Author's response to reviews

**Title:** DEK is a potential marker for aggressive phenotype and irinotecan-based therapy response in metastatic colorectal cancer

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**Author's response to reviews:** see over
Dear Editor,

Thank you very much for your letter and comments on our manuscript (1245482012146008).

We do appreciate the suggestions made by the reviewers for the improvement of the manuscript; all comments have been taken into consideration and properly answered following each query. We hope that after this revision it will be suitable for reevaluation and publication in BMC Cancer. The manuscript has been carefully reviewed following the “Guidelines for Submission of Manuscripts”.

Thanks for the opportunity to consider our work potentially acceptable for publication in BMC Cancer. I am looking forward to hearing from you soon.

Sincerely,

Dr. Jesús García-Foncillas
Responses to comments of Reviewers

We are pleased to know that the reviewers find this study potentially interesting. After revising the manuscript, we agree with the reviewers that there are some points in the manuscript that needed to be clarified.

Referee #1 (Comments to the Author):

Comments: Overall, this is a sound piece of research, which may potentially suggest DEK overexpression as a crucial event for the emergence of an aggressive phenotype in colorectal cancer and its potential role as biomarker for irinotecan response in those patients with KRAS wild-type status. However, there are minor points where the manuscript needs to be revised before it will be ready for a publication.

1. Your results from patient samples and cell line clearly show that increased DEK in colorectal cancer promotes cell proliferation. However, the conclusion that “Knock-down of DEK on DLD1 and SW620 cell lines decreased cell migration and invasion” is not fully supported by your results. Similarly, for the correlation between the abnormal DEK expression and irinotecan induced apoptosis in colorectal cancer cell lines, the authors only showed the IHC results. It’s not enough to verify it. I suggest to do the Western blot for some apoptosis related proteins, for example Caspase 3/8/9.

Effectively, as the reviewer has commented, our results only show cell migration but not invasion. Boyden chamber assay was performed with FBS as chemotactant. We appreciate this suggestion and we have modified the text accordingly throughout the manuscript.

Following the suggestion of the reviewer, we have determined cleaved Caspase 3 after DEK downregulation in our CRC cell lines. Since cleaved Caspase 3 is an executioner factor, we have analyzed it by Western Blot to compare its expression between control and siDEK cells. This result is shown in Figure 4B and correlates with the published results by Lin et al. 2014 where cleaved Caspase 3 increased after DEK downregulation.

After modification of Figure 4, representative IHC images and survival analysis are shown in a new Figure (Figure 5).

2. Please confirm the DEK antibody dilution for IHC staining [1:5000 dilution of DEK antibody (610948, BD Biosciences)--Page 8, Line 15]. According to our previous study, the dilution is 1:50~100. Additionally, a positive control is needed for the IHC staining of DEK in paraffin-embedded tissues.

We appreciate this reviewer suggestion and we admit that it is a type mistake and the dilution is 1:50.

Our positive control for IHC was FFPE preparation of the CRC cell line SW620 in which the highest DEK expression was previously confirmed by Western Blot (see below).

We do appreciate this remark, because our results show that silencing DEK induced apoptosis through cleaved Caspase 3, and decreased cell viability. These results are in accordance to those described by Lin et al. 2014. The reference has been included and properly argued in the discussion section.

4. "KRAS and BRAF mutation status have achieved significant recent success associated to anti-EGFR antibody therapy. For the commonly used drugs, 5-FU, irinotecan, and oxaliplatin, markers of efficacy have not reached enough clinical usefulness" (Page 14, Line 11~13) are not the conclusion of this manuscript, please cancel it.

We are grateful for this comment and we absolutely agree with reviewer suggestion. This paragraph has been eliminated.

Referee #3 (Comments to the Author):

The authors described the roles of DEK as a crucial event for the emergence of an aggressive phenotype in colorectal cancer and its potential role as biomarker for irinotecan response in those patients with KRAS wild-type status. This is an interesting study which could impact the currently inadequate prognostic
biomarkers for metastatic CRC. The experiments are thorough and generally well-done. However, the following needs to revise before publication:

1. The authors concluded that “Knock-down of DEK on DLD1 and SW620 cell lines decreased cell migration and invasion”, however, the results are not enough to support the results. It's better to verify the relationship between DEK expression level and EMT.

We appreciate this suggestion of the reviewer. As we have previously answered to Reviewer 1, the experiment using Boyden Chamber assay was carried out with FBS as chemoattractant. So effectively, the obtained results only show the effect on cell migration.

2. The authors verified the relationship between DEK expression level and apoptosis by using IHC result for DEK protein staining. I suggest that it’s better to do Western blot for Caspases.

According to reviewer suggestion, we have analyzed Caspase 3 by Western Blot. This result has been included as Figure 4B. siDEK increases cleaved Caspase 3 levels in both cell lines. This result is in agreement with the results recently published by Lin et al. 2014. The reference has been included in the text.

After modification of Figure 4, representative IHC images and survival analysis are shown in a new Figure (Figure 5).

3. Could you please confirm the DEK antibody dilution for IHC staining on page 8?

We appreciate this reviewer comment and we admit that is a typo error and real dilution is 1:50.

We hope that these additional explanations and changes have improved the manuscript and now it may be suitable for publication in BMC Cancer.