FOXM1 as chemo-sensitizing target in neuroendocrine lung tumors.

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Background:
Bronchopulmonary neuroendocrine neoplasms (BP-NEN) represent a heterogeneous subgroup among lung tumors. Due to their clinical and molecular characteristics BP-NEN are grouped into low-grade typical carcinoids (TC), intermediate-grade atypical carcinoids (AC), and high-grade large-cell neuroendocrine lung carcinomas (LCNEC) and small-cell lung carcinomas (SCLC). For the highly malignant carcinomas combined chemotherapy is the standard therapy option, but shows its limitations in recurring tumors [1]. For advanced carcinosarcoma tumors somatostatin-analogues are first-line treatment, since they are often insensitive to chemotherapy [2]. FOXM1 is a typical proliferation-associated transcription factor and is involved in cell cycle control and DNA damage response. FOXM1 has been implicated in tumor progression and is therefore a promising target in many cancer types [3]. Additionally, inhibition of FOXM1 has been shown to enhance sensitivity to chemotherapy [4].

Aim:
To investigate the role of FOXM1 in BP-NEN, it was analyzed whether inhibition of this transcription factor, either by DNA interference or proteasome inhibition, has a chemosensitizing effect. Proteasome inhibitors have been shown to target FOXM1 either directly or indirectly [5]. Here, the clinically relevant immunomodulator bortezomib was used, since it has been proven promising in SCLC [6].

Material and Methods:
BP-NEN cell lines (NCI-H7777/TC, NCI-H810/LCNEC, NCI-
H69/SCLC) and the non-neuroendocrine NSCLC cell line A549 were transfected with siRNA against FOXM1 or control siRNA and subsequently treated with cisplatin. Additionally, the same cell lines were treated with bortezomib, cisplatin or a combination of both, and analyzed via cell proliferation assay (WST-1 cell proliferation assay, Roche). Molecular effects of FOXM1 knockdown as well as the inhibitors bortezomib and cisplatin were investigated by flow cytometry (cell cycle: propidium iodide staining for DNA content and phospho-Histone H3 for mitotic index, apoptosis: JC-1 assay for detection of mitochondrial membrane polarization), western blot and multiplex gene expression assay (nCounter® PanCancer pathway panel, Nanostring technologies, T70 genes).

Results:
Bortezomib is highly effective in BP-NEN cell lines and exerts synergistic effects with cisplatin (figure 1). It induces G2 arrest, and in combination with cisplatin a slight shift to apoptosis is seen in NCI-H7777 and NCI-H810 (figure 2). NCI-H69 cells show no induction of apoptosis after knockdown of FOXM1, whereas the NSCLC (wild-type p53) cell line is highly apoptotic (figure 3). The DNA-damaging agent cisplatin leads to increased DNA repair mechanisms, which are decreased after inhibition of FOXM1 (figures 4 and 5).

Conclusion:
Inhibition of FOXM1 sensitizes BP-NEN cell lines to apoptosis induced by DNA damaging agents. The described effects are much stronger in the p53 wild-type cell line A549, whereas BP-NEN are often p53 mutated. It might be beneficial to improve the mode of application, since cisplatin exerts its main effects in G1 and S phase, which might be counteracted by the increased G2 arrest after inhibition of FOXM1. An application route with induction of DNA damage and S phase arrest before DNA repair is impaired by inhibition of FOXM1, might increase the apoptotic response. Nevertheless, inhibition of FOXM1 is a promising approach to re-sensitize BP-NEN to chemotherapy with cisplatin, which is especially relevant in high-grade BP carcinomas. In this context, bortezomib seems a promising clinically relevant agent to facilitate FOXM1 inhibition.

References: