Chao Rong
Dr. sc. Hum

Estrogen receptor-related signaling networks as prognostic biomarkers and therapeutic targets in head and neck squamous cell carcinoma

Fach/Einrichtung: Hals-Nasen-Ohrenheilkunde
Doktorvater: Prof. Dr. rer. nat. Jochen Hess

Head and neck squamous cell carcinoma (HNSCC) is one of most common human malignancies worldwide with high tumor-related morbidity and mortality. Unraveling molecular mechanism of treatment resistance and identifying new biomarkers for HNSCC patients at high risk for treatment failure is urgently needed. Previous data have provided an experimental evidence that a subpopulation of radiotherapy resistant tumor cells reveals co-expression of estrogen receptor 2 (ESR2) and submaxillary gland androgen regulated protein 3A (SMR3A) after fractionated irradiation (IR). In first part of the present study, ESR2 expression was assessed by immunohistochemistry (IHC) staining on tissue microarrays (TMAs) containing tumor specimens of OPSCC patients, which were treated with either definitive or post-surgical radiotherapy with or without adjuvant chemotherapy. Statistical analysis revealed that a subgroup of patients with positive ESR-2 and high SMR3A had an unfavorable clinical outcome as compared to those with low SMR3A expression. Furthermore, a new cell culture model of fractionated irradiation provided compelling experimental evidence for the existence and expansion of a subpopulation of radioresistant tumor cells, which were characterized by ESR2, opiorphin genes (SMR3A, OPRPN) expression. Nevertheless, the protein expression of OPRPN, another member of the opiorphin gene family, and clinical outcome of patients with OPSCC were not significantly correlated. Combined expression of ESR2 and OPRPN or SMR3A was associated with an unfavorable clinical prognosis post definitive or adjuvant radiotherapy, suggesting that opiorphin-related genes serve as surrogate markers for HNSCC cells with intrinsic radioresistance. Treatment with 4-hydroxytamoxifen or fulvestrant, two well-established antagonists of estrogen receptor signaling, increased the sensitivity of HNSCC cell lines upon fractionated irradiation in vitro. These data provide a strong evidence in which evaluation of ESR2 and opiorphin gene co-expression in primary tumor samples supports the identification of HNSCC patients with a higher risk for treatment failure after radiotherapy, who might benefit from an adjuvant treatment with antagonists of estrogen receptor signaling.

In this study, several cell culture models have been established to address the impact of drugs targeting the EGFR-MEK-MAPK pathway (cetuximab and MEK1/2 inhibitor) on tumor cell survival and clonal expansion as well as expression of ESR2 and immune checkpoint inhibitor PD-L1. A

concentration-dependent induction of ESR2 was detected in FaDu cells after long-term treatment with cetuximab. Blocking ERK signaling, one of the downstream pathways of EGFR activation, can activate ERS2 signaling in FaDu cell under normal growth condition. These data provide the evidence that ESR2 interacts with the EGFR signaling pathway as alternate signaling mechanisms. The impact of cetuximab on PD-L1 after short or long-term inhibition has been clarified, which tends to be heterogenous but independent of ERK phosphorylation. The majority of HNSCC cell lines with long-term treatment regain expression of ERK phosphorylation as compared to short-term, and up-regulation of PD-L1 was observed in the most resistant cell line with long-term treatment. These results provide a proof-of-concept that up-regulation of PD-L1 might be a potential mechanism of resistance to cetuximab treatment in HNSCC, suggesting potential benefits of combining cetuximab with immunotherapy. Moreover, the experimental data not only reveal that PD-901, a MEK1/2 inhibitor, increases the radiosensitivity of HNSCC cell lines, providing potential clinical benefit from combination treatment of MEK1/2 inhibitor and radiotherapy but also confirmed that PD-L1 regulation in HNSCC cell lines is mainly independent of MEK-ERK signaling. Taken together, these novel findings suggest a complex and context-dependent regulation of ESR2 as well as PD-L1 upon inhibition of the EGFR-MEK-MAPK signaling cascade, may provide potential molecular targets for HNSCC treatment in the future.