BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

Name of Journal: ***World Journal of Biological Chemistry***

ESPS Manuscript NO: 20116

Manuscript Type: Minireviews

Answering reviewers

The manuscript has been prepared in accordance with the suggestions of the Editor:

1. The format has been updated.
2. New Table 1 and Figures (Figures 1 and 2) have been prepared and Figure 3 (previously Figure 1) has been prepared as an editable PowerPoint file (20116-revised Figures.ppt).

Revisions have also been made to address the Reviewers' suggestions and all of the added and modified parts of the manuscript are indicated in yellow.

Reviewed by 00504183

This is a well written review on the role of caldecrin in regulation of osteoclast formation and function. It would be interesting to give some perspectives of the potential use of caldecrin in cancer diagnosis/treatment/prognosis/prediction of response to cancer treatments, with particular focus on the RANKL-dependent regulation of ostelytic metastases of tumors.

ANSWER

We appreciate the Reviewer's comment. We have described the role of caldecrin in cancer and cited relevant literature in the '**OTHER BIOLOGICAL ASPECTS**' paragraph of the revised manuscript.

Reviewed by 01404215

The authors isolated caldecrin for the first time in 1995. Therefore, they know very much about the structure and function of this new molecule and it seems appropriate to publish present mini-review. But before publication of the manuscript, authors should modify it, attending to the present suggestions.

ANSWER

We appreciate the Reviewer's comments, and have addressed these in the revised manuscript.

Major Points:

1) The paragraphs comparing the structure of caldecrin with that of the elastases are confusing. Sometimes, they propose caldecrin is identical to elastase IV, and at other times, they do not. The same occurs when they compare it with elastase III A and B or elastase II. Authors should clarify what is sequence identity and what is homology.

ANSWER

This issue is addressed more clearly in the revised manuscript, where we indicate that the sequence of a newly identified PCR clone referred to as elastase IV was reported by Kang et al. (1992). The new Figure 1 compares the nucleotide and amino acid sequences of caldecrin and elastase IV. One nucleotide deletion and one nucleotide insertion were observed in rat elastase IV, as compared to rat caldecrin, resulting in a 23-amino acid change caused by a frameshift. Partial amino acid sequencing showed that purified caldecrin protein was identical to the amino acid sequence deduced for rat caldecrin, but not for elastase IV. Recombinant elastase IV showed low protein stability and rat genomic sequencing did not identify the elastase IV sequence. We therefore consider caldecrin and elastase IV to be the same gene product and the PCR clone referred to as elastase IV might be a cloning artifact or mutant caldecrin.

We have added a new Table 1 comparing the amino acid sequences of caldecrin and other members of the chymotrypsin and elastase family, showing sequence identity and similarity. Sequence identity is percent of same amino residues in a sequence alignment between 2 sequences. Sequence similarity is percent amino acid sequence identity and percent positive substitutions between 2 sequences. We have changed 'homology' to 'similarity' in the revised manuscript.

2) To facilitate comprehension about identities, a Scheme comparing data of sequences of cDNAs would be very useful. Readers could then conclude for themselves whether

caldecrin is more or less identical with such or such elastases. The same applies to the comparison of the genes

ANSWER

To address this, we have added Table 1 showing the similarity between the amino acid sequences of caldecrin and other elastase family enzymes. As this manuscript is a minireview, we chose to focus on the mechanism underlying caldecrin's suppression of osteoclast differentiation and function. Inclusion of more detailed comparisons of individual enzymes would therefore be disproportionate, although we hope to address this topic in a future review article.

3) Now that it is known that caldecrin is identical in primary structure to chymotrypsin C, the name caldecrin could be omitted from the article, maintaining the other.

ANSWER

The official name for chymotrypsin C, caldecrin, and elastase IV is chymotrypsin C (caldecrin), according to the HUGO Gene Nomenclature Committee. Therefore, we have used 'CTRC (caldecrin), CTRC, or caldecrin' according to the title of relevant reference in this minireview.

4) It is said throughout the manuscript that caldecrin is a pancreatic peptide. However, its expression in other tissues has not been shown. A new Figure showing the expression in several tissues (albeit minimal) would help to better define the origin of the peptide

ANSWER

To address this, we have added a northern blot analysis of caldecrin expression (Figure 2A).

5) The manuscript lacks a figure showing the double-barrel structure deduced from its sequence, and the how interaction of cysteine produces the final architecture in the space.

ANSWER

We have added the three-dimensional structure of human chymotrypsin C (4H4F), which was reported by Batra J et al, in Figure 2C. This shows the double-barrel structure and disulfide bonds.

*6) Figure 1 does not show that caldecrin might be an inductor of the small pathway of NFκB, Cfos and MAPK. It only shows that caldecrin down regulates the Ca++ pathway. Figure 1 should be greatly impro*ved.

ANSWER

In order to clarify this point, we have added information indicating that caldecrin suppresses NFATc1 activity, but not NFκB, c-Fos, and MAPK signal pathways, in the paragraph entitled '**CALDECRIN AND BONE METABOLISM**'. Figure 3 (previously Figure 1) has also been improved by the addition of arrows indicating activation or inhibition of these signaling pathways.

Minor points 1) the manuscript uses several abbreviations, which are not commonly used in articles of biochemistry. RANKL, NFATc1, ELA4, CTRC, DFP, M-CSF, Syk, PLC????PX and others, are for most readers difficult to interpret. An Abbreviation List could facilitate the reading of the article.

ANSWER

We have added a list of abbreviations at the beginning of the article.

Thank you again for considering our manuscript for publication in the *World Journal of Biological Chemistry*.

Sincerely yours,

Mineko Tomomura, PhD, Associate Professor

Meikai Pharmaco-Medical Laboratory, MPL, and Division of Biochemistry, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry, 1-1 Keyakidai, Sakado, Saitama 350-0283 Japan

E-mail: mineko-t@dent.meikai.ac.jp

Telephone: +81-49-279-2766

Fax: +81-49-271-2503