AAO Foundation Awards
Final Report
6/28/2014

1. Type of Award (Check One):

___ Orthodontic Faculty Development Fellowship Award
___ Postdoctoral Fellowship Award
_√_ Biomedical Research Award
___ Center Award
___ Educational Innovation Award
___ Program Award
___ Research Aid Award

2. Name(s) of Principal Investigator(s): Guoqiang Guan

3. Institution: SUNY Buffalo

4. Period of AAOF support: 07-01-13 to 06-30-14

5. Amount of AAOF Funding: $25,000

Summary/Abstract (250 word maximum)

Bmp4 is a key signaling molecule that regulates multiple developmental processes, including tooth formation. It is expressed in a specific spatiotemporal pattern throughout tooth development. The dental specific transcriptional regulation of the *Bmp4* gene is difficult to study because of the vast regulatory region, which spans more than 400 kb in human and mouse genomes. *Takifugu* (fugu, pufferfish) has a highly compact genome approximately one-tenth the size of the human genome, yet it contains a similar repertoire of genes. Fugu pharyngeal teeth are histologically and developmentally comparable to oral teeth in other vertebrates. Bmp4 has been shown to be expressed during fish pharyngeal tooth development in a tissue-specific manner. In this project, we used the Fugu genome to evaluate the 15kb promoter region upstream of the Fugu *bmp4* gene. By DNA segmental cloning and luciferase assay with two dental odontoblast-like cell lines, a dental ameloblast-like cell line, and a kidney fibroblast cell line, we identified a 485 bp *cis*-regulatory enhancer between -4,213 to -3,728 bp of the Fugu *bmp4* gene. This enhancer showed strong transcriptional activity in all three dental cell lines and, to a lesser extent, also in kidney fibroblast cells. Though not located in an evolutionary conserved region, the enhancer activity for the DNA segment is intense. This is the first time a *bmp4* enhancer sequence with activity in mesenchymal cells has been identified, which will help to decode the mechanism of tooth development in vertebrates. This information may eventually be used to

develop strategies to rescue tooth agenesis, perhaps by replacing faulty embryonic odontogenic cells with stem cells engineered to express essential genes for tooth formation.

Response to the following questions:

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

We evaluated the 15kb promoter region upstream of the Fugu *bmp4* gene with a luciferase reporter system. By analyzing three dental cell lines (two dental odontoblast-like cell lines, a dental ameloblast-like cell line) and a kidney fibroblast cell line, we identified *cis*-regulatory elements in the Fugu *bmp4* promoter, which may involve in tooth development. These experiments demonstrated that parts of the -15kb promoter are specific for tooth development. After tested the promoter/enhancer activity of this 15 kb area in cell culture systems we completed part of the *in vivo* experiments using transgenic zebrafish as we proposed in our proposal and we are actively working on the potential future projects open these results. Most importantly, to our knowledge, this is the first time a *bmp4* enhancer sequence with activity in mesenchymal cells has been identified, which will help to decode the mechanism of tooth development in vertebrates.

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

A manuscript has been submitted to *Archives of Oral Biology* in the last September.
"**Identification and analysis of a novel *bmp4* enhancer in Fugu genome"**
Y. Wang[1], L. Hutson[2], Y. Chen[3], G. Guan[1*]
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After a minor revision, the manuscript was sent to the Editor's office in the last April for final decision.

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?

Part of the results was presented to the journal of Archives of Oral Biology for publication. We are planning to present the other data in the future.

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

The funding from AAOF has been encouraging me to keep the research projects on going, especially in the current decrement of NIH budget. The continued AAOF support will help me and my colleagues prepared to compete for future NIH grants; eventually, it will benefit our orthodontic profession to provide better oral health care to our patients.

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