Dear Ellie,

First of all, I would like to sincerely thank you for your time and effort in addressing this matter.

This is to confirm that the authors categorically deny any deliberate attempt to manipulate the data presented in the original publication. While we do not agree with the conclusions drawn by the contributors on PubPeer, we regret that we no longer have access to the original data, as the paper in question was published almost 20 years ago (*Cell* 2005). Given this, we acknowledge the possibility of unintentional errors during the preparation of the figures or the potential occurrence of similarities, as explained below.

Regarding the alleged image duplication pointed out by the pubeer, I would like to emphasize that the "color overlay" method used for band comparison is widely criticized by several imaging experts as being subjective, unreliable, and prone to misinterpretation. This method assesses band similarity based on both the volume and spatial distribution of bands, which can be influenced by multiple factors, including:

- The mesh of the gel,
- The size of the gel,
- The buffer composition,
- Migration time,
- The nature of the cell extract,
- The type of target protein and the antibodies used.

It is important to note that for nearly all our experiments, we used the same acrylamide provider, with consistent gel percentages, identical buffer systems, and uniform gel sizes. Furthermore, many of the figures in question involved the same cell extracts, target proteins, and antibodies. As such, it is entirely reasonable that the bands might appear similar, and any similarities detected by the "color overlay" method are to be expected.

In addition, manipulations performed by PubPeer commentators—such as horizontal or vertical flipping, stretching, and other alterations—could amplify these perceived similarities, leading to misleading interpretations when evaluated through the "color overlay" method.

Please find my detailed comments on each paper below. I deeply appreciate your time and consideration in reviewing this response.

I-hnRNP K: An HDM2 Target and Transcriptional Coactivator of p53 in Response to DNA Damage

Abdeladim Moumen,[1] Philip Masterson,[2] Mark J. O'Connor,[2,*] and Stephen P. Jackson[1,2,*]

[1] The Wellcome Trust and Cancer Research UK Gurdon Institute and Department of Zoology, Cambridge University, Tennis Court Road, Cambridge CB2 1QN, United Kingdom

[2] KuDOS Pharmaceuticals Ltd., 327 Cambridge Science Park, Milton Road, Cambridge CB4 OWG, United Kingdom *Contact: mjoconnor@kudospharma.co.uk (M.J.O.); s.jackson@gurdon.cam.ac.uk (S.P.J.)

DOI 10.1016/j.cell.2005.09.032

This paper has now been published for nearly 20 years, and all of its findings were pioneering contributions to the scientific literature. Specifically:

- We were the first to demonstrate that hnRNP K plays a role in the DNA damage response.
- We were the first to show that hnRNP K is rapidly induced following DNA damage.
- We were the first to report that hnRNP K interacts with HDM2 and is regulated through an HDM2- and ubiquitin-dependent mechanism.
- We were the first to establish that hnRNP K undergoes regulation by ubiquitination.
- We were the first to identify the interaction between hnRNP K and p53.
- We were the first to demonstrate that hnRNP K regulates p53-dependent transcription after DNA damage, including the induction of p21 and other target proteins.

All these findings have been reproduced and confirmed by numerous studies published in subsequent years. I have provided a list of references below, which demonstrate how these results have been validated by independent research.

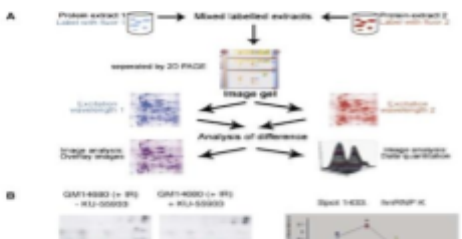
Discussion of the allegations

1-HnRNP K is induced following DNA damage

Figure 1, composed of six panels, demonstrates the induction of hnRNP K following DNA damage, progressing from proteomic analysis to the final panel (F). We were the first to report this regulatory mechanism, which has since been confirmed and validated by other independent studies (see references below).

The first allegation on PubPeer claims that the tubulin control in panel F was taken from panel E. I acknowledge that, after the manipulations performed by the PubPeer commentators, the tubulin bands in both panels appear similar. However, I maintain that such similarity is plausible, as both tubulin bands were generated using the same antibody and derived from highly similar cell extracts.

Since the original data is no longer available (given that the paper was published nearly 20 years ago), I cannot definitively rule out the possibility of an unintended error during the preparation of the figure (which remains a likely scenario). For this, I sincerely apologize. Regardless, this potential mistake does not alter the conclusions of the figure, as the key findings are supported by the other panels. Therefore, there was no motive or need to falsify the tubulin control.



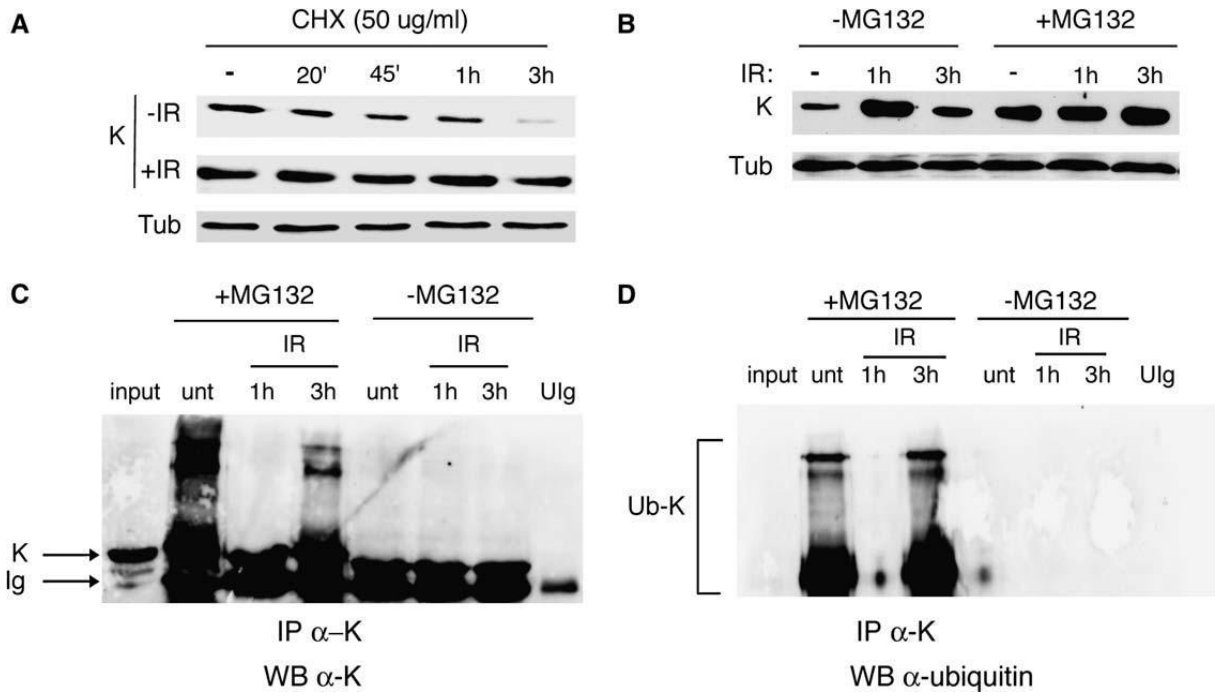
In addition, the induction of hnRNP k following DNA damage is also demonstrated on Figure 2 of the paper which is constituted by 4 panels.

2-HnRNP K Stabilization following DNA Damage Reflects Inhibition of Its Proteasomal Degradation

This figure, consisting of four panels, demonstrates that hnRNP K is regulated by the ubiquitin-dependent proteasome pathway. Panels B, C, and D clearly show that hnRNP K undergoes ubiquitination and degradation in a proteasome-dependent manner. We were the first to report this regulatory mechanism, and it has since been confirmed by multiple independent studies (see references below).

In the PubPeer comments, it is claimed that the tubulin band in panel A of this figure resembles the tubulin from Figure 1E, but only after a horizontal flip manipulation. While I personally find it difficult to see the similarity—particularly the section highlighted by the red circle—I no longer have access to the original data, making it impossible to verify whether an error occurred during figure assembly.

That said, there was no need to fabricate or manipulate the tubulin band in this figure, as the conclusions drawn from the results in this panel are not critical to the main findings. Furthermore, the regulation of hnRNP K levels following cycloheximide treatment has been independently confirmed by other researchers (see references below).

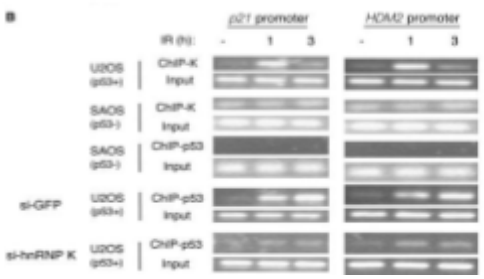


3-hnRNP K Interacts with p53, and the Two Proteins Associate with p53-Dependent Promoters in an Interdependent Manner

Panel B in Figure 7 demonstrates that hnRNP K is recruited to the p53-dependent promoters, including p21 and HDM2, and is required for p53 binding to these promoters. The key conclusion is based on experiments conducted in U2OS cells, which express p53. In these cells, the siRNA-mediated downregulation of hnRNP K significantly reduces p53 recruitment to its target promoters. In contrast, in SAOS cells (which are p53-deficient), hnRNP K is still recruited, but only at a basal level.

The PubPeer commentator claims that some bands from the SAOS cell experiments appear similar. On carefully examining enlarged images for panel B.of Figure 3, it is not clear to us that the images are actual duplications, but if they are, this could be due to an unintentional mistake during image assembly. Unfortunately, I no longer have access to the original data to verify this.

That said, there was no need to fabricate or manipulate this part of the figure, as the main conclusion is already supported by the U2OS experiments. Thus, any potential error would not impact the validity of the overall findings.



4-Mdm2 mediates ubiquitination of hnRNP k and this is interrupted by DNA damage

A series of experiments presented in Figures 3 and 4 (9 panels in total) clearly demonstrate that hnRNP K is ubiquitinated in an HDM2-dependent manner and that this ubiquitination is inhibited by DNA damage. These findings are shown both ex vivo (in cell-based experiments: panels A, B, C, and D of Figure 3, and panels C, D, and E of Figure 4) and in vitro using purified proteins (panel F of Figure 4).

Our study was the first to demonstrate the interaction between HDM2 and hnRNP K (panels A and B of Figure 4) as well as its HDM2-mediated ubiquitination. These results have been successfully reproduced and validated by other independent studies, both ex vivo and in vivo (including mouse models), as referenced below.

However, the PubPeer commentators criticized panel E of Figure 4, claiming that the images appear similar. In my view, such similarity is expected since both images originate from the same type of cells (Mdm2-deficient MEFs) and were generated using the same hnRNP K antibody for immunoprecipitation. On carefully examining enlarged images for panel E of Figure 4, it is not clear to us that the images are actual duplications, but if they are, this may be due to an inadvertent mistake during the image assembly process. Unfortunately, I no longer have access to the original data to verify this.

That said, there was no need to manipulate or fabricate these images, as the main conclusion of the figure remains intact and is strongly supported by the other experiments.

figure 3, in cell experiments showing that hnRNP K Stabilization following DNA Damage Reflects Inhibition of Its Proteasomal Degradation. it shows its ubiquitination.

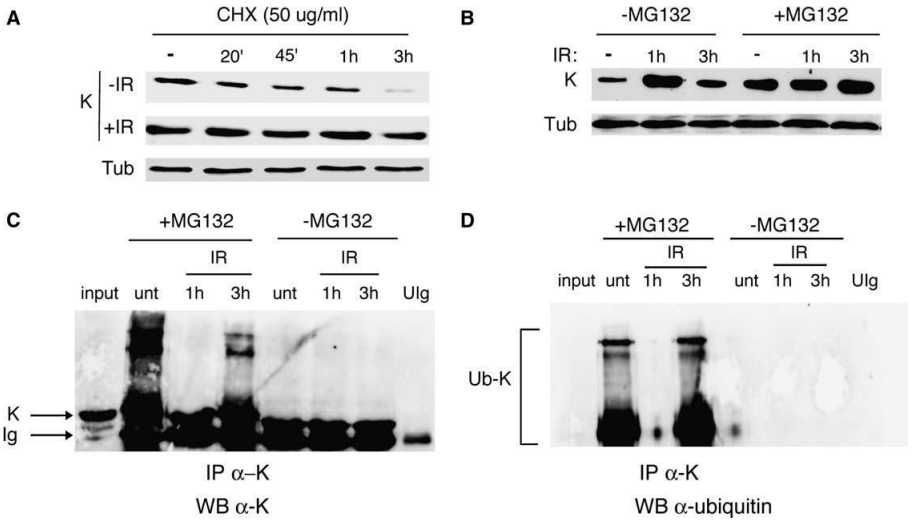
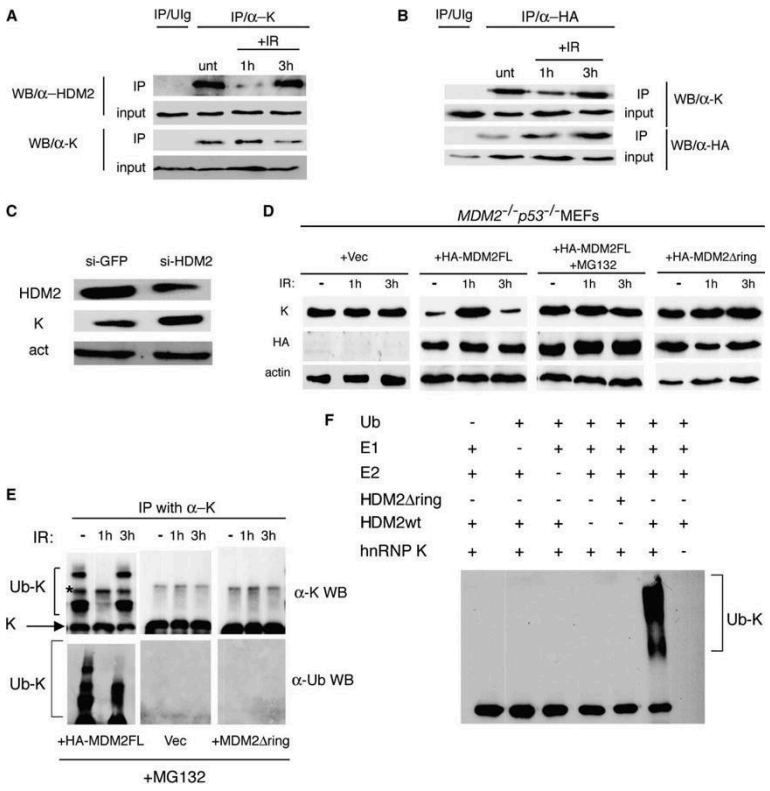


Figure 4, shows that HDM2 Mediates Ubiquitin-Dependent Degradation of hnRNP K in both in cell and in vitro conditions. it shows also that hnRNP k interacts with HDM2 and this interaction is interrupted with DNA damage.



5- hnRNP K/P53 Interaction and hnRNP K/HDM2 interaction

All the other figures criticized on PubPeer—specifically Figures 4, 6, 7, 8, 9, 10, and 11—present experiments demonstrating the interactions between hnRNP K and P53, as well as hnRNP K and HDM2. The concerns raised by the commentators primarily focus on western blot bands, which were heavily manipulated by the pubeer commentators (e.g., through horizontal flipping, vertical compression, stretching, etc.) to create the appearance of similarity.

However, the data in these figures have been independently reproduced and confirmed in multiple published studies (see references below). Notably, the interactions between P53/hnRNP K and HDM2/hnRNP K, which were first reported in our paper and depicted in the criticized figures, have been repeatedly demonstrated and validated by subsequent research.

Since the paper was published 20 years ago, I no longer have access to the original data to address these specific criticisms. Nevertheless, the reproducibility of our findings by others highlights the integrity and validity of the conclusions presented in the paper.

REFERENCES REPRODUCING AND CONFIRMING OUR RESULTS.

I-HnRNP K is induced following DNA damage

1- Arginine methylation of hnRNPK negatively modulates apoptosis upon DNA damage through local regulation of phosphorylation

Jen-Hao Yang[1], Yi-Ying Chiou[2], Shu-Ling Fu[3], I-Yun Shih[2], Tsai-Hsuan Weng[1], Wey-Jinq Lin[2] and Chao-Hsiung Lin[1,2,4,*]

Nucleic Acids Research, (2014) 1 *doi: 10.1093*

2-hnRNP K Is a Haploinsufcient Tumor Suppressor that Regulates Proliferation and Differentiation Programs in Hematologic Malignancies.

Gallardo M, Lee HJ, Zhang X, Bueso-Ramos C, Pageon LR, McArthur M, Multani A, Nazha A, Manshouri T, Parker-Thornburg J, Rapado I, Quintas-Cardama A, Kornblau SM et al

Cancer Cell. (**2015**); 28:486–99

3-Knockdown of hnRNPK leads to increased DNA damage after irradiation and reduces survival of tumor cells

Nadine Wiesmann[†], Judith Strozynski[†], Carina Beck, Nadine Zimmermann, Simone Mendler, Rita Gieringer, Irene Schmidtmann[1] and Jürgen Brieger*

***Carcinogenesis*, (2017)**, Vol. 38, No. 3, 321–328

4-Expression levels of hnRNP K and p21$^{WAF1/CIP1}$ are associated with resistance to radiochemotherapy independent of p53 pathway activation in rectal adenocarcinoma

WASSILIKI dASKALAKI[1], EVA WARdELMANN[1], MATTHIAS PORT[2], KATHARINA STOcK[1], JULIE STEINESTEL[3], SEBASTIAN HUSS[1], JAN SPERVESLAGE[1], KONRAd STEINESTEL[1,4*] and STEFAN EdER[2*]

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 42: 3269-3277, (2018)

5-SUMOylation of hnRNP-K is required for p53-mediated cell-cycle arrest in response to DNA damage

Seong Won Lee, Moon Hee Lee, Jong Ho Park, Sung Hwan Kang, Hee Min Yoo, Seung Hyun Ka, Young Mi Oh, Young Joo Jeon* and Chin Ha Chung*

Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul, Korea

The EMBO Journal (2012) 31, 4441–4452

II-HnRNP K Stabilization following DNA Damage Reflects Inhibition of Its Proteasomal Degradation and MDM2 interaction

1-HnRNPK/miR-223/FBXW7 feedback cascade promotes pancreatic cancer cell growth and invasion

De He[1,*], Cheng Huang[1,2,*], Qingxin Zhou[3,*], Dawei Liu[1,2], Longhui Xiong[1], Hongxia Xiang[1], Guangnian Ma[1], Zhiyong Zhang[4]

Oncotarget, (2007), Vol 8, (N 12), pp: 20165-20178

2-SUMOylation of hnRNP-K is required for p53-mediated cell-cycle arrest in response to DNA damage

Seong Won Lee, Moon Hee Lee, Jong Ho Park, Sung Hwan Kang, Hee Min Yoo, Seung Hyun Ka, Young Mi Oh, Young Joo Jeon* and Chin Ha Chung*

Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul, Korea

The EMBO Journal (2012) 31, 4441–4452

3-DNA Damage-induced Heterogeneous Nuclear Ribonucleoprotein K SUMOylation Regulates p53 Transcriptional Activation

Federico Pelisch[1,2], Berta Pozzi[3], Guillermo Risso[3], Manuel Javier Muñoz[2], and Anabella Srebrow[2,4]

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL.287,NO.36,pp.30789–30799,August31, (2012)

4-p53 Modulation as a Therapeutic Strategy in Gastrointestinal Stromal Tumors

Joern Henze[1], Thomas Mu¨hlenberg[1], Susanne Simon[1], Florian Grabellus[2], Brian Rubin[5], Georg Taeger[3], Martin Schuler[1], Juergen Treckmann[4], Maria Debiec-Rychter[6], Takahiro Taguchi[7], Jonathan A. Fletcher[8], Sebastian Bauer[1]*

PLoS ONE May (2012) Volume 7, Issue 5

5-Transcriptional regulation and ubiquitination-dependent regulation of HnRNPK oncogenic function in prostate tumorigenesis

Huan-Lei Wu[1†], Sen-Mao Li[2,3†], Yao-chen Huang[2], Qi-Dong Xia[2], Peng Zhou[2], Xian-Miao Li[2], Xiao Yu[2], Shao-Gang Wang[2], Zhang-Qun Ye[2] and Jia Hu[2*]

Cancer Cell International (2021) 21:641 https://doi.org/10.1186/s12935-021-02331-x

III-hnRNP K Interacts with p53, and the Two Proteins Associate with p53-Dependent Promoters in an Interdependent Manner

1- Increased expression of the heterogeneous nuclear ribonucleoprotein K in pancreatic cancer and its association with the mutant p53

Renyuan Zhou[1,3], Renee´ Shanas[2], Mark A. Nelson[2], Achyut Bhattacharyya[2] and Jiaqi Shi[3]

Int. J. Cancer: 126 , 395–404 (2010)

2-A large intergenic non-coding RNA induced by p53 mediates global gene repression in the p53 response

Maite Huarte[1,2,*], Mitchell Guttman[1,3], David Feldser[3,4], Manuel Garber[1], Magdalena J. Koziol[1,2], Daniela Kenzelmann-Broz[5], Ahmad M. Khalil[1,2], Or Zuk[1], Ido Amit[1], Michal Rabani[1], Laura D. Attardi[5], Aviv Regev[1,3], Eric S. Lander[1,3,6], Tyler Jacks[3,4], and John L. Rinn1,2,*

Cell. (2010) August 6; 142(3): 409–419

3-Aurora-A phosphorylates hnRNPK and disrupts its interaction with p53

Kai-Wei Hsueh [a], Shu-Ling Fu [b], Chi-Ying F. Huang [c], Chao-Hsiung Lin [a,⇑]

FEBS Letters 585 (2011) 2671–2675

4-SUMOylation of hnRNP-K is required for p53-mediated cell-cycle arrest in response to DNA damage

Seong Won Lee, Moon Hee Lee, Jong Ho Park, Sung Hwan Kang, Hee Min Yoo, Seung Hyun Ka, Young Mi Oh, Young Joo Jeon* and Chin Ha Chung*

Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul, Korea

The EMBO Journal (2012) 31, 4441–4452

5-DNA Damage-induced Heterogeneous Nuclear Ribonucleoprotein K SUMOylation Regulates p53 Transcriptional Activation

Federico Pelisch[1,2], Berta Pozzi[3], Guillermo Risso[3], Manuel Javier Muñoz[2], and Anabella Srebrow[2,4]

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL.287 .30789–30799, August 31, (2012)

6-Expression levels of hnRNP K and p21$^{WAF1/CIP1}$ are associated with resistance to radiochemotherapy independent of p53 pathway activation in rectal adenocarcinoma

WASSILIKI DASKALAKI[1], EVA WARDELMANN[1], MATTHIAS PORT[2], KATHARINA STOCK[1], JULIE STEINESTEL[3], SEBASTIAN HUSS[1], JAN SPERVESLAGE[1], KONRAD STEINESTEL[1,4*] and STEFAN EDER[2*]

7-LincRNA-p21 Activates p21 In cis to Promote Polycomb Target Gene Expression and to Enforce the G1/S Checkpoint

Nadya Dimitrova,[1] Jesse R. Zamudio,[1] Robyn M. Jong,[1] Dylan Soukup,[1] Rebecca Resnick,[1] Kavitha Sarma,[2] Amanda J. Ward,[3] Arjun Raj,[4] Jeannie T. Lee,[2] Phillip A. Sharp,[1] and Tyler Jacks[1,5,*]

Molecular Cell *54*, 777–790, June 5, 2014

8-hnRNP K Is a Haploinsufficient Tumor Suppressor that Regulates Proliferation and Differentiation Programs in Hematologic Malignancies

Miguel Gallardo,[1] Hun Ju Lee,[2] Xiaorui Zhang,[1] Carlos Bueso-Ramos,[3] Laura R. Pageon,[4] Mark McArthur,[4] Asha Multani,[5] Aziz Nazha,[1] Taghi Manshouri,[1] Jan Parker-Thornburg,[5] Inmaculada Rapado,[6] Alfonso Quintas-Cardama,[1] Steven M. Kornblau,[1] Joaquin Martinez-Lopez,[6] and Sean M. Post[1,*]

Cancer Cell *28*, 486–499, October 12, (2015).

9-HNRNPK inhibits gastric cancer cell proliferation through p53/ p21/CCND1 pathway

Hao Huang[1,*], Yong Han[2,3,4,*], Xingjiu Yang[1], Mengyuan Li[1], Ruimin Zhu[1], Juanjuan Hu[1], Xiaowei Zhang[5], Rongfei Wei[1], Kejuan Li[1] and Ran Gao[1]

Oncotarget, (2017), Vol. 8, (No. 61), pp: 103364-103374

10-Regulation of the p53 expression profile by hnRNP K under stress conditions

Agata Swiatkowska , Mariola Dutkiewicz , Piotr Machtel , Damian Janecki , Martyna Kabacinska , Paulina Zydowicz-Machtel & Jerzy Ciesiołka

RNA Biology DOI: 10.1080/15476286.2020.1771944 (2020)

11- **Long Noncoding RNA p53-Stabilizing and Activating RNA Promotes p53 Signaling by Inhibiting Heterogeneous Nuclear Ribonucleoprotein K deSUMOylation and Suppresses Hepatocellular Carcinoma**.

Qin G, Tu X, Li H, Cao P, Chen X, Song J, Han H, Li Y, Guo B, Yang L, Yan P, Li P, Gao C, Zhang J, Yang Y, Zheng J, Ju HQ, Lu L, Wang X, Yu C, Sun Y, Xing B, Ji H, Lin D, He F, Zhou G.

Hepatology. 2020 Jan;71(1):112-129. doi: 10.1002/hep.30793. Epub 2019 Aug 12. PMID: 31148184.

Conclusion

We were the first to show the involvement of hnRNP k in the DNA damage response, its involvement in the P53 pathway, its regulation by the ubiquitination pathway under HDM2. now it is well established that hnRNP k accomplishes these functions by other either in vitro, in cell and in vivo setting. In addition, thanks to our work, hnRNP k becomes a very attractive target for cancer treatment (see references above and other papers are not presented here). furthermore, before our paper and as far as we know there were no other work that has described the implication of hnRNP k in the DNA damage response. Furthermore, all the critics are subjective and scientifically unfounded. Indeed, and as far as we are aware, there were no other scientific published work in the literature that shown results contrasting ours.

In addition, all the concerned parts of the figures criticized do not compromise the final conclusions, therefore there was no need to manipulate these figures and mainly the tubulin bands. Tubulin band are very easy to create if we needed so.
Therefore, we deny any attempt to deliberately fabricate or duplicate any data presented in this paper or others. We confirm that any potential duplication, if presented, in this paper could be due definitely to a mistake occurred when the images were assembled for publication, otherwise this is a completely wrong interpretation of the image.

ATM-dependent phosphorylation of heterogeneous nuclear ribonucleoprotein K promotes p53 transcriptional activation in response to DNA damage

Abdeladim Moumen,[1,†] Christine Magill,[2] Katherine L. Dry[2] and Stephen p. Jackson[2,*]

The authors deny any attempt to deliberately manipulate the data presented in the original publication in question. Whilst the authors do not concur with the conclusions drawn by the Pubpeer contributor(s), the authors do not have the original data for comparison since the paper in question has been published for now more than 9 years. Without this, the authors acknowledge the possibility of unintended error having been made during compilation of the figures in question.

Figure1: ATM-dependent phosphorylation of hnRNP k

in U2OS cells the ATM mediated phosphorylation of hnRNP K using a phospho-ATM/ATR substrate antibody (antiP-S/T) was assessed. this antibody recognizes specifically targets phosphorylated by ATM or ATR. The specific ATM mediated hnRNP K phosphorylation in a DNA damage dependent manner is demonstrated in this figure on its three panels (A,B and D) using this antibody. The commentator on Pubpeer criticized the figure 1 panel A by pretending that the first four lines are similar. For me it is very well expected that this bands to be similar because these are background from the same cell extract with the same antibody. Indeed, if you look to the same figure, the two bands on the right-hand side (sample 4A) are also looking similar to the bands on the left-hand side (vector and WT+ATMi) (see circled parts)

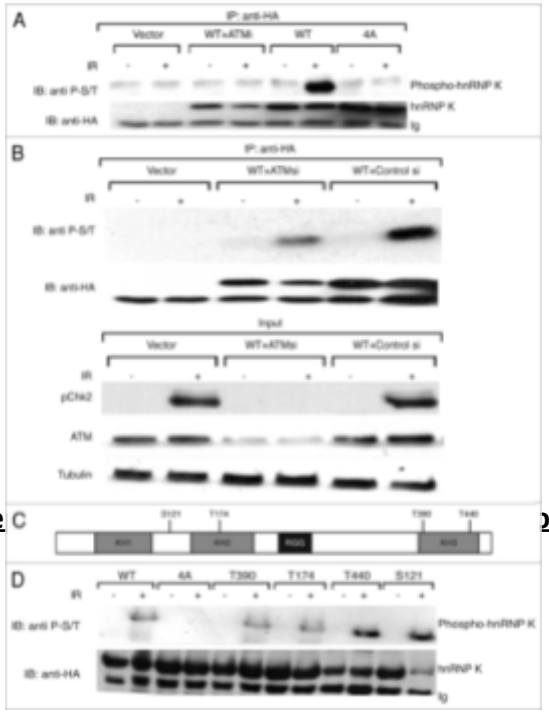


Figure 3: ATM-de omotes P53 transcriptional activity after IR.

both siRNA resistant HA-tagged WT hnRNP K (WT/siR) and siRNA resistant HA-tagged 4A mutant (4A/siR) were successfully expressed in U2OS cells to assess the effect of ATM-mediated phosphorylation of hnRNP K on the P53 activity following DNA damage. The commentator on Pubpeer criticized the figure 3 panel B by pretending that the band on the right-hand side representing the expression of 4A/siR is added manually. **The expression of this protein (4A/siR) is already made efficiently as it is shown on the panel A of the figure 3. Therefore, there were no reason to add it manually on the panel B as it is pretended**. The splice appearing is more likely due to a bad transfer of the protein from the gel to the membrane. This is something frequent in western blotting. Indeed, as you can see on the figure 4 of the Cell paper (see below), we have this kind of splice appearing although this is an integral gel. This demonstrates that the apparent splice could be due to a bad transfer during the western blotting process.

Figure 3 (cell cycle 2011)

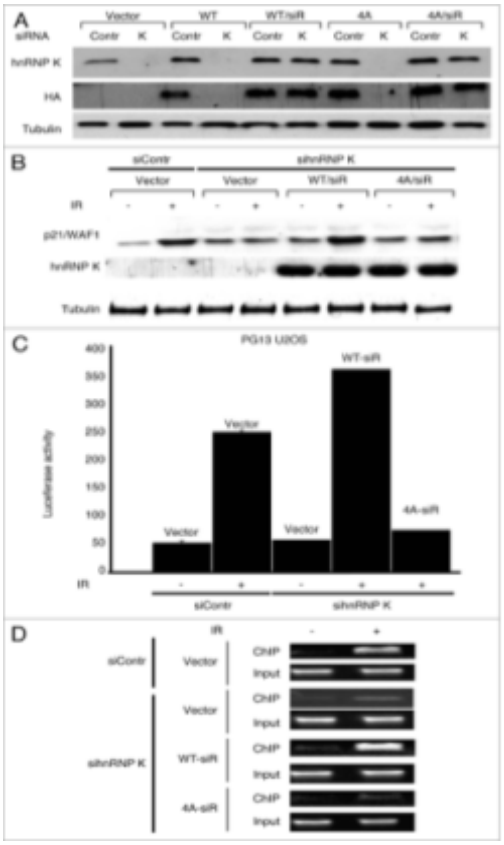


Figure 4 (Cell 2005)