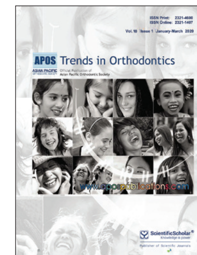
www.apospublications.com

ScientificScholar® Knowledge is power
Publisher of Scientific Journals

APOS Trends in Orthodontics



Original Article

Age-related osteogenesis on lateral force application to rat incisor – Part I: Premaxilla suture remodeling

Young-Yuhn Choi

Department of Dental Science and Oral Medicine, Yonsei University, Seoul, Republic of Korea.

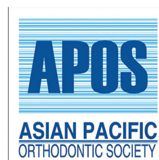
***Corresponding author:**
Young-Yuhn Choi,
Department of Dental Hygiene, Suwon Science College, 288 Seja-ro, Jeongnam-myun, Hwaseong-si, Geonggi-do, Korea.

yuhn17@naver.com

Received : 06 March 20
Accepted : 22 March 20
Published : 30 March 20

DOI
10.25259/APOS_27_2020

Quick Response Code:



ABSTRACT

Objectives: The suture is a fibrous tissue intervening two adjacent bone segments, existing only in the craniofacial region. In spite of wide use of palatal expansion in various ages, the age-dependent cellular mechanism for osteogenesis is largely unknown. The aim of this study was to examine the proliferation and differentiation pattern of the suture cells on lateral expansion in rats depending on the ages.

Materials and Methods: Calibrated lateral tensile stress of 50 g was given to the male Sprague-Dawley rat incisors using a double helix in 30 young (10 weeks) and another 30 aged (52 weeks) group, respectively. Each group was subdivided into control, 1, 3, 7, 14, and 21 days, with five animals in each group. Premaxilla area was retrieved from each animal for further histologic analyses including H and E, Masson's trichrome, and immunohistochemical staining using antibodies against phospho-extracellular signal-regulated kinase, proliferating cell nuclear antigen (PCNA), and fibroblast growth factor receptor-2 (FGFR2). Positive cell counts in the region of interest were conducted.

Results: Gross suture separation and subsequent bone formation on the sutural side bone surface were observed in both groups, characterized as active collagen turnover, remarkable woven bone projection toward the sutural mesenchyme and subsequent maturation in 3 weeks. Increase in PCNA- and FGFR2-postive cell proportions were comparable in both groups, indicating similar time- and area-specific proliferation and osteogenic differentiation patterns in the stretched suture regardless of the age groups.

Conclusion: According to the results, it can be implicated that the tensile stress applied to the suture in the adult group may induce active bone formation similar to that in young group, in associated with FGFR2 and Erk signaling cascade. Mesenchymal cells in the premaxillary suture appear to retain remarkable potential for further proliferation and differentiation even in aged subjects.

Keywords: Suture, Premaxilla, Osteogenesis, Phospho-extracellular signal-regulated kinase, Proliferating cell nuclear antigen, Fibroblast growth factor receptor-2

INTRODUCTION

Sutures are loose connective tissues that connect each bone, primarily functioning as shock absorber as a rigid type of articulation[1] and are characteristic structures found only in the skull. More importantly, undifferentiated mesenchymal cells are reserved in suture area and are readily recruited for further proliferation and differentiation, which is widely used in dentofacial orthopedics.[2,3] Due to the pathogenesis of the uncontrolled suture growth, such as craniosynostosis, ample molecular evidences have been found to be incorporated in the suture growth represented as bone growth by visualizing various osteoblast markers such as

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.
©2020 Published by Scientific Scholar on behalf of APOS Trends in Orthodontics

type I collagen, alkaline phosphatase, osteopontin, and osteocalcin.[4-8] Among them, the fibroblast growth factor/ receptor (FGF/FGFR) signaling molecules have been brought into attention since the finding of FGFR mutations causing various syndromic craniosynostoses. FGFRs are expressed in the osteoblasts and exerts biologic activities through various intracellular signaling molecules, such as extracellular signal-regulated kinases (Erks).[9-11] Erks are the members of the family of intracellular serine–threonine kinases (mitogen-activated protein kinases) and are known as essential mediators of the growth-factor-induced cell proliferation and differentiation in various cell types including osteoblasts.[12]

Unlike the calvarial sutures showing obliteration at various ages,[1,13] facial suture has been shown to maintain the patency even at late phase in the lifetime.[14] Contact with the dura mater is exclusive in the cranial sutures, implicating the regulation by dura mater.[3,15] Regardless of the core regulatory mechanism, these facts allow orthopedic correction for orthodontic purpose, including maxillary expansion and protraction. Unlike the regular fracture healing, osteogenesis in the sutures according to tensile stress has been demonstrated in the literature, leading to new suture formation tend to make bones according to tensile stress.[16-19]

Hence, it may be worthwhile to investigate the cellular behavior in the suture in response to a constant tensile stress. Underlying bone formation potential of the mesenchymal cells in the patent sutures in the relatively "mature" individuals may be interesting and possibly provide some information for possible orthopedic correction in adults.

This study was aimed to investigate the age-dependent osteogenic differentiation of the premaxillary suture cells in response to lateral tensile stress *in vivo* and to visualize the cellular markers to elucidate the related intracellular signaling.

MATERIALS AND METHODS

Animal

A total of 60 male Sprague-Dawley rats were used in this study. They were divided into two groups of 30 rats each according to age – young group (10 weeks) and aged group (52 weeks), respectively. Each group was divided into five subgroups according to experiment time, control, 3 days, 7 days, 14 days, and 21 days with five animals in each subgroup. All rats were acclimatized for 1 week before initiation of experiment. Body weight was measured every week throughout the experiment. The animals were kept in separate cages in 12-h light/dark cycles and at constant temperature of 23°C.

Appliance setup

A double helical spring was fabricated with 0.014" round stainless steel wire and was heat treated at 450°C for 30 s, to provide constant light expansion force [Figure 1]. General anesthesia was performed by intraperitoneal injection of Rumpun (0.1 ml, Bayer Korea) and Zoletil (0.4 ml, Zoletil 50, Virbac Lab, Carros, France). High-speed 1/4 round bur was used to place symmetric hole on upper central incisors. Double helical springs were inserted in the holes and precalibrated 50 g of force was given by the appliance. Maintenance of force level was confirmed before and after experiment period.

Tissue preparation

At the end of the experimental period, all rats were euthanized in the carbon dioxide chamber. Following the skin dissection in the head, the premaxilla area was carefully removed from the head using a high-speed handpiece. The specimens were fixed in 4% paraformaldehyde solution for 24 h, embedded in the paraffin block. Each specimen in the block was then sectioned at 5 μm thickness along the plane of sectioning [Figure 1], to examine the area of interest including the premaxillary suture and its vicinity. The prepared slides were then deparaffinized using xylene, rehydrated with descending series of alcohol and H-E staining was conducted in the conventional manner [Figure 2].

Masson's trichrome staining

The sectioned slides were deparaffinized using xylene and rehydrated with a descending series of alcohol. The slides were then stained with Weigert's iron hematoxylin and washed with tap water, followed by destaining with 1% HCl-alcohol and washing with water. Background staining was performed with Ponceau acid fuchsin for 10 min and washed with 0.2% acetic acid. Phosphomolybdic acid orange G staining for 5 min, detaining, and washing with 0.2% acetic acid were then followed. Collagen was stained light green and non-bound light green stain was then removed using

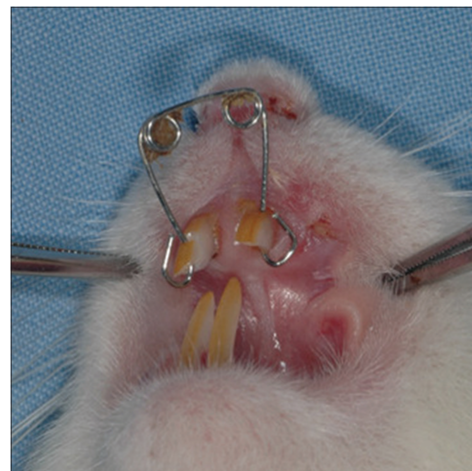


Figure 1: Double helical spring inserted in rat incisors.

0.2% acetic acid. Finally, slides were then examined using light microscope.

Immunohistochemical staining for phospho-extracellular signal-regulated kinase (p-Erk), proliferating cell nuclear antigen (PCNA), and fibroblast growth factor receptor-2 (FGFR-2).

The premaxillary coronal paraffin sections were examined with immunohistochemical staining for the expression pattern of p-Erk, PCNA, and FGFR-2, respectively. As reported previously, following deparaffinization and rehydration using descending series of alcohol to facilitate the immunological reaction of antibodies with antigens in fixed tissue, the slides were then treated with 0.5% Triton X-100 (for PCNA) or proteinase-K (for FGFR-2) for antigen retrieval. The slides were then incubated in 0.3% H_2O_2 for 5 min to deactivate the endogenous peroxidase. To block non-specific binding, 5% bovine serum albumin was applied to the specimens for 30 min. After several washing, the sections were then incubated with rabbit polyclonal primary antibodies against phospho-Erk (p-Erk, Erk1/2, Cell Signaling Tech Inc., USA), PCNA (FL-261, Santa Cruz Co., USA), and FGFR-2 (Bek sc-122, Santa Cruz, California, USA) at room temperature for 1 h and again at 4°C cold room for overnight, diluted at 1:100, respectively. After several washing in phosphate-buffered saline, the specimens were treated with a 2nd antibody-conjugated polymer (Envision kit, DAKO Co., Denmark). The specimens were subsequently treated with diaminobenzidine tetrahydrochloride (Zymed; San Francisco, USA) for 2–3 min to visualize immunoreaction. They were then counterstained with Gill's Hematoxylin (Lab Vision; California, USA), dehydrated with ascending series of alcohol and finally mounted with cover slide.

Immunoreactive cell counting

From the crestal bone, both coronal (crestal) and apical (nasal) 200 μm space were excluded due to the inherent anatomical variations. In the middle part of the premaxillary suture, positively stained cells for PCNA and p-Erk, respectively, were counted in each section. Percentage of the positive cells relative to total number of cells within the suture was collected to construct a histogram, for visualization of the time- and group-dependent change in the cell number. Means and standard errors were calculated at each time point.

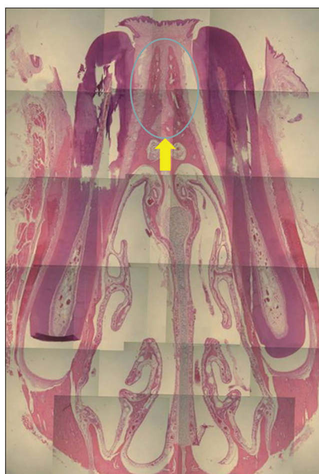


Figure 2: Cross section of premaxillary area and area of interest (yellow arrow).

RESULTS

Active bone formation on the osteogenic front in the suture

On force application, gross suture widening and subsequent bone formation on the sutural side bone surface were observed in both groups along the time course [Figure 3]. In detail, significant suture widening was found in 3 days in both groups, indicating effective transduction of expansion force to the suture area. Woven bone projection started to develop along the sutural mesenchyme at 1 week, which was more prominent in young group. Increase in number, as well as extension and enlargement of the bony projection, was remarkable at 2 weeks in both groups, and subsequent maturation of the bony projection was noticed in 3 weeks of time. The height of bone projection on the suture side was greatest at 3 weeks in young group, while the projection was blunting in adult group, displaying more bone maturation within the suture.

Collagen turnover in the suture according to the force application

Masson's trichrome staining indicates the region and degree of active collagen turnover. In the young group, active turnover shown as green was initiated in the crestal region on day 3, compared to the quiescent status before expansion. Green-colored region was then spread on the osteogenic front on day 7 and along the elevated bony projection on day 14 [Figure 4].

Spatiotemporal expression of p-Erk, PCNA, and FGFR2 in the sutural mesenchyme

In the young control group, basal level p-Erk-positive cells were found evenly in the suture and within the bone proper as well. On force application, a population of Erk-positive cells was intensively localized in the suture area at 3 days [Figure 5]. Before force application, PCNA-positive cell layer was not very evident around the suture area. However, remarkable increase in the PCNA-positive cells characterized as dense nuclear staining was found at a vicinity of the bone surface at 3 days. FGFR2, a cell membrane-bound tyrosine kinase receptor, can be recognized as strong cytosolic

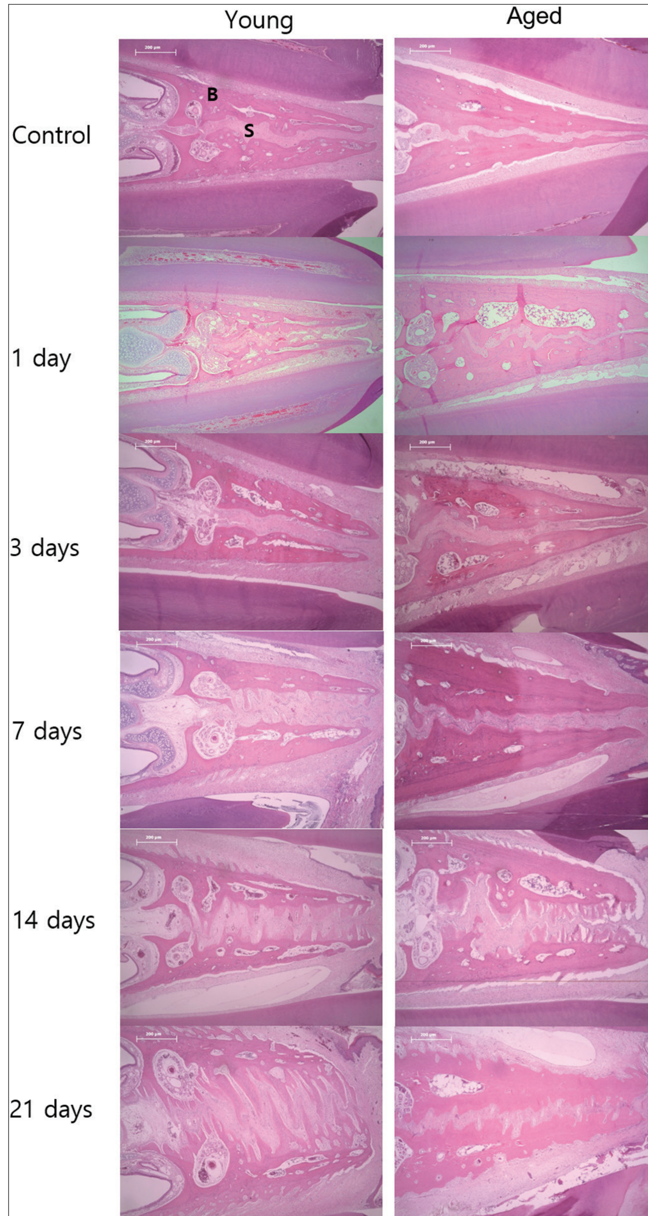


Figure 3: Hematoxylin and eosin staining of premaxillary suture on constant tensile stress (×40, scale bar = 200 μm).

staining. Cells expressing FGFR2 were intensely localized on the osteogenic front at day 3. The expression was sustained throughout the experiment period in both groups up to 2 weeks. At 3 weeks, the staining intensity was reduced but still high relative to the control group, indicating possible osteogenic activities [Figure 6]. The positive cell counts indicated similar expression intensity between both groups, implying remarkable osteogenic potential in aged groups [Figure 6]. Expression pattern of PCNA with time resembled that of the young group, with significantly greater expression in aged groups at 3 weeks. FGFR2 expression pattern did not show group difference at each time point.

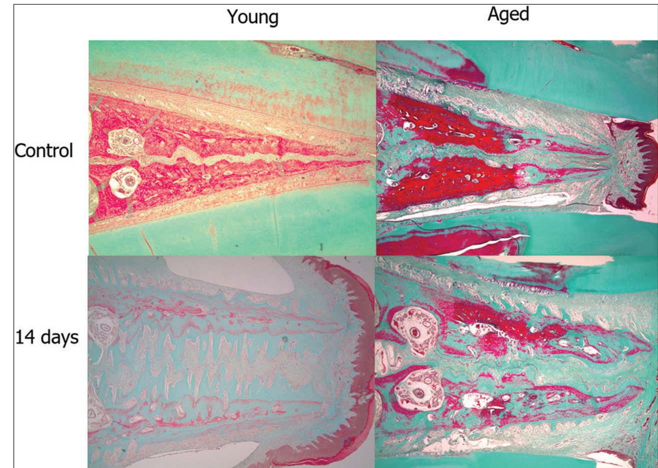


Figure 4: Masson's trichrome staining in young and aged groups according to the time points.

DISCUSSION

In the present study, an *in vivo* animal study was used to simulate the microenvironment of the suture. The murine models have been favored as the animals for the investigation of the craniofacial sutures due to the similarities and easy manipulation, such as the premaxillary,[20] maxillary,[21] and calvarial sutures.[22] Unlike the human, however, murine calvarial sutures remain patent for their lifetime.[23,24] Accordingly, it was considered reasonable to investigate one of the facial sutures as a homologous structure and the results may reflect the circumstance in human facial sutures, not necessarily the calvarial sutures.

The rats normally reach sexual maturity at 40–60 days of age.[25] Hence, a 10-week-old young group in this study may have reached sexual maturity, while a 52-week-old group is normally comparable to middle ages in human.[20] Nevertheless, the aging and related biological activity of rats differs from those in humans. For example, 3–4 rat days correspond to 1 human year during pubertal growth, while 10–17 rat days appear to be equal to 1 human year,[26] meaning that the human ages may not be simply extrapolated from the rat ages. These discrepancies must be taken into consideration in the interpretation.

In terms of force application, either fixed "quantity of expansion"[20,27,28] or fixed "magnitude of force"[21,22] has been recruited to deliver tensile force to the suture. For the fixed quantity of expansion, relatively heavy and interrupted force is used and the actual stress sensed by the cells in the suture may be relatively instant. Furthermore, the amount of force required for identical amount of expansion between two groups may be greater in adults due to the increased density of bone, which may adversely affect the consistency of the experiment. Since this study was aimed to examine the

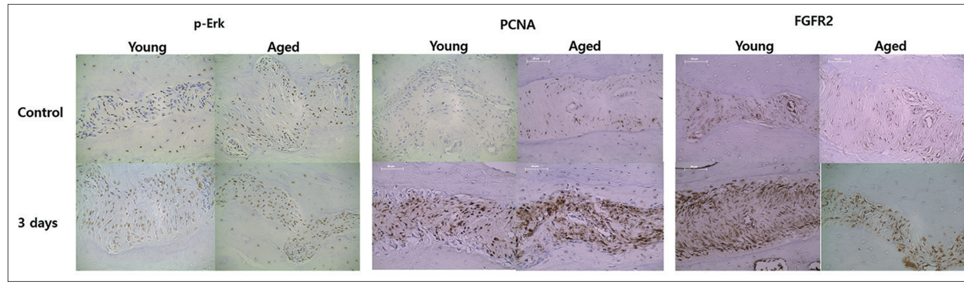


Figure 5: Immunohistochemical observations on p-Erk, PCNA, and FGFR2 (×400) in young and aged group at control and day 3 (×400, scale bar = 20 μm). p-Erk: Phospho-extracellular signal-regulated kinase, PCNA: Proliferating cell nuclear antigen, FGFR2: Fibroblast growth factor receptor-2.

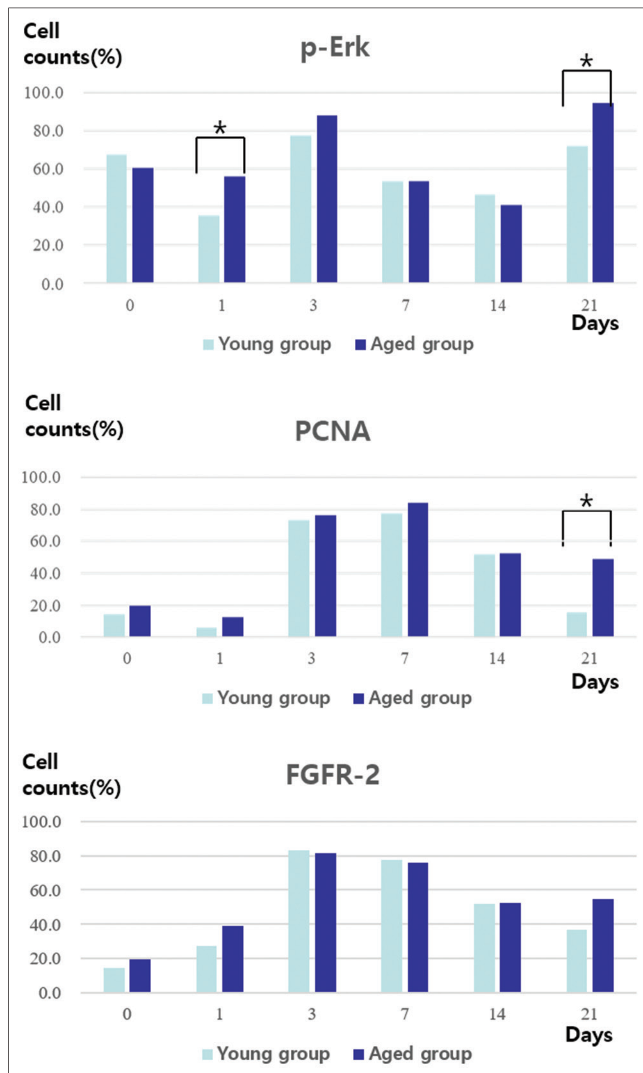


Figure 6: Positive cell counts for p-Erk, PCNA, and FGFR-2 and group comparison (*$P < 0.05$, *t*-test). p-Erk: Phospho-extracellular signal-regulated kinase, PCNA: Proliferating cell nuclear antigen, FGFR2: Fibroblast growth factor receptor-2.

cellular behavior according to a constant tensile stress, fixed magnitude of force was used in spite of the differences in the actual amount of expansion between two groups.

As for the force magnitude, it has been shown that the cellular response is not proportionate to the magnitude of force on beyond 100 g of force.[29] Accordingly, a 50 g of constant force was used. However, due to the indirect force delivery through incisors, tension from the extended periodontal ligament is secondarily transmitted to the suture area and the force deduction sensed by the suture cells also had to be considered. Admitting those weaknesses of the current approach, this indirect method was expected to reproduce a constant stress *in vivo*, similar to in other *in vitro* models.[28]

Gross bone responses in both groups were similar in both groups, displaying active matrix formation at around 1 week of time, regardless of the actual amount of suture separation. The rate of collagen turnover, however, was slower in aged group according to the time course, which is similar to the previous findings.[20,30] In contrast, Shimpo *et al*. claimed that the compensatory bone formation related to orthodontic tooth movement in old rats aged 60 weeks was almost comparable to that in young rats.[31] The reduced osteogenesis in adult group may be associated with either slow cellular response causing delayed matrix mineralization process or smaller cell population ready for maturation in response to signals delivered through the gap junction due to the increased stiffness of bone.[32]

Our immunohistochemical staining revealed some interesting aspects of the suture cells. First, a noticeable change was the immediate increase in the FGFR2 expression along the bony margins named as osteogenic front at day 1 on force application. FGFR2 has been shown to be expressed in the proliferating osteoprogenitor cells in the growing suture.[6,11,33] Yu et al. reported that the FGF2, a universal ligand for various FGFRs,[34] was immediately in response to extrinsic tension.[35] Considering the crucial roles of the FGF2 such as cell migration, proliferation, and differentiation,[36-40] it is likely that the FGF/FGFR2 may be one of the initial signs for the tension-related osteogenesis in the suture.

The redistribution and localization of the p-Erk-positive cells within the suture in 3 days were contrasted by the basal level expression pattern in suture and marrow space. Erk is

known to be essential for growth and differentiation of the osteoblastic cells by modulation of receptor tyrosine kinase activities, including those of FGFRs.[12,41,42] Moreover, it affects the integrin expression for cell attachment.[43] Unlike the *in vitro* environment, bone formation *in vivo* accompanies population of cells through chemotaxis and attachment on the osteogenic front, as well as their proliferation and differentiation. The capillaries found in the suture after force application reflects the recruitment of cells from vicinity of the suture, for which the chemotaxis may be important. The Erk has been shown to play an important role both in "outside-in" signal transduction for osteoblast growth and differentiation, and also the "inside-out" signal transduction for the integrin levels on cell surface for the cell adhesion, spreading, and migration. Hence, the redistribution and concentration of (phospho-) Erk-positive cells, a novel finding in this study, may be one of the initial important steps preceding actual bone formation. Interestingly, the p-Erk was declined below the baseline level after 1 week in both groups, implying the presence of possible feedback mechanism or refractory period.[44] Marshall has proposed a possible role of Erk as a regulator of cellular response depending on the duration of its activation.[45] Associated with that, Jackson *et al*. have demonstrated suppression of the phosphorylation of Erk1/2 on a long-term loading.[46] Further molecular investigations would be needed to reveal the underlying intracellular signaling. In terms of timing, there was no remarkable difference between the two groups, indicating the instantaneousness of the suture cell response *in vivo*, regardless of host's age.

The elevation of Erk is induced by many ways, including the growth factors, BMPs, and the mechanical stress.[12] In terms of the types of mechanical stress, neither repeated activation nor increased magnitude appears to be effective to induce "more" cellular response. Clinically, mechanical stress is easy to apply and less costly, than using any chemical agents and a single constant activation may be sufficient to activate the potential for growth and differentiation.

The PCNA expression was similar in the two groups, in terms of timing and location, with early elevation in 3 days, sustaining for around a week. In the suture, it has been shown that the osteogenic front is lined with rather early osteoblasts.[2,5,47] Above this layer, less differentiated osteoblastic cells are expected to proliferate before they actually produce extracellular matrices. The location of the PCNA-positive cells reflects this ontogeny of the osteoblasts in suture. Similar to the FGFR2 expression, PCNA expression was rather cyclic and reduced to baseline level in 3 weeks of time. The change in the proportions of PCNA-positive cells on force application was similar in the two groups, implicating that the differences in the mineralization might be attributed by the size of the initial cell population, not by the individual cell behavior [Figure 6]. Relative reduction of PCNA- and FGFR2- positive cells in 3 weeks may not confirm the termination of the osteogenesis but reflect the single cycle of bone formation according to a constant tensile stress. Other typical osteoblast markers, such as osteopontin, bone sialoprotein, or osteonectin, may be used to further confirm the viable osteoblasts and their activity.

The results of this study implicate that the cellular mechanism for osteogenesis in the suture on tensile force application may not be significantly different in aged individual. This also implies the possible orthopedic correction in adults for various skeletal discrepancies. Namely, the timely response of the suture cells and molecular reactivity of the suture cells in adult rats did not appear to be dissimilar to those in young rats. However, adequate manipulation of the mesenchymal cells, i.e., mechanical or chemical triggering is supposedly the prerequisite for the expression of the potential. Further research on the ontogeny of the aged suture cells needs to be followed.

CONCLUSION

According to the results, it can be implicated that the tensile stress applied to the suture mesenchyme in the aged group may induce active bone formation associated with a coordinated activity between FGFR2 and Erk signaling cascade. Subsequent proliferation and differentiation of the bone cells were comparable in both groups. Mesenchymal cells in the premaxillary suture appear to retain remarkable potential for further proliferation and differentiation, implying significant bone formation activity.

Declaration of patient consent

Patient's consent not required as patient's identity is not disclosed or compromised.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Cohen MM Jr. Sutural biology and the correlates of craniosynostosis. Am J Med Genet 1993;47:581-616.
2. Opperman LA. Cranial sutures as intramembranous bone growth sites. Dev Dyn 2000;219:472-85.
3. Opperman LA, Sweeney TM, Redmon J, Persing JA, Ogle RC. Tissue interactions with underlying dura mater inhibit osseous obliteration of developing cranial sutures. Dev Dyn 1993;198:312-22.
4. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K,

Deguchi K, *et al*. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 1997;89:755-64.
5. Kim HJ, Rice DP, Kettunen PJ, Thesleff I. FGF-, BMP- and Shh-mediated signalling pathways in the regulation of cranial suture morphogenesis and calvarial bone development. Development 1998;125:1241-51.
6. Rice DP, Rice R, Thesleff I. Molecular mechanisms in calvarial bone and suture development, and their relation to craniosynostosis. Eur J Orthod 2003;25:139-48.
7. Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. Nat Genet 1994;8:98-103.
8. Wilkie AO. Craniosynostosis: Genes and mechanisms. Hum Mol Genet 1997;6:1647-56.
9. Katz S, Boland R, Santillan G. Modulation of ERK 1/2 and p38 MAPK signaling pathways by ATP in osteoblasts: Involvement of mechanical stress-activated calcium influx, PKC and Src activation. Int J Biochem Cell Biol 2006;38:2082-91.
10. Hill PA, Tumber A, Meikle MC. Multiple extracellular signals promote osteoblast survival and apoptosis. Endocrinology 1997;138:3849-58.
11. Iseki S, Wilkie AO, Morriss-Kay GM. Fgfr1 and Fgfr2 have distinct differentiation and proliferation-related roles in the developing mouse skull vault. Development 1999;126:5611-20.
12. Kapur S, Chen ST, Baylink DJ, Lau KH. Extracellular signal-regulated kinase-1 and -2 are both essential for the shear stress-induced human osteoblast proliferation. Bone 2004;35:525-34.
13. Kokich VG. Age changes in the human frontozygomatic suture from 20 to 95 years. Am J Orthod 1976;69:411-30.
14. Pritchard JJ, Scott JH, Girgis FG. The structure and development of cranial and facial sutures. J Anat 1956;90:73-86.
15. Levine JP, Bradley JP, Roth DA, McCarthy JG, Longaker MT. Studies in cranial suture biology: Regional dura mater determines overlying suture biology. Plast Reconstr Surg 1998;101:1441-7.
16. Burger EH, Klein-Nulend J, Veldhuijzen JP. Modulation of osteogenesis in fetal bone rudiments by mechanical stress *in vitro*. J Biomech 1991;24 Suppl 1:101-9.
17. Burger EH, Klein-Nulend J, Veldhuijzen JP. Mechanical stress and osteogenesis *in vitro*. J Bone Miner Res 1992;7 Suppl 2:S397-401.
18. Sato M, Ochi T, Nakase T, Hirota S, Kitamura Y, Nomura S, *et al*. Mechanical tension-stress induces expression of bone morphogenetic protein (BMP)-2 and BMP-4, but not BMP-6, BMP-7, and GDF-5 mRNA, during distraction osteogenesis. J Bone Miner Res 1999;14:1084-95.
19. Heller JB, Gabbay JS, Wasson K, Mitchell S, Heller MM, Zuk P, *et al*. Cranial suture response to stress: Expression patterns of Noggin and Runx2. Plast Reconstr Surg 2007;119:2037-45.
20. Kanekawa M, Shimizu N. Age-related changes on bone regeneration in midpalatal suture during maxillary expansion in the rat. Am J Orthod Dentofacial Orthop 1998;114:646-53.
21. Kobayashi ET, Hashimoto F, Kobayashi Y, Sakai E, Miyazaki Y, Kamiya T, *et al*. Force-induced rapid changes in cell fate at midpalatal suture cartilage of growing rats. J Dent Res 1999;78:1495-504.
22. Lee K, Sugiyama H, Imoto S, Tanne K. Effects of bisphosphonate on the remodeling of rat sagittal suture after rapid expansion. Angle Orthod 2001;71:265-73.
23. Lee K, Ratisoontorn C, Baik HS, Park Y, Park K, Nah H. Generation of transgenic mouse model to study cranial suture developement: Apert syndrome. Kor J Orthod 2003;33:485-97.
24. Ignelzi MA Jr., Liu YH, Maxson RE Jr., Snead ML. Genetically engineered mice: Tools to understand craniofacial development. Crit Rev Oral Biol Med 1995;6:181-201.
25. Kohn DF, Clifford CB. Biology and diseases of rats. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory Animal Medicine. 2nd ed. New York: Academic Press; 2002. p. 121-65.
26. Quinn RD. Comparing rat's to human's age: How old is my rat in people years? Nutrition 2005;21:775-77.
27. Chang HN, Garetto LP, Potter RH, Katona TR, Lee CH, Roberts WE. Angiogenesis and osteogenesis in an orthopedically expanded suture. Am J Orthod Dentofacial Orthop 1997;111:382-90.
28. Ma J, Wu Y, Zhang W, Smales RJ, Huang Y, Pan Y, *et al*. Up-regulation of multiple proteins and biological processes during maxillary expansion in rats. BMC Musculoskelet Disord 2008;9:37.
29. Zahrowski JJ, Turley PK. Force magnitude effects upon osteoprogeniotr cells during premaxillary expansion in rats. Angle Orthod 1991;62:197-202.
30. Ren Y, Maltha JC, Van't Hof MA, Kuijpers-Jagtman AM. Age effect on orthodontic tooth movement in rats. J Dent Res 2003;82:38-42.
31. Shimpo S, Horiguchi Y, Nakamura Y, Lee M, Oikawa T, Noda K, *et al*. Compensatory bone formation in young and old rats during tooth movement. Eur J Orthod 2003;25:1-7.
32. Cowin SC, Moss-Salentijn L, Moss ML. Candidates for the mechanosensory system in bone. J Biomech Eng 1991;113:191-7.
33. Rice DP, Rice R, Thesleff I. Fgfr mRNA isoforms in craniofacial bone development. Bone 2003;33:14-27.
34. Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, *et al*. Receptor specificity of the fibroblast growth factor family. J Biol Chem 1996;271:15292-7.
35. Yu JC, Lucas JH, Fryberg K, Borke JL. Extrinsic tension results in FGF-2 release, membrane permeability change, and intracellular Ca++ increase in immature cranial sutures. J Craniofac Surg 2001;12:391-8.
36. Naski MC, Ornitz DM. FGF signaling in skeletal development. Front Biosci 1998;3:d781-94.
37. Nagai H, Tsukuda R, Mayahara H. Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats. Bone 1995;16:367-73.
38. Moore R, Ferretti P, Copp A, Thorogood P. Blocking endogenous FGF-2 activity prevents cranial osteogenesis. Dev Biol 2002;243:99-114.
39. Mancilla EE, De Luca F, Uyeda JA, Czerwiec FS, Baron J. Effects of fibroblast growth factor-2 on longitudinal bone growth. Endocrinology 1998;139:2900-4.
40. Li CF, Hughes-Fulford M. Fibroblast growth factor-2 is an immediate-early gene induced by mechanical stress in osteogenic cells. J Bone Miner Res 2006;21:946-55.
41. Matsushita T, Chan YY, Kawanami A, Balmes G, Landreth GE,

Murakami S. Extracellular signal-regulated kinase 1 (ERK1) and ERK2 play essential roles in osteoblast differentiation and in supporting osteoclastogenesis. Mol Cell Biol 2009;29:5843-57.
42. Chaudhary LR, Avioli LV. Extracellular-signal regulated kinase signaling pathway mediates downregulation of Type I procollagen gene expression by FGF-2, PDGF-BB, and okadaic acid in osteoblastic cells. J Cell Biochem 2000;76:354-9.
43. Lai CF, Chaudhary L, Fausto A, Halstead LR, Ory DS, Avioli LV, *et al*. Erk is essential for growth, differentiation, integrin expression, and cell function in human osteoblastic cells. J Biol Chem 2001;276:14443-50.
44. Lee KJ, Park YC, Yu HS, Choi SH, Yoo YJ. Effects of continuous and interrupted orthodontic force on interleukin-1beta and prostaglandin E2 production in gingival crevicular fluid. Am J Orthod Dentofacial Orthop 2004;125:168-77.
45. Marshall CJ. Specificity of receptor tyrosine kinase signaling: Transient versus sustained extracellular signal-regulated kinase activation. Cell 1995;80:179-85.
46. Jackson RA, Kumarasuriyar A, Nurcombe V, Cool SM. Long-term loading inhibits ERK1/2 phosphorylation and increases FGFR3 expression in MC3T3-E1 osteoblast cells. J Cell Physiol 2006;209:894-904.
47. Rice DP, Aberg T, Chan Y, Tang Z, Kettunen PJ, Pakarinen L, *et al*. Integration of FGF and TWIST in calvarial bone and suture development. Development 2000;127:1845-55.

How to cite this article: Choi Y. Age-related osteogenesis on lateral force application to rat incisor – Part I: Premaxilla suture remodeling. APOS Trends Orthod 2020;10(1):38-45.