Turning off the switch in medulloblastoma: The inhibitory acetylation of an oncogene

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Thirty-five years ago it was observed for the first time that sodium butyrate inhibits tumor cell growth by inducing histone acetylation.[1,2] Twenty years later the first histone deacetylase (HDAC1) was cloned[3] and it was understood that the anticancer effect of butyrate was due to its ability to inhibit HDACs' activity. Since then, a massive amount of studies have investigated the therapeutic potential of the various HDAC inhibitors (HDACi) on different tumors, providing evidence that these compounds may be considered effective tools against several cancers. Currently, only one HDAC inhibitor, the suberoylanilide hydroxamic acid (SAHA or Vorinostat), has been approved by FDA for the treatment of cutaneous T cell lymphoma, and other inhibitors have entered clinical trias in other malignancies.[4]

One potential limitation to the use of HDAC inhibitors is that they have a broad spectrum of action, which results in the onset of undesirable side effects at higher doses. For this reason, a lot of effort has been made to understand the molecular basis of HDACi action, in order to develop therapeutic strategies aimed at targeting the selective epigenetic alterations involved in each tumor. To this end, several studies have been conducted to characterize the acetylated substrates following HDACi administration. It is now widely accepted that, in addition to histones, the originally identified substrates, inhibition of HDACs also results in acetylation of non-histone proteins.[5]

The anti cancer effect of the HADC inhibitors has been largely attributed to their ability to modulate the acetylation status of chromatin, thereby inducing or repressing the expression of genes involved in cell cycle, apoptosis, angiogenesis and immune response. Alternatively, HDAC inhibitors have been shown to exert their anti cancer effect by an acetylation-mediated enhancement of transcription factors promoting cell cycle arrest or apoptosis, such as p53, p73, E2F1, pRb and NFκB.[6] Recently, it has been shown an alternative and unexpected mechanism by which HDAC inhibition promotes acetylation of an oncogene, thereby preventing its transcriptional activation.[7] This inhibitory event counteracts the growth of medulloblastoma, a malignant brain tumor of the childhood that often arises from inappropriate activation of the Hedgehog signaling pathway.[8] Starting from the observation that loss of class I HDAC disrupts the transcriptional response to Hh activation, HDAC1 and 2 are shown to bind and deacetylate the oncogenic transcription factor Gli1, the most potent effector of the Hh pathway. HDAC-dependent deacetylation causes enhanced Gli1 transcriptional activity, whereas ectopic expression of HAT/p300 has an opposite inhibitory effect.

Gli1 is acetylated at a single lysine (K518) that is conserved in Gli2 but not in Gli3 and K518R mutation, which mimics a status of constitutive deacetylation and confers increased transcriptional activity. Therefore, acetylation functions as an inhibitory switch that negatively affects the transcriptional activity of Gli1 and Gli2. Mechanistically, it is unclear how Gli activity is inhibited by acetylation. A change in Gli DNA binding affinity, intracellular localization or turnover rate has been ruled out and it seems plausible that acetylation affects Gli transactivation potential, an effect that could be due to changes in binding affinity for transcriptional co-activators or co-repressors. Unfortunately, the current knowledge of the mechanisms leading to transcriptional activation of Gli-target genes are only poorly understood and a better knowledge of the proteins that mediate the Gli-dependent transcriptional process is needed to properly address this issue.

Gli acetylation is finely regulated by two opposite mechanisms. One mechanism is triggered by activation of the Hh pathway and leads to increased HDAC1 mRNA and protein levels, and consequent Gli1 deacetylation and Hh-target gene activation. Since Gli1 activates its own expression, this mechanism turns on a positive feedback loop between Gli deacetylation and HDAC1/Gli1 upregulation. The second, inhibitory mechanism is regulated by the tumor suppressor REN^{KCTD11}, a protein that was initially identified as a Hedgehog inhibitor, encoded by a gene located in chromosome 17p, deleted in most of medulloblastomas.[9] REN turns off the positive Gli1/HDAC1 feedback loop by downregulating HDAC1 levels. Indeed, REN functions as an endogenous HDAC inhibitor that binds Cul3, a component of the SCF-like E3 ubiquitin ligase complex, and targets HDAC1 to ubiquitination and subsequent proteasomal degradation. Consistent with its role as a HDAC inhibitor, REN promotes Gli1 acetylation, thereby inhibiting the expression of Hh-target genes. Therefore, activation of Hedgehog and REN seem to work in an opposite and coordinated fashion, by regulating HDAC1 levels and, ultimately, Gli1 acetylation.

What are the consequences of an imbalance of this finely orchestrated equilibrium? The prediction is that any perturbation of the mechanisms that govern Gli acetylation will have an impact on cell

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proliferation and, eventually, may lead to malignant transformation. Indeed, constitutively deacetylated Gli1 mutants cause increased cell proliferation and transformation, and increased HDAC1 levels and REN downregulation are observed in Hh-dependent medulloblastoma.

Thus, acetylation of Gli1 represents a finely-tuned transcriptional checkpoint in neuronal cells and is the first example of an oncogene whose activation is inhibited by its own acetylation. This mechanism works in concert with the activating acetylation of oncosuppressors since they all share a common final outcome: the inhibition of tumor cell growth. As such, Gli1 acetylation may be considered a novel and promising new target for pharmacological screenings aimed at the identification of novel drugs to treat medulloblastoma and other Hh-dependent tumors.

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