Ptpn11 deletion in a novel progenitor causes metachondromatosis by inducing hedgehog signalling

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The tyrosine phosphatase SHP2, encoded by PTPN11, is required for the survival, proliferation and differentiation of various cell types1,2. Germline activating mutations in PTPN11 cause Noonan syndrome, whereas somatic PTPN11 mutations cause childhood myeloproliferative disease and contribute to some solid tumours. Recently, heterozygous inactivating mutations in PTPN11 were identified in monocytes, macrophages and osteoclasts (lysozyme M-Cre; LysMCre) or in cathepsin K (Ctsk)-expressing cells, previously thought to be osteoclasts. LysMCre;Ptpn11fl/fl mice had mild osteopetrosis. Notably, however, CtskCre;Ptpn11fl/fl mice developed deformities3,4. The detailed pathogenesis of this disorder has remained unclear. Here we use a conditional knockout (floxed)Ptpn11 allele (Ptpn11fl) and Cre recombinase transgenic mice to delete Ptpn11 specifically in monocytes, macrophages and osteoclasts (lysozyme M-Cre; LysMCre) or in cathepsin K (Ctsk)-expressing cells, previously thought to be osteoclasts. LysMCre;Ptpn11fl/fl mice had mild osteopetrosis. Notably, however, CtskCre;Ptpn11fl/fl mice developed features very similar to metachondromatosis. Lineage tracing revealed a novel population of CtskCre-expressing cells in the perichondrial groove of Ranvier that display markers and functional properties consistent with mesenchymal progenitors. Chondroid neoplasms arise from these cells and show decreased extracellular signal-regulated kinase (ERK) pathway activation, increased Indian hedgehog (Ihh) and parathyroid hormone-related protein (Pthrp, also known as Pthlh) expression and excessive proliferation. Shp2-deficient chondroprogenitors had decreased fibroblast growth factor-evoked ERK activation and enhanced Ihh and Pthrp expression, whereas fibroblast growth factor receptor (FGFR) or mitogen-activated protein kinase kinase (MEK) inhibitor treatment of chondroid cells increased Ihh and Pthrp expression. Importantly, smoothed inhibitor treatment ameliorated metachondromatosis features in CtskCre;Ptpn11fl/fl mice. Thus, in contrast to its pro-oncogenic role in haematopoietic and epithelial cells, Ptpn11 is a tumour suppressor in cartilage, acting through a FGFR/MEK/ERK-dependent pathway in a novel progenitor cell population to prevent excessive Ihh production.

Cartilage tumours, including exostoses, enchondromas and chondrosarcomas, comprise ~20% of skeletal neoplasms3. Benign and malignant cartilaginous tumours can arise sporadically, but cartilage tumour syndromes, including hereditary multiple exostoses, the multiple enchondromas, including hereditary multiple exostoses, the multiple enchondromatosis disorders (Ollier disease and Maffucci syndrome) and metachondromatosis, also exist4. The cellular and molecular pathogenesis of most cartilage tumours is incompletely understood.

Metachondromatosis is an autosomal dominant tumour syndrome featuring multiple exostoses and enchondromas5,6. Recently, heterozygous early frameshift or nonsense mutations in PTPN11 were identified in >50% of metachondromatosis cases4,7. PTPN11 encodes the non-receptor protein tyrosine phosphatase SHP2, which is required for RAS/ERK pathway activation in most receptor tyrosine kinase, cytokine receptor, and integrin signalling pathways1,2. Germline activating

Figure 1 | Ptpn11 deletion in Ctsk-expressing cells causes metachondromatosis. a, Schemes for generating Ctsk-KO, LysM-KO and control mice. b, c, Gross images (b) and Faxitron/-CT radiographs (c) of 12-week-old Ctsk-KO mice showing dwarfism and scoliosis (b ii, white arrow; c ii, black arrow), increased bone mineral density (c ii, iv; arrows) and multiple exostoses of knees, ankles and metatarsals (b iv and c ii, iv, vi, vii; arrows) with joint destruction. d, Sagittal sections of metatarsal joints stained with haematoxylin and eosin (H&E; i–iii), Safranin O (iv–vi) and Alcian Blue (vii–ix) showing cartilaginous exostoses and enchondromas (arrows) in Ctsk-KO mice. Images in i, iv, vii are magnified ×2, in ii, v, vii ×10 and in ii, vi, ix ×20. Images in ii, vi, vii and iii, vi, ix are magnified ×10 views of boxed areas in i, iv, vii and ii, vii, respectively. Data shown are representative images; each analysis was performed on at least five mice per genotype.

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mutations in PTPN11 cause Noonan syndrome, whereas mutations that impair SHP2 catalytic activity cause LEOPARD syndrome (an acronym for multiple lentigines, ECG conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth and sensorineural deafness), both of which can feature skeletal abnormalities8. Somatic activating mutations in PTPN11 are the most common cause of juvenile myelomonocytic leukaemia and contribute to other leukaemias.

Global Ptpn11 deletion results in early embryonic lethality9,10, whereas postnatal deletion has context-dependent effects1,2. To assess the role of Shp2 in osteoclasts, we crossed Ptpn11fl/fl;LysMCre and Ptpn11fl/fl;CtskCre (hereafter, LysM-control and LysM-knockout (KO)) and Ctsk-KO mice, respectively (Fig. 1a).

Neither LysM-control nor Ctsk-control mice had a discernible phenotype, so we focused all subsequent analyses on LysM-KO and Ctsk-KO mice. Shp2 levels were reduced by >80% in bone-marrow-derived macrophages and osteoclasts in LysM-KO and Ctsk-KO mice (Supplementary Fig. 1a and data not shown). LysM-KO and Ctsk-KO mice were born at the expected Mendelian ratios and appeared to be normal for the first 3 weeks after birth. Subsequently, LysM-KO mice developed mild, age-related osteoporosis (Supplementary Fig. 1b and data not shown). By contrast, within 8 weeks after birth, Ctsk-KO mice exhibited a dramatic skeletal phenotype, comprising decreased body length, increased bone mineral density, scoliosis, metaphyseal exostoses and markedly decreased mobility (Fig. 1b–d and Supplementary Video 1).

Sections of hindlimb paw and knee joints from 12-week-old Ctsk-KO mice revealed multiple exostoses and enchondromas at the metaphyses of their metatarsals and phalanges (Fig. 1d), tibiae and femurs (Supplementary Fig. 1c, d), and other bones (data not shown), features reminiscent of metachondromatosis. As heterozygous PTPN11 frameshift mutations cause metachondromatosis3,4, these findings indicate that PTPN11 is a cartilage tumour-suppressor gene, and suggest that loss (or silencing) of the remaining PTPN11 allele is required for tumour formation.

To identify the cells responsible for metachondromatosis-like disease in Ctsk-KO mice, we first injected bone marrow from 6-week-old Ctsk-KO and Ctsk-control mice (C57BL/6; CD45.2) into lethally irradiated 3-week-old recipients (B6.SJL; CD45.1). Recipient mice exhibited high chimaerism (Supplementary Fig. 2a, b), but did not develop cartilage tumours in over 12 months of observation. Consistent with the osteopetrosis seen in LysM-KO mice, recipients had increased bone mineral density (Supplementary Fig. 2c). Clearly, however, cartilage tumours in Ctsk-KO mice are not due to altered osteoclast development or function.

Next, we performed lineage-tracing studies using Rosa26-lox-stop-lox-lacZ (R26-LSL-lacZ) or Rosa26-LSL-YFP (R26-LSL-YFP) Cre reporter mice. Notably, CtskCre, but not LysMCre, was expressed in a subset of perichondrial cells within the so-called groove of Ranvier (Fig. 2a). Sections from knee joints collected at postnatal day (P)10 revealed expansion of a cluster of Alcian Blue/Safranin O-positive cells in this region in Ctsk-KO, mice, but not in controls (Fig. 2b, boxed region and Supplementary Fig. 1c). By postnatal week 2, the yellow fluorescent protein (YFP) population had increased and differentiated into ectopic cartilaginous tissue in compound Ctsk-KO;YFP reporter mice (Fig. 2c, boxed region). Exostoses were palpable at 6 weeks and visible by 8–12 weeks. In compound Ctsk-KO;YFP reporter mice, these lesions consisted of YFP chondroid cells at various stages of development, including proliferating, pre-hypertrophic and hypertrophic

**Figure 2** | Skeletal tumours in Ctsk-KO mice originate from perichondrial groove of Ranvier cells. a. 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) staining of knee joint sections from 1-week-old R26-LSL-lacZ;CtskCre and R26-LSL-lacZ;LysMCre reporter mice showing that the Ctsk (but not the LysM) promoter is active not only in osteoclasts, but also in a subset of cells from the perichondrial groove of Ranvier (arrows). Images are magnified ×4. b. Haematoxylin and eosin and Safranin O staining of knee joint sections from P10 Ctsk-control (i, iv) and Ctsk-KO (ii, iii, v, vi) mice showing expansion of cells within the perichondrial groove of Ranvier region in Ctsk-KO mice. Images i, iv, v are magnified ×4. Images in iii and vi are magnified (×10) views of boxed areas in ii and v, respectively. c. Haematoxylin and eosin- and Safranin O (SO)-stained sections (magnified ×2) showing expanding YFP population within the perichondrial groove of Ranvier (boxed region in top panels, magnified ×10 below) that also stains with Safranin O, indicative of cartilage. Dashed line marks boundary between marrow/ growth plate and perichondrial groove. d. Frozen section of an exostosis from the metatarsal joint of Ctsk-KO;YFP mice showing co-localization of YFP reporter with cartilaginous tumour cells (boxed area). SO panel, ×4 magnification; all other panels, ×10 magnification. Note that the lesion is enriched in proliferating and pre-hypertrophic chondrocytes, as shown by overlapping Col2a1 and Col10a1 immunostaining. Each panel is a representative image from one mouse; each analysis was performed on at least three mice per genotype. DAPI, 4′,6-diamidino-2-phenylindole.
chondrocytes, as revealed by cell morphology and Col2a1 and Col10a1 immunostaining (Fig. 2c, d and data not shown). Notably, nearly all chondroid tumour cells were YFP⁺ (Fig. 2c and Supplementary Fig. 2d). Hence, cartilaginous tumours in Ctsk-KO mice (and, by analogy, most likely in metachondromatosis) result from cell-autonomous lack of Shp2 in Ctsk⁺ cells from the perichondrial groove of Ranvier.

The perichondrial groove of Ranvier is believed to contain chondroprogenitors responsible for circumferential cartilage growth, but these cells are not well-characterized. We used flow cytometry to analyse epiphyseal cartilage cells collected from the distal femurs and proximal tibiae of Ctsk-control;YFP and Ctsk-KO;YFP mice at P10–12. Compared with controls, the frequency of YFP⁺ cartilage cells in Ctsk-KO;YFP mice was increased by -fivefold (Fig. 3a). Within the YFP⁺ cell population, the percentage of cells expressing CD44, CD90 and CD166 (mesenchymal progenitor markers), but not CD31 (endothelial cell marker), also was increased (Fig. 3b). Staining for the stromal cell antigen Stro-1 in addition to jagged 1, markers associated with presumptive chondroprogenitors in the groove on the basis of BrdU and cell antigen Stro-1 in addition to jagged 1, markers associated with presumptive chondroprogenitors in the groove on the basis of BrdU (Fig. 2a, b and data not shown). Note enhanced intensity of Stro-1 and jagged 1 staining in Ctsk-KO cells. Data consistent with our qRT–PCR data, Ihh messenger RNA and protein were increased in Shp2-deficient CCPs (Fig. 4a). Ihh antibody specificity was confirmed by immunostaining of growth plate cartilage (Supplementary Fig. 3c).

CCPs are rare, rendering their detailed biochemical analysis unfeasible. We therefore tested the effects of Shp2 depletion in ATDC5 chondroid cells by stably expressing either of two short hairpin RNAs (shRNAs) targeting mouse Ptpn11. As in Ctsk-KO mice (Fig. 4a, 4b), evoked Erk activation was decreased, whereas Ihh and Pthrp levels were increased in Shp2-deficient cells (Fig. 4b). Conversely, FGFR (PD173074) or MEK (UO126) inhibition led to enhanced Ihh and Pthrp expression in parental ATDC5 cells (Fig. 4c).

Ihh signalling evokes Pthrp production. Our data, in addition to previous studies, suggested that increased Ihh levels might be pathogenic in metachondromatosis. If so, then blocking or attenuating Ihh signalling might slow and/or prevent the disease. To test this hypothesis, control (wild-type) and Ctsk-KO mice (9 per group) were gavaged daily with the smoothened inhibitor PF-04449913 (SMO1, 100 μg g⁻¹ body weight) or vehicle control (0.5% methylcellulose), beginning at 5 weeks of age (when early lesions were present) and continuing for the succeeding 4 weeks. Skeletal phenotype was assessed by X-ray, micro-computed tomography (μ-CT) and histology. Notably, SMO1 treatment significantly reduced the number of exostoses in Ctsk-KO

![Figure 3](image-url)

**Figure 3 | Ptpn11 deletion in Ctsk-expressing cells causes expansion of novel chondroprogenitor cell population within the perichondrial groove of Ranvier.** a. Flow cytometric analysis showing YFP⁺ cells from pooled epiphyseal cartilage from 5–7 Ctsk-control;YFP mice; note increased percentage of such cells in 2-week-old Ctsk-KO;YFP mice. CC, chondroid cells. b. Flow cytometric analysis of YFP⁺ perichondrial cells showing staining for CD31, CD44, CD90 and CD166. Data in panels a and b are from a single experiment; similar results were obtained in two additional experiments. c. Immunofluorescence micrograph (magnified ×20) showing Stro-1 and jagged 1 expression in YFP⁺ perichondrial cells. Nuclei are stained with DAPI. Note enhanced intensity of Stro-1 and jagged 1 staining in Ctsk-KO cells. Data shown are from single mice of each genotype; two additional mice were analysed for each genotype with similar results. d. CCPs give rise to cartilage, fat and bone. Fluorescent-activated cell sorting (FACS)-purified YFP⁺ cells from 5–7 mice were subjected to differentiation assays in triplicate. After 2–3 weeks of culture (see Methods), cells were fixed and stained with Alcian Blue, Oil Red O and Alizarin Red to visualize the formation of cartilage, fat and bone tissue (magnified ×20), respectively.
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**Figure 4 | Shp2 deficiency impairs Erk activation but promotes Ihh and Pthrp expression.** a. Top left, qRT–PCR showing increased Col2a1, Col10a1, Ihh and Pthrp expression in laser-captured cartilaginous cells from exostoses in four mice per genotype, compared with normal articular cartilage cells (mean ± s.d.; *P < 0.05, two-tailed Student’s t test). Top right, immunostaining of representative paraffin sections from perichondrial groove of Ranvier region of Ctsk-KO and control mice. Note the decreased number of p-Erk+ cells (75.4% in Ctsk-control versus 32.2% in Ctsk-KO; n = 3 mice). Bottom, note the increased Ihh expression but unchanged p-Akt staining in Ctsk-KO, compared with control, mice. b. Top left, immunoblot showing Shp2 in ATDC5 knockdown (KD) cells, stably expressing shRNAs against mouse Ptpn11 (ATDC5-KD1, ATDC5-KD2) or scrambled control hairpin. Top right, Representative blot showing that Shp2 deficiency decreases Erk activation in response to Fgf18; data from multiple experiments (n = 3) showing p-Erk levels (compared with control at 5 min, mean ± s.d.; *P < 0.05, two-tailed Student’s t test) are quantified below (bottom right). qRT–PCR (bottom left) shows increased Ihh and Pthrp expression in Shp2-deficient ATDC5 cells (mean ± s.d.; n = 3, *P < 0.05, two-tailed Student’s t test) are quantified below (bottom right). qRT–PCR (bottom left) shows increased Ihh and Pthrp expression in Shp2-deficient ATDC5 cells (mean ± s.d.; n = 3, *P < 0.05, two-tailed Student’s t test). c. FGFRI (PD173074, 10 nM) or MEK (U0126, 1 μM) inhibitor treatment (FGFRi and UO, respectively) of parental ATDC5 cells enhances Ihh and Pthrp expression, as shown by qRT–PCR (mean ± s.d.; n = 3, *P < 0.05, two-tailed Student’s t test). d. Fux-Fax radiographs showing that hedgehog pathway blockade following administration of the SMOi (100 μg g−1 body weight) to Ctsk-KO mice ameliorates tumour formation, compared with vehicle control (0.5% methylcellulose)-treated mice. Images of representative posterior paws (i–iv) and knees (v–viii) taken before (i, iii, v, vii) and after treatment with vehicle (ii, vi) or SMOi (iv, viii) for 4 weeks. Note continued development of exostoses and enchondromas in vehicle-treated mice, and their amelioration in SMOi-treated group (arrows). Also see Supplementary Figs 4–7 and Supplementary Video 1.

Our findings strongly suggest that metachondromatosis results from loss of SHP2, specifically in CCPs, a heretofore poorly characterized population within the perichondrial groove of Ranvier, which is believed to function as a stem–cell niche for joints and a reservoir for the germlinal layer cells of the growth plate. Cells within the groove of Ranvier express high levels of FGFFR3 (ref. 25), and their removal prevents longitudinal bone growth. Emerging evidence shows that groove of Ranvier cells can migrate into articular cartilage, implicating them in maintaining cartilage homeostasis and possibly in degenerative joint diseases, such as osteoarthritis. Indeed, in lineage-tracing studies of normal mice, we noticed YFP+ cells migrating towards articular cartilage (Supplementary Fig. 9, arrows, and data not shown). On the basis of our mouse metachondromatosis model, we propose that SHP2, acting downstream of FGFFR3 and upstream of the RAS/ERK pathway, regulates CCP proliferation and chondrogenic differentiation. Consequently, PTPN11 deficiency in these cells promotes excessive proliferation, chondrogenic differentiation and cartilage tumours.

Metachondromatosis is associated with heterozygous inactivating mutations in PTPN11, yet Ctsk-control mice are normal, whereas Ctsk-KO mice exhibit metachondromatosis-like features. Although PTPN11 gene dosage effects could differ in mouse and man (and thus 50% reduction in SHP2 level might cause metachondromatosis in humans but not in mice), we think it is more likely that loss of the remaining PTPN11 allele (for example, by loss of heterozygosity or silencing) is required to cause cartilage tumours in metachondromatosis. If so, then unlike its oncogenic role in juvenile myelomonocytic leukaemia, other hematologic malignancies and solid tumours, PTPN11 is a tumour suppressor in cartilage. Liver-specific Ptpn11 deletion reportedly results in hepatocellular carcinoma. However, we have not seen liver tumours in our Ptpn11 conditional knockout mice crossed to the same Cre line (F.H. & B.G.N., manuscript in preparation), nor is PTPN11 mutated in human hepatocellular carcinoma. Moreover, our biochemical and pharmacological analysis, together with previous studies, provide a parsimonious and attractive explanation for the
apparently paradoxical pro- and anti-oncogenic effects of PTPN11. In both cases, SHP2 is a critical regulator of ERK. The activating PTPN11 mutations associated with cancer promote proliferation and survival, at least in part via increased ERK activation. Similarly, overexpression or increased activation of normal SHP2 binding proteins such as GAB2, or the presence of pathologic SHP2 binding proteins such as Helicobacter pylori CagA, can hyperactivate ERK and contribute to various malignancies. Conversely, SHP2 deficiency is oncogenic in CCGs because in these cells, ERK normally represses the expression of the growth stimulator IHH (which, in turn, stimulates PTHR production). Future studies should focus on better defining the properties of CCGs, determining whether PTPN11 also acts as a tumour suppressor in other cartilage neoplasms, including chondrosarcoma, and most importantly, on testing the effects of smoothed inhibition in metachondromatosis patients. Finally, given our proposed mechanism of metachondromatosis pathogenesis, our results call for caution in the long-term use of MEK or ERK inhibitors.

METHODS SUMMARY

Ptpn11 floxed (Ptpn11flox)18, cathepsin K-Cre (Ctsk-Cre)19, Rosa26-LSL-LacZ (R26-LSL-lacZ)20, and Rosa26-LSL-YFP (R26-LSL-YFP)18 Cre reporter mice were on C57BL/6 background. PCR genotyping was performed as described21,12,23,29,30; conditions are available from W.Y. Animal studies were approved by the Institutional Animal Care and Use Committee at Rhode Island Hospital. Mice of both genders are available from W.Y. Animal studies were approved by the Institutional Animal Care and Use Committee at Rhode Island Hospital. Mice of both genders were used for this study. Antibodies and reagents are detailed in Methods. CCGs isolated from embryonic cartilage of 2-week-old Csk-control/YFP and Csk-KO/YFP mice (detailed in Methods) were analysed by flow cytometry or subjected to multiple lineage differentiation assays. For flow cytometry, cells were stained with fluorescence-labelled antibodies, and analysed on a BD LSR II flow cytometer using FlowJo software (TreeStar). YFP+ cells were purified using a BD Influx cell sorter (BD Bioscience). ATDC5 cells stably expressing mouse Ptpn11 shRNAs or a scrambled control were generated in pSuper( Retro)/puro (Oligogene). RNA was extracted from cells or laser-dissected lesions using RNeasy (Qiagen), com-

Published online 17 July 2013.

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Supplementary Information is available in the online version of the paper.

Acknowledgements We thank S. Kato for Ctsk-Cre mice, A. Craft for review of the manuscript, X. Wang and P. Monfils for help with histology and J. Cao for helping with analysis. We thank S. Kato for Ctsk-Cre mice, A. Craft for review of the manuscript, X. Wang and P. Monfils for help with histology and J. Cao for helping with analysis. We thank S. Kato for Ctsk-Cre mice, A. Craft for review of the manuscript, X. Wang and P. Monfils for help with histology and J. Cao for helping with analysis.
METHODS

Ptpn11 floxed (Ptpn11<sup>fl/fl</sup>)<sup>a</sup>, cathepsin K-Cre (Ctsk-Cre)<sup>b</sup>, Rosa26-LSL-lacZ (R26-LSL-lacZ)<sup>c</sup>, and Rosa26-LSL-YFP (R26-LSL-YFP)<sup>d</sup> Cre reporter mice were on C57BL/6 background.

**Antibodies and reagents.** The following antibodies were purchased: monoclonal anti-p-tyrosine (4G10) was from Millipore; polyclonal antibodies against Ptpn11, Ptpn11<sup>a</sup> were from Santa Cruz Biotechnology and Abcam, respectively; fluorescence-labelled antibodies against CD31, CD44, CD45, CD90 and CD166 were purchased from eBioscience; and antibodies against Ihh, Col2<sup>a</sup>, Erk2, p-Akt(Ser 473), Akt, Shp2, p-Stat1 (Tyr 701) and Stat1 were from Cell Signaling; antibodies against Ihh, Col2<sup>a</sup> and Col2<sup>a</sup> were purchased from eBioscience; and Alexa 488-labelled goat anti-rabbit IgG and Alexa 594-labelled antibodies against Stro-1 and jagged 1 were purchased from Invitrogen and Epi-Immunosignaling; antibodies against Ihh, Col2<sup>a</sup>, Erk2, p-Akt(Ser 473), Akt, Shp2, p-Stat1 (Tyr 701) and Stat1 were from Cell Signaling; antibodies against Ihh, Col2<sup>a</sup> and Col2<sup>a</sup> were purchased from eBioscience; and Alexa 488-labelled goat anti-rabbit IgG and Alexa 594-labelled antibodies against Stro-1 and jagged 1 were purchased from Invitrogen. Pgf18 was purchased from PeproTech. UO126 and PD173074 were from Calbiochem and Epi-Immunosignaling, respectively. Alexa 488-labelled goat anti-rabbit IgG and Alexa 594-labelled antibodies against Stro-1 and jagged 1 were purchased from Invitrogen.

**Cell isolation and culture.** Cells were isolated from 2-week-old Ctsk-control;YFP and Ctsk-KO;YFP mice, and digested with hyaluronidase (2.5 mg ml<sup>−1</sup>, Sigma) and trypsin-EDTA (0.25%, Invitrogen) to remove soft tissues, and then with collagenase D (2.5 mg ml<sup>−1</sup>, Roche) for 4–6 h to release all cartilage cells. After washing in PBS, cells were stained with fluorescence-labelled antibodies (using concentrations recommended by the manufacturers), and analysed by flow cytometry, or YFP<sup>+</sup> cells were purified by FACS and placed in short-term cultures (3–4 days) in murine mesenchymal culture medium (StemCell Technologies) containing 10% FBS.

Parental ATDC5 cells were obtained from C. Phornphutkul (Brown University) and cultured in complete DMEM/F12 medium (1:1) (Invitrogen), as described<sup>13</sup>. shRNAs against mouse Ptpn11 (KD1: 5′-GATCTTACAACACTGAGGACTTCA AGAGAGTCCCCAGTGTTCTGAATC-3′; KD2: 5′-GAGTAAACCTGGAGAC TTCTTCAAGAGAGTCCCCAGTGTTCTGAATC-3′), or a scrambled control for KD1 (5′-TAGTACAAAGATCCAGGGCTTCAAGAGAGTCCCCAGTGTTCTGAATC-3′), were introduced into the retroviral vector pSuper(retro)puromycin (Oligoengine). Viral supernatants were collected from 293T cells co-transfected with each retroviral vector and Ecopack, and used to infect ATDC5 cells, which were then selected with puromycin<sup>13</sup>.

**Differentiation assays.** CCPS (~2 × 10<sup>6</sup>), purified by FACS (for YFP) from 10–14-day-old Ctsk-R26-LSL-YFP reporter mice, were cultured in differentiation medium for chondrocytes (DMEM with 10% FBS, 0.1 mM dexamethasone, 0.1 mM ascorbic acid, 10 mM glycerol 2-phosphate, 1 ng ml<sup>−1</sup> TGF-β1), adipocytes (DMEM with 10% FBS, 0.1 mM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 10 µg ml<sup>−1</sup> insulin), or osteoblasts (DMEM with 10% FBS, 0.1 mM dexamethasone, 0.2 mM ascorbic acid, 10 mM glycerol 2-phosphate, 10 ng ml<sup>−1</sup> rhBMP2), respectively. After culturing for 2 (adipogenic or chondrogenic differentiation) or 3 weeks (osteogenesis), cells were fixed and stained with Alcian Blue, Red Oil O or Alizarin Red to visualize the formation of cartilage, fat, and bone tissue, respectively.

**qRT-PCR.** RNA was extracted from cultured cells or cartilage lesions enriched by laser-capture using the RNeasy kit (Qiagen). cDNA was synthesized using iScriptDNA Synthesis Kit (Bio-Rad), and qRT-PCR was performed by using the IQ SYBR Green qPCR kit. All values were normalized to Gapdh levels, and qRT-PCR data were expressed as fold-increases compared with controls. Primer sequences and PCR conditions are available from W.Y. upon request.

**Flow cytometry and FACS.** Epiphysial cartilage cells were stained with fluorescence-labelled antibodies, as described<sup>14</sup>, and analysed on a BD LSR II flow cytometer. YFP<sup>+</sup> cells were purified by FACS using a BD Influx cell sorter (BD Bioscience). Flow cytometric data were analysed with FlowJo software (TreeStar).

**Histology.** Ctsk-control and Ctsk-KO mice were euthanized at the indicated ages, and femurs, tibiae and paws were removed and fixed in 4% PFA overnight at 4 °C. Postnatal skeletal tissues were decalcified in 0.5 M EDTA before embedding. Tissue sections (5 µm) were stained with haematoxylin and eosin, Alcian blue or Safranin O. Immunofluorescence staining was carried out using secondary antibodies conjugated to the indicated fluorophores at concentrations recommended by their manufacturers. Immunohistochemistry was performed using fluorescence- or peroxidase-coupled anti-rabbit, -mouse or -goat secondary antibodies, as per the manufacturer’s instructions, with dianisobenzidine serving as the substrate. X-gal staining was performed as described<sup>12</sup>.

**Drug treatment.** Two trials were performed using the smoothened inhibitor PF-04499193. In a pilot study, groups (five mice per each group) of knockout mice were treated with SMOi (100 µg kg<sup>−1</sup> body weight) or vehicle control (0.5% methylcellulose), beginning at 5 weeks of age (at which time early lesions were present) and continuing for the succeeding 4 weeks. Mice were randomized by alternate assignment to control (vehicle) or drug treatment arms. The pilot experiment showed a significant difference in number of exostoses (assessed radiographically) in the SMOi group, and led to a second study (again involving five mice each) to confirm these findings and also assess additional parameters (µ-CT, histology, gene expression). Two mice (one each from control and experimental groups, respectively) died for unknown reasons during the second trial, and were excluded from the analysis because they were removed from cages and could not be recovered. All surviving mice from both studies were included in the analyses shown in the text.

**Microcomputed tomography (µ-CT) and X-ray analysis.** X-ray images of the entire skeleton, knees, metatarsals and phalanges were obtained immediately after euthanasia using a Faxitron X-ray system (Wheeling). After fixation in 4% PFA, µ-CT images of skeletal tissues were scanned with a desktop microcomputer graphic imaging system (µ-CT40, Scanco Medical AG). The number of exostoses was measured from these radiographic images, as indicated in the figure legends. For these studies, mice were assigned a code number by the animal technician, and blinded quantification was carried out by W.Y.

**Immunoblotting.** Cells were lysed in modified NP-40 buffer (0.5% NP-40, 150 mM NaCl, 1 mM EDTA, 50 mM Tris (pH 7.4)), supplemented with a protease inhibitor cocktail (1 mM PMSF, 1 mM NaF, 1 mM sodium orthovanadate, 10 µM aprotinin, 0.5 mg ml<sup>−1</sup> antipain and 0.5 mg ml<sup>−1</sup> pepstatin), as described<sup>16</sup>. For immunoblotting, cell lysates (10–50 µg) were resolved by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and incubated with primary antibodies for 2 h or overnight at 4 °C (according to the manufacturer’s instructions), followed by horseradish peroxidase-conjugated secondary antibodies. Detection was by enhanced chemiluminescence (Amersham). Signals were quantified using NIH ImageJ.

**Statistical analysis.** Differences between groups were evaluated by Student’s t test. A p value of <0.05 was considered significant. For all of these experiments, between-group variances were similar and data were symmetrically distributed. All analyses were performed by using Excel (Microsoft) and Prism 3.0 (GraphPad).

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Corrigendum: Ptpn11 deletion in a novel progenitor causes metachondromatosis by inducing hedgehog signalling

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Nature 499, 491–495 (2013); doi:10.1038/nature12396

After publication of this Letter, we became aware that we had not reported the details of construction and validation of our floxed conditional deletion Ptpn11 allele. These details are presented in the Supplementary Methods and Supplementary Fig. 1 of this Corrigendum. We also stated in our manuscript that PTPN11 is not mutated in human hepatocellular carcinoma, but it has been brought to our attention that a low frequency of copy number abnormalities involving the PTPN11 locus in this disorder has been reported. However, comparable numbers of amplifications and deletions were observed, making it difficult to conclude from the data in ref. 1 that PTPN11 acts as a tumour suppressor gene in hepatocellular carcinoma.

Supplementary Information is available in the online version of the Corrigendum.

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