Author's response to reviews

Title: IL6 secreted by Ewing Sarcoma tumor microenvironment confers anti-apoptotic and cell-disseminating paracrine responses in Ewing Sarcoma cells

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Dear Editor,

thank you for the detailed and positive review of our manuscript. You will find the answers to the reviewers' questions on a point-by-point response below.

As a consequence to the reviewers' suggestions we have performed additional experiments such as RT-PCR and flow cytometry for the detection of IL6 in now all Ewing cell lines used, as well as additional immunohistochemical stains in Ewing tumors, and testing of additional serum samples for IL6. These additional experiments strengthen the main message of the paper that IL6 is produced by tumor fibroblasts in Ewing Tumors and that Ewing sarcoma cells have an intact IL6 signaling pathway which inhibits apoptosis and promotes cell dissemination.

The major part of these additional experiments has been performed by Dr. Anna Makowska and therefore we would like to add her to the list of coauthors.

We appreciate your suggestion that the paper could be strengthened by the addition of mouse experiments. Since there is no syngeneic mouse-ES model, animal studies are conducted in xenograft models. The xenograft model would have - even if applying coculture of Ewing cells with MSC or TAF – limitations in analyzing the role of tumor microenvironment due to possible invasion of mouse fibroblasts and mouse endothelial cells. The effects of mouse cytokines, especially mouse IL6, on human ES cells have not been well described and treatment of animals with rhIL6 would be necessary. Taking this into account we decided for the CAM assay in order to analyze for migration and invasion of ES tumor cells. Our experiments describing the biological effects of IL6 on Ewing
sarcoma cells are conducted with externally added recombinant IL6. This might mimic the effects of IL-6 from an external source like the tumor-microenvironment. We have stained tissue fibroblasts in ES primary tumors and show that IL6 is mainly found in the stromal septa but not tumor cells strongly suggesting that a possible biological effect of IL6 in Ewing tumors is mediated via paracrine action. We therefore agree with you that autocrine action in ES is of minor relevance.

Yours sincerely

Udo Kontny, M.D.