André Nollert

Dr. med.

Characterisation of molecular mechanisms leading to treatment failure of squamous cell carcinoma of the head and neck

Hals-Nasen-Ohrenheilkunde

Prof. rer. nat. Jochen Hess

An inverse correlation between gene promoter methylation and expression of the aldehyde dehydrogenase 1 subfamily A2 (ALDH1A2), which encodes the rate-limiting enzyme in the cellular synthesis of the vitamin A metabolite retinoic acid (RA), has been identified as a common feature of oropharyngeal squamous cell carcinoma (OPSCC). Moreover, low ALDH1A2 expression was associated with an unfavorable prognosis of OPSCC patients. However, the causal link between reduced ALDH1A2 function and characteristic features of cancer cells, which cause treatment failure has not been addressed so far.

The impact of ALDH1A2-RAR signaling on tumor-relevant processes was investigated in established tumor cell lines in vitro and in an orthotopic mouse xenograft model in vivo. These studies revealed that inhibition of ALDH1A2-RAR signaling induced a mesenchymal-like phenotype, which was characterized by prominent vimentin expression. Prolonged inhibition of ALDH1A2-RAR signaling in FaDu *cells* resulted in anoikis resistance and tumorsphere formation, resembling characteristic traits of recurrent tumor initiating cells. Re-attachment of tumorspheres after withdrawel of ALDH1A2-RAR inhibitors or administration of retinoids demonstrated the revesible nature of tumorsphere formation, and supported the assumption that a retinoid based therapy might be beneficial for HNSCC with impaired ALDH1A2 expression and/or function.

The mesenchymal-like phenotype and tumorsphere formation after inhibition of ALDH1A2-RAR signaling were accompanied by reduced expression of the Kallikrein-related peptidase 6

(KLK6), which was previously shown to serve as a key regulator of cancer cell plasticity and prognostic biomarker for HNSCC patients. Vice versa loss of ALDH1A2 protein levels were found in FaDu cells after silencing of KLK6 expression (FaDu-shKLK6). These data provide experimental evidence for the existence of a more complex ALDH1A2-RAR-KLK6 cascade, which is under the control of feedback regulation.

Reduced ALDH1A2 expression, accelerated tumor growth and an EMT-like phenotype of FaDu-shKLK6 cells as compared to mock controls were confirmed in an orthotopic mouse xenograft model.

In summary, presented data of this work implicate that a subgroup of HNSCC patients at high risk for treatment failure under currently established therapeutic regimens might benefit from an adjuvant treatment with retinoids to restore ALDH1A2-RAR signaling in tumor cells.

In line with the concept of personalized oncology, expression of the Kallikrein-related peptidase 6 (KLK6) in an ALDH1A2/KLK6-negative patient cohort, which is under the control of feedback regulation, could serve as a predictor of retinoid-based therapy response.

Furthermore, elucidation of the mechanisms and functional interactions between ALDH1A2-RA- KLK6 signaling and other signaling cascades, promoting EMT and metastasis, is essential in order to gain a better understanding of the molecular effects leading to therapeutic resistance of tumors of the head and neck.