HHS Public Access

Author manuscript

Musculoskelet Regen. Author manuscript; available in PMC 2015 June 04.

Published in final edited form as:

Musculoskelet Regen. 2015 ; 1(1): . doi:10.14800/mr.814.

A mouse model of craniofacial bone lesion of tuberous sclerosis complex

Fang Fang[1], **Xiaoxi Wei**[1,2], **Min Hu**[2], and **Fei Liu**[1]

[1]Department of Biologic and Materials Sciences Division of Prosthodontics, University of Michigan School of Dentistry, Ann Arbor, MI 48109, USA

[2]Orthodontic Department, Jilin University College of Dentistry, Changchun, Jilin 130012, China

Abstract

The mammalian/mechanistic target of rapamycin (mTOR) signaling pathway plays critical roles in skeletal development. The impact and underlying mechanisms of its dysregulation in bone homeostasis is poorly defined. The best known and characterized mTOR signaling dysregulation in human disease is called Tuberous Sclerosis Complex (TSC). TSC is an autosomal dominant neurocutaneous syndrome with a high frequency (>66%) of osseous manifestations such as sclerotic lesions in the craniofacial region. TSC is caused by mutations of TSC1 or TSC2, the heterodimer protein inhibitor of mTORC1 signaling. The underlying mechanism of bone lesions in TSC is unclear. We generated a TSC mouse model with TSC1 deletion in neural crest derived (NCD) cells, which recapitulated the sclerotic craniofacial bone lesion in TSC patients. We demonstrated that TSC1 null NCD osteoblasts overpopulated the NCD bones and the resultant increased bone formation is responsible for the sclerotic bone phenotype. Mechanistically, osteoblast number increase is due to the hyperproliferation of osteoprogenitor cells at an early postnatal stage. Noteworthy, administration of rapamycin, an mTORC1 inhibitor at early postnatal stage can completely rescue the excess bone acquisition, but late treatment cannot. Altogether, our data suggested that enhanced mTORC1 signaling in NCD cells can enlarge the osteoprogenitor pool and lead to the excess bone acquisition, which is likely the underlying mechanism of sclerotic bone lesion observed in TSC patients.

Keywords

mice; craniofacial; osteoblast; tuberous sclerosis; Tsc1; neural crest; mTOR

The mammalian target (also known as the mechanistic target) of rapamycin (mTOR) signaling pathway could sense and integrate intracellular and extracellular signals, such as insulin, hormones, growth factors and nutrients to modulate cell growth, homeostasis and

Correspondence: Fei Liu, feiliu@umich.edu.

Conflict of Interest

The author declares that they have no conflict of interest.

survival. The serine/threonine kinase mTOR is present in two distinct protein complexes: mTOR complex1 (mTORC1) and mTOR complex2 (mTORC2). There are two critical upstream regulators of mTORC1: Akt signaling and a heterodimer protein inhibitor TSC1/2, which consists of tuberous sclerosis 1 (TSC1, also known as harmatin) and TSC2 (also known as tuberin). TSC1/TSC2 complex is upstream inhibitor of mTORC1, and thus in the absence of either TSC1 or TSC2, mTORC1 signaling is chronically increased [1].

mTOR signaling pathway regulates many major cellular processes and is implicated in an increasing number of pathological conditions including bone lesions in Tuberous Sclerosis Complex (TSC) disease. TSC is an autosomal dominant neurocutaneous syndrome characterized by the presence of benign congenital tumors in multiple organs, such as brain, lung, liver, kidney, heart, and alimentary tract. TSC is caused by inactivating mutations of TSC1 or TSC2 and with an estimated prevalence at 1/5800 at birth [2]. Osseous manifestation of TSC has a high frequency (>66%) and is very diverse, including sclerotic lesion, cyst like lesion, osteoporotic lesion [3]. 40–60% TSC patients have sclerotic bone lesions but its etiology and mechanisms are poorly defined. Sclerotic lesions vary in size, shape and location. It can occur in cranial bone, hand, feet, vertebrae, rib and long bone with the highest incidence in calvaria (40–50%) [4, 5]. The frontal and parietal bones are most often involved. Hyperostosis on the inner table of calvaria and sclerotic islands in the vault diploe are commonly seen [4]. Microscopic change shows osteosclerosis [4]. Cranial sclerosis is not seen in very young age, about half cases will present in their first decade of life [6]. Occasionally, the calvaria can be generalized sclerosis and thickening [7, 8].

Despite the high relevance to human disease, we have limited knowledge about how mTOR signaling regulates bone metabolism *in vivo*. Studies using rapamycin, an mTORC1 inhibitor, have showed both positive [9–11] and negative [12–15] effects on osteogenesis *in vitro*. Some recent reports demonstrated the important roles of mTOR signaling in skeletal development [16–18]. However, despite the high prevalence of bone lesions in TSC patient, the underlying bone lesion mechanism is largely unknown, which is mainly due to the lack of appropriate animal models of TSC bone lesions. Because TSC is caused by the loss-of-function mutation in either TSC1 or TSC2, TSC1 or TSC2 knockout mouse models are the logic models to investigate this disease mechanism. However, the embryonic lethality of conventional TSC1 or TSC2 knockout mice prevented us from utilizing these models to study the postnatal sclerotic bone lesions [19, 20].

Recently, we generated a viable TS bone lesion mouse model by deleting TSC1, the negative regulator of mTORC1, with P0-Cre, which specifically targets neural crest derived cells[21]. $TSC1^{flox/flox}$;P0-Cre mice (CKO) had dramatically increased thickness of neural crest derived frontal bone but not of mesoderm derived parietal bones. Of note, the frontal bone thickness of $TSC1^{flox/+}$; P0-Cre mice (cHet) mice is comparable to control ($TSC1^{flox/flox}$). Besides the sclerotic alteration in bones formed by intramembranous ossification, cranial base bones with neural crest origin (presphenoid and basisphenoid bone) formed by endochondral ossification also had increased bone mass (Xiaoxi Wei and Fei Liu, unpublished). Interestingly, mutant bones had not only increased mass but also increased tissue mineral density (Xiaoxi Wei and Fei Liu, unpublished). Interestingly, TSC2 deletion in mature osteoblasts also leads to increased calvaria thickness [16]. However, the

disorganized bone structure was reported in that study, which is clearly different from the highly mineralized bone in our model. TSC patients are often reported to have sclerotic calvaria island, which is more similar to the sclerotic bone phenotype in our mouse model.

Histomorphometry analysis of one-month-old CKO and control mice demonstrated enhanced bone formation rate on both extracranial and intracranial surfaces of frontal bone in CKO mice. TRAP staining showed similar osteoclast number and surface on frontal bone of CTR and CKO mice, indicating that increased bone mass in CKO mice is due to increased osteoblast bone formation but not altered osteoclastogenesis. To determine the effect of TSC1 deletion on osteoblast differentiation, we examined the osteoblast differentiation marker gene expression. The only difference we observed is slightly increased expression of Osteocalcin, whose inactivation or over-expression does not affect extracellular matrix mineralization [22, 23].

The relatively low P0-Cre activity (<30%) revealed by reporter mice analysis and high TSC1 deletion efficiency (60%–90%) shown by western blot prompted us to hypothesize that TSC1 null cell may overpopulate the mutant bone. To test this hypothesis, we performed lineage tracing experiment. We found significantly increased P0-Cre targeted cells in neural crest derived frontal bones in one-week-old skulls of CKO mice compared to cHet mice. This strongly supported the notion that TSC1 null neural crest derived cells overpopulated neural crest derived bones. Using the anti-osterix (an osteoblast marker protein) antibody, we found significantly increased osterix positive osteoblast in the periosteum of CKO frontal bone. In addition, we found an increase in osteocyte density in CKO frontal bone. All these data suggested that TSC1 deletion by P0-Cre leads to increased osteoblast lineage cells. To account for the increased osteoblasts in CKO, we found a striking increase of proliferation in frontal bone periosteum of CKO mice at one week old but not at one month old. The mechanism of increased proliferation in CKO osteoblast progenitor cells is under further investigation. Altogether, these data indicated that the excess bone acquisition in CKO mice is due to increased osteoblast progenitor proliferation.

Interestingly, when we treated CTR and CKO mice with rapamycin, an mTORC1 inhibitor, from one-week-old to two-month-old, the increased calvaria thickness phenotype was completely rescued in CKO mice. However, when rapamycin was delivered after one-month-old, the high bone mass bone phenotype in CKO mice cannot be rescued, suggesting that the causative change of sclerotic bone phenotype has already occurred at one month of age. This prompted us to hypothesize that up-regulated mTORC1 signaling in neural crest cells can enlarge the osteogenic stem cell number during early postnatal stage. The enlarged osteogenic stem cell pool is responsible for the subsequently increased osteoblast lineage cells and increased bone mass acquisition. To support this notion, we observed increased proliferation in cranial periosteum (rich in osteoprogenitors) in CKO mice at early (one week) but not late (one month) postnatal stage. We are currently using mouse models with TSC1 deletion in cranial osteogenic stem cells to further test this hypothesis.

In summary, we established the first mouse model recapitulating the craniofacial sclerotic bone lesions in TSC patients. Mechanistically, enhanced mTORC1 signaling in neural crest cells can promote the expansion of osteoprogenitor pool at an early stage, which is likely the

underlying mechanism of the sclerotic bone lesion observed in TSC patients. The molecular mechanisms of osteoprogenitor cell proliferation regulation by TSC1/mTOR remains to be determined.

Acknowledgments

FL was supported by the National Institute of Dental and Craniofacial Research of the National Institutes of Health under Award Number R03DE021718, the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under award number R01AR062030, and the Office of Vice President Research Grant of University of Michigan.

References

1. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol. 2003; 13:1259–1268. [PubMed: 12906785]
2. Osborne JP, Fryer A, Webb D. Epidemiology of tuberous sclerosis. Ann N Y Acad Sci. 1991; 615:125–127. [PubMed: 2039137]
3. Umeoka S, Koyama T, Miki Y, Akai M, Tsutsui K, Togashi K. Pictorial review of tuberous sclerosis in various organs. Radiographics. 2008; 28:e32. [PubMed: 18772274]
4. Holt JF, Dickerson WW. The osseous lesions of tuberous sclerosis. Radiology. 1952; 58(1):1–8. [PubMed: 14883368]
5. Lagos JC, Holman CB, Gomez MR. Tuberous sclerosis: neuroroentgenologic observations. Am J Roentgenol Radium Ther Nucl Med. 1968; 104:171–176.
6. Medley BE, McLeod RA, Houser OW. Tuberous sclerosis. Semin Roentgenol. 1976; 11:35–54. [PubMed: 766190]
7. Tod PA. Radiological findings in tuberose sclerosis. J Coll Radiol Australas. 1962; 6:42–48. [PubMed: 13921485]
8. Morris BS, Garg A, Jadhav PJ. Tuberous sclerosis: a presentation of less-commonly encountered stigmata. Australas Radiol. 2002; 46:426–430. [PubMed: 12452918]
9. Ogawa T, Tokuda M, Tomizawa K, Matsui H, Itano T, Konishi R, et al. Osteoblastic differentiation is enhanced by rapamycin in rat osteoblast-like osteosarcoma (ROS 17/2. 8) cells. Biochem Biophys Res Commun. 1998; 249:226–230. [PubMed: 9705862]
10. Lee KW, Yook JY, Son MY, Kim MJ, Koo DB, Han YM, et al. Rapamycin promotes the osteoblastic differentiation of human embryonic stem cells by blocking the mTOR pathway and stimulating the BMP/Smad pathway. Stem Cells Dev. 2010; 19:557–568. [PubMed: 19642865]
11. Martin SK, Fitter S, Bong LF, Drew JJ, Gronthos S, Shepherd PR, et al. NVP-BEZ235, a dual pan class I PI3 kinase and mTOR inhibitor, promotes osteogenic differentiation in human mesenchymal stromal cells. J Bone Miner Res. 2010; 25:2126–2137. [PubMed: 20499346]
12. Isomoto S, Hattori K, Ohgushi H, Nakajima H, Tanaka Y, Takakura Y. Rapamycin as an inhibitor of osteogenic differentiation in bone marrow-derived mesenchymal stem cells. J Orthop Sci. 2007; 12:83–88. [PubMed: 17260122]
13. Singha UK, Jiang Y, Yu S, Luo M, Lu Y, Zhang J, et al. Rapamycin inhibits osteoblast proliferation and differentiation in MC3T3-E1 cells and primary mouse bone marrow stromal cells. J Cell Biochem. 2008; 103:434–446. [PubMed: 17516572]
14. Faghihi F, Baghaban Eslaminejad M, Nekookar A, Najar M, Salekdeh GH. The effect of purmorphamine and sirolimus on osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. Biomed Pharmacother. 2013; 67:31–38. [PubMed: 23228449]
15. Shoba LN, Lee JC. Inhibition of phosphatidylinositol 3-kinase and p70S6 kinase blocks osteogenic protein-1 induction of alkaline phosphatase activity in fetal rat calvaria cells. J Cell Biochem. 2003; 88:1247–1255. [PubMed: 12647306]
16. Riddle RC, Frey JL, Tomlinson RE, Ferron M, Li Y, DiGirolamo DJ, et al. Tsc2 is a molecular checkpoint controlling osteoblast development and glucose homeostasis. Mol Cell Biol. 2014; 34:1850–1862. [PubMed: 24591652]

17. Chen J, Long F. mTORC1 signaling controls mammalian skeletal growth through stimulation of protein synthesis. Development. 2014; 141:2848–2854. [PubMed: 24948603]
18. Chen J, Holguin N, Shi Y, Silva MJ, Long F. mTORC2 signaling promotes skeletal growth and bone formation in mice. J Bone Miner Res. 2015; 30:369–378. [PubMed: 25196701]
19. Kobayashi T, Minowa O, Kuno J, Mitani H, Hino O, Noda T. Renal carcinogenesis, hepatic hemangiomatosis, and embryonic lethality caused by a germ-line Tsc2 mutation in mice. Cancer Res. 1999; 59:1206–1211. [PubMed: 10096549]
20. Kobayashi T, Minowa O, Sugitani Y, Takai S, Mitani H, Kobayashi E, et al. A germ-line Tsc1 mutation causes tumor development and embryonic lethality that are similar, but not identical to, those caused by Tsc2 mutation in mice. Proc Natl Acad Sci U S A. 2001; 98:8762–8767. [PubMed: 11438694]
21. Fang F, Sun S, Wang L, Guan JL, Giovannini M, Zhu Y, et al. Neural Crest-Specific TSC1 Deletion in Mice Leads to Sclerotic Craniofacial Bone Lesion. J Bone Miner Res. 201510.1002/jbmr.2447
22. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, et al. Increased bone formation in osteocalcin-deficient mice. Nature. 1996; 382:448–452. [PubMed: 8684484]
23. Murshed M, Schinke T, McKee MD, Karsenty G. Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. J Cell Biol. 2004; 165:625–630. [PubMed: 15184399]