The histone acetyltransferase hMOF is frequently downregulated in primary breast carcinoma and medulloblastoma and constitutes a biomarker for clinical outcome in medulloblastoma

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Loss of H4 lysine 16 (H4K16) acetylation was shown to be a common feature in human cancer. However, it remained unclear which enzyme is responsible for the loss of this modification. Having recently identified the histone acetyltransferase human MOF (hMOF) to be required for bulk H4K16 acetylation, here we examined the involvement of hMOF expression and H4K16 acetylation in breast cancer and medulloblastoma. Analysis of a recent mRNA expression profiling study in breast cancer (n = 100 cases) and an array-CGH screen in medulloblastomas (n = 102 cases), revealed downregulation in 40% and genomic loss in 11% of cases, respectively. We investigated hMOF protein expression as well as H4K16 acetylation in large series of primary breast carcinomas (n = 298) and primary medulloblastomas (n = 180) by immunohistochemistry. In contrast to nontransformed control tissues, significant fractions of both primary breast carcinomas and medulloblastomas showed markedly reduced hMOF mRNA and protein expression. In addition, hMOF protein expression tightly correlated with acetylation of H4K16 in all tested samples. For medulloblastoma, downregulation of hMOF protein expression was associated with lower survival rates identifying hMOF as an independent prognostic marker for clinical outcome in univariate as well as multivariate analyses.

Key words: medulloblastoma; hMOF; MYST1; epigenetic; histone acetylation

Histone acetylation is one of the best characterized epigenetic modifications and is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). The histone H4 tail contains 4 closely spaced lysines (K5, K8, K12, K16) that are subject to acetylation by various HATs. Histone acetylation is generally regarded as an activating modification but has also been shown to play a role in gene repression, DNA repair, DNA replication and recombination. Considering the involvement of histone acetylation in such fundamental processes, it is not surprising that perturbation of the physiological acetylation status has been implicated in human cancer. Mutations in the p300 acetyltransferase, which acetylates many lysine residues in the histone tails, were found in primary tumors and cell lines. Analysis of global histone modifications, including several acetylated lysines, was shown to help to direct the search for potential drug targets. Interestingly, about 60% of total histone H4 is monomethylated, mostly at lysine 16, in normal human cells, whereas this acetylation is frequently lost in cancer. More recently, hMOF specifically acetylates H4K16 in mammalian cells, and that depletion of hMOF leads to global reduction of H4K16 acetylation in HeLa cells. In addition, hMOF depleted cells showed an impaired DNA repair response following ionizing radiation, and hMOF was also shown to be involved in ATM function. More recently, hMOF was found to be able to acetylate the tumor suppressor protein p53, and this acetylation is able to influence the behavior of p53 in response to DNA damage. Together this suggests a role for hMOF in transcriptional regulation, cell proliferation, differentiation and the DNA repair response.

Since hMOF is involved in these important cellular processes with obvious links to cancer, we were interested in understanding whether hMOF expression is lost in tumors and whether loss of hMOF is the reason for the decrease in the levels of H4K16Ac in tumors. We therefore investigated hMOF protein expression and H4K16 acetylation status by immunohistochemistry (IHC) on tissue microarrays (TMAs) in 298 primary breast carcinomas and 180 medulloblastomas, and corresponding normal tissues.

Material and Methods

Tumor material, patient characteristics and preparation of TMAs

A commercially available human breast cancer TMA (CBA-5-SBC) including 40 primary samples of breast carcinomas and 8 controls of normal mammary gland was purchased from Biocat (Heidelberg, Germany). Details on collection and preparation of specimens can be obtained at www.biocat.de.

Two custom TMAs, one each for the investigation of breast carcinomas and medulloblastomas, were constructed. Hematoxylin and eosine stained sections from all paraffin blocks were prepared to define representative tumor regions as previously described.

All tumors were arrayed in duplicate.

For the breast TMA consisting of 258 breast cancer samples, 46 mastopathies and 53 normal glandular breast samples, specimens...