Acetylation: A Novel Link between Double-Strand Break Repair and Autophagy

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Abstract
Histone deacetylase (HDAC) inhibitors are clinically relevant because they are used as anticancer drugs. Recent evidence sheds light on an intriguing connection among the DNA damage response (DDR), protein acetylation, and autophagy. HDAC inhibitors have been shown to counteract key steps in the cellular response to double-strand break formation by affecting checkpoint activation, homologous recombination–mediated repair of DNA lesions, and stability of crucial enzymes involved in resection of DNA ends. The degradation of the resection factors depends on autophagy, which plays a detrimental role when cells are in a hyperacetylated state and experience treatment with radiomimetic anticancer drugs. Future work will be required to further investigate the mechanisms underlying the link between acetylation, autophagy, and the DDR, as well as the significance of mTORC1 inhibitors, which are potent inducers of autophagy that are now used in cancer treatment. Cancer Res; 72(6); 1332–5. ©2012 AACR.

Introduction
One hallmark of cancer and aging cells is an unstable genome, which results from DNA damage. Eukaryotic cells developed intricate surveillance mechanisms to maintain their genome integrity and to repair DNA lesions. Following genotoxic stress, the evolutionary conserved DNA damage response (DDR) is activated and, in turn, coordinates DNA repair with cell-cycle events. In Saccharomyces cerevisiae, the DDR is mediated by two phosphoinositide 3-kinases: Mec1 (ATR in humans) and Tel1 (ATM in humans). ATR senses single-stranded DNA (ssDNA) coated with replication protein A (RPA), whereas ATM monitors the presence of DNA breaks. This mediation of the DDR results in activation of signal transduction pathways, which involves the Rad53 (Chk2 in humans) and Chkl (Chkl in humans) kinases (1). These cascades end in phosphorylation of several substrates involved in cell-cycle arrest and DNA repair (2). Defects in the DDR pathway components can lead to genome instability and cancer. In fact, several studies showed that replication stress and double-strand breaks (DSB), which lead to activation of DDR, occur in early tumor lesions (3, 4).

DSBs can arise spontaneously as a consequence of certain metabolic reactions, can be induced by extrinsic genotoxic events, such as ionizing radiation, or can occur in a programmed manner as in meiosis. These lesions must be promptly repaired to prevent loss of genetic material and maintain genome integrity. Induction of a single DSB is sufficient to activate the DDR in mitotically growing cells (5). In G2, DSBs are typically repaired by the homologous recombination (HR) pathway, which is initiated by 5′-end resection followed by the coating of 3′-ssDNA overhangs with RPA. The RPA–ssDNA nucleofilaments are thought to be the signal for ATR activation (6). S. cerevisiae has been instrumental in elucidating the conserved pathways involved in DSB repair. Several proteins mediate the resection process, including the MRX complex that consists of Mre11-Rad50-Xrs2, Sae2, Exo1, Sgs1, as well as Dna2 (7). RPA is then exchanged with Rad51, the RecA strand exchange protein, which in turn promotes DNA recombination processes. Inefficient and/or faulty DSB repair inevitably lead to genome rearrangements and cancer. A new phenomenon, called chromothripsis, has recently been described in cancer cells (8). It involves the rearrangement of broken chromosomes and can be acquired from one cellular catastrophic event. Remarkably, cells can survive such a crisis by selectively rearranging their genomes, although the consequence is cancer.

Regulation of expression and activity of key DNA repair and checkpoint proteins is essential and is mediated partly by posttranslational modifications (PTM), such as acetylation, phosphorylation, or ubiquitination. Cross-talk between different PTMs is key for regulation of DNA repair pathways. Acetylation, which is mediated by acetyltransferases (HAT) and histone deacetylases (HDAC), not only affects gene expression through its modulation of histone tails but also has recently been implicated in regulating nonhistone proteins. Intriguingly, a large class of acetylated proteins is involved in...
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HDAC inhibitors are currently used in cancer treatment (9). To investigate the importance of protein acetylation in the yeast DSB repair pathway, Robert and colleagues (10) used valproic acid (VPA), a U.S. Food and Drug Administration–approved antiepileptic drug shown to be an HDAC class I and II inhibitor and, therefore, now used in cancer clinical trials (11). Cells experiencing a single irreparable DSB were treated with VPA to induce a hyperacetylated state. Intriguingly, VPA treatment counteracted DDR activation. The authors then dissected the steps of DSB repair that are defective in VPA-treated cells and found that DSB resection rates were slower in VPA. They found that, whereas loading of Mre11 at the DSB site was unaffected, its unloading seemed to be defective in VPA. This phenotype resembled that of sae2 cells (12), raising the possibility that Sae2 endonuclease might be nonfunctional in VPA. Indeed, the levels of Sae2 protein in VPA-treated cells significantly declined. This finding not only applies to Sae2 but also to Exo1, suggesting that the DSB repair defect in VPA is due to the absence of at least these 2 critical nucleases implicated in resection. Consistently, Sae2 protein is hyperacetylated in VPA-treated cells.

A previous study by Kaidi and colleagues showed that CtIP, the mammalian counterpart of Sae2, is also acetylated in mammalian cells (13), indicating that acetylation as a PTM on Sae2/CtIP is highly conserved. Interestingly, also in mammals, a hyperacetylated state induced by treatments with HDAC inhibitors led to DSB repair defects, although CtIP degradation was not reported. Although acetylation of Exo1 was described in mammals (9), it was not found in yeast (10).

A Role for Autophagy in DNA Damage Response

An interesting study by Jeong and colleagues showed that mutant Huntington (Htt) protein, which undergoes acetylation, is degraded by macroautophagy (referred to as autophagy hereon; ref. 14). Autophagy is a highly conserved catabolic process involved in degradation of cytoplasmic components in the vacuole (lysosomes in humans). During autophagy, a double-membrane vesicle, named the autophagosome, forms and nonspecifically engulfs the cytoplasmic material that typically includes organelles, pathogens, or protein aggregates. Upon fusion of the autophagosome with the vacuole, the content is released and consequently gets degraded by lysosomal enzymes. This degradation allows recycling of amino acids and nutrients that are necessary for survival of cells under stress conditions.

Much of our knowledge of the molecular mechanisms underlying autophagy comes from S. cerevisiae (15). Although autophagy largely occurs on a nonspecific basis, several selective types of autophagy also exist; examples include pexophagy, mitophagy, and the cytoplasm to vacuole (CvT) pathway. CvT takes place in nutrient-rich conditions and mediates the transport of the precursor vacular hydrolases aminopeptidase 1 and α-mannosidase from the cytoplasm into the vacuole. In S. cerevisiae, 34 autophagy (ATG) genes have been identified to date, some of which are shared among all autophagy subpathways and constitute the core machinery needed for membrane formation. Autophagy is initiated by activation of the essential Ser/Thr kinase Atg1 and is induced most commonly by nutrient starvation and in response to several stimuli, such as treatment with the mTORC1 inhibitor rapamycin, which is currently used in the clinic as an anticancer agent, suggesting that inhibition of mTOR might have important therapeutic values in cancer prevention (16).

The link between mutant Htt protein acetylation and its autophagy-dependent degradation suggested that autophagy could also account for Sae2 degradation. Indeed, genetic and chemical inactivation of autophagy, more specifically the CvT pathway, rescued Sae2 degradation in VPA. Moreover, induction of autophagy by rapamycin resulted in Sae2 degradation. These lines of evidence support the notion that autophagy negatively influences DSB repair in a hyperacetylated state.

Sae2 Is Regulated by Gcn5, Hda1, and Rpd3

To uncover the identity of HDACs and HATs that mediate regulation of Sae2 acetylation status, the authors monitored Sae2 stability in strains deleted for both HDAl (class 1) and RPD3 (class 2). Indeed, Sae2 is destabilized in hda1 rpd3 cells. This finding recapitulates the effect of VPA, validating the mechanism of action of VPA through HDAC inhibition and indicating that Hda1 and Rpd3 are at least 2 HDAC targets of VPA involved in the DDR. Gcn5 HAT, a member of the SAGA HAT family in mammals, opposes the effect of Hda1 and Rpd3 (17). Consistently, deletion of GCN5 resulted in the suppression of Sae2 degradation in VPA and rapamycin. These findings suggest that interplay among Gcn5, Hda1, and Rpd3 regulates the acetylation status of Sae2 and, thus, its stability during DSB repair. Interestingly, in mammals, the SIRT6 HDAC has been implicated in CtIP acetylation and in DSB repair (13). SIRT6 belongs to class III HDACs, suggesting a role of other HDACs in the DDR pathways.

A Working Model

Robert and colleagues proposed the following model on the basis of their study (Fig. 1A): upon DSB formation, the MRX complex is recruited to DSB sites, followed by Sae2 and Exo1, which remain in a deacetylated state in an Hda1/Rpd3-dependent manner. Once resection takes place, Gcn5 (and likely other HATs) mediate hyperacetylation of Sae2 (Ac-Sae2), triggering its degradation by autophagy. Because autophagy takes place in the cytoplasm, it is likely that Ac-Sae2, along with Exo1 and additional unknown targets, is exported into the cytoplasm through the nuclear pore. Piecemeal microautophagy of nucleus (PMN) has been reported in S. cerevisiae and has been shown to take place at nucleus–vacuole junctions (18). PMN depends on several auotophagy genes, including ATG1. Another possibility, which remains to be addressed, is that Ac-Sae2 degradation takes place in a PMN-dependent manner.

DNA damage and repair; examples include p53, ATM, Ku70, and Exo1 (9). The biologic significance of protein acetylation is largely not understood.
A future challenge is to further elucidate the contribution of the different autophagy pathways in regulating the DDR. Autophagy is involved in degradation of long-lived proteins and in recycling of macromolecular components. Perhaps acetylated Sae2 and other DSB repair proteins (including Exo1) form large complexes and, thus, difficult substrates for ubiquitylation. Hence, after their use, autophagy might represent the only degradation pathway mediating their recycling. In fact, the acetylome study conducted in human cells showed that large protein complexes undergo acetylation (9), which may signal their degradation by autophagy.

Increasing evidence in the literature shows that autophagy is induced in response to DNA damage (19). In addition to the recent link between autophagy and DNA damage in S. cerevisiae (10), Dyavaiah and colleagues (20) showed that a subunit of ribonucleotide reductase Rnr1 is also regulated in an autophagy-dependent manner (20). It is important to note that DNA-damaging agents also cause protein damage (21), which in turn may induce stress pathways such as autophagy as a survival mechanism to degrade damaged proteins or aggregates.

The physiologic role of autophagy has recently been a topic of debate (22). Autophagy has a cytoprotective role and is needed for survival during development, aging, and pathogen invasion. Defects in autophagy genes lead to predisposition to cancer and neurodegenerative diseases (22). In fact, a strong correlation exists between autophagy induction and tumor suppression. On the other hand, autophagy can have a cytotoxic effect because tumor cells can rely on it for survival or healthy cells can be killed by excessive autophagy. The yeast studies suggest that autophagy has a negative impact on cell survival when cells in a hyperacetylated state are exposed to radiomimetic drugs (10).

The study by Robert and colleagues has raised many questions. Pathways that regulate autophagy are well characterized and include TOR1, Ras-PKA, Snf1 AMP kinase (AMPK in humans), as well as Sch9 kinase (S6K and/or PKB in humans; ref. 23). It will be interesting to investigate the mechanism(s) underlying autophagy induction by VPA or other HDAC inhibitors that are currently in clinical development. In mammalian cells, VPA activates autophagy in an mTOR-independent manner (24, 25); however, in yeast, the target pathway of VPA is still unknown. Moreover, the precise mechanism underlying the CvT-mediated degradation of Sae2 and Exo1 (and likely other targets) should be explored further. Cross-talk between autophagy and the proteasome has been reported (26), and whether it takes place during DSB repair should be investigated.

One study conducted in breast cancer cells showed that rapamycin affects DSB repair pathways, HR, and nonhomologous end joining (27). Consistently, the report by Robert and colleagues further pinpoints that the DDR can be counteracted by rapamycin and HDAC inhibitors. Rapamycin and its analogs, rapalogues, have effective antitumor activity and are currently used either alone or in combination with anticancer drugs (16). Ongoing clinical trials that rely on combining rapamycin with genotoxic drugs should be evaluated in light of these recent findings.

Although much attention has been granted to DNA damage repair pathways, protein damage likely plays a significant role in response to genotoxic stress. Thus, the theme of nutritional...
pathways such as autophagy will become more common in the genome integrity field. From the therapeutic perspective, mechanistic studies aimed at understanding the interplay among autophagy, DSB resection and acetylation, and drug intervention designed for autophagy and/or DDR cross-talk might aid in the development of more effective cancer treatments.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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