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AUTOPHAGIC PUNCTUM

Autophagy modulator plays a part in UV protection

Yongfei Yang, Christine Quach, and Chengyu Liang

Department of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA, USA

ABSTRACT

Ultraviolet (UV)-induced DNA damage is a major risk factor for skin cancers including melanoma. UVRAG, originally identified to complement UV sensitivity in xeroderma pigmentosum (XP), has since been implicated in modulating macroautophagy/autophagy, in coordinating different intracellular trafficking pathways, and in maintaining chromosomal stability. Intriguingly, our recent study has demonstrated that UVRAG plays an essential role in protecting cells from UV-induced DNA damage by activating the nucleotide excision repair (NER) pathway. Since NER is the major mechanism by which cells maintain DNA integrity against UV insult, the inactivation of UVRAG seen in some melanoma may impart these cells with an ability to accumulate high-load UV mutagenesis, leading to cancer progression. Thus, this property of UVRAG has untapped potential to be of fundamental importance in understanding the genetics and pathogenesis of human skin cancer.

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Autophagy, which mediates lysosome-dependent bulk degradation and recycling of cytoplasmic materials, has been the subject of an exploding wave of investigation in the past decade, leading to the discovery of a plethora of autophagy-related genes that govern both its induction and execution. Meanwhile, it has become clear that many proteins initially described for their essential role in autophagy also exploit multiple metabolic and immune pathways, often independent of their autophagy modulation. Such functional heterogeneity of autophagy modulators highlights the intersection of autophagy with other cell signaling networks and also suggests that the unconventional roles of autophagic factors might be eminently selected in specific pathophysiological settings, as can be envisioned in our recent study on the UV-protecting aspect of UVRAG (UV radiation resistance associated gene).

UVRAG was initially isolated in a genetic screen for partially rescuing the UV sensitivity in XP cells. Unfortunately, no study further explored or validated this characteristic, nor is the molecular basis of UVRAG in UV protection known. Rather, UVRAG is generally accepted as an autophagy promoter. Suppression of UVRAG levels or overexpression of a dominant-negative form causes failure of autophagy, a feature sufficient to disturb homeostasis and fuel uncontrolled cell proliferation. In addition to its autophagy-promoting role in the cytoplasm, it has been found that UVRAG functions in centrosome stability and in double-strand DNA damage repair in the nucleus, a finding with practical relevance toward cancer therapy. Our recent work further extended the functional scope of nuclear UVRAG by showing its direct role in alleviating UV-like mutagenesis, a function that is of particular significance to skin tumors, whereby UV-induced DNA damage is considered to be a major risk factor.

Exposure of the skin to UV radiation, such as excessive and cumulative sun exposure, induces DNA photodamages, which is removed by NER. If left unrepaired, this damage leads to the accumulation of "UV-signature" mutations, mainly C to T or G to A transitions at dipyrimidine sites, and induction of cancer. We discovered that skin melanoma patients with lower levels of UVRAG tend to have higher amounts of UV-signature in their genomes, suggesting a potential link of UVRAG to photolesion protection. Indeed, removing UVRAG from melanoma cells sensitizes these cells to UV and UV-mimetic drugs. Although autophagy is implicated in UV protection, abrogating the autophagy apparatus cannot erase the UV-protecting effect of UVRAG. Similarly, a melanoma-associated mutant UVRAG, defective in photolesion repair, is as capable as wild-type UVRAG in autophagy promotion. This suggests that the autophagic aspect of UVRAG is not directly involved in control of UV-induced DNA damage. In fact, UVRAG itself accumulates around the sites of photolesions soon after UV exposure—a characteristic shared by many NER factors that respond to UV-induced damage, yet one that had never been reported for any of the key factors in autophagy.

To address the mechanism of this phenomenon, we demonstrated a critical role of the UV-damage sensor, the DDB1-DDB2 heterodimer (UV-DDB), in recruitment of UVRAG to photolesions, which influences the outcome of the NER response to UV-induced damage. Removing UV-DDB delocalizes UVRAG from the damaged sites and abolishes its effect in UV protection, whereas silencing UVRAG results in defective signal transfer from UV-DDB to the downstream NER effector XPC. Next, we purified the protein from UV-irradiated cells and identified the interacting partner for UVRAG. We found that DDB1 bridges UVRAG to form a complex

CONTACT Chengyu Liang chengyu.liang@usc.edu Department of Molecular Microbiology and Immunology, University of Southern California, Room 5517, MC NRT 9605, 1450 Biggy Street, Los Angeles, CA 90033, USA.

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with both DDB2 and the CUL4A (cullin4A)-RBX1/Roc1 ubiquitin-ligase complex. CUL4A-RBX1 is a key enzyme involved in global-genomic NER (GG-NER), a process that repairs photolesions regardless of the gene transcriptional status. We found that UVRAG binds the DDB1-DDB2-CUL4A-RBX1 ($CRL4^{DDB2}$) complex at the photolesions, and that this interaction is stronger in response to UV exposure. Removal of UVRAG or expression of a mutant form of the protein that cannot bind DDB1 leads to cells defective in performing GG-NER, whereas overexpression of UVRAG lowers the threshold for triggering GG-NER. A detailed mechanistic analysis revealed that UVRAG interaction facilitates the assembly of a stable $CRL4^{DDB2}$ E3 complex as well as the activation of $CRL4^{DDB2}$ for NER.

A mechanism that cullin-based E3-ligase has in common is the cyclic attachment to and removal from the cullin subunit of NEDD8, which turns on and off the E3-ligase, respectively. Unlike CAND1 (cullin-associated and neddylation dissociated 1), which preferentially interacts with an unmodified CUL4A and prevents it from binding DDB1, UVRAG behaves in an opposite manner. This raised a mechanistic possibility that UVRAG-mediated activation of $CRL4^{DDB2}$ in NER may be, at least in part, through CAND1 inhibition. Indeed, excessive UVRAG liberates CUL4A from CAND1 sequestration, allowing more catalytically active CUL4A complex formation. Thus, UVRAG is not only a direct UV-DDB-CUL4A-RBX1 assembly factor, but also functions as a CAND1 antagonizer, both of which converge to elicit a robust NER response through $CRL4^{DDB2}$ activation.

Although the $CRL4^{DDB2}$ complex does not have any DNA excision or ligation activity, it is critical for chromatin structure remodeling around UV lesions, allowing repair proteins access to DNA. On the one hand, histone H3 and H4 ubiquitination by $CRL4^{DDB2}$ allows more open chromatin conformation to expose photolesions, which, however, are barely detectable in UVRAG-deficient cells. Moreover, DDB2, which recognizes the chromatin-associated signals of UV damage, is polyubiquitinated by $CRL4^{DDB2}$ and subsequently removed from the destabilized chromatin, presumably to release space around the photolesion to load downstream effectors for the cascade to proceed. However, the absence of UVRAG extends the turnover rate of DDB2 and prolongs NER execution. On the other hand, $CRL4^{DDB2}$-mediated ubiquitination of XPC stimulates its DNA-binding activity, providing a verification signal for DNA damage to recruit downstream repairing factors. Similarly, XPC fails to be recruited to the damaged site and ubiquitinated when UVRAG is silenced. It is evident that photolesion-associated UVRAG is part of the machinery that governs NER response and prevents cells from UV-induced genetic instability, reducing cancer predisposition and progression. Consistent with this, melanoma patients with lower UVRAG expression are associated with worse clinical outcome, accompanied by increased UV-signature loads.

Overall, this study establishes a previously unknown mechanism of UVRAG in promoting UV-induced DNA damage repair through interaction with NER core machinery, which also poses interesting questions about its mechanism of action. Given that the DDB1-containing CUL4A-based E3 ligase is involved in many developmental and physiological processes, it remains to be shown whether UVRAG assembles with DDB1 in cellular processes besides NER. Our work on the interaction between UVRAG, autophagy, and NER reveals an intriguing new route for tumor suppression, and associates a mechanistic function with its name.