

ANSWERING REVIEWERS

June, 20th, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: manuscript_revised.docx). We appreciate the helpful comments and modified the manuscript according to the reviewer's suggestions. Modifications in the text are marked by applying the track-changes option of Microsoft Word.

Title: Establishment, functional and genetic characterization of ultra-low passage cell lines from early and late stage chromosomal instable colorectal cancer

Author: Claudia Maletzki, Michael Gock, Martin Randow, Ernst Klar, Maja Huehns, Friedrich Prall, Michael Linnebacher

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11466

The manuscript has been improved according to the suggestions of reviewers:

Response to reviewer 1:

1. The reviewer remarked the missing information regarding potential treatment of patients either by surgery or chemotherapy prior to surgical resection. None of the patients received any kind of pretreatment. We thank the reviewer for this helpful comment and very likely included the missing information in the text (material & methods section, page 6).
2. Next, we were asked to include data on the manufactures of reagents and kits employed throughout this study. Here again, we thank the reviewer for this kind hint and incorporated missing data in the text body of the manuscript.
3. Finally, the reviewer wanted to know how statistical analysis on in vitro chemosensitivity analysis was done. We used the Sigmaplot 12.5 software for determining dose-responses. This information has been included as a subsection at the end the material and methods part ("Statistics", page 11).

Response to reviewer 2:

1. Firstly, the reviewer asked about a rationale for applying "HLA typing" and "wound healing" assays in this study and possible conclusions drawn from obtained results. Both methods were included into a panel of methods for comprehensively characterizing our novel tumor models and to provide well-characterized tumor material associated with the CIN⁺ phenotype for follow-up studies: HLA typing of tumor cells was done for analyses focusing more on immunological strategies. For subsequent development of immunotherapeutic approaches, aiming at identification of immunogenic epitopes from novel candidate antigens, detailed knowledge on HLA-status is crucial. The wound healing assay is a well-developed method to analyze directional cell migration and interaction *in vitro* and to give an idea on *in vivo* growth behavior. This assay is particularly

suitable for studying the regulation of cell migration by cell interaction with the extracellular matrix (ECM). Hence, this is a great advantage compared to other methods, like the Boyden chamber assay, in which cell preparation before the assays disrupts cell-cell and cell-ECM interactions. Nonetheless, we thank the reviewer for this kind advice and included some information on the meaning of obtained results in the discussion part (pages 17/18).

2. Secondly, the reviewer wished to describe the method and materials used for cell line establishment in more detail. This information has been incorporated in the main text (pages 6).

Response to reviewer 3:

1. We were asked to describe the practical benefits and potential limitations of cell lines from xenograft of individual patients more clearly in the discussion. This has been done accordingly (pages 16/17).
2. Lastly, the reviewer wished to include criteria of drug selection for predicting response and to provide appropriate references in the methods. Likewise, this information has been incorporated in the main text (page 10).

We are very confident that the enhanced version now matches the requirements for publication in the *World Journal of Gastroenterology*.

In the name of all authors,

Sincerely yours,



Michael Linnebacher