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The Role and Regulation of Ryanodine Receptor 2 in the Pathogenesis of Head and Neck Squamous Cell Carcinoma

Fach/Einrichtung: Hals-Nasen-Ohrenheilkunde

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In an integrated omics approach, based on whole-exome sequencing and global DNA methylation array data of tumor samples from the HIPO-HNC cohort, loss of RYR2 expression and/or function by somatic mutation and DNA hypermethylation was identified as a common event in head and neck squamous cell carcinoma (HNSCC). The RYR2 gene, encoding for the endoplasmic calcium-channel ryanodine receptor 2 (RYR2), showed frequent somatic mutations and high promoter methylation, latter one preferentially found in HPV-related tumors. Immunohistochemical staining of tissue sections from HIPO-HNC and an independent validation cohort revealed a gradual loss of RYR2 expression from normal mucosa via dysplastic lesion to HNSCC in line with the dedifferentiation of the transformed cells. 80% of all HNSCC samples showed low or no RYR2 expression whereas 20% kept high protein levels throughout malignant transformation and progression. Reduced RYR2 expression in the adjacent tissue of HNSCC was associated with a reduced progression-free survival. Heterogenous RYR2 expression was also confirmed for HNSCC cell lines *in vitro*. Protein and transcript level were inversely correlated with variable promoter methylation, evident in HNSCC cell lines and in datasets from TCGA-HNC. More epithelial-like cells demonstrated weaker staining with only a subset of RYR2-positive cells, while mesenchymal-like cells showed abundant basal RYR2 levels. Treatment with the DNA methyltransferase inhibitor Decitabine partially restored RYR2 expression in cell lines with silenced hypermethylated promoter region, which was accompanied by an increase of protein and transcript level. Functionality of RYR2 was proven with SCC4 cells by calcium measurements after stimulation and inhibition of RYR2 with Caffeine, 4-chloro-m-cresol and ryanodine. Treatment with the potent RYR2 agonist Caffeine impaired clonal expansion of all HNSCC cell lines. A higher basal RYR2 level was associated with a greater response, represented by a significant decrease of colony number already at low concentrations and overall lower relative survival fraction in a

colony-forming assay. Moreover, Caffeine treatment could sensitize all HNSCC cell lines to fractionated irradiation. A scratch wounding assay with FaDu and Cal27 cells established a potential link between cellular motility and RYR2 expression. Investigation of the genetically modified cell line FaDu-shKLK6 not only confirmed a correlation between RYR2 and epithelial to mesenchymal transition (EMT), but also revealed a decrease in RYR2 promoter methylation during EMT. Treatment of these cells with Blebbistatin and Y-27632, both impairing actin-myosin contractility, not only restored an epithelial phenotype, but significantly reduced RYR2 protein level while RNA level remained unchanged.

In summary, the data of this thesis strongly suggest that impaired RYR2 function by either somatic mutation or epigenetic silencing is a common event in the pathogenesis of HNSCC, whereas elevated RYR2 levels in advanced HNSCC are associated with EMT. Detection of RYR2 expression or gene promoter methylation might serve as an early biomarker for risk assessment of malignant conversion of dysplastic lesions or as indicator for adequate treatment decision in advanced HNSCC. Reactivation and stimulation of RYR2 was shown to be possible. If this is also viable, considering the potential link to EMT, needs to be clarified. Future experiments need to focus on context dependency of RYR2 by addressing two major topics: the role of RYR2 in normal healthy keratinocytes, e.g. in wound healing, on the one side and the potential involvement of RYR2 in EMT on the other. To underline the validity of the hypothesis above, larger collectives of patient data and more complex preclinical models need to be investigated.