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Wnt5a Induces Homodimerization and Activation of Ror2 Receptor Tyrosine Kinase

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ABSTRACT

Wnts are secreted glycoproteins that control vital biological processes, including embryogenesis, organogenesis and tumorigenesis. Wnts are classified into several subfamilies depending on the signaling pathways they activate, with the canonical subfamily activating the Wnt/β-catenin pathway and the non-canonical subfamily activating a variety of other pathways, including the Wnt/calcium signaling and the small GTPase/c-Jun NH2-terminal kinase pathway. Wnts bind to a membrane receptor Frizzled and a co-receptor, the low-density lipoprotein receptor related protein. More recently, both canonical and non-canonical Wnts were shown to bind the Ror2 receptor tyrosine kinase. Ror2 is an orphan receptor that plays crucial roles in skeletal morphogenesis and promotes osteoblast differentiation and bone formation. Here we examine the effects of a canonical Wnt3a and a non-canonical Wnt5a on the signaling of the Ror2 receptor. We demonstrate that even though both Wnt5a and Wnt3a bound Ror2, only Wnt5a induced Ror2 homo-dimerization and tyrosine phosphorylation in U2OS human osteoblastic cells. Furthermore, Wnt5a treatment also resulted in increased phosphorylation of the Ror2 substrate, 14-3-3β scaffold protein, indicating that Wnt5a binding causes activation of the Ror2 signaling cascade. Functionally, Wnt5a recapitulated the Ror2 activation phenotype, enhancing bone formation in the mouse calvarial bone explant cultures and potentiating osteoblastic differentiation of human mesenchymal stem cells. The effect of Wnt5a on osteoblastic differentiation was largely abolished upon Ror2 down-regulation. Thus we show that Wnt5a activates the classical receptor tyrosine kinase signaling cascade through the Ror2 receptor in cells of osteoblastic origin. J. Cell. Biochem. 105: 497–502, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: Wnt5A; ROR2; Wnt SIGNALING; RECEPTOR TYROSINE KINASE SIGNALING

The Ror2 and its homologue Ror1 comprise a small family within the super-family of receptor tyrosine kinases (RTKs) (reviewed in [Forrester, 2002; Billiard et al., 2007]). Ror2 plays key roles in developmental morphogenesis, particularly of the cartilage-derived skeleton. In addition, Ror2 has been identified as a pro-survival kinase in HeLa cervical carcinoma cells [MacKeigan et al., 2005] and shown to modulate neurite extension in central neurons [Paganoni and Ferreira, 2005]. We recently demonstrated that Ror2 initiates osteoblastic lineage commitment of human mesenchymal stem cells (hMSC) that have ability to differentiate into several distinct lineages [Liu et al., 2007a]. Ror2-induced osteogenesis results in production of mineralized extracellular matrix indicating formation of fully functional osteoblasts in this system.

In addition to the intracellular tyrosine kinase domain, the Ror receptors possess an extracellular cysteine-rich domain that mediates Wnt binding of Frizzled receptors and a variety of other proteins. Wnts are a family of 19 secreted glycoproteins that mediate vital biological processes, including embryogenesis, cell proliferation, migration, and differentiation [Wodarz and Nusse, 1998; Huelsken and Birchmeier, 2001]. Wnts are classified into several sub-families depending on the signaling pathways they activate. The Wnt1 sub-family (e.g. Wnt1, Wnt3a, Wnt8) activates the canonical Wnt/β-catenin pathway whereas the Wnt5a subfamily (e.g. Wnt5a, Wnt11) activates the non-canonical pathways, including the G protein-mediated Wnt/calcium pathway [Kuhl et al., 2000] and the small GTPase/c-Jun NH2-terminal kinase (JNK) pathway [Weston and Davis, 2002]. Ror2 has been shown to bind several canonical and non-canonical Wnts, including Wnt3a and Wnt5a [Hikasa et al., 2002; Oishi et al., 2003; Billiard et al., 2005; Mikels and Nusse, 2006]. Functionally, Ror2 potentiates or inhibits canonical Wnt signaling depending on the cell type and the specific Wnts examined [Billiard et al., 2005; Mikels and Nusse, 2006; Li et al., 2008]. However, it is the interaction between Ror2 and the non-canonical Wnt5a that received the most attention in recent years. Ror2−/− and Wnt5a−/− mice exhibit strikingly similar phenotypes, including dwarfism, shortened limbs, facial abnormalities, ventricular septal defects in the heart, and abnormalities in lung development [Oishi et al., 2003]. Mouse and

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Xenopus Ror2 synergize with non-canonical Wnts to inhibit convergent extension in Xenopus embryos [Hikasa et al., 2002; Oishi et al., 2003]. In mammalian cells, Ror2 synergizes with Wnt5a in activating JNK [Oishi et al., 2003] and mediates Wnt5a-induced inhibition of the canonical Wnt signaling [Mikels and Nusse, 2006; MacLeod et al., 2007].

Whereas effects of Ror2 on Wnt signaling have been documented rather extensively, effects of Wnts on the Ror2 signaling pathway have not been well examined. Here we investigated the effects of Wnt3a and Wnt5a binding on the Ror2 signaling pathway, including receptor homo-dimerization, tyrosine phosphorylation, and phosphorylation of its intracellular substrate, the 14-3-3β scaffold protein [Liu et al., 2007b]. We found that even though both Wnt3a and Wnt5a are known to bind Ror2, only Wnt5a induced Ror2 homo-dimerization. Furthermore, only Wnt5a was capable of signaling through a chimeric receptor consisting of the extracellular domain of Ror2 fused to the transmembrane and cytoplasmic domains of the TrkB RTK. Wnt5a induced phosphorylation of Ror2 on tyrosine residue(s) and increased phosphorylation of the Ror2 substrate, 14-3-3β scaffold protein, indicating that Wnt5a binding results in activation of the Ror2 signaling cascade. Wnt5a potentiated osteogenic differentiation of hMSC in a Ror2-dependent manner and enhanced bone formation in the mouse calvarial bone explant cultures. In summary, we demonstrate that Wnt5a, but not Wnt3a, is able to activate signaling through the Ror2 RTK and to induce functional consequences of Ror2 signaling. Whether or not Wnt5a is the only or the most potent ligand for Ror2 remains to be determined.

MATERIALS AND METHODS

MATERIALS AND TISSUE CULTURE

Except where noted, tissue culture reagents were purchased from Invitrogen Corporation (Carlsbad, CA); other reagents and chemicals were purchased from either Sigma Chemical Co. (St. Louis, MO) or Invitrogen. Anti-flag M2 mouse monoclonal antibody and anti-flag M2 affinity agarose were obtained from Sigma; anti-14-3-3β and anti-his rabbit polyclonal antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); anti-human Ror2 goat polyclonal antibody, control goat IgG, and recombinant mouse Wnt3A and Wnt5A were purchased from R&D Systems (Minneapolis, MN). Agarose-conjugated anti-phosphotyrosine antibody (4G10) was obtained from Upstate Cell Signaling Solutions (Charlottesville, VA); anti-phosphothreonine rabbit polyclonal antibody and immobilized phosphotyrosine antibody P-Tyr-100 were from Cell Signaling Technologies (Beverly, MA); and anti-phosphoserine rabbit polyclonal antibody was from Invitrogen. The horseradish peroxidase-conjugated secondary antibodies were from Santa Cruz Biotechnology. GSK-3 inhibitor (Lilly 603281-31-8) was synthesized as described [Engler et al., 2004]. All plasmids and adenoviruses have been described previously [Liu et al., 2007b].

The hMSC were purchased from Cambrex, Inc. (Baltimore, MD) and maintained at 37°C in a 5% CO_2–95% humidified air incubator using hMSC growth medium (MSCGM, Cambrex). The U2OS human osteosarcoma cells were maintained in McCoy's 5A Modified Medium, containing 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2 mM glutaMAX-I. The HEK293 human embryonic kidney cell line was maintained in DMEM, containing 10% heat-inactivated FBS, 1% penicillin-streptomycin, and 2 mM glutaMAX-I. Generation of the HEK293 CRE-luc Ror2-TrkB was previously described [Liu et al., 2007b]. The cells were grown at 37°C in a 5% CO_2–95% humidified air incubator in DMEM, containing 10% FBS, 1% non-essential amino acids, 1% penicillin-streptomycin, 2 μg/ml puromycin, 250 μg/ml G418 and 350 μg/ml hygromycin.

TRANSIENT TRANSFECTIONS

U2OS cells were plated at 75,000 cells/cm^2 and transfected 24 h later with 0.3–0.5 μg/cm^2 of total plasmid DNA using FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN) per manufacturer's instructions.

IMMUNOPRECIPITATION AND WESTERN IMMUNOBLOTTING

Cells were solubilized in lysis buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 1% Triton X100) supplemented with protease and phosphatase inhibitor cocktails (Sigma) and the extracts were clarified by centrifugation. For flag immunoprecipitation, 1 mg of total cell lysates was incubated with 30 μl of M2 flag affinity agarose for 1 h at 4°C. The beads were collected by centrifugation and washed three times in lysis buffer containing 350 mM NaCl and three times in lysis buffer. For phosphotyrosine precipitation, 1–1.5 mg of cellular extract was added to 100 μl of G410 beads and allowed to attach for 3 h at 4°C. At this time, P-Tyr-100 immobilized antibody (100 μl) was added to the mix for an additional 3 h. At the end of immunoprecipitation reactions, the beads were boiled in 30–50 μl of 2× LDS-PAGE buffer with reducing agent, and the solubilized proteins were separated by SDS–PAGE. The gels were transferred onto 0.45 μm nitrocellulose membrane before detection with each specific antibody.

REPORTER GENE ANALYSIS

HEK293 CRE-luc Ror2-TrkB cells were plated at 60,000 cells/well in 96-well plates (Falcon 35–3072) with 150 μl/well of phenol red-free DMEM, containing 5% FBS, 1% non-essential amino acids, and 1% penicillin-streptomycin. After 24 h, 50 μl of the plating medium was replaced with the same medium containing the indicated treatments at threefold the final concentrations. Cells were lysed 24 h later, and extracts were assayed for luciferase activity using Bright-Glo Luciferase Assay System (Promega, Madison, WI) as suggested by the manufacturer. Light emission was measured by the TopCount NXT luminometer (PerkinElmer, Shelton, CT).

HUMAN MSC VIRAL INFECTION AND HISTOCHEMICAL STAINING

Human MSC were seeded at 6,000 cm^{-2} in 12-well plates and allowed to adhere and proliferate overnight. Cells were further cultured in MSCGM supplemented with 0.05 mM ascorbic acid (AA), 10 mM β-glycerophosphate (β-GP), 100 nM dexamethasone (dex) and indicated treatments. For Ror2 knock-down in hMSC, cells were infected with adenoviruses containing Ror2-specific or EGFP-specific shRNA for 72 h in 0.4 ml/cm^2 MSCGM at MOI = 4,000 in presence of hCAR (MOI = 750). After 72 h, cells were washed once in PBS and placed in MSCGM supplemented with 0.05 mM AA, 10 mM

β-GP, 100 nM dex and 1 μg/ml Wnt5A. Medium was changed every 3 days. Formation of mineralized nodules was determined by alizarin red-S histochemical staining. To quantify the level of alizarin red-S staining, the dye was eluted with 1 ml/well of 10% (w/v) Cetylpyridinium Chloride. Alizarin red-S in the eluted samples was quantified (vs. a standard curve of 0–800 μM dye) at 562 nm with a microplate reader.

CALVARIAL ORGAN CULTURE AND INFECTION

The mice were used in accordance with the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals. Calvariae were excised from 4-day-old mouse littermates, cut along the sagittal suture and incubated for 24 h in serum-free BGJ medium containing 0.1% bovine serum albumin (BSA). Each half of the calvaria was then placed with the concave surface downward on a stainless steel grid (Small Parts Inc., Miami, FL) in a well of a 12-well plate. After 24 h, the medium was replaced with fresh medium containing 15 μg/ml calcein and 1 μg/ml of BSA or Wnt5a. The medium, calcein, and treatments were changed after 4 days.

After 7 days of culture, the bones were fixed in 10% neutral phosphate-buffered formaldehyde and stained with hematoxylin-eosin. Consistent 300 μm stretches (450 μm away from frontal sutures) were subjected to histomorphometric analysis using Bioquant-osteo (Bioquant Image Analysis Corp., Nashville, TN).

STATISTICAL ANALYSIS

Data are presented as mean ± S.E. Statistical significance was determined using two-tail Student's *t*-test. Results were considered statistically different when $P < 0.05$.

RESULTS

To investigate if Wnt binding has any effect on Ror2 signaling, we first used a chimeric receptor consisting of the extracellular domain of Ror2 fused to the transmembrane and intracellular domains of TrkB. The TrkB signaling is activated by receptor homo-dimerization and includes phosphorylation of ERK and stimulation of the cAMP response element (CRE)-mediated transcription. HEK293 cells stably over-expressing 3xCRE promoter-luciferase reporter gene and the Ror2-TrkB chimeric receptor (HEK293 CRE-luc Ror2-TrkB) showed an increase in luciferase activity in response to treatment with the dimerizing Ror2 antibody confirming that this system gives a functional read-out for Ror2 activation (Fig. 1). Figure 1 shows that treatment with Wnt5a, but not Wnt3a, resulted in a dose-dependent increase in luciferase activity compared to a control protein (BSA). These findings suggest that although Wnt3a and Wnt5a bind Ror2 equally well [Billiard et al., 2005], only Wnt5a activates signaling through this receptor.

Since Ror2 is activated by dimerization [Liu et al., 2007b], we tested the ability of Wnt5a and Wnt3a to dimerize this receptor. Flag-tagged and his-tagged Ror2 receptor constructs were expressed in U2OS osteosarcoma cells and the cells were treated for 1 h at 37°C with BSA (control), Wnt3a, or Wnt5a. Upon incubation, total cellular proteins were precipitated on anti-flag affinity agarose and

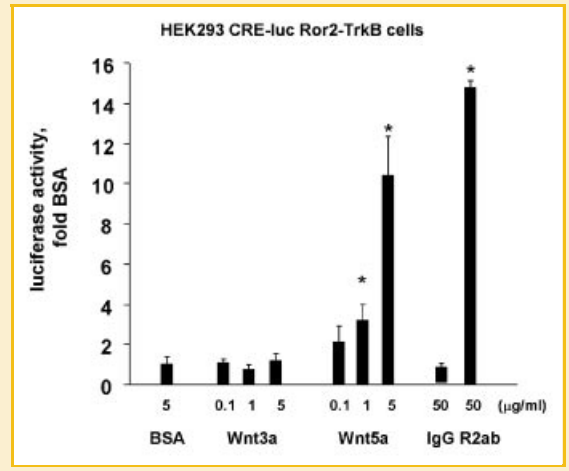

Fig. 1. Wnt5a, but not Wnt3a signals through Ror2-TrkB chimeric receptor. HEK293 cells stably transfected with 3xCRE-luciferase and Ror2-TrkB chimera (HEK293 CRE-luc Ror2-TrkB) were treated with BSA (control), Wnt3a, Wnt5a, anti-Ror2 antibody or non-specific IgG at indicated concentrations for 24 h. Luciferase activity was assessed as described in Materials and Methods and the signal obtained in BSA-treated sample was set at 1. The results are means ∀ S.E. of n = 8 (*$P < 0.05$).

subjected to immunoblotting with anti-his antibody. As shown in Figure 2A, under control conditions of BSA treatment, there was some association between his-tagged and flag-tagged Ror2 receptors indicating that Ror2 forms homo-dimers upon over-expression in U2OS cells. Consistent with the activation of the Ror2-TrkB chimera, the Ror2 dimerization was strongly enhanced upon treatment with Wnt5a, but not Wnt3a. The specificity of interactions was demonstrated by the fact that anti-flag antibody failed to immunoprecipitate Ror2-his in absence of Ror2-flag, and that anti-his antibody did not recognize the Ror2-flag protein (Fig. 2A).

To address if Wnt5a activated the Ror2 tyrosine kinase, we treated U2OS cells with 5 μg/ml Wnt5a or BSA for 1 h at 37°C, isolated the whole-cell proteins, and precipitated all tyrosine phosphorylated proteins on anti-phosphortyrosine agarose. Figure 2B demonstrates that treatment with Wnt5a resulted in significant increase in tyrosine phosphorylation of the Ror2 kinase as well as in phosphorylation of its substrate, 14-3-3β. These data provide strong evidence that Wnt5a dimerizes and activates the Ror2 receptor. Treatment of U2OS cells with 5 μg/ml Wnt3a did not induce phosphorylation of either Ror2 or 14-3-3β (data not shown).

Recent published evidence indicates that Ror2 is phosphorylated on serine/threonine residues by glycogen synthase kinase-3 (GSK-3) in response to Wnt5a in NIH3T3 and HeLa-S3 cells [Yamamoto et al., 2007]. We found that in human U2OS cells, Wnt5a treatment induced marginal, if any, serine/threonine phoshporylation of Ror2 above the background (Fig. 3A). However, GSK-3α/β inhibitor (Lilly 603281-31-8, GSKi) significantly inhibited the level of serine/threonine phosphorylation (Fig. 3B), indicating that GSK-3 is at least partially responsible for phosphorylating Ror2 on serine/threonine in U2OS cells as well. The nature of multiple bands observed in these experiments remains to be determined, but they were consistently

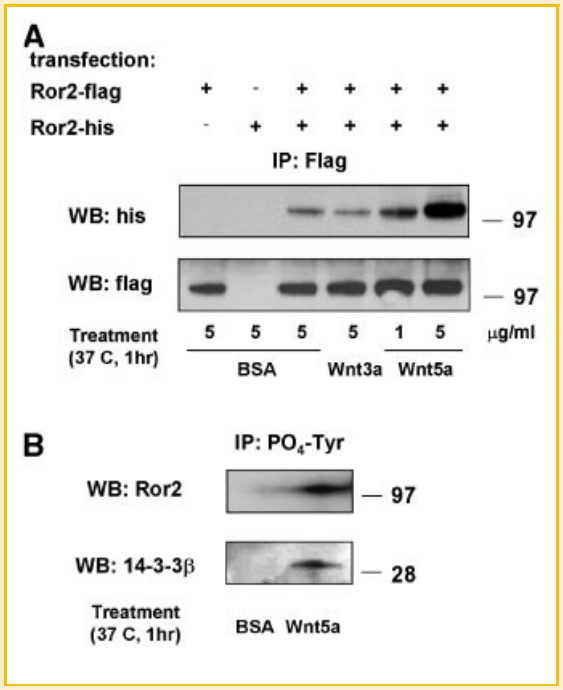

Fig. 2. Wnt5a induces Ror2 dimerization and promotes receptor activation. A: U2OS cells were transiently transfected with the indicated Ror2 constructs and treated with BSA (control), Wnt3a, or Wnt5a at indicated concentrations for 1 h at 37°C. Cell lysates were precipitated on anti-flag agarose and subjected to immunoblotting with anti-his antibody (top panel). In the bottom panel, 10% of the precipitated material was immunoblotted with anti-flag antibody to verify equal expression and precipitation. B: U2OS cells were treated with 5 μg/ml BSA or Wnt5a for 1 h at 37°C. Cell lysates were precipitated on anti-phospho-tyrosine agarose and subjected to immunoblotting with anti-Ror2 (top) or anti-14-3-3β (bottom) antibody.

present in multiple repetitions of the assay. To test if serine/threonine phoshorylation is a pre-requisite for the tyrosine phosphorylation of Ror2, we treated U2OS cells with Wnt5a in absence and presence of GSKi. We observed no change in the extent of Wnt5a-induced tyrosine phosphorylation of Ror2 (Fig. 3B) upon treatment with GSKi. Thus tyrosine phosphorylation of Ror2 in response to Wnt5a is independent of GSK-3 activation.

We next asked if Wnt5a-induced dimerization and activation of Ror2 affects functional consequences of Ror2 signaling. We have previously shown that Ror2 initiates commitment of hMSC to osteoblastic lineage and promotes differentiation at early and late stages of osteoblastogenesis [Liu et al., 2007a]. Since hMSC do not express Ror2 unless differentiated towards the osteogenic phenotype [Billiard et al., 2005], we induced Ror2 expression by treatment with osteogenic cocktail (MSCGM supplemented with 0.05 mM AA, 10 mM β-GP, and 100 nM dex) and added increasing amounts of BSA or Wnt5a. After 9 days of incubation, the degree of mineralized matrix formation was assessed with alizarin red-S histochemical staining. As shown in Figure 4A, Wnt5a dose-dependently increased the extent of calcified matrix formation in hMSC. To test if this Wnt5a effect was mediated through the Ror2 receptor, we performed Wnt5a treatment when endogenous Ror2 expression was inhibited.

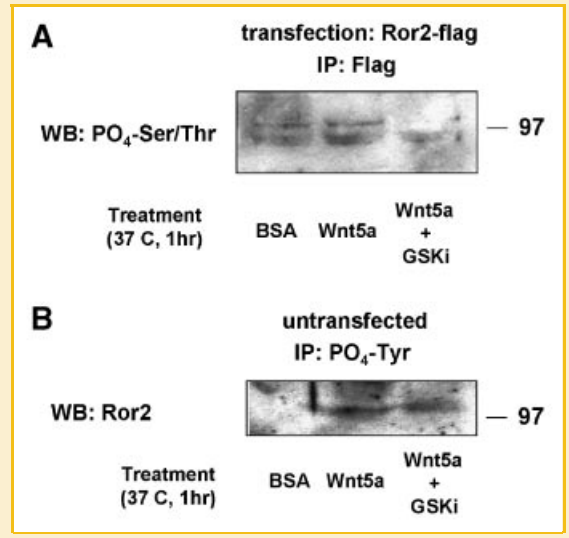

Fig. 3. Ror2 tyrosine phosphorylation is independent of the canonical Wnt signaling. A: U2OS cells were treated with 5 μg/ml BSA (control), 5 μg/ml Wnt5a, or 5 μg/ml Wnt5a and 20 μM GSK inhibitor (GSKi) for 1 h at 37°C. The whole-cell protein extracts were precipitated on anti-phospho-tyrosine agarose and immunoblotted with anti-Ror2 antibody. B: U2OS cells were transiently transfected with Ror2-flag and treated with 5 μg/ml BSA (control), 5 μg/ml Wnt5a, or 5 μg/ml Wnt5a and 20 μM GSKi for 1 h at 37°C. Cell lysates were precipitated on anti-flag agarose and subjected to immunoblotting with anti-phospho-serine and anti-phospho-threonine antibodies.

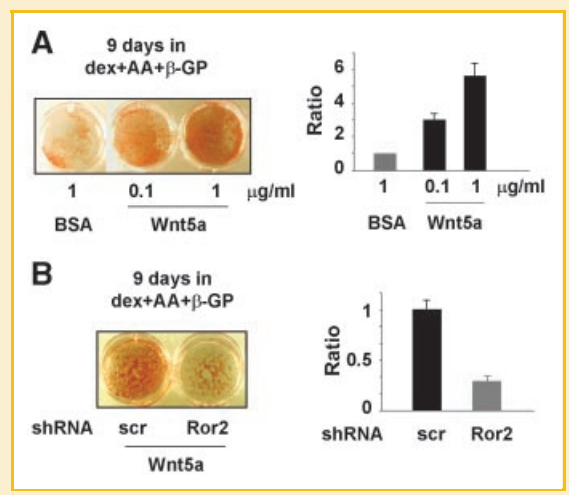

Fig. 4. Wnt5a promotes mineralized matrix formation in hMSC through Ror2 receptor activation. A: Human MSC were cultured in MSCGM supplemented with 0.05 mM ascorbic acid (AA), 10 mM β-glycerophosphate (β-GP) and 100 nM dexamethasone (dex) in presence of indicated concentrations of BSA or Wnt5a. After 9 days, cells were subjected to alizarin red-S staining for matrix mineralization (left panel). The graph on the right provides quantification of the alizarin red-S staining from three independent experiments. B: Human MSC were infected with adenoviruses containing shRNA specific for Ror2 or EGFP (control) and incubated in MSCGM supplemented with 1 μg/ml Wnt5a, 0.05 mM AA, 10 mM β-GP and 100 nM dex. After 9 days, cells were subjected to alizarin red-S staining for matrix mineralization. The graph on the right provides quantification of the alizarin red-S staining from three independent experiments.

Human MSC were infected with the adenovirus containing Ror2-specific shRNA, which strongly inhibited the dex-induced rise in Ror2 protein expression when compared to control shRNA [Liu et al., 2007a]. Infection with Ror2 shRNA resulted in ~70% reduction in the extent of Wnt5a-induced matrix mineralization (Fig. 4B), suggesting that Wnt5a effect is mediated, at least in part, through the Ror2 receptor. Thus Wnt5a can dimerize and activate the Ror2 receptor and promote Ror2-mediated calcified matrix formation in hMSC.

We then tested if in vitro effects of Wnt5a can translate into increased bone formation in ex vivo organ culture. Calvarial bones of 4-day-old mouse littermates were treated for 7 days with 1 μg/ml of BSA or Wnt5a, stained with hematoxylin-eosin and subjected to histomorphometric analysis. Treatment with Wnt5a resulted in 30% increase in total bone area (Fig. 5) indicating that Wnt5a promotes osteogenesis in calvarial organ cultures.

DISCUSSION

Our data demonstrate that Wnt5a activates Ror2 receptor signaling pathway, including receptor dimerization, its phosphorylation on tyrosine residue(s), and phosphorylation of its cellular substrate. Following these signaling events, Wnt5a activates functional consequences of Ror2 signaling.

Despite the fact that both Wnt3a and Wnt5a bind Ror2 equally well in immunoprecipitation experiments [Billiard et al., 2005], only Wnt5a was able to induce dimerization and phosphorylation of the receptor and phosphorylation of its cellular substrate. This suggests that only Wnt5a, but not Wnt3a, activates the classical RTK signaling through the Ror2 receptor. However, classical RTK pathway is not the only mode of Ror2 signaling since a Ror2 mutant lacking the entire cytoplasmic domain retains some signaling ability [Hikasa et al., 2002]. Furthermore, Ror2 associates with several other membrane receptors, including BRI-b [Sammar et al., 2004] and Frizzled [Li et al., 2008], raising a possibility that it may be activated without homodimerization. It is possible therefore that Wnt3a activates other, non-RTK signaling events through the Ror2 receptor.

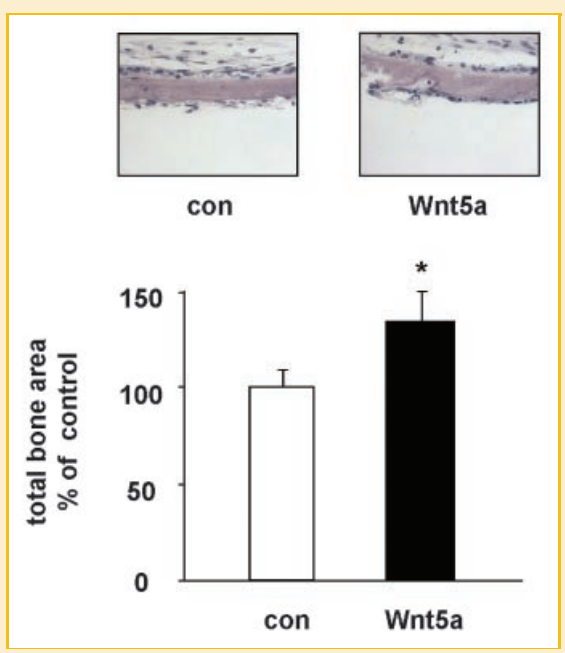

Fig. 5. Wnt5a treatment promotes new bone formation ex vivo. Calvariae from 4-day-old mouse pups were excised and treated with BSA or Wnt5a antibody (1 μg/ml). After 7 days of incubation, the total bone area was assessed as described in Materials and Methods. Values obtained in BSA-treated cultures were set at 100%. The results are means ∀ S.E. of 4–5 calvariae per condition (*$P < 0.05$).

Wnt5a has been previously shown to stimulate serine/threonine phosphorylation of Ror2 by GSK-3 in NIH3T3 mouse fibroblasts [Yamamoto et al., 2007]. Interestingly, Wnt5a did not induce tyrosine phosphorylation of Ror2 in this system [Yamamoto et al., 2007]. The situation appears to be reversed in human U2OS osteosarcoma cells used here, with Wnt5a inducing tyrosine, but not serine/threonine phosphorylation of Ror2 (Figs. 2 and 3). Wnt5a has also been shown to activate Jnk phosphorylation in NIH3T3 cells [Oishi et al., 2003] and to stimulate CDC42 and PI3K pathways in xenopus [Schambony and Wedlich, 2007]. We did not detect activation of either of these pathways by Wnt5a in U2OS cells (no change in Jnk or Jun phosphorylation or in phosphorylation of Akt, data not shown). Thus effects of Wnt5a on the downstream Ror2 signaling are species- and cell type-dependent.

We show that Wnt5a promotes functional consequences of the Ror2 signaling, including enhanced osteogenic differentiation of hMSC and increased bone formation in mouse calvarial organ culture model. Furthermore, the ability of Wnt5a to promote osteogenic differentiation of hMSC is largely dependent on signaling through the Ror2 receptor as it is inhibited by Ror2 shRNA. Previously, Wnt5a has been shown to enhance osteogenic differentiation of ST2 cells through activation of nemo-like kinase, leading to transcriptional repression of PPARγ and induction of Runx2 expression [Takada et al., 2007]. Whether or not this mechanism is operative in hMSC in response to Wnt5a-induced Ror2 activation remains to be determined. In contrast, Wnt3a that activates canonical β-catenin signaling, suppresses osteogenesis in adult hMSC [Boland et al., 2004; de Boer et al., 2004], raising a possibility that Wnt5a signals through Ror2 to suppress canonical Wnt signaling in hMSC as was suggested in HT-29 adenocarcinoma and HEK293 cells [MacLeod et al., 2007; Pacheco et al., 2007].

In summary, we have shown that Wnt5a, but not Wnt3a, activates classical RTK signaling through the Ror2 receptor and potentiates osteoblast differentiation and ex vivo bone formation. It is becoming increasingly clear that the presence of the Ror2 receptor conveys a variety of new consequences on Wnt5a signaling in several cell systems.

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