Author's response to reviews

Title: FOXP1 inhibits cell growth and reduces tumorigenicity of neuroblastoma

Authors:

Sandra Ackermann (sandra.ackermann@uk-koeln.de)
Hayriye Kocak (hayriye.kocak@uk-koeln.de)
Barbara Hero (barbara.hero@uk-koeln.de)
Volker Ehemann (Volker.Ehemann@med.uni-heidelberg.de)
Yvonne Kahlert (yvonne.kahlert@uk-koeln.de)
André Oberthür (andre.oberthuer@uk-koeln.de)
Frederik Roels (frederik.roels@uk-koeln.de)
Jessica Theißen (jessica.theissen@uk-koeln.de)
Margarete Odenthal (m.odenthal@uni-koeln.de)
Frank Berthold (frank.berthold@uk-koeln.de)
Matthias Fischer (matthias.fischer@uk-koeln.de)

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Author's response to reviews: see over
Dear Dr. Rao,

thank you for giving us the opportunity to re-submit our manuscript “FOXP1 inhibits cell growth and reduces tumorigenicity of neuroblastoma” (MS: 5908954131316238) to BMC Cancer. We appreciate the comments of the two reviewers and have addressed all the issues raised by the reviewers point by point as indicated below and in our manuscript.

In detail, the following changes in the manuscript have been made to address the issues raised by the reviewers:

1. Analysis of FOXP1 target genes:
To gain insights into the molecular processes occurring upon FOXP1 re-expression, as suggested by both reviewers, we performed series of time-resolved gene expression measurements in FOXP1 transgenic neuroblastoma cells. Microarray expression profiles of IMR-32, CHP-212 and SK-N-BE(2) cells were generated 0, 12, 24 and 72 hours after transgene induction and gene set enrichment analyses were performed. In line with the results of the in vitro analyses, we found a significant up-regulation of pro-apoptotic genes such as DIABLO, CDC42 and DAPK1 in FoxP1-induced IMR-32 and CHP-212, but not in the p53 mutant cell line SK-N-BE(2). The results of this gene set enrichment analysis have been added to the results section and are presented in the new Figure 7. Furthermore, we observed a significant down-
regulation of cell motility-associated genes such as MAPK12, PLCG2 and NCF4 in all three FoxP1 transgenic neuroblastoma cell lines analyzed, thus confirming the inhibition of cell migration in the in vitro analyses. The results of this gene set enrichment analysis have been added to the results section and are presented in the new Figure 8.

2. Analysis of proliferation:
According to the suggestion of reviewer #2, we performed additional experiments to validate the results obtained in the Trypan Blue cell viability assay. Cell proliferation of IMR-32, CHP-212 and SK-N-BE(2) cells was measured six days after FOXP1 induction using the Alamar Blue assay. Consistent with the results of the Trypan Blue assay, we observed a significant reduction of cell proliferation in all three cell lines, confirming the anti-proliferative effect of FoxP1 in neuroblastoma cells. These results have been added to the results section and are presented in Figure 5b.

Thank you very much for your kind consideration of our work. I hope that the revised manuscript is now acceptable for publication in BMC Cancer.

We look forward to hearing from you.

Sincerely,

Sandra Ackermann, Ph.D.
University Children’s Hospital of Cologne
Department of Pediatric Oncology
E-mail: sandra.ackermann@uk-koeln.de