Antagonism of interferon signaling by fibroblast growth factors promotes viral replication

Luigi Maddaluno, Corinne Urwyler, Theresa Rauschendorfer, Michael Meyer, Debora Stefanova, Roman Spoerri, Mateusz Wietecha, Luca Ferrarese, Diana Stoycheva, Daniela Bender, Nick Li, Gerhard Strittmatter, Khondokar Nasirujjaman, Hans-Dietmar Beer, Peter Staeheli, Eberhard Hildt, Annette Oxenius and Sabine Werner

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Transaction Report:

20th Dec 2019

Dear Sabine,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript.

You will see below that the three referees find the study interesting and well done. While ref. #1 is enthusiastic and only requests supportive experiments, quantitation, more controls and explanations about statistics and sampling, ref. #2 and #3 share a similar concern and would like to see more mechanism. In addition ref. #2 asked whether the data can be applied to other cell types. Overall, we agree that all these requests would make the study stronger and I'd like to invite you to address those comments in a major revision of your work.

We would therefore welcome the submission of a revised version within three to six months for further consideration and would like to encourage you to address all the criticisms raised as suggested to improve conclusiveness and clarity. Please note that EMBO Molecular Medicine strongly supports a single round of revision and that, as acceptance or rejection of the manuscript will depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

Please also contact us as soon as possible if similar work is published elsewhere. If other work is published we may not be able to extend the revision period beyond three months.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript.

Happy Holidays!

Celine Carret

Celine Carret, PhD
Senior Editor
EMBO Molecular Medicin

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

the authors have confirmed the results in the human keratinocyte cell line HaCaT and have also conducted individual experiments in primary keratinocytes. In addition, functional assays were performed to confirm the biological relevance of their data.

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In the present study Maddaluno et al. investigated the effect of FGF on interferon signaling in keratinocytes. For this purpose, mice without FGFR1, FGFR2 or both receptors in keratinocytes of the epidermis and hair follicles were used to identify FGF targets in the epidermis. Further analyses included the genetic or pharmacological inhibition of FGF receptors in primary mouse keratinocytes or the human keratinocyte cell line HaCaT. In addition, the results in primary human keratinocytes were partially confirmed. They found that inhibition of the FGF receptor promotes expression of interferon-stimulated genes (ISG) and proteins in vitro and in vivo. In accordance with these results FGF7 or FGF10 treatment of keratinocytes suppressed ISG expression both basally and after IFN or Poly(I:C) treatment. Further, by infection studies of keratinocytes with HSV-1, ZIKV, or lymphocytic choriomeningitis virus they demonstrated that the effect of FGF on ISG is of biological relevance: Stimulation with FGF increased the expression of genes of the virus, while inhibition of FGFR signaling blocked HSV-1 replication in cultured human keratinocytes and mice.
The study is well conducted and clearly written. In addition, it is also of interest to more than one professional audience. Nevertheless, there are a few minor points that the authors should address:

1. The panel of IFN-stimulated genes whose expression was analyzed in this study should be more consistent between the different but related experiments to facilitate comparison. For example, in Fig. 1H HaCaTs were analyzed for ISG15 expression, but not for IRF7 and Oasl2, whose expression was previously shown in mouse analysis. Moreover in Fig 2B HaCaTs were analyzed for IRF1, IRF9 and SOCS3 but therefor no data was shown in mice whereas mice were analysed for STAT2 and Ifit1 which was not the case in HaCaTs.
Please also use the same please also use the same order of presentation in your graphics, this will make it easier to read and understand the graphics (in some cases this has already been done).
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In order to increase the significance of the western blot, a densiometric evaluation of all westernblots should be carried out and their evaluation should be presented as a graph in addition to the existing exemplary image.

a. Fig. 3C the differences in pSTAT1 activation between IFNa and INFa+FGF7 are hardly to see, this may be improved by densiometric analysis (see above).
b. Fig. 4B the western blot for IRF3 and pIRF3 is not convincing . The manuscript also mentions

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c. Fig. 6E the western blot is unfortunately not convincing because of the GapDH levels, which are much lower in the FGF7 stimulated cells than in the KCtrl or HSV-1 cells.
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7. Human keratinocytes: Mean n=6 that keratinocytes from 6 different donors were investigated or are they replicates? Please indicate it clearly in the legend
8. Statistical analysis: which test was used to test if the values come from a Gaussian distribution? Two-way ANOVA assumes that your replicates are sampled from Gaussian distributions, especially with small sample sizes this is important and should be taken into account.

Referee #2 (Remarks for Author):

Maddaluno and colleagues report the very interesting, important and original observation that signaling through FGFR1 and FGFR2 inhibits interferon stimulated gene (ISG) expression. They further show that this is functionally relevant in the context of viral infection. They propose that FGFR inhibitors could be used as broadly active antivirals.

The experiments are very well done, and the results support the conclusions.

I have two major considerations:

1. The big missing part of this exciting story is the mechanism. How does FGF7 inhibit both tonic and induced ISG expression?

The authors provide some insights with the proteasome inhibitor experiments (Figures 2G, 2H, S2G). They speculate that "The effect of FGF7 is likely to involve proteasomal degradation of a protein required for ISG expression, since it was abolished in the presence of different proteasome inhibitors." They further speculate that this mechanism could involve the well known ISG transcriptional regulators IRF9, IRF1, IRF7 and STAT1 and STAT2.

There is indeed a slight reduction of the protein levels of this transcription factors (shown in figures 2C, 3C, and 6E) but in parallel there is a reduction of the corresponding mRNAs that could very well explain the reduced protein levels. The experiments with the proteasome inhibitor provide only circumstantial evidence. The authors should further explore this. What is known about regulation of the proteasome pathway by FGF7? Is the presumed FGF7 induced proteasome activation non-specific, or specifically/preferentially targeting ISG transcriptional regulators such as IRF7 and IRF1? What about mTORC1, a central regulator of proteasome assembly (and a potential target of FGF signaling downstream of Akt)?

The authors also provide some mechanistic insights by showing that blockade of the major FGFR signaling pathways, the PI3-kinase/Akt and Erk1/2 pathways, do not suppress the effect of FGF7 on ISG expression. They conclude that "other pathways or pathway combinations are involved." What about PLCγ or STATs? Is the FGFR kinase activity required the observed effects on ISG expression?

2. How generally valid is this observation? The authors focus their study on keratinocytes. What about other cells in the body? This seems an important question, since the authors propose FGFR inhibition as an exciting new antiviral strategies, also for viruses that do not only replicate in keratinocytes.

Specific points:

1. SOCS1 rather than SOCS3 is the canonical inhibitor of IFNAR signaling. Is SOCS1 regulated by FGFR2?

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5. The panels of Figure 7 are partially overlapping and seem to be dislocated.

Referee #3 (Comments on Novelty/Model System for Author):

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Referee #3 (Remarks for Author):

This MS reports the finding that Mice with a KO of FGF receptors (FGFR) 1 and 2 in their skin have high levels of expression of IFN stimulated genes (ISG). Further investigation of this initial observation leads to the conclusion that FGF signaling inhibits the expression of ISGs in mouse and human keratinocytes and, as such, promotes the replication of HSV1 and other viruses in the same cells. This notion is supported by a variety of experiments using FGF7 treatment of Keratinocytes, by showing that ISG expression is stimulated by FGFR inhibitors, and that FGF signaling competes with IFN or Poly:IC treatment in regulating ISG expression. Finally it is shown that the replication of HSV1, LMC and zika viruses is promoted by FGF signaling by dampening the IFN response and that inhibition of FGF signaling adversely affects HSV 1 replication.
The results presented here are quite convincing and the major conclusion of the MS, i.e. that FGF signaling antagonizes IFN signals is amply documented.
The major weakness of this report is however that it does not identify or even investigate the mechanisms responsible for the phenomenon described. Furthermore I noticed that, in most

experiments testing the expression of the ISGs in response to FGF treatment (figs. 1,2,3,4, 6), the effect on the RNA levels of the genes are much stronger than the effect on protein levels, that in some cases show no difference. Unfortunately the genes whose expression is determined at the RNA level and those whose protein expression is shown are often not the same, making difficult to ascertain how general is this phenomenon. This discrepancy has to be discussed or explained if this MS has to be accepted for publication.

Two minor points: 1) it would be better to measure viral titers, in Infectious units, rather than DNA levels.

2)The Ms is extremely long and could be trimmed considerably. The discussion is rambling, never discusses mechanisms and some of the therapeutic implications could be toned down.

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The authors have confirmed the results in the human keratinocyte cell line HaCaT and have also conducted individual experiments in primary keratinocytes. In addition, functional assays were performed to confirm the biological relevance of their data.

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The study is well conducted and clearly written. In addition, it is also of interest to more than one professional audience. Nevertheless, there are a few minor points that the authors should address:

1. The panel of IFN-stimulated genes whose expression was analyzed in this study should be more consistent between the different but related experiments to facilitate comparison. For example, in Fig. 1H HaCaTs were analyzed for ISG15 expression, but not for IRF7 and Oasl2, whose expression was previously shown in mouse analysis. Moreover in Fig 2B HaCaTs were analyzed for IRF1, IRF9 and SOCS3 but therefor no data was shown in mice whereas mice were analysed for STAT2 and Ifit1 which was not the case in HaCaTs. Please also use the same please also use the same order of presentation in your graphics, this will make it easier to read and understand the graphics (in some cases this has already been done).

<u>Our reply:</u> We showed that almost all ISGs that we tested were regulated by FGF signaling in mouse and human keratinocytes. The basal expression of different ISGs and the efficiency of FGFs in their regulation was, however, slightly different for the mouse and human cells and the promoter regions of the mouse and human ISGs are also different. Therefore, we have not always used the same ISGs in our analysis. Nevertheless, we now extended our analysis and we show the results for different ISGs in a similar order.

2. Figure S2: Inhibition of Erk1/2, PI3K or Akt does not affect the FGF7-mediated suppression of ISG expression, while inhibition of proteasomal activity abrogates the FGF7 effect. Please insert a positive control for the inhibitor so that you can be sure that the inhibitor itself works.

Our reply: Positive controls for Erk1/2 or Akt inhibition had been shown in Fig. S2C and D, previous version (Western blot analysis of pErk1/2 and pAkt). Therefore, the activity of these inhibitors was confirmed. Similar experiments were performed for additional inhibitors that we tested for the revision. These results are now shown in Appendix Fig. S1. The activity of the proteasome inhibitors was confirmed by Western blot analysis of the NRF2 transcription factor, which is normally rapidly degraded via the ubiquitin/proteasome pathway. NRF2 protein levels strongly increased in the presence of all proteasome inhibitors. This result is now shown in Appendix Fig. S2B.

3. Westernblots: it's not clear from the legend how high the n-number is. Means one experiment =n=1 or one experiment with n=?

Our reply: We now explain this in more detail in the individual legends. In addition, we repeated some of these experiments and analyzed each condition in triplicate. The data were quantified (see Fig. 3C and 4B).

In order to increase the significance of the western blot, a densiometric evaluation of all westernblots should be carried out and their evaluation should be presented as a graph in addition to the existing exemplary image.

a. Fig. 3C the differences in pSTAT1 activation between IFNa and INFa+FGF7 are hardly to see, this may be improved by densiometric analysis (see above).

b. Fig. 4B the western blot for IRF3 and pIRF3 is not convincing . The manuscript also mentions STAT3, which is not shown in the figure.

Our reply: The Western blot analyses of IFN-α- and Poly(I:C)-treated cells were repeated using triplicates for each condition and the data were quantified by densitometry. The new results are shown in Fig. 3C and 4B. STAT3 was not analyzed – this has been removed from the text.

c. Fig. 6E the western blot is unfortunately not convincing because of the GapDH levels, which are much lower in the FGF7 stimulated cells than in the KCtrl or HSV-1 cells.

Our reply: We agree that the GAPDH levels are lower in the protein lysates, which were also analyzed for Glyc-D. This even results in an underestimation of the effect of FGF7. In the other lysates there was no difference in the GAPDH levels between FGF7-treated and non-treated samples. To address this point we scanned the blots and we normalized each protein value to the GAPDH value of the same lysate. The results are now shown graphically in this figure.

4. Fig 6G und follows: please explain the legend of the figure. Stain 976 of ZIKV? PF13/251013-18?

Our reply: This has been done (see new legend to this figure)

5. Fig7: Please control Fig 7. Some of the graphs are shifted.

<u>Our reply:</u> We apologize for this problem, which seems to have happened during the file conversion. The figure displayed correctly in the final pdf document that was checked before submission.

6. Diskussion: Nectin-1 was shown to be involved in HSV-1 entry in keratinocytes (Charlotte L. Sayersa and Gillian Elliott 2016). Is the expression of nectin-1 influenced by FGF treatment?

<u>Our reply:</u> As suggested by the reviewer we analyzed if nectin-1 expression is affected by FGF7 in keratinocytes. However, its expression was not up-regulated, but rather mildly down-regulated, strongly suggesting that promotion of viral infection by FGF7 is not mediated via nectin-1. These new data are now shown in Fig. 5J. We also cite the Sayers and Elliott paper as well as another paper (Petermann et al., 2015) that also showed an involvement of Nectin-1 in HSV-1 entry into keratinocytes.

7. Human keratinocytes: Mean n=6 that keratinocytes from 6 different donors were investigated or are they replicates? Please indicate it clearly in the legend.

<u>Our reply:</u> These were 6 replicates from two experiments with two donors. We now clarify this in the legend to Fig. 3D.

8. Statistical analysis: which test was used to test if the values come from a Gaussian distribution? Two-way ANOVA assumes that your replicates are sampled from Gaussian distributions, especially with small sample sizes this is important and should be taken into account.

<u>Our reply:</u> For comparison of more than two groups, we used Brown-Forsythe test to verify equal variance between groups, followed by one-way or two-way ANOVA and Tukey's multiple comparisons post-hoc tests.

Referee #2 (Remarks for Author):

Maddaluno and colleagues report the very interesting, important and original observation that signaling through FGFR1 and FGFR2 inhibits interferon stimulated gene (ISG) expression. They further show that this is functionally relevant in the context of viral infection. They propose that FGFR inhibitors could be used as broadly active antivirals.

The experiments are very well done, and the results support the conclusions. I have two major considerations:

1. The big missing part of this exciting story is the mechanism. How does FGF7 inhibit both tonic and induced ISG expression?

<u>Our reply:</u> We are aware that we have not fully explored the mechanism underlying this novel activity of FGFs, in particular since we focused on the consequences of the findings for viral infection/replication. Nevertheless, we already provided some insight into the mechanism: We showed that this effect is downstream of interferon receptors, that it occurs

at the transcriptional level, and that it requires proteasomal activity. In response to the comments of the reviewer we performed additional experiments (see below) and we now provide further mechanistic insight. However, we would like to point out that this is an entirely novel finding and it will clearly require extensive follow-up studies to further explore the molecular mechanisms.

The authors provide some insights with the proteasome inhibitor experiments (Figures 2G, 2H, S2G). They speculate that "The effect of FGF7 is likely to involve proteasomal degradation of a protein required for ISG expression, since it was abolished in the presence of different proteasome inhibitors." They further speculate that this mechanism could involve the well known ISG transcriptional regulators IRF9, IRF1, IRF7 and STAT1 and STAT2. ,There is indeed a slight reduction of the protein levels of this transcription factors (shown in figures 2C, 3C, and 6E) but in parallel there is a reduction of the corresponding mRNAs that could very well explain the reduced protein levels. The experiments with the proteasome inhibitor provide only circumstantial evidence. The authors should further explore this.

What is known about regulation of the proteasome pathway by FGF7?

Our reply: To our knowledge, there is as yet no published study describing an effect of FGF7 on the proteasome. A paper from 2006 (PMID 16720300) reported that FGF2 induces degradation of the phosphatase HD-PTP via the proteasome, but it was not determined if FGF2 affects proteasome activity.

In fact, our data do not necessarily imply that FGF7 activates the proteasome – rather, it may promote ubiquitination of one or more proteins, followed by their proteasomal degradation (see discussion). For example, FGFR activation was shown to activate the ubiquitin ligase c-Cbl, which is involved in receptor degradation. C-Cbl also targeted Sprouty 2 to the ubiquitin-dependent proteasome pathway in response to FGF stimulation of fibroblasts (PMID 12593796). We therefore tested if knock-down of c-Cbl affects the effect of FGF7 on ISGs, but this was not the case (our unpublished data). Therefore, it seems likely that other ubiquitin ligases are responsible, which need to be identified in an unbiased approach.

Although we favor an effect of FGF7 on protein ubiquitination, we had of course planned to test a potential direct effect of FGF7 on proteasome activity in keratinocytes as suggested by the reviewer. Our first attempts to address this issue failed due to a faulty kit, and the quality problem was confirmed by the company that sells the kit. An alternative kit was ordered, but due to the shut-down of our laboratories during the SARS-CoV-2 pandemic we were so far unable to perform these experiments. Therefore, we can unfortunately not provide these data at this stage.

Is the presumed FGF7 induced proteasome activation non-specific, or specifically/preferentially targeting ISG transcriptional regulators such as IRF7 and IRF1?

Our reply: As mentioned above, there is as yet no information on a potential activating effect of FGF7 on the proteasome. This effect may well be regulated at the level of ubiquitination. Nevertheless, it is of course very interesting to determine if the rapid suppression of gene expression by FGF7 and the rescue by proteasome inhibitors is specific for certain ISGs, for ISGs in general or if it also affects other genes (non-ISGs).

The inhibition of the FGF7-induced down-regulation by MG132 was not only observed for *IRF1* and *IRF7*, but also for *RSAD2*, *ISG15* and *STAT1* (other ISGs were not tested). We show

the *IRF1* and *IRF7* data in the manuscript, because we analyzed these genes in experiments with different proteasome inhibitors. We could certainly show the other genes as well upon request.

Our microarray data from isolated epidermis of ctrl and K5-R1/R2 mice demonstrate that other genes, which have not been described as ISGs, are upregulated in the FGFR-deficient mice, although the vast majority of the most highly regulated genes are ISGs. This is now mentioned on page 5, first paragraph. We have preliminary data showing that some of the other upregulated genes are directly (and negatively) regulated by FGFs. However, we do not yet know if they are also regulated via proteasomal degradation. The question regarding specificity for ISGs is indeed very interesting and important, but we feel that it goes beyond the scope of this initial publication. We will certainly test this in future studies.

What about mTORC1, a central regulator of proteasome assembly (and a potential target of FGF signaling downstream of Akt)?

Our reply: mTORC1 regulation by receptor tyrosine kinases usually occurs via PI3K/Akt signaling and neither inhibition of PI3K nor of Akt alone abolished the suppression of ISGs by FGF7.

Nevertheless and as suggested by the reviewer, we checked if inhibition of mTORC1 (by rapamycin) inhibits the FGF7-induced ISG suppression. We performed one experiment and the effect of FGF7 on two different ISGs was not affected. Unfortunately, we could not repeat the experiment because of the shut-down of our lab, but we have included the result of the first experiment as "additional information for reviewer 2" (first page)".

The authors also provide some mechanistic insights by showing that blockade of the major FGFR signaling pathways, the PI3-kinase/Akt and Erk1/2 pathways, do not suppress the effect of FGF7 on ISG expression. They conclude that "other pathways or pathway combinations are involved." What about PLCγ or STATs? Is the FGFR kinase activity required the observed effects on ISG expression?

Our reply: We also tested inhibitors for PLCγ, but they had no effect on the FGF7-mediated ISG suppression. These data are now shown in Fig. EV2D. Although we did not find a strong phosphorylation of STAT3 in keratinocytes upon FGF7 treatment (see for example "additional information for reviewer 2" (second page), we also tested a STAT3 inhibitor. However, it only marginally affected the FGF7-mediated ISG suppression. Since we could not repeat this experiment because of the lab shut-down, we provide this information as "additional information for reviewer 2" (second page). Most importantly, however, we include new data demonstrating that the combined inhibition of Erk1/2 and PI3K signaling inhibited the effect of FGF7. These new and repeated data are now shown in Fig. EV2C.

We already showed in Fig. 7A that FGFR kinase inhibitors suppress the effect of FGF7 on HSV-1 replication. We now also tested the effect of these inhibitors on FGF7-induced ISG expression in primary murine keratinocytes and in human HaCaT keratinocytes, and it was indeed inhibited. These new data are now shown in Fig. EV2A and B.

2. How generally valid is this observation? The authors focus their study on keratinocytes. What about other cells in the body? This seems an important question, since the authors

propose FGFR inhibition as an exciting new antiviral strategies, also for viruses that do not only replicate in keratinocytes.

Our reply: In response to this comment we also tested the effect of FGF7 on Caco-2 colon cancer cells, which express the high affinity receptor for FGF7 (FGFR2b) (PMID 19326389). Indeed, FGF7 also suppressed the expression of ISGs in these cells. These data are now shown in Fig. EV1B. In addition, we also mention in the Discussion that treatment of primary human lung epithelial cells reduced the expression of some ISGs (PMID 11459923). Therefore, the effect is not specific for keratinocytes, but occurs at least in other epithelial cells that express FGFR2b.

Specific points:

1. SOCS1 rather than SOCS3 is the canonical inhibitor of IFNAR signaling. Is SOCS1 regulated by FGFR2?

We checked the effect of FGF7 on *SOCS1* expression and its expression was also suppressed. These data are now shown in Fig. EV1A.

2. In many figures, qPCR values are shown as relative mRNA levels. Relative to what? Please indicate in each case in the figure legend or the figure.

Our reply: The reference genes had been specified in Materials and Methods. We now added this information to the Legends. We further specify in each legend that the mean value of the control samples was set to 1, and the relevant control is mentioned in each legend.

3. There is also another concern about showing these relative mRNA levels. For example in Figure 2D, Ifr7 mRNA is relatively lower in IFNAR KO compared to wildtype. In Figure 2E, untreated wild type and IFNAR KO have the same mRNA level of Irf7 (around 1), and in both cases it goes down after FGF7 treatment. Showing all results in one figure would probably be preferable to avoid apparent inconsistencies between Fig 2D and 2E.

Our reply: We show these results separately, since the absolute expression levels in the knockout cells are of course much lower. Therefore, we would not be able to see the effect of FGF7 in a combined figure. However, we provide all raw data in Dataset EV2.
We would also like to point out that the mean expression level in non-treated WT cells in the left graph was set to 1 and in the other graphs the mean expression level in the non-treated IFNR knockout mice was set to 1. Therefore, the graphs show expression levels relative to non-treated control and not absolute expression levels. Thus, the results shown in Fig. 2D and 2E are not contradictory. We have further clarified this in the text and in the legend to this figure.

4. The figure legends are often un-sufficient. Length of treatments and concentration of reagents are often missing. Y axis description often missing. Authors should please go carefully through figures and legends.

Our reply: We had provided all concentrations in Materials and Methods, but we agree that the legends should also include this and additional information. Therefore, we extended the legends as requested. Y axis description has been included for all figures.

5. The panels of Figure 7 are partially overlapping and seem to be dislocated.

Our reply:
We apologize for this problem, which seems to have happened during the file conversion. The figure displayed correctly in the final pdf document that was checked before submission.

Referee #3 (Comments on Novelty/Model System for Author):

frankly i am not too confident about the therapeutic applications of these findings

Our reply: We show that FGF7 regulates replication of different viruses. We now further show that this effect is not restricted to keratinocytes. Therefore, our findings may well have therapeutic implications (as also acknowledged by the other reviewers). Nevertheless, we now discuss this more carefully (see last point of the reviewer).

Referee #3 (Remarks for Author):

This MS reports the finding that Mice with a KO of FGF receptors (FGFR) 1 and 2 in their skin have high levels of expression of IFN stimulated genes (ISG). Further investigation of this initial observation leads to the conclusion that FGF signaling inhibits the expression of ISGs in mouse and human keratinocytes and, as such, promotes the replication of HSV1 and other viruses in the same cells. This notion is supported by a variety of experiments using FGF7 treatment of Keratinocytes, by showing that ISG expression is stimulated by FGFR inhibitors, and that FGF signaling competes with IFN or Poly:IC treatment in regulating ISG expression. Finally it is shown that the replication of HSV1, LMC and zika viruses is promoted by FGF signaling by dampening the IFN response and that inhibition of FGF signaling adversely affects HSV1 replication.
The results presented here are quite convincing and the major conclusion of the MS, i.e. that FGF signaling antagonizes IFN signals is amply documented.

The major weakness of this report is however that it does not identify or even investigate the mechanisms responsible for the phenomenon described.

Our reply: We are aware that we have not fully explored the mechanism of this novel finding, in particular since we focused on the consequences of the FGF7-mediated suppression of IFN and ISGs for viral replication. Nevertheless, we already provide some insight into the mechanism: We show that this effect is downstream of the interferon receptors, that it occurs at the transcriptional level, and that it requires proteasomal activity. In response to the comments of reviewers 2 and 3 we performed additional experiments and we now provide further mechanistic insight (see below). However, we would like to point out that this is an entirely novel finding and it will clearly require extensive follow-up studies to further explore the molecular mechanisms.
To address the concern of the reviewer, we performed additional experiments and we now show that the effect of FGF7 is dependent on FGFR kinase activity. Furthermore, we show

that combined inhibition of Erk1/2 and PI3K abolishes the effect of FGF7 on ISG expression, while inhibition of other pathways had no effect. These new data are now shown in Fig. EV2.

Furthermore I noticed that, in most experiments testing the expression of the ISGs in response to FGF treatment (figs. 1,2,3,4, 6), the effect on the RNA levels of the genes are much stronger than the effect on protein levels, that in some cases show no difference.

Our reply: We repeated the Western blot experiments using triplicates from each condition and we quantified the data. These data are now shown in Fig. 3C and 4D. We agree that the effect on the RNA is often stronger than on the protein level, but this was also dependent on the time point. Nevertheless, we clearly observed significant effects of FGF7 on IRF1 and IRF9 in the basal state and a strong suppression of the poly(I:C)-induced STAT1 and STAT2 phosphorylation by FGF7.

Unfortunately the genes whose expression is determined at the RNA level and those whose protein expression is shown are often not the same, making difficult to ascertain how general is this phenomenon. This discrepancy has to be discussed or explained if this MS has to be accepted for publication.

Our reply: The ISG products that we show in the Western blots are also regulated at the transcriptional level by FGF7. Unfortunately, we could not analyze the protein levels of all ISGs that are regulated at the RNA level because of the insufficient quality of the antibodies. Nevertheless, we believe that it is sufficient to show a selection of ISG products at the protein level.

Two minor points: 1) it would be better to measure viral titers, in Infectious units, rather than DNA levels.

Our reply: We show viral titers in Fig. 7C and they strongly correlated with viral DNA and protein levels.

2)The Ms is extremely long and could be trimmed considerably. The discussion is rambling, never discusses mechanisms and some of the therapeutic implications could be toned down.

Our reply: As requested by the reviewer, we shortened the manuscript, included a discussion about mechanisms and toned down the potential therapeutic implications.
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17th Jun 2020

Dear Sabine,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

Please address the minor comments of referee 1 and address the statistical questions to the best of your ability. I'm not sure whether you will be able to add more primary cells analysis from a different donor, if you don't still make sure to indicate the number of donors in the legend and discuss the limitation of data as obtained from only 2 donors.

Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses to their comments (as Word file).

Please submit your revised manuscript within two weeks.

I look forward to reading a new revised version of your manuscript as soon as possible.

Yours sincerely,

Celine Carret

Celine Carret, PhD
Senior Editor
EMBO Molecular Medicine

*** Instructions to submit your revised manuscript ***

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When submitting your revised manuscript, please include:

1) a .docx formatted version of the manuscript text (including Figure legends and tables)

2) Separate figure files*

3) supplemental information as Expanded View and/or Appendix. Please carefully check the authors guidelines for formatting Expanded view and Appendix figures and tables at https://www.embopress.org/page/journal/17574684/authorguide#expandedview

4) a letter INCLUDING the reviewer's reports and your detailed responses to their comments (as Word
file).

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

the authors have confirmed the results in the human keratinocyte cell line HaCaT and have also conducted individual experiments in primary keratinocytes. In addition, functional assays were performed to confirm the biological relevance of their data.

Referee #1 (Remarks for Author):

Most of the concerns were well taken up by the authors. However, there are still some minor points that the authors should address:

1. Of course, there are cell type and species-specific differences in the regulation of ISG by FGF. But these differences are also of interest, especially for the assessment of a possible potential of FGF receptor inhibitors as therapeutic targets for viral infections. Even if no common panel of ISGs for mouse and human was measurerd/is shown, these differences should at least be mentioned and discussed in the manuscript.

2. In Figure 4B the graph showing the quantification of western blot analysis is missing.

3. Using primary human cells, the number of donors should be increased to get a robust power of your findings. The number of two different donors is too low. Furthermore, you should at least perform 3 independent experiments. Making statistics from only two donors even they were measured as triplicates has no power. The number of different donors (primary human keratinocytes) should be added to each figure legend.

Referee #2 (Remarks for Author):

The authors answered my questions and concerns in their point-to-point reply and improved the manuscript.

The authors performed the requested editorial changes.

Reviewer's comments

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Our reply: This has been done (see page 7, first paragraph). We would also like to point out that we verified the effect of FGF7 on almost all ISGs that we showed to be regulated in mouse keratinocytes also with human HaCaT keratinocytes. We just did not analyze all ISGs in all experiments, because the regulation of the different ISGs was so well reproducible. The only exception was *Oasl2*, because the human orthologue *OASL* is quite different with regard to sequence and function (Eskildsen et al., 2003). We also mention on page 7, last paragraph and page 8, first paragraph, that *RSAD2* was not expressed in Caco-2 cells and that *IRF7* expression was not regulated by FGF7. This shows the cell-type specific differences in ISG expression.

2. In Figure 4B the graph showing the quantification of western blot analysis is missing.

Our reply: The graph was/is shown in Figure EV3 as mentioned in the legend to Fig. 4B.

3. Using primary human cells, the number of donors should be increased to get a robust power of your findings. The number of two different donors is too low. Furthermore, you should at least perform 3 independent experiments. Making statistics from only two donors even they were measured as triplicates has no power.

We agree that only two donors were used in the experiments shown in Fig. 3D and 5E, but the data points obtained with both donors clustered very well in both cases. The two donors were analyzed in independent experiments and therefore, the results were well reproducible. Furthermore, the human primary keratinocytes used in the experiments shown in Fig. 3D and 5E were from different donors. We now mention the limitation in the Results (page 10, first paragraph) and we also mention the number of donors in the legends (this was already done in the previous version for the results shown in Fig. 3D). We would like to point out that the data were also reproduced with primary mouse keratinocytes and with HaCaT keratinocytes and they were all consistent. Finally, we used primary

keratinocytes from a third donor and showed that poly(I:C)-induced IFN expression is suppressed by FGF7 (so far performed with cells from one donor, and therefore not included in the manuscript). We show this result below for the information of the reviewer.

Figure for reviewers removed

It was unfortunately not possible to obtain cells from a third donor for a third repetition within the 2-week time frame that was given for the revision, but we believe that the results described above clearly show the reproducibility of the FGF7 effect with different types of keratinocytes and with cells from different donors.

The number of different donors (primary human keratinocytes) should be added to each figure legend.

Our reply: The number of donors had already been mentioned in the legend to Fig. 3D. We now included this information in the legend to Fig. 5E.

Referee #2 (Remarks for Author):

The authors answered my questions and concerns in their point-to-point reply and improved the manuscript.
ms and turned down the potential therapeutic implications.

Our reply: We thank the reviewer for his/her positive comments.

30th Jun 2020

Dear Sabine,

Thank you for amending the article. We are very pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

Please read below for additional IMPORTANT information regarding your article, its publication and the production process.

Congratulations on your interesting work and all the best,

Celine

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Senior Editor
EMBO Molecular Medicine

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ⬇

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Luigi Maddaluno and Sabine Werner
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2019-11793

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures
1. Data
The data shown in figures should satisfy the following conditions:

➜ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
➜ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
➜ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
➜ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
➜ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

➜ a specification of the experimental system investigated (eg cell line, species name).
➜ the assay(s) and method(s) used to carry out the reported observations and measurements
➜ an explicit mention of the biological and chemical entity(ies) that are being measured.
➜ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

➜ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
➜ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
➜ a statement of how many times the experiment shown was independently replicated in the laboratory.
➜ definitions of statistical methods and measures:
  • common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  • are tests one-sided or two-sided?
  • are there adjustments for multiple comparisons?
  • exact statistical test results, e.g., P values = x but not P values < x;
  • definition of 'center values' as median or average;
  • definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ⬇ (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was estimated based on previous experience from us and from others, who perform similar types of experiments. Experiments were repeated with more samples when the result was close to statistical significance.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Sample size was estimated based on previous experience from us and from others, who perform similar types of experiments.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals/samples were excluded from the analysis
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Epidermal sheets and cell cultures were randomly assigned to different treatment groups. For the animal experiments we compared mice of two genotypes and all available mice of the correct age and genotype were used for the virus infection experiments.
For animal studies, include a statement about randomization even if no randomization was used.	See above
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Analysis of stained skin sections or epidermal sheets was performed blinded by the investigators. Group allocation of the mutant mice could not be performed blinded, because the phenotype of the K5-R1/R2 mice is obvious macroscopically and histologically.
4.b. For animal studies, include a statement about blinding even if no blinding was done	See above
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Most of the data were not normally distributed and therefore, we used Mann-Whitney test for comparisons in such cases
Is there an estimate of variation within each group of data?	yes

Is the variance similar between the groups that are being statistically compared?	yes - For comparison of more than two groups we used Brown-Forsythe test to verify equal variance between groups

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Catalogue numbers and/or clone numbers are provided.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HaCaT cells were directly obtained from the owner, Prof. Petra Boukamp, DKFZ Heidelberg. They are routinely authenticated there. All cells were regularly tested for mycoplasma contamination

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	This is specified in Materials and Methods. The age of the animals is also specified in the Results section and in the Figure Legends.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	This statement is included in Materials and Methods (all animal experiments were approved by the local authorities (Kantonales Veterinäramt Zürich)).
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	All animal experiments were performed according to Swiss Law and approved after in-depth review and approval by the Kantonales Veterinäramt Zürich.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Not applicable
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not applicable
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Not applicable
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not applicable
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not applicable
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not applicable

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	This section has been included: The microarray data have been deposited in NCBI's Gene Expression Omnibus and are accessible via the following link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111274. All original Western blots and other original data (including exact P-values) are available in Dataset EV2.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	The microarray data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE111274 (reviewer token: olcncgyqvpihdkr). All original Western blots and other original data (including exact P-values) are available in Dataset EV2.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	Not appicable
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Not appicable

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	Not applicable