Reply to the E-mail of Prof. K. L. Chopra, President, SSV

1. Does the Director, NCCS, and the two enquiry committees that examined the case agree that there exists a clear basis for a prima-facie case, even if they do not agree on the evidence for it?

(a) No, as far as I am aware.

(b) The allegation was made by a pseudonymous e-mail dated 16th May, 2006. The Director constituted an internal committee to enquire into the allegation. The said committee did not provide any finding to my knowledge. As I mentioned to you earlier, no opportunity or time was given to me to properly respond to the allegations. I requested for more time to contact my student, Ms. Hema Rangaswami who was pursuing her post-doctoral work in UCSD, USA and to collect all the original, supportive and computer-analyzed data. I was pressurized and under duress, I was forced to make some submission to the committee. Soon there after, I promptly represented to the Director bring out the fact that my submission was made under duress and the same be held in abeyance. In the mean time, a member of the committee (Dr. Sanjeev Galande) also came under the allegation of the scientific misconduct.

(c) The Director, NCCS, thereafter decided to refer the entire issue to an external National level Fact Finding Committee of the eminent Scientists in the country headed by Prof. G. Padmanaban, Former Director, Indian Institute of Science, Bangalore and currently Distinguished Scientist, Department of Biotechnology, Government of India. The committee examined the data, documentary evidence and interrogated the person against whom the allegations were made including Ms. Hema Rangaswami who came all the way from USA to depose before the committee. The committee came to unanimous conclusion that there was no basis for the allegations against the concerned persons that is myself, Ms. Hema Rangaswami and Dr. Sanjeev Galande. A copy of office memorandum issued by the Controller of Administration, NCCS dated 17th August, 2006 conveying the findings of the committee is enclosed for your reference.

2. Whether one committee recommended retraction of the papers from the Journal of Biological Chemistry.

No recommendation was made to retract the papers by the first or second committee, but a forced retraction was imposed on 23th May, 2006 without going through all the original and supportive data and conducting a thorough investigation. This retraction was withdrawn.

3. What necessitated the second enquiry committee, on what terms of reference, and what were their findings?

The second committee became necessary because: (i) As I understand the first committee did not reach any conclusion; (ii) I have represented to the Director that my submission to the first committee was secured under duress, which may not be taken into consideration and be held in abeyance; and that the basis of the pseudonymous complaints and data were not verified by this committee. (iii) That a member of the committee itself came under the suspicion of scientific misconduct.

From the O.M. of NCCS dated 17th August, 2006, it is seen that the terms of reference of the second Fact Finding Committee were " to investigate the matter mentioned in the e-mail dated 16th May, 2006 from Shivaji Bode1 and e-mail dated 22nd June, 2006 received from Ganapati Mahabeleshwar".

As regards to the finding of the committee, kindly refer to the O.M. of NCCS dated 17th August, 2006 and a copy of which is enclosed.

4. What more did the second committee do, that was not done by the first committee, for eg., did it conduct forensic examination of the record books, published pictures etc?

The first committee did not examine any reports and made any recommendation. The second committee had verbal examinations of all the authors of the papers and the detailed examination of the raw and original data used in the papers. Hema Rangaswami who was the first author of both these papers came all the way from USA to appear before the committee and presented all the raw, original, and supportive and computer analyzed data that was not seen by the first committee.

5. Did the second committee acknowledge that there were some strikingly similar (if not identical) photographs in both the papers? On what basis did the Director, NCCS (or any other authority concerned) find the findings of the second enquiry more acceptable than the first.

The second committee looking at the original data and figures used in both these papers came to the conclusions that although some of the blots with different treatment appear superficially similar, they are indeed different. The recommendations of the second committee were based on proper investigation and therefore were accepted.

6. Whether the editor(s) of the Journal of Biological Chemistry was contacted for comments regarding the papers in question and what was the response.

Somebody sent the same email to JBC and they have contacted to me for response. I have submitted all the raw, original, and supportive and computer analyzed data to JBC. The office of JBC did not retract the papers till date.

7. Whether these and any other such publications submitted were considered for the Bhatnagar award conferred upon Dr. Kundu.

Both these papers were published after the submission of the Bhatnagar nomination.

Case Summary:

1. The strips used in Fig. 1B of paper I and Fig. 2B in paper II are the same, but denote different proteins, NIK and MEKK1.
2. The strips used in Fig. 2A of paper I and Fig. 1A in paper II are the same, but denote different proteins, actin and MEKK1.
3. The same strip from Fig. 3 B (top panel from paper I) was also used for Fig. 1c in paper I, again denoting different proteins.
4. The strip in Fig. 3 B (top) are the same in both paper I and paper II.
5. The strips used in the lower panels of Fig 3 A&B are flip-flops of each other, though they represent the same protein.
6. Both the strips in the figure 7C of Paper I have been reused in fig. 6A of paper II. While actin is common to the bottom panel in both above cases, in the top panel, the same strip was used to denote ERK in one case and MEKK in another case, with different values.
7. The top panels of Fig. 6A and 8A of paper II use the same strip to denote upa and jun.

Response:

The two JBC papers represent two very similar pathways regulated by osteopontin upon binding to integrin receptor. The experimental design and the methodology used to examine the two pathways are very similar; both sets of experiments were performed in B16F10 cells. This explains why some figures in the two JBC papers show similar patterns.

The first paper has total of **11** figures, which contain **66** individual illustrations containing *in vitro* and *in vivo* blots, bar diagrams, table, nude mice photographs and schematic model. There is clear experimental evidences in the first paper that describe how nuclear factor inducing kinase (NIK) plays crucial role in osteopontin-induced MAPK/IκBα kinase dependent NFκB-mediated pro MMP-9 activation in B16F10 cells. Similarly, the second paper has total of **9** figures, which contain **53** individual illustrations containing *in vitro* and *in vivo* blots, bar diagrams, table and schematic model. In this paper, we have demonstrated how JNK1 differentially regulates osteopontin-induced NIK/MEKK1-dependent AP-1-mediated pro MMP-9 activation in same B16F10 cells. **Both these papers are highly cited and other groups that support our findings publish similar papers.**

Ques. 1.: The strips used in Fig. 1B of paper I and Fig. 2B in paper II are the same, but denote different proteins, NIK and MEKK1.

Point 1. The effects of αvβ3 integrin blocking antibody and the two peptides (GRGDSP and GRGESP) on OPN-induced NIK phosphorylation **(Paper I, Figure 1B)** were analyzed in B16F10 cells. The lower panel in the figure represents the NIK, which is used as the loading control.

The **Figure 2B in Paper II** represents a JNK kinase assay using c-Jun as the substrate. We have demonstrated that OPN-induced JNK activity is NIK independent by transfecting the cells with wild type and kinase negative NIK followed by treatment with OPN. The middle and

the lower panels in the figure showed the levels of JNK1 and NIK expressions as controls. **The level of MEKK1 has not been studied in this experiment (Paper II, Figure 2 B).**

There are absolutely no similarities between Fig. 1B of paper I and Fig. 2B in paper II.

Ques. 2: The strips used in Fig. 2A of paper I and Fig. 1A in paper II are the same, but denote different proteins, actin and MEKK1.

Point 2. In **Figure. 2A of Paper I**, we have performed a NIK kinase assay using IKK α/β as the substrate. The middle and the lower panels indicate the levels of NIK and IKK α/β expression as controls. **The level of actin has not been represented in this experiment (Paper I, Figure 2 A).**

The **Figure 1A in Paper II** shows the effect of OPN in regulating MEKK1 phosphorylation. The cells were treated with 5 μM OPN for various time points. The cell lysates were immunoprecipitated with anti-MEKK 1 antibody and analyzed by western blot using anti-pSerine antibody. The same blot was reprobed with anti-MEKK1 as loading control.

There are absolutely no similarities between Fig. 2A of paper I and Fig. 1A in paper II.

Ques. 3: The same strip from Fig. 3 B (top panel from paper I) was also used for Fig. 1c in paper I, again denoting different proteins.

Point 3. In this experiment, we have studied the effect of αvβ3 integrin antibody, GRGDSP and GRGESP on OPN-induced MEK 1 phosphorylation (**Figure 3B, top panel in Paper I**). The level of MEK1 was analyzed by western blot as loading control in the lower panel.

The **Figure 1 C in paper I** show the effect of TNFα on NIK phosphorylation (upper panel). The level of NIK (middle panel) and actin (lower panel) were also detected by western blot as loading controls.

There are absolutely no possible similarities between these two figures.

Ques. 4: The strip in Fig. 3 B (top) are the same in both paper I and paper II.

Point 4. The effect of αvβ3 integrin antibody, GRGDSP and GRGESP on OPN-induced MEK1 phosphorylation (**Figure 3B in Paper I**) and c-Jun expression (**Figure 3B in Paper II**) were analyzed by western blot in B16F10 cells. **The original blots and blots of two independent experiments along with the loading controls showed that αvβ3 and GRGDSP but not GRGESP inhibit OPN-induced MEK1 phosphorylation and c-Jun expression and all these original and supportive data are available.** Our computer analyses data have clearly shown the distinct differences between both these two blots.

Ques. 5: The strips used in the lower panels of Fig 3 A&B are flip-flops of each other, though they represent the same protein.

Point 5. The accusation that the loading control blots in Figure 3 A and B in Paper I have been "flipped" is very strange. **We have original blots and supportive experimental data.** It has been proven that this is not so using the computer analysis. The enlarged versions of the blots

clearly showed that the supposedly related lanes have totally different signal intensities. The artifact dot below lane 4 is different from the artifact below lane 1.

Ques. 6: Both the strips in the figure 7C of Paper I have been reused in fig. 6A of paper II. While actin is common to the bottom panel in both above cases, in the top panel, the same strip was used to denote ERK in one case and MEKK in another case, with different values.

Point 6. These blots examine the roles of ERK1/2 (**Figure 7C in Paper I)** and MEKK1 and c-Jun (**Figure 6A in Paper II**) in regulating the OPN-induced uPA expression using the wild type and dominant negative constructs. **We have original blots for both these figures.** Moreover, computer analyses results have clearly shown the distinct differences between both the blots. **These strips are not denoting the ERK in one case and MEKK in another case with different values.**

Ques. 7: The top panels of Fig. 6A and 8A of paper II use the same strip to denote upa and jun.

Point 7. The Figure 6A in Paper II shows the effect of MEKK1 and c-Jun in regulating OPN-induced uPA expression. The Figure 8A in Paper II is an *in vivo* data showing the effects of NIK and MEKK in regulating OPN-induced c-Jun expression in the tumor extracts of mice injected with transfected (wt/mut NIK and wt/mut MEKK1) B16F10 cells. **We have original blots of both these figures.** The computer analyses results have clearly shown the distinctive differences between these two blots.

NATIONAL CENTRE FOR CELL SCIENCE, PUNE

Ref : NCCS/Admin/2006 Date : 17th August, 2006

OFFICE MEMORAMDUM

This has reference to letter No. NCCS/Adm/36(7) dated 26th July, 2006 intimating Dr. G. C. Kundu, Scientist 'E' regarding constitution of the Fact Finding Committee under the chairmanship of Prof. G. Padmanaban, Department of Biochemistry, IISC, Bangalore to investigate the matters mentioned in the email dated 16th May, 2006 received from Shivaji Bode 1 & email dated 22nd June, 2006 received from Ganapati Mahabaleshwar.

In this connection, Dr. G. C. Kundu, Scientist 'E', is hereby informed that the Fact Finding Committee has submitted its report to the Director. **The Committee has reported that there is no substance in the allegations made in the above emails.**

18/8/06

B. G. Acharya
Controller of Administration

To,
Dr. G. C. Kundu,
Scientist 'E',
NCCS, Pune.