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Autophagy is Involved in HDAC6 Mediated Ciliary Loss, and Increases Malignancy in Cholangiocarcinoma Models

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Primary cilia are cellular organelles involved in different signaling pathways. Its malfunction has been linked with diseases. The reduced expression of cilia has been reported in different tumors, including cholangiocarcinoma, and experimental ciliary loss in cultured normal cholangiocytes induces a malignant phenotype. HDAC6 is involved in the process of ciliary disassembly, and its inhibition in tumor cells attenuates malignancy, but the mechanisms are unknown. We hypothesize that autophagy may be related to cilia disassembly.

Therefore, we performed electron-microscopy on CCA patient's tissues, and immunofluorescence on normal and tumor cell lines to visualize autophagosomes and ciliary components. CCA patient samples and cell lines showed increased number of autophagosomes compared to normal, and colocalization with ciliary components were observed. Western blot analysis showed that LC3 levels in CCA cell lines were increased after inhibition of HDAC6 suggesting an inhibition of the autophagic flux. Ciliary frequency increased after treatment with the autophagy inhibitor chloroquine, ACY-1215 (HDAC6 inhibitor) or the combination of both inhibitors as measured with immunofluorescence using specific antibodies for cilia, correlating with decreased proliferation. Furthermore, ciliary lengths were measured after siRNA inhibition of different autophagy cargo proteins, CALOCOCO2 and NBR1, and increased lengths were observed. Finally, treatment with chloroquine decreased tumor growth in a rat ortothopic model of CCA.

This results suggest that ciliary disassembly is mediated by an HDAC6-regulated autophagic process, i.e ciliophagy. The inhibition of ciliophagy may decrease malignancy in cholangiocarcinoma and may be further investigated as a potential therapeutic approach.