The aldehyde hydrogenase inhibitor Disulfiram (Antabuse®) reverses stem cell and EMT features of HNSCC-lines

Wenhai Yao¹, Sebastian Ochsenreither², Veit M. Hofmann¹, Andreas M. Kaufmann³, Andreas E. Albers¹

¹Otorhinolaryngology, Head&Neck Surgery, ²Department of Hematology and Oncology, ³Clinic for Gynecology - Charité-Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt Universität zu Berlin, und Berlin Institute of Health, Campus Benjamin Franklin, 12200 Berlin, Germany

Background
Radio-chemo resistance leading to disease relapse is one major challenge to improve outcome in squamous cell cancer of the head and neck (HNSCC). Cancer stem cells (CSCs) are increasingly being implicated in therapy-resistance and cancer recurrence. Conventional radio-chemo therapy is targeting proliferating cancer cells, however, the undifferentiated population of CSCs are often unaffected and retain their self-renewal properties. We have evaluated Disulfiram (DSF) for its efficacy to inhibit CSCs in HNSCC cell lines and the correlation between CSC-features and drug resistance.

Objective
To explore the inhibitory effect of Disulfiram (DSF) and DSF/Cu²⁺ on cancer stem cells in HNSCC cell lines.

Methods
Three HNSCC cell lines were used (UM-SCC9, UM-SCC47, UM-SCC11B). Spheroids were generated from adherently growing cell lines by forced anchorage-independent growth, then they were collected and treated with DSF and DSF/Cu²⁺. Cell viability was assessed using MTT-Assay. ALDH activity was determined by ALDEFLUOR and FACS-sorting. Expression of stemness-related transcription factors Sox2, Oct3/4 and Nanog was quantified by real-time PCR, cell self-renewal by sphere- and colony-formation assay, and migration by wound healing assay and E-Cadherin expression.

Results
We demonstrate that the ALDH⁺⁰ cell population show resistance to Cisplatin but are sensitive to DSF and the DSF/Cu²⁺ complex. DSF and DSF/Cu²⁺ synergistically enhanced cytotoxicity of Cisplatin in ALDH⁺⁰ cells (Fig. 1). Our results also show that the levels of three stemness-related nuclear transcription factors (TF), Sox2, Oct3/4 and Nanog, were higher in SDCs than MDCs (Fig. 2). The mRNA levels of these TF in all three HNSCC cell lines were significantly decreased in SDCs after DSF and DSF/Cu²⁺ treatment (Fig. 3). Moreover, DSF and DSF/Cu²⁺ treatment significantly reduced the capacity for colony- (Fig. 4) and sphere-formation and migration (covered area after 18h: control: 88.82%, DSF/Cu²⁺: 23.77%, Fig. 6). DSF and DSF/Cu²⁺ also induced E-Cadherin expression (control: 50%, DSF/Cu²⁺: 75.1%, Fig. 7) as well.

Conclusion
Our data demonstrate that DSF and DSF/Cu²⁺ block ALDH enzymatic function, and are able to inhibit CSC- and EMT-properties in HNSCC cell lines. Moreover, DSF and DSF/Cu²⁺ enhance cytotoxicity and thereby may allow perspectives a reduction of Cisplatin or increased effectiveness of current standard radio-chemo therapy regimen in HNSCC. Inhibition of EMT and migration may lead to a reduction of metastasis formation.