AAO Foundation Award Final Report
(a/o 5/31/2012)

Please prepare a report that addresses the following:

Type of Award

Orthodontic Faculty Development Fellowship Award

Name(s) of Principal Investigator(s)

Jianjun Hao

Title of Project

Initial project at University of Illinois at Chicago: Mutant DSPP protein accumulation in endoplasmic reticulum induces dentinogenesis imperfect II reversed by proteostasis regulators

Current project at University of Connecticut Health Center: Generation of Fam20C-GFP (topaz) transgenic mice

Period of AAOF Support

07-01-11 to 12-31-2012

Amount of Funding

$15,000

Summary/Abstract (250 word maximum)

Fam20C is critical and essential for murine bone and tooth development. Fam20C enriched in Golgi fraction possesses casein kinase (GEF-CK) activities and phosphorylates secretory pathway proteins of bone and teeth. Mutation of Fam20C causes lethal osteosclerotic bone dysplasia (Raine syndrome). Thus, it is imperative to characterize regulatory elements and promoter of Fam20C that regulates its specific expression in mineralized tissues.
Objectives: Generate a reporter transgenic mouse to express green fluorescence protein (GFP) driven by Fam20C promoter in mineralized tissues.
Methods: Recombineering was used to insert a 15 kb of mouse Fam20C genomic fragment containing the 5'UTR, promoter and upstream sequence directly into a vector with the topaz variant of green fluorescent protein (GFP-tpz) and bovine growth hormone polyadenylation sequence (bGHpolyA) by gap repair.

Transgene fragment was microinjected into C57BL6j one-cell embryos for the generation of transgenic animals. Potential founders and F1 progeny were identified by PCR using primer pair specific to the Fam20C 5'UTR and GFP. GFP expression was evaluated by histomorphmetry in cryosections.
Results: Fluorescence was evident in the odontoblasts and dental pulp of incisor and molars. The intensity levels were variable, showing different levels of expression among odontoblasts and dental pulp cells. We also detected GFP in the osteoblasts in alveolar bone.
Conclusions: We generated a transgenic mouse model that expresses a GFP marker under the control of a 15 kb promoter of Fam20C. As expected, the transgene was active in the mineralized tissues of odontoblasts and alveolar bones.

Response to the following questions

1. Were the original, specific aims of the proposal realized?

The original, specific aims were not realized because the proposal was changed. The current proposal has been completed after extension.

2. Were the results published? If not, are there plans to publish? If not, why not?

The results are published as abstracts in 2012 ASMBR (American Society of Bone and Mineral Research) meeting and 2013 IADR/AADR (International Association of Dental Research/American Association of Dental Research) meeting (see attachment).
The manuscript is under preparation. It will be submitted to "BONE" in the middle of this year (see attachment).

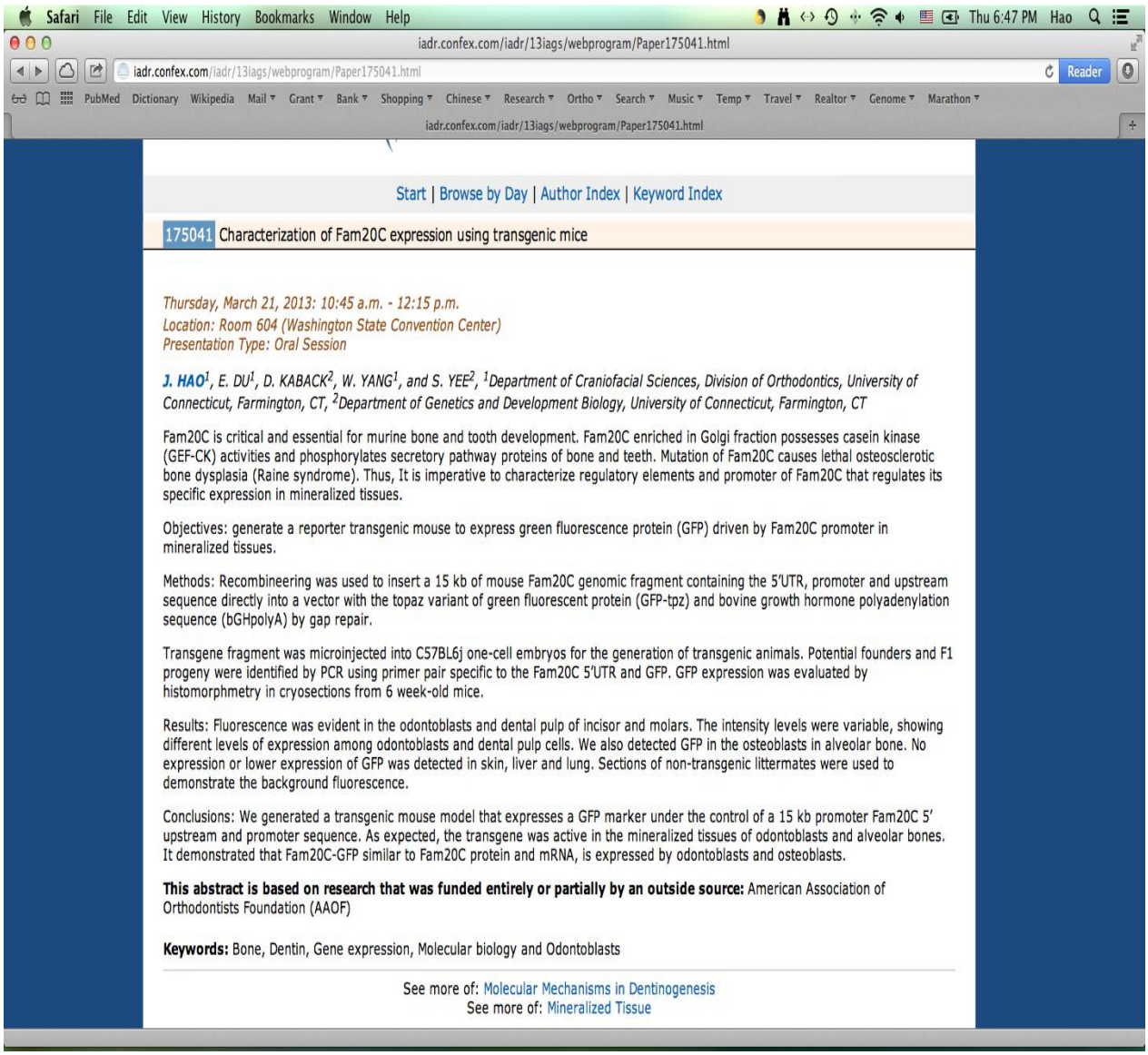
3. Have the results of this proposal been presented? If so, when and where? If not, are there plans to do so? If not, why not?

The results have been presented as a poster in 2012 ASBMR meeting in Minneapolis, MN on October 12, 2012.
The results will be presented as an oral presentation in Seattle IADR meeting on March 21, 2013.

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

I applied for AAOF Biomedical Research Award in Dec. 2012.
I will apply for an NIDCR R03 grant in Feb. 2013 with the preliminary data accumulated through the support of AAOF. It will help me to become an independent researcher in the future.

Please mail hard copy to AAOF and also send electronically
(as a Word document and e-mail attachment) to
aaofevp@aaortho.org



175041 Characterization of Fam20C expression using transgenic mice

Thursday, March 21, 2013: 10:45 a.m. - 12:15 p.m.
Location: Room 604 (Washington State Convention Center)
Presentation Type: Oral Session

J. HAO[1], *E. DU*[1], *D. KABACK*[2], *W. YANG*[1], *and S. YEE*[2], *[1]Department of Craniofacial Sciences, Division of Orthodontics, University of Connecticut, Farmington, CT, [2]Department of Genetics and Development Biology, University of Connecticut, Farmington, CT*

Fam20C is critical and essential for murine bone and tooth development. Fam20C enriched in Golgi fraction possesses casein kinase (GEF-CK) activities and phosphorylates secretory pathway proteins of bone and teeth. Mutation of Fam20C causes lethal osteosclerotic bone dysplasia (Raine syndrome). Thus, It is imperative to characterize regulatory elements and promoter of Fam20C that regulates its specific expression in mineralized tissues.

Objectives: generate a reporter transgenic mouse to express green fluorescence protein (GFP) driven by Fam20C promoter in mineralized tissues.

Methods: Recombineering was used to insert a 15 kb of mouse Fam20C genomic fragment containing the 5'UTR, promoter and upstream sequence directly into a vector with the topaz variant of green fluorescent protein (GFP-tpz) and bovine growth hormone polyadenylation sequence (bGHpolyA) by gap repair.

Transgene fragment was microinjected into C57BL6j one-cell embryos for the generation of transgenic animals. Potential founders and F1 progeny were identified by PCR using primer pair specific to the Fam20C 5'UTR and GFP. GFP expression was evaluated by histomorphmetry in cryosections from 6 week-old mice.

Results: Fluorescence was evident in the odontoblasts and dental pulp of incisor and molars. The intensity levels were variable, showing different levels of expression among odontoblasts and dental pulp cells. We also detected GFP in the osteoblasts in alveolar bone. No expression or lower expression of GFP was detected in skin, liver and lung. Sections of non-transgenic littermates were used to demonstrate the background fluorescence.

Conclusions: We generated a transgenic mouse model that expresses a GFP marker under the control of a 15 kb promoter Fam20C 5' upstream and promoter sequence. As expected, the transgene was active in the mineralized tissues of odontoblasts and alveolar bones. It demonstrated that Fam20C-GFP similar to Fam20C protein and mRNA, is expressed by odontoblasts and osteoblasts.

This abstract is based on research that was funded entirely or partially by an outside source: American Association of Orthodontists Foundation (AAOF)

Keywords: Bone, Dentin, Gene expression, Molecular biology and Odontoblasts

See more of: Molecular Mechanisms in Dentinogenesis
See more of: Mineralized Tissue

ASBMR 2012

[SU0094] Generation of FAM20C-GFP Transgenic Mice

Jianjun Hao, University of Connecticut Health Center, USA

October 14, 11:30 AM - 01:30 PM
Discovery Hall-Hall B/Minneapolis Convention Center

Session: Poster Session II and Poster Tours

Abstract

Generation of FAM20C-GFP Transgenic Mice

Author(s)
Jianjun Hao, Erxia Du, Deborah Kaback, Wuchen Yang, Siu-Pok Yee

Formation of calcified tissues is a well-regulated process balanced by promoters and inhibitors of biomineralization. Interruption of the balance in bone and other mineralized tissues could generate a wide range of pathologic conditions. Lethal osteosclerotic bone dysplasia (Raine syndrome), one of the neonatal osteosclerotic dysplasias, is an inherited disease clinically manifested by increased calcification and distinctive craniofacial abnormities. Our previous study has clearly demonstrated that mutations of FAM20C gene are associated with Raine syndrome. As a first step to investigate the functional role of FAM20C, we have generated FAM20C transgenic mice to determine its spatiotemporal expression during development. To this end, we have prepared a transgene with 15 kb of FAM20C upstream promoter sequence to direct expression of the GFPtpz reporter. We anticipate that FAM20C-GFP similar to the FAM20C mRNA and protein is highly expressed in odontoblasts, ameloblasts, cementoblasts, osteoblasts and osteocytes during tooth and bone development. The FAM20C fluorescence labeled transgenic mice will provide powerful experimental models for identification and isolation of tooth and bone forming cells in mineralized tissues.

Supported by the American Association of Orthodontists Foundation

Disclosures:

Jianjun Hao has nothing to disclose.