSM/mLIF versus control and mOSM versus control in OsMr-null cells. This allowed us to account for the difference in magnitude of effect between hOSM/mLIF and OsMr−/− cells. This revealed that STAT3-responsive genes were up-regulated by mOSM in OsMr−/− cells at both 1 and 6 h (Fig. 1, B and C, and Table 1). At 1 h of treatment, eight of the top 10 genes disproportionately up-regulated by mOSM-LIFR were Socs3 (26), Cxcl1 (27), Zfp36 (28), Mt2 (29), Sbn2 (30), Bcl3 (31), Junb (32), and Fos (33) (Fig. 1B). The only “non-STAT3 targets” in this top 10 were Cish, a STAT5-responsive gene (34), and Ras11a, a putative small Ras GTPase (35). At 6 h of treatment, the five most strongly regulated probes, Bcl3, Mmp13 (36), Socs3, and Mt2 (Fig. 1C and Table 1), were also STAT3 targets. We confirmed strong enrichment of STAT3 target genes in those retained by mOSM signaling in OsMr−/− cells by comparison with the available STAT3 gene sets from the Molecular Signatures Database (MSigDB); significant enrichment was observed from a number of STAT3 target data sets at 1 h, including DAUER_STAT3_TARGETS_UP (five of 66 genes, p = 3.5E−14), ST_STAT3_PATHWAY (two of 15 genes, p = 7.7E−07), and BAKER_HEMATOPOIESIS_STAT3_TARGETS (two of 16 genes, p = 8.8E−07), and at 6 h, including DAUER_STAT3_TARGETS_UP (four of 66 genes, p = 8.9E−10) and NUMATA_CSFSIGNALING_VIA_STAT3 (two of 23 genes, p = 9.1E−06).

In contrast, in the top 10 genes most highly regulated by the canonical (hOSM/mLIF)-LIFR pathway that were not regulated by mOSM-mLIFR were known STAT1/IFN-γ-responsive genes (Fig. 1, B and C, and Table 2). At 1 h, the top nine known genes regulated were all STAT1/IFN-γ targets: Mx2 (37), Igrom1 (38), Ccl2 (39), Serpin3f (40), Socs1 (41), Cxcl10 (39), Ifi47 (42), Trim35 (42), and Cxcl9 (43). At 6 h, the top 10 known probes were all STAT1/IFN-γ-responsive genes: Gbp2, Gbp3 (44), Gbp5 (45), Cxcl9 (43), Osas2 (46), Serpin3f (40), Igt7 (47), Psmb9 (48), Dhx58 (49), and Osas1g (49). A subset of genes was confirmed by quantitative real time PCR (qPCR) in a further three independent sets of cultures from OsMr−/− and WT cells (Fig. 1, D and E). This indicates that the interaction of mOSM with LIFR, unlike canonical mLIF/LIFR signaling, did not induce STAT1-responsive genes but retained the ability to induce STAT3 target genes, albeit at a lower level.

Tnfsf11 (the gene for RANKL) was significantly stimulated downstream of mOSM-LIFR in contrast to our earlier PCR-based analysis. As we reported previously, Tnfsf11 was not up-regulated by mOSM in OsMr−/− cells at 1 h after treatment (7), but at 6 h after treatment Tnfsf11 was up-regulated by mOSM 2.8-fold. This response was 9.6-fold lower than the response observed to mOSM in WT cells (p = 2.43 E-14) and less than the response in OsMr−/− cells treated with hOSM/mLIF (Tnfsf11 was increased 6.1-fold for both hOSM and mLIF).

Our hypothesis was that osteoblastic genes promoting osteoblast differentiation would be regulated through mOSM-LIFR, whereas genes supporting osteoclast formation (catabolic) would be regulated by mOSM through OSMR. To identify those genes regulated by only mOSM through OSMR (mOSM-OSMR) and not by mOSM through LIFR, we determined genes differentially expressed in WT cells treated with mOSM versus control that were not differentially expressed in OsMr−/− cells treated with mOSM versus control; mOSM-OSMR targets numbered 2669 genes (supplemental Table 1). This gene set was compared with those differentially expressed in mLIF/
hOSM-treated WT cells (mLIF/hOSM-LIFR targets) which identified 172 regulated genes in common between mLIF/mOSM-LIFR and mOSM:OSMR, and 2497 genes unique for mOSM:OSMR (Fig. 1F; supplemental Table 1) were identified. mOSM:OSMR target genes included some previously identified to mediate actions of OS in cartilage degradation (Adams4) and osteoclastogenesis (Il33) (50, 51) (rank 47, log₂-fold change (log₂FC) 2.96, \(p = 9.17E^{-13}\)). Although previously we could detect no osteoblastic response to IL-31, IL-31α receptor (Il31ra) mRNA was detected and found to be elevated by mOSM:OSMR.

The comparison between target genes revealed that mOSM:OSMR does not stimulate a purely catabolic gene set. Some genes known to suppress osteoblast differentiation and promote adipogenesis, including Zfp521 and Zfp467 (52–54), were down-regulated through mOSM in wild type cells (rank, log₂FC, and \(p\) values: Zfp521, 69, \(-1.6\), \(p = 2.77E^{-13}\); Zfp467, 602, \(-1.65\), \(p = 8.39E^{-8}\)). Indeed, genes supportive of osteoblast commitment and differentiation, including Sost, Sp7, Ifitm5, Zfp521, Zfp467, Mef2c, and Cebp, were regulated through multiple pathways as were genes supportive of osteoclast differentiation or precursor migration, such as Tnfsf11, Cxcl1, Wnt16, and Il33 (Fig. 1F). This indicated that there is no purely anabolic gene response to mOSM-LIFR compared with (hOSM/mLIF)-LIFR or mOSM-mOSMR.

Confirmation of LIFR-dependent STAT3 Induction—Western blotting analysis showed that mOSM, hOSM, and mLIF treatment of WT primary calvarial osteoblasts stimulated STAT1 and STAT3 phosphorylation (Fig. 2, A and B). Although Osmr\(^{-/-}\) cells responded to hOSM and mLIF with phosphorylation of the lower molecular weight form of STAT1, no STAT1 phosphorylation was detected in response to mOSM (Fig. 2A). STAT3 phosphorylation at tyrosine 705 was robustly stimulated by mOSM, hOSM, and mLIF in both WT cells and Osmr\(^{-/-}\) cells at 15 and 30 min (15 min shown in Fig. 2B), indicating an intact STAT3 phosphorylation response in the absence of OSMR. To confirm that the mOSM-induced increase in STAT3 phosphorylation was mediated by LIFR, we tested the response when cells were pretreated with a specific LIFR antagonist (LA) (55). This blocked the induction of STAT3 phosphorylation by mOSM, hOSM, and mLIF in Osmr\(^{-/-}\) cells (Fig. 2C), showing that STAT3 phosphorylation in the absence of OSMR was mediated by LIFR. In wild type cells, LA blocked the effects of hOSM and mLIF but not mOSM, indicating that mOSM acts preferentially through OSMR.

Use of a specific STAT3 inhibitor (ML116) (56) validated that the Socs3 gene response to mOSM-LIFR was STAT3-dependent (Fig. 3, A and B). In WT cells, Socs3 mRNA levels induced by mLIF and mOSM were inhibited only by the highest dose of ML116 (10 \(\mug/ml\)), whereas the increased Socs3 mRNA levels in Osmr\(^{-/-}\) cells treated with mOSM were completely blocked by even the lowest dose of ML116 (Fig. 3A). Phosphorylation of STAT3 in response to mOSM in Osmr\(^{-/-}\) cells was reduced by ML116 treatment as was the response to mLIF in both wild type and Osmr\(^{-/-}\) cells (Fig. 3, B and C).

Because STAT3 phosphorylation at serine 727 and tyrosine 705 may result in different downstream activities (57), we sought to determine whether mOSM-LIFR influences only a subset of mLIF-LIFR-responsive genes because it phosphorylates only tyrosine 705 (Tyr-705) and not serine 727 (Ser-727). Phosphorylation analysis indicated STAT3 phosphorylation occurred at both Tyr-705 and Ser-727 in response to mOSM in Osmr\(^{-/-}\) primary calvarial osteoblasts (Fig. 4). Levels of phosphorylation in mLIF-LIFR-responsive genes were found to be equivalent between hOSM/mLIF-LIFR and mLIF/mOSM-LIFR within the dose range tested. Levels of phosphorylation in mLIF-LIFR-responsive genes were found to be equivalent between hOSM/mLIF-LIFR and mLIF/mOSM-LIFR within the dose range tested.

![Table 2](https://example.com/table2.png)

**TABLE 2**

Top probes regulated by (hOSM/mLIF)-LIFR but lost by mOSM-LIFR

The top probes ordered by mLIF-fold change lying below the cyan dashed lines in Fig. 1C and D, respectively, are shown. At 1 h, the selected genes have log₂FC response to mLIF <27.5% of that in hOSM/mLIF. At 6 h, the selected genes have log₂FC response to mOSM <30% of that in hOSM/mLIF.
were also comparable with those induced by mLIF and hOSM in Osmr−/− and WT primary calvarial osteoblasts (Fig. 4). This suggests that the difference in genes transcribed in response to mOSM-LIFR versus mLIF-LIFR does not relate to a difference in STAT3 phosphorylation patterns.

To determine whether differences in gp130 and LIFR phosphorylation could be responsible for the bias toward STAT3 signaling of mOSM-LIFR versus mLIF-LIFR, we assessed phosphorylation of these receptor subunits in response to each ligand (Fig. 5, A–C). In WT cells, mOSM treatment resulted in robust OSMR and gp130 phosphorylation (Fig. 5, A and B). OSMR was not phosphorylated in response to mLIF (Fig. 5B) and was not detected in Osmr−/− cells as expected (Fig. 5B). LIFR phosphorylation was robustly detected in response to mLIF in WT and Osmr−/− cells (Fig. 5C). Although mLIF and hOSM both induced phosphorylation of gp130 in Osmr−/− cells, gp130 phosphorylation was barely detected in response to mOSM and in some experiments could not be detected at all (Fig. 5A). In addition, LIFR phosphorylation in response to mOSM in Osmr−/− cells was also only barely detected (Fig. 5C). This suggests that, in Osmr−/− cells, although mLIF induces phosphorylation of both gp130 and LIFR, resulting in phosphorylation of both STAT3 and STAT1, the ability of mOSM to activate STAT3 without STAT1 signaling may relate to reduced phosphorylation of gp130 and LIFR (Fig. 5D).

Assessing Possible Therapeutic Benefit in the Skeleton—To assess whether shifting the balance of signaling toward STAT3 rather than STAT1 might promote bone formation, we analyzed the bone phenotype of 12-week-old male global Stat1−/− mice but observed no significant difference in trabecular bone volume (Fig. 6A), trabecular number (Fig. 6B), or trabecular separation (Fig. 6D). A significant increase in periosteal circumference without any change in femoral length was observed, suggesting elevated periosteal bone formation when STAT3 signaling is greater than STAT1 (mean periosteal circumference ± S.E.: WT, 7.50 ± 0.14; Stat1−/−, 8.05 ± 0.12; p = 0.0042; mean femoral length ± S.E.: WT, 13.33 ± 0.13; Stat1−/−, 13.48 ± 0.09).

To further explore the implications of biased STAT3 signaling in a disease setting, we used a previously described mouse model of osteopenia caused by hyperactivation of Stat1/3 signaling downstream of gp130 by deletion of the binding site for the negative regulators SOCS3 and SHP2 (gp130Y757F) (58). When these mice were crossed with Stat1−/− mice so that only STAT3 signaling downstream of gp130 was hyperactivated (36), the gp130Y757F osteopenic phenotype was rescued; trabecular bone volume, trabecular number, trabecular thickness, and trabecular separation all returned to normal (Fig. 6, E–I). This was not reproduced when gp130Y757F mice were crossed with Stat3−/− mice, a cross that normalizes STAT3 signaling downstream of gp130 (36). Osteoclast and
osteoblast numbers remained significantly elevated in gp130<sup>Y757F/Y757F</sup>/Stat1<sup>/H11002/H11002</sup> mice but not in gp130<sup>Y757F/Y757F</sup>/Stat3<sup>/H11001/H11002</sup>. Numbers of osteoblasts/bone perimeter (mean ± S.E.) were as follows: wild type, 9.1 ± 1.7; gp130<sup>Y757F/Y757F</sup>, 24.0 ± 0.7<sup>*</sup>; gp130<sup>Y757F/Y757F/Stat1<sup>/H11002/H11002</sup></sup>, 29.3 ± 4.1<sup>*</sup>; gp130<sup>Y757F/Y757F/Stat3<sup>/H11002/H11002</sup></sup>, 11.9 ± 5.7<sup>(, p < 0.05)</sup>. Numbers of osteoblasts/bone perimeter (mean ± S.E.) were as follows: wild type, 2.23 ± 0.45; gp130<sup>Y757F/Y757F</sup>, 3.97 ± 0.37<sup>*</sup>; gp130<sup>Y757F/Y757F/Stat1<sup>/H11002/H11002</sup></sup>, 4.31 ± 0.71<sup>*</sup>; gp130<sup>Y757F/Y757F/Stat3<sup>/H11001/H11002</sup></sup>, 2.38 ± 1.10<sup>(, p < 0.05)</sup>. These results indicate a beneficial effect of promoting STAT3 signaling over STAT1 downstream of gp130 in this low bone mass condition.

**Discussion**

In this study, we report a full set of gene responses to mOSM in the absence of OSMR indicating that mOSM, which we have previously shown to promote bone formation independently of OSMR (7), regulates multiple gene targets downstream of mLIFR. Unlike canonical signaling of mLIF and hOSM where both STAT1 and STAT3 are phosphorylated downstream of mLIFR, mOSM induces phosphorylation of STAT3 but not STAT1 and stimulates a STAT3-responsive subset of genes downstream of mLIFR. This may be explained by formation of a signaling complex with very low levels of gp130 and LIFR.
pathway, favoring STAT3 over STAT1 signaling downstream of gp130 protected against osteopenia in a mouse with hyper-responsiveness to IL-6 family cytokines (gp130<sup>Y575F/Y757F</sup>). This suggests such a pathway could be exploited in skeletal pathologies where IL-6 family signaling is elevated, such as inflammatory or metastatic bone loss.

Reduced trabecular bone volume due to hyperactivation of STAT1/3 signaling downstream of gp130 in the gp130<sup>Y575F/Y757F</sup> mice has been reported previously to result from a high level of bone remodeling (58). Although both bone resorption and bone formation are elevated, the level of resorption outstrips that of formation, resulting in an osteopenic phenotype, a similar etiology to bone loss associated with estrogen deficiency (60). Elevated signaling by gp130-dependent cytokines has been postulated not only to cause osteoporosis after menopause (61) but also to play a role in focal and systemic bone loss that results from colitis (62), inflammatory arthritis (63, 64), multiple myeloma (65), Gorham-Stout disease (66), Paget’s disease (67), and breast cancer metastasis to bone (68). The osteopenic phenotype of gp130<sup>Y575F/Y757F</sup> mice was rescued by blocking STAT1 signaling. Although bone remodeling remained high, the balance was shifted to favor bone formation, and bone mass was protected. STAT1 signaling blockade may therefore provide a therapeutic benefit in patients with bone loss related to elevated IL-6 family cytokine signaling. The greater level of periosteal bone formation in Stat1<sup>−/−</sup> mice may relate to the loss of STAT1-mediated suppression of periosteal development; STAT1 is expressed at high levels in the developing perichondrium (69). The limited benefit of STAT1 deletion without hyperactivation of STAT3 (i.e. only periosteal bone formation was increased in Stat1<sup>−/−</sup> mice) suggests that this approach might not benefit all forms of bone fragility.

When IL-6 family cytokines form a receptor-ligand complex, it is phosphorylation of the intracellular domain of the receptor subunits, both gp130 (70, 71) and LIFR (72), that provides docking sites for STAT proteins. These are subsequently phosphorylated by JAKs on specific residues allowing homo- or heterodimerization and translocation to the nucleus to modify transcription. This study identifies that the low affinity interaction of mOSM with mLIFR results in transcription of a subset of genes downstream of canonical (mLIF/hOSM)-mLIFR signaling. These are known STAT3-responsive genes, including Socs3 (26), Bcl3 (31), Junb (32), Zfp36 (28), Rgs16 (28), Cxcl1 (27), Mt2 (29), Sbno2 (30), Mmp13 (36), and c-fos (33). In contrast, the mLIF/hOSM-responsive genes that did not respond to mOSM in the absence of OSMR included STAT1 target genes: Mx2 (37), Irgm1 (38), Socs1 (41), Ccl2 (39), Serpina3f (40), Ifi47 (42), Trim35 (42), Cxcl9 (43), Gbp3 (44), Gbp5 (45), Cxcl9 (43), Oasl2 (46), Serpina3f (40), Igtp (47), Psmb9 (48), Dlx5/6 (49), and Oas1g (49). Thus, STAT1 target gene responses to canonical mLIFR signaling were lost when mOSM interacted with mLIFR. This pattern of STAT3, rather

FIGURE 4. mOSM phosphorylates STAT3 on Tyr-705 and Ser-727 downstream of the LIFR. Phosphorylation of STAT3 Tyr-705 and Ser-727 in 22–24-day WT and Osmr<sup>−/−</sup> primary calvarial osteoblasts is shown. A and B, histograms depicting a shift in MFI when WT (A) or Osmr<sup>−/−</sup> (B) cells were stained for AF488 phosho-STAT3 Tyr-705, AF647 phosho-STAT3 Ser-727, and phycoerythrin total STAT3 protein. C and E, quantification of MFI for phosho-STAT3 Tyr-705 (C), phosho-STAT3 Ser-727 (D), and total STAT3 (E) in 22–24-day-differentiated osteoblasts after 15-min stimulation with mOSM, HOSM, or mLIF (50 ng/ml). Graphed data are mean -fold change versus untreated of three independent experiments; error bars represent S.E. Two-way ANOVA indicated significant treatment effects for all three cytokines at both phosphorylation sites but no significant interaction between treatment and genotype of the cells. Ctrl, control.
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The question of how mOSM can act through gp130-LIFR to phosphorylate STAT3 at both Ser-727 and Tyr-705 but not to phosphorylate STAT1 in response to mOSM in Osmr−/− cells.

The only gene that was not a classical STAT3 target in the top 10 genes was Cish, which is usually regarded as a STAT5-responsive gene (34). Previously we did not detect STAT5 phosphorylation in response to mOSM in Osmr−/− osteoblasts (7), and other STAT5 target genes, such as Pim1 and Bcl2 (73), were not in the mOSM-LIFR gene set, suggesting that Cish transcription may occur in response to a broader range of STAT proteins.

The question of how mOSM can act through gp130-LIFR to regulate only a subset of those genes regulated by mLIF/hOSM through the same receptor complex is common to all receptors with multiple ligands that produce different biological effects within a single cell type. These include not only LIFR and OSMR (1, 74, 75) but also type I interferons (76). In the case of mOSM, our data suggest that impaired gp130 and LIFR phosphorylation may be responsible for the bias toward STAT3 signaling, but how receptor phosphorylation level is controlled remains unknown. It may relate to altered binding conformation, binding affinity, or binding stability compared with mLIF/hOSM. This could be better understood by structural studies as achieved with the binding of mLIF to LIFR (75). No structural data yet have defined the binding of mOSM or hOSM to either OSMR or LIFR; it remains structurally unclear why hOSM can bind to both LIFR and OSMR, whereas mOSM binds with higher affinity to OSMR (1). One possible model is that mOSM engages LIFR and gp130 in a manner that leads to incomplete transactivation of JAKs so that the STAT3 docking site on LIFR is phosphorylated, but STAT1 and SOCS3 binding sites are not. This would lead to selective STAT3 signaling and possibly its enhancement by lack of SOCS3 feedback even at low affinity interactions.

STAT3 docking sites on LIFRβ are phosphorylated at Tyr-765, Tyr-812, Tyr-904, and Tyr-914 in mouse (Tyr-981, Tyr-1001, and Tyr-1028 in human), whereas STAT3 docking sites on gp130 are phosphorylated at Tyr-767, Tyr-814, Tyr-905, and Tyr-915 (75). Any one or a combination of these may be modified. In addition, the single SOCS3 binding site on gp130 is phosphorylated at Tyr-757 in mouse (Tyr-759 in human), and a similar SOCS3 binding site on LIFR is phosphorylated at Tyr-915 (75). Any one or a combination of these may be modified. In addition, the single SOCS3 binding site on gp130 may also be phosphorylated. The focus of mOSM-LIFR action on STAT3 signaling is a similar effect on gene expression as that described previously for hOSM-hLIFR in human breast cancer cell lines (3). In that work, a LIF antagonist was used to distinguish shared and distinct signaling pathways of hOSM-hOSMR and hOSM-hLIFR.

They reported that hOSM action through both hOSMR and hLIFR resulted in changes in genes downstream of STAT3 and STAT1 (e.g. Socs3 and Irf1), but regulation of STAT1 genes was lost when hOSM interacted with hLIFR. The similarities we
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To conclude, this study identifies that mOSM acting through the mLIFR phosphorylates STAT3 but not STAT1, resulting in specific regulation of STAT3-responsive genes, thereby activating a more specific intracellular signaling pathway than those induced by hOSM or hLIF acting through the same receptor. Our data suggest that this specificity relates to reduced gp130

This study was conducted in primary calvarial osteoblasts because genetic deletion of OSMR in these cells allowed us to identify mOSM-dependent regulation of sclerostin (7), an inhibitor of bone formation, in the absence of OSMR. Whether mOSM is capable of initiating STAT3 phosphorylation in other OSMR-deficient cell types is not known. We could detect no STAT3 phosphorylation response to mOSM in Ba/F3 cells expressing mgp130 and mLIFR. This suggests that accessory factors that promote mOSM-LIFR complex formation may be present in calvarial osteoblasts. One possibility is sortilin, which has been shown to promote ciliary neurotrophic factor and LIF signaling (77) and which we have detected by qPCR in primary calvarial osteoblasts (data not shown). However, addition of sortilin to mgp130/mLIFR-positive Ba/F3 cells did not promote STAT3 phosphorylation.

This study began with the aim of identifying the pathway through which mOSM-mLIFR is able to promote bone formation without stimulating bone resorption. Although in our earlier work we observed that mOSM could promote bone formation in the absence of OSMR, and its effect on promoting RANKL (Tnfsf11) transcription was not detectable in OSMR-deficient cells, the present study detected a delayed, but still significant, increase in Tnfsf11 mRNA in Osmr<sup>−/−</sup> cells in response to mOSM. Although our hypothesis that mOSM-LIFR might promote an exclusively “anabolic” gene signature whereas mOSM-OSMR promoted an exclusively “catabolic” gene signature was not upheld, the rescue of the gp130<sup>Y757F/Y757F</sup>/Stat1<sup>−/−</sup> mice, and high alkaline phosphatase activity in cultured osteoblasts with constitutive STAT3 activation (16).

FIGURE 6. Reducing STAT1 relative to STAT3 signaling rescues gp130-dependent osteopenia. A–E, trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) measured in the distal femur of 12-week-old male wild type (wt) and Stat1<sup>−/−</sup> mice (n = 9–10). F–H, trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) measured in the proximal tibiae of 12–14-week-old wild type (wt), gp130<sup>Y757F/Y757F</sup>/Stat1<sup>−/−</sup>, and gp130<sup>Y757F/Y757F</sup>/Stat1<sup>−/−</sup>/Stat3<sup>+/−</sup> mice (n = 4–6/group). Values are means; error bars represent S.E. (*, p < 0.05; **, p < 0.01; ***, p < 0.001 versus wild type; +, p < 0.05, ++, p < 0.001 versus gp130<sup>Y757F/Y757F</sup>/Stat1<sup>−/−</sup> mice. Black, bone; scale bar, 200 μm.
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and LIFR phosphorylation by mOSM-mLIFR. Targeted activation of STAT3 signaling downstream of gp130 without promoting STAT1 signaling provides a protective effect in the skeleton, suggesting that mimics of the mOSM-mLIFR interaction or of this pattern of STAT3-specific activation could provide benefit for skeletal fragility.

Experimental Procedures

Cell Culture and Microarray—Osmr−/− mice (6) were obtained from Prof. Atsushi Miyajima (The University of Tokyo), backcrossed onto C57BL/6 for six generations, and maintained as a heterozygous breeding colony at our institute for >5 years. Primary calvarial osteoblasts were generated as described previously (79) from homozygous litters of Osmr−/− and wild type cousin-bred mice. On three occasions, WT and Osmr−/− cells were defrosted, expanded, and differentiated for 17 days in osteoblast differentiation medium. At 17 days, when these cells express elevated levels of sclerostin, they were serum-starved overnight and then treated with 10 ng/ml hOSM (R&D Systems), mOSM (R&D Systems), or mLIF (Chemicon). Cell digests were collected at 1 and 6 h after treatment commenced. mRNA was prepared as described previously (7) and hybridized to Illumina MouseWG-6 V2 BeadChips. Raw probe intensities for both regular and control probes were exported from Illumina’s Genome Studio software.

Bioinformatics analysis was conducted using the limma software package (80). The raw intensity values were background-corrected and normalized using the neqc function, which performs “normexp” background correction and quantile normalization using parameters estimated from the control probes (81). Probes were filtered as not expressed if they failed to achieve a detection p value of 0.05 for at least three samples. The Illumina manifest file (MouseWG-6_V2_0_R1_11278593_A) was used for probe annotation. Gene symbol aliases were converted to current official gene symbols using the Bioconductor organism package org.Mm.eg.db. Linear models were used to test for expression differences between treatments while adjusting for batch effects associated with the date on which the cell cultures were performed. Differential expression between treatments was assessed using empirical Bayes moderated t statistics (82), allowing for an intensity-dependent trend in the standard errors. The mLIF and hOSM responses were combined and compared with others using appropriate linear model contrasts. The false discovery rate was controlled at less than 0.05 using the method of Benjamini and Hochberg (59). Biological correlations between log -fold change profiles were estimated by limma’s genas function, which estimates genuine associations between profiles, adjusting for any technical component of the correlation. Lists of STAT3 target genes were obtained from the Molecular Signatures Database v5.1 (83), and enrichment was tested using limma’s kega function. The microarray data are available as Gene Expression Omnibus (GEO) series GSE83418.

Phosflow and Western Blotting—Samples for Phosflow were generated by culturing mouse primary calvarial osteoblasts from C57BL/6 mice for 22–24 days in differentiation medium on three separate occasions. Cells were seeded and grown to 80–90% confluence in culture in complete medium and serum-starved overnight in αMEM supplemented with 2% heat-inactivated fetal bovine serum (FBS) + 50 µg/ml ascorbate. The following day cells were stimulated with 50 ng/ml mOSM, hOSM, or mLIF for 15 min in low serum conditions. Cells were rinsed twice with PBS, fixed in 1% formalin at 37 °C for 10 min, and permeabilized in 90% methanol for 30 min on ice. After overnight storage at −80 °C, cells were washed twice in Stain Buffer (PBS plus 1% FBS, 0.09% sodium azide, 0.5 mM EDTA) and stained with the following antibodies: AF647 pSTAT3 Ser-727, AF488 pSTAT3 Tyr-705, and phycocyanin total STAT3 (all BD Biosciences). Cells were washed, resuspended in Stain Buffer, and analyzed on an LSR Fortessa (BD Biosciences). Mean fluorescence intensity (MFI) was determined for all three biological replicates using FlowJo software. Gating was based on fluorescent signal above background such that all signals were included for cytokine induction of pSTAT3 Tyr-705 and total STAT3; signals in the secondary peak were included for cytokine induction of pSTAT3 Ser-727.

Whole cell lysates were prepared for Western blotting analysis from wild type or Osmr−/− primary calvarial osteoblasts maintained in normal growth medium (αMEM + 10% FBS) (JRH Biosciences batch numbers 074, 414, and 379) or under differentiating conditions (αMEM + 15% FBS (JRH Biosciences batch numbers 074, 414, and 379; Assay Matrix catalogue number A137A111) and 50 µg/ml ascorbate (Sigma)) for 21 days in 10-cm² dishes. Cultures were serum-starved in 2% FBS overnight prior to cytokine treatment. Cells were treated with mOSM (R&D Systems catalogue number 495-MO, lot number TX0313011), hOSM (R&D Systems catalogue number 295-OM, lot number DY0808111), or mLIF (Merck Millipore catalogue number LIF2010, lot number 2561112) at 50 ng/ml for 15 or 30 min. LA (55) was used at 2.5 µg/ml for 30 min prior to the addition of cytokines; STAT3 inhibitor (ML116) (56) was used for 1 h prior to the addition of cytokines at 0.1, 1, or 10 µg/ml. Cells were then washed twice with ice-cold PBS before cell lysates were prepared by shaking at 4 °C with modified radio-immune precipitation assay buffer (84), proteinase inhibitor (Sigma), and phosphatase inhibitor (Dako) before lysates were centrifuged. Protein concentration was determined according to the manufacturer’s protocol (Pierce). 20–50 µg of protein were loaded onto 4–12% gradient gels (Invitrogen) under reducing conditions for electrophoresis before being transferred to nitrocellulose membranes (iBlot, Invitrogen) and probed with pSTAT1(Tyr-701) (Cell Signaling Technology catalogue number 9171, lot number 8), STAT1 (Cell Signaling Technologies catalogue number 9175, lot number 13), pSTAT3(Ser-727) (Cell Signaling Technology catalogue number 9136, lot number 13), pSTAT3(Tyr-705) (Cell Signaling Technology catalogue number 9131, lot number 30), STAT3 (Cell Signaling Technology catalogue number 9139, lot number 8), pan-actin Ab-5 (Neomarkers catalogue number 1295-P0, lot number 1295P1501N). Protein bands were detected with ECL chemiluminescence (GE Healthcare catalogue number RPN2209) and film (Fujifilm catalogue number 4741019289) according to the manufacturers’ instructions. ImageJ was used to measure pixel intensity of phospho-STAT3 relative to total STAT3 and corrected for relative response magnitude within
TABLE 3

| Gene   | Primer sequence          | GenBank™ accession no. |
|--------|--------------------------|------------------------|
| B2m    | 5'-TTACACCCACGATGATGTGAG-3' | NM_009773.3 (88)       |
| Becl3  | 5’-CCCTTGGATCCGAAATATTCTTACC-3’ | NM_033601              |
| Gby2   | 5’-CAGCGCTTGCTCTGACGTG-3’ | NM_010260              |
| Hprt1  | 5’-TGTGGCTCCCTGTTGAGTCCAG-3’ | NM_013556 (89)        |
| Oas1   | 5’-AGAAGGCAAGACAGCAGGAAC-3’ | NM_01185                |
| Sbn2   | 5’-AGAGTGTCCTGATGAGAAGA-3’ | NM_183426              |
| Socs1  | 5’-GCTCGTCATCATCATA-3’     | NM_09989 (90)          |
| Socs3  | 5’-TGACGCTCAAGACCCAGCGTGC-3’ | NM_007707 (90)        |

each experiment by expressing as a percentage of the level of STAT3 induction reached by mOSM.

Immunoprecipitation was performed by dividing the volume of lysate collected equally into three, one lysate per antibody. Immunoprecipitation was carried out using 30 μl of lysate collected equally into three, one lysate per antibody.

Quantitative Real Time PCR—cDNA synthesis of 1 μg of DNase-treated (Ambion) RNA was performed using AffinityScript (Agilent Technologies catalogue number 600559) according to the manufacturer’s instructions. Stock cDNA was diluted 1:5–1:10, and qPCR was performed using either an in-house master mix or 10× AmpliTaq Gold with Brilliant II SYBR Green Mastermix (Agilent Technologies catalogue number 600828). Primers used (Table 3) were designed using NCBI Primer Blast or PrimerQuest. Samples were dispensed into an 8-tube run on a Stratagene Mx3000P PlateViewer (version 1.4.4), and CT Analyzer (version 1.11.8.0). The femoral trabecular analysis region of interest was determined by identifying the intracondylar notch at the distal end of the femur and calculating 15% of the total femur length toward the femoral midshaft where we then analyzed a region of interest of 15% of the total femur length; a threshold of 43–255 was used to define trabecular bone. Tibial samples from gp130^Y757F/Y757F mice were analyzed by histomorphometry in the proximal secondary spongiosa using the OsteoMeasure system (Osteometrics, Decatur GA) after embedding in methylmethacrylate resin as described previously (87). Microscope images were collected on a Leica DMRB microscope coupled to an Olympus DP72 camera and CellSens software under a 4.0 Plan objective.

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References

1. Herrmanns, H. M. (2015) Oncostatin M and interleukin-31: cytokines, receptors, signal transduction and physiology. Cytokine Growth Factor Rev. 26, 545–558
Defining mOSM Action through mLIFR

2. Zarling, J. M., Shoyab, M., Marquardt, H., Hanson, M. B., Lioubin, M. N., and Todaro, G. J. (1986) Oncostatin M: a growth regulator produced by differentiated histiocytic lymphoma cells. Proc. Natl. Acad. Sci. U.S.A. 83, 9739–9743

3. Underhill-Day, N., and Heath, J. K. (2006) Oncostatin M (OSM) cytokostasis of breast tumour cells: characterization of an OSM receptor β-specific keratin. Cancer Res. 66, 10891–10901

4. Bolin, C., Tawara, K., Sutherland, C., Redshaw, J., Aranda, P., Moselhy, I., Anderson, R., and Jorcyk, C. L. (2012) Oncostatin m promotes mammary tumor metastasis to bone and osteolytic bone degradation. Genes Cancer 3, 117–130

5. Nair, B. C., DeVico, A. L., Nakamura, S., Copeland, T. D., Nicholson, G. C., Zhang, J. G., Nicola, N. A., Gillespie, M. T., Martin, T. J., and Sims, N. A. (2010) Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. J. Clin. Investig. 120, 582–592

6. Walker, E. C., McGregor, N. E., Poulton, I. J., Solano, M., Pompolo, S., Fernandes, T. J., Constable, M. J., Nicholson, G. C., Zhang, J. G., Nicola, N. A., Gillespie, M. T., Martin, T. J., and Sims, N. A. (2010) Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. J. Clin. Investig. 120, 582–592

7. Walker, E. C., McGregor, N. E., Poulton, I. J., Solano, M., Pompolo, S., Fernandes, T. J., Constable, M. J., Nicholson, G. C., Zhang, J. G., Nicola, N. A., Gillespie, M. T., Martin, T. J., and Sims, N. A. (2010) Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. J. Clin. Investig. 120, 582–592

8. Mozzafarian, A., Brewer, A. W., Trueblood, E. S., Luzina, I. G., Todd, N. W., Atamas, S. P., and Arnett, H. A. (2008) Mechanisms of oncostatin M-induced pulmonary inflammation and fibrosis. J. Immunol. 181, 7423–7425

9. Pöling, J., Gajawada, P., Lörchner, H., Polyakova, V., Szibor, M., Böttger, T., Warnecke, H., Kubin, T., and Braun, T. (2012) The Janus face of OSM-mediated cardiomyocyte dedifferentiation during cardiac repair and disease. Cell Cycle 11, 439–445

10. Taniya, T., Tanaka, S., Yamaguchi-Kabata, Y., Hanaoka, H., Yamasaki, C., Maekawa, H., Barrero, R. A., Lenhard, B., Datta, M. W., Shimoyama, M., Bumgarner, R., Chakraborty, R., Hopkinson, I., Jia, L., Hide, W., et al. (2012) A prioritization analysis of disease association by data-mining of functional annotation of human genes. Genomics 99, 1–9

11. Simpson, J. L., Baines, K. J., Boyle, M. J., Scott, R. J., and Gibson, P. G. (2009) Oncostatin M (OSM) is increased in asthma with incompletely reversible airflow obstruction. Exp. Lung Res. 35, 781–794

12. Pradeep, A. R., Manojkumar, S. T., Garima, G., and Raju, A. (2010) Serum levels of oncostatin m (a gp130 cytokine): an inflammatory biomarker in periodontal disease. Biomarkers 15, 277–282

13. Sims, N. A., and Walsh, N. C. (2010) GP130 cytokines and bone remodeling in health and disease. BMB Rep. 43, 513–523

14. Mosley, B., De Imus, C., Friend, D., Boiani, N., Thoma, B., Park, L. S., and Cosman, D. (1996) Dual oncostatin M (OSM) receptors. Cloning and characterization of an alternative signaling subunit conferring OSM-specific receptor activation. J. Biol. Chem. 271, 32635–32643

15. Ichihara, M., Hara, T., Kim, H., Murate, T., and Miyajima, A. (1997) Oncostatin M and leukemia inhibitory factor do not use the same functional receptor in mice. Blood 90, 165–173

16. Nicolaidou, V., Wong, M. M., Redpath, 23, 400–401

17. Silva, A. R., Nakamura, T., Nakao, K., Arai, T., Katsuki, M., Heike, T., and Yokota, T. (1999) STAT3 activation is sufficient to maintain an undifferentiated state of breast tumor cells: characterization of an OSM receptor promoter. J. Biol. Chem. 274, 3357–3367

18. Li, S., Chen, X., Xu, S., Sundstedt, A., Paulsson, K. M., Anderson, P., Karlsson, S., Jörnvall, H., and Wang, P. (2000) Cytokine-induced SRC homology 2 protein (Cis) promotes T cell receptor-mediated proliferation and prolongs survival of activated T cells. J. Exp. Med. 191, 985–994

19. Louro, R., Nakaya, H. I., Paquaio, A. C., Martins, E. A., da Silva, A. M., Verjovski-Almeida, S., and Reis, E. M. (2004) RASL11A, member of a novel small monomeric GTPase gene family, is down-regulated in prostate tumors. Biochem. Biophys. Res. Commun. 316, 618–627
36. Ernst, M., Najdovska, M., Grail, D., Lundgren-May, T., Buchert, M., Tye, H., Matthews, Y. B., Armes, I., Bhathal, P. S., Hughes, N. R., Marcussen, E. G., Karras, J. G., Na, S., Sedwick, J. D., Hertzog, P. J., et al. (2008) STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. J. Clin. Investig. 118, 1727–1738

37. Ho, H. H., and Ivashkiv, L. B. (2006) Role of STAT3 in type I interferon responses. Negative regulation of STAT1-dependent inflammatory gene activation. J. Biol. Chem. 281, 14111–14118

38. King, K. Y., Baldrige, M. T., Welsberg, D. C., Chambers, S. M., Lukov, G. L., Wu, S., Noes, C. J., Jung, S. Y., Qin, J., Liu, D., Songyang, Z., Eissa, N. T., Taylor, G. A., and Goodell, M. A. (2011) Igml1 protects hematopoietic stem cells by negative regulation of IFN signaling. Blood 118, 1525–1533

39. Buttmann, M., Berberich-Siebelt, F., Serfling, E., and Rieckmann, P. (2007) Interferon-β is a potent inducer of interferon regulatory factor-1-dependent IL-1β- and -independent pathways in IFN-γ/H9253-dependent signaling. J. Exp. Med. 204, 1733–1742

40. Achtman, A. H., Pilat, S., Law, C. W., Lynn, D. J., Janot, L., Mayer, M. L., Auer, N. S., Corbin, J. E., Cornish, A. L., Darwiche, R., Owezarek, C. M., Kay, N. S., Boyce, B., Broxmeyer, H., and Manolagas, S. C. (1992) Increased bone resorption precedes increased bone formation in the ovariectomized rat. J. Clin. Investig. 89, 1219–1229

41. Alexander, W. S., Starr, R., Fenner, J. E., Scott, C. L., Handman, E., Sprigg, N. S., Corbin, J. E., Cornish, A. L., Darwiche, R., Owezarek, C. M., Kay, N. S., Boyce, B., Broxmeyer, H., and Manolagas, S. C. (1992) Increased bone resorption precedes increased bone formation in the ovariectomized rat. J. Clin. Investig. 89, 1219–1229

42. Ramana, C. V., Gil, M. P., Schreiber, R. D., and Stark, G. R. (2002) STAT1-dependent and -independent pathways in IFN-γ-dependent signaling. Trends Immunol. 23, 96–101

43. Marquis, L., Bruet, M., Lloberas, J., and Celada, A. (2004) STAT1 Regulates Lipopolysaccharide- and TNF-α-dependent expression of transporter associated with antigen processing 1 and low molecular mass polypeptide 2 genes in macrophages by distinct mechanisms. J. Immunol. 173, 1103–1110

44. Dickensheets, H., Sheikh, F., Park, O., Gao, B., and Donnelly, R. P. (2013) Interferon-α (IFN-α) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes. J. Leukoc. Biol. 93, 377–385

45. Saleh, H., Eeles, D., Hodge, J. M., Nicholson, G. C., Gu, R., Pompolo, S., Gillespie, M. T., and Quinn, J. M. (2011) Interleukin-33, a target of parathyroid hormone and oncostatin M, increases osteoblastic matrix mineral deposition and inhibits osteoclast formation in vitro. Endocrinology 152, 1911–1922

46. Rogerson, F. M., Chung, Y. M., Deutscher, M. E., Last, K., and Fonang, A. J. (2010) Cytokine-induced increases in ADAMS-4 messenger RNA expression do not lead to increased aggrecanase activity in ADAMS-5-deficient mice. Arthritis Rheum. 62, 3365–3373

47. Quach, J. M., Walker, E. C., Allan, E., Solano, M., Yokoyama, A., Kato, S., Sims, N. A., Gillespie, M. T., and Martin, T. J. (2011) Zinc finger protein 467 is a novel regulator of osteoblast and adipocyte commitment. J. Biol. Chem. 286, 4186–4198

48. Kang, S., Akerblad, P., Kiviranta, R., Gupta, R. K., Kajimura, S., Griffin, M. J., Min, J., Baron, R., and Rosen, E. D. (2012) Regulation of early adipsion commitment by Zfp521. PLoS Biol. 10, e1001433

49. Wu, M., Hesse, E., Morvan, F., Zhang, J. P., Correa, D., Rowe, G. C., Kiviranta, R., Neff, L., Philbrick, W. M., Horne, W. C., and Baron, R. (2009) Zfp521 antagonizes Runtx2, delays osteoblast differentiation in vitro, and promotes bone formation in vivo. Bone 44, 528–536

50. Fairlie, W. D., Ubolf, A. D., McCoubrie, J. E., Wang, C. C., Lee, E. F., Yao, S., De Souza, D. P., Mifsud, S., Metcalf, D., Nicola, N. A., Norton, R. S., and Baca, M. (2004) Affinity maturation of leukaemia inhibitory factor and conversion to potent antagonists of signaling. J. Biol. Chem. 279, 2125–2134

51. Madoux, F., Koenig, M., Sessions, H., Nelson, E., Mercer, B. A., Cameron, M., Roush, W., Frank, D., and Dodder, P. (2010) Modulators of STAT transcription factors for the targeted therapy of cancer (STAT3 inhibitors), in Probe Reports from the NIH Molecular Libraries Program, National Center for Biotechnology Information, Bethesda, MD

52. Qin, H. R., Kim, H. J., Kim, J. Y., Hurt, E. M., Klarmann, G. J., Kawasaki, B. T., Duhamon Serrat, M. A., and Farrar, W. L. (2008) Activation of signal transducer and activator of transcription 3 through a phosphomimetic serine 727 promotes prostate tumorigenesis independent of tyrosine 705 phosphorylation. Cancer Res. 68, 7736–7741

53. Sims, N. A., Jenkins, B. J., Quinn, J. M., Nakamura, A., Glatt, M., Gillespie, M. T., Ernst, M., and Martin, T. J. (2004) Glycoprotein 130 regulates bone turnover and bone size by distinct downstream signaling pathways. J. Clin. Investig. 113, 379–389

54. Benjamini, Y., and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Series B Stat. Methodol. 57, 289–300

55. Sims, N. A., Morris, H. A., Moore, R. J., and Durbridge, T. C. (1996) Increased bone resorption precedes increased bone formation in the ovariectomized rat. Calcif. Tissue Int. 59, 121–127

56. Ilkka, R. L., Hangoc, G., Girasole, G., Passeri, G., Williams, D. C., Abrams, J. S., Boyce, B., Broxmeyer, H., and Manolagas, S. C. (1992) Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257, 88–91

57. Mitsu, K., Toyonaga, A., Sasaki, E., Ishida, O., Ikeda, H., Tsuruta, O., Harada, K., Tateishi, H., Nishiyama, T., and Tanikawa, K. (1995) Soluble interleukin-6 receptors in inflammatory bowel disease: relation to circulating interleukin-6. Gastroenterology 109, 45–49

58. Kotake, S., Sato, K., Kim, K. J., Takahashi, N., Udagawa, N., Nakamura, I., Yamaguchi, A., Kishimoto, T., Suda, T., and Kashiwazaki, S. (1996) Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J. Bone Miner. Res. 11, 89–95

59. Wang, P. K., Quinn, J. M., Sims, N. A., van Nieuwenhuijze, A., Campbell, I. F., and Wicks, I. P. (2006) Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis. Arthritis Rheum. 54, 158–168

60. Kyrtois, M. C., Dedoussis, G., Zervas, C., Perifanis, V., Baxevanis, C., Stamatelou, M., and Maniatis, A. (1996) Soluble interleukin-6 receptor (sl-6R), a new prognostic factor in multiple myeloma. Br. J. Haematol. 93, 398–400

61. Devlin, R. D., Bone H. G., 3rd, and Roodman, G. D. (1996) Interleukin-6: a paracrine factor in Paget's disease of bone. J. Clin. Endocrinol. Metab. 81, 1893–1897

62. Roodman, G. D., Kurihara, N., Ohsaki, Y., Kukita, A., Hosking, D., Demulder, A., Smith, J. F., and Singer, F. R. (1992) Interleukin 6. A potent autocrine/paracrine factor in Paget's disease of bone. J. Clin. Investig. 89, 46–52

63. Thomas, R. J., Guise, T. A., Yin, J. J., Elliott, J., Holwood, N. J., Martin, T. J., and Gillespie, M. T. (1999) Breast cancer cells interact with osteoblasts to promote osteoclast formation. Endocrinology 140, 4451–4458
Defining mOSM Action through mLIFR

69. Retting, K. N., Song, B., Yoon, B. S., and Lyons, K. M. (2009) BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. Development 136, 1093–1104

70. Schnitz, J., Dahmen, H., Grimm, C., Gendo, C., Müller-Newen, G., Heinrich, P. C., and Schaper, F. (2000) The cytoplasmic tyrosine motifs in full-length glycoprotein 130 have different roles in IL-6 signal transduction. J. Immunol. 164, 848 – 854

71. Haan, S., Hemmann, U., Hassiepen, U., Schaper, F., Schneider-Mergener, J., Wollmer, A., Heinrich, P. C., and Gröttzinger, J. (1999) Characterization and binding specificity of the monomeric STAT3-SH2 domain. J. Biol. Chem. 274, 1342–1348

72. Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., Jr., and Yancopoulos, G. D. (1995) Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science 267, 1349–1353

73. Mitchell, T. J., Whittaker, S. J., and John, S. (2003) Dysregulated expression of COOH-terminally truncated Stat5 and loss of IL2-inducible Stat5-dependent gene expression in Sezary syndrome. Cancer Res. 63, 9048–9054

74. Sims, N. A. (2015) Cardiotrophin-like cytokine factor 1 (CLCF1) and neuropoietin (NP) signalling and their roles in development, adulthood, cancer and degenerative disorders. Cytokine Growth Factor Rev. 26, 517–522

75. Nicola, N. A., and Babon, J. J. (2015) Leukemia inhibitory factor (LIF). Cytokine Growth Factor Rev. 26, 533–544

76. Piewler, J., Thomas, C., Garcia, K. C., and Schreiber, G. (2012) Structural and dynamic determinants of type I interferon receptor assembly and their functional interpretation. ImmunoL Rev. 250, 317–334

77. Larsen, J. V., Hansen, M., Muller, B., Madsen, P., Scheller, J., Nielsen, M., and Petersen, C. M. (2010) Sortilin facilitates signaling of ciliary neurotrophic factor and related helical type 1 cytokines targeting the gp130/leukemia inhibitory factor receptor β heterodimer. Mol. Cell. Biol. 30, 4175–4187

78. Tajima, K., Takaishi, H., Takito, J., Tohmonda, T., Yoda, M., Ota, N., Kosaki, N., Matsumoto, M., Ikeyama, H., Nakamura, T., Kimura, T., Okada, Y., Horiuchi, K., Chiba, K., and Toyama, Y. (2010) Inhibition of STAT1 accelerates bone fracture healing. J. Orthop. Res. 28, 937–941

79. Takyar, F. M., Tonna, S., Ho, P. W., Crimeen-Irwin, B., Baker, E. K., Martin, T. J., and Sims, N. A. (2013) EphrinB2/EphB4 inhibition in the osteoblast lineage modifies the anabolic response to parathyroid hormone. J. Bone Miner. Res. 28, 912–925

80. Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., and Smyth, G. K. (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43, e47

81. Shi, W., Oshlack, A., and Smyth, G. K. (2010) Optimizing the noise versus bias trade-off for Illumina whole genome expression BeadChips. Nucleic Acids Res. 38, e204

82. Smyth, G. K. (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3, Article3

83. Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., and Mesirov, J. P. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U.S.A. 102, 15545–15550

84. Koolpe, M., Burgess, R., Dail, M., and Pasquale, E. B. (2005) EphB receptor-binding peptides identified by phage display enable design of an antagonist with ephrin-like affinity. J. Biol. Chem. 280, 17031–17037

85. Meraz, M. A., White, J. M., Sheehan, K. C., Bach, E. A., Rodrig, S. J., Dighe, A. S., Kaplan, D. H., Riley, J. K., Greenlund, A. C., Campbell, D., Carver-Moore, K., DuBois, R. N., Clark, R., Aguet, M., and Schreiber, R. D. (1996) Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell 84, 431–442

86. Johnson, R. W., McGregor, N. E., Brennan, H. J., Crimeen-Irwin, B., Poulton, I. J., Martin, T. J., and Sims, N. A. (2015) Glycoprotein130 Gp130/interleukin-6 (IL-6) signalling in osteoclasts promotes bone formation in periosteal and trabecular bone. Bone 81, 343–351

87. Sims, N. A., Clément-Lacroix, P., Da Ponte, F., Bouali, Y., Binart, N., Moriggi, R., Goffin, V., Coschigano, K., Gaillard-Kelly, M., Kopchick, J., Baron, R., and Kelly, P. A. (2000) Bone homeostasis in growth hormone receptor-null mice is restored by IGF-I but independent of Stat5. J. Clin. Investig. 106, 1095–1103

88. Winkler, I. G., Hendy, J., Coughlin, P., Horvath, A., and Lévesque, J.-P. (2005) Serine protease inhibitors serpina1 and serpina3 are down-regulated in bone marrow during hematopoietic progenitor mobilization. J. Exp. Med. 201, 1077–1088

89. Kartsgoannis, V., Sims, N. A., Quinn, J. M., Ly, C., Cipetic, M., Poulton, I. J., Walker, E. C., Saleh, H., McGregor, N. E., Wallace, M. E., Smyth, M. J., Martin, T. J., Zhou, H., Ng, K. W., and Gillespie, M. T. (2008) Osteoclast inhibitory lectin, an immune cell product that is required for normal bone physiology in vivo. J. Biol. Chem. 283, 30850–30860

90. Wormald, S., Zhang, J. G., Krebs, D. L., Mielke, L. A., Silver, J., Alexander, W. S., Speed, T. P., Nicola, N. A., and Hilton, D. J. (2006) The comparative roles of suppressor of cytokine signaling-1 and -3 in the inhibition and desensitization of cytokine signaling. J. Biol. Chem. 281, 11135–11143