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UVRAG

At the crossroad of autophagy and genomic stability

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UVRAG is a promoter of the autophagy pathway, and its deficiency may fuel the development of cancers. Intriguingly, our recent study has demonstrated that this protein also mediates the repair of damaged DNA and patrols centrosome stability, mechanisms that commonly prevent cancer progression, in a manner independent of its role in autophagy signaling. Given the central role of UVRAG in genomic stability and autophagic cleaning, it is speculated that UVRAG is a bona fide genome protector and that the decrease in UVRAG seen in some cancers may render these cells vulnerable to chromosomal damage, making UVRAG an appealing target for cancer therapy.

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Autophagy mediates lysosome-dependent bulk degradation and recycling of cytoplasmic materials, and thus constitutes the cell's quality control program and allows cells to respond correctly to stress. Considering that autophagy represents, intrinsically, a survival mechanism of cells, it seems counterintuitive that autophagy often acts in tumor suppression, as mutations in the essential autophagy genes, such as *BECN1*, promote tumor growth in both clinical samples and genetically-engineered mice. To reconcile the paradoxical effects of autophagy in the promotion and control of cancer, recent findings demonstrate that cells with defective autophagy, though impaired in metabolic stress recovery, are prone to genomic instability with increased DNA damage and aneuploidy. For the first time, these findings integrate autophagy's roles in cancer suppression by promoting genetic stability, yet the evidence in support of autophagy as an immediate or direct genome protector is confined largely to a phenotypic association between loss of an autophagy-related gene(s) and gain of genetic instability in cells. It remains unclear whether the dysfunction of autophagy per se suffices to drive genomic instability or serves as a 'second hit' in the process of cancer transformation, and thus the mechanism(s) underlying autophagy- or autophagy factor-mediated genome protection is still uncharted.

The ultraviolent (UV) irradiation resistance-associated gene (*UVRAG*), initially identified in a genetic screen for rescuing the UV sensitivity in xeroderma pigmentosum (XP) cells, is an autophagy factor. Overexpression of UVRAG activates autophagy and suppresses tumor cell growth, whereas silencing of *UVRAG* or overexpression of a dominant-negative form of UVRAG decreases autophagy levels and triggered uncontrolled cell proliferation. Notably, UVRAG forms distinct complexes with BECN1 and the class C-Vps complex at different stages of the autophagy pathway, enhancing the overall flux of autophagic degradation. However, our recent work shows that UVRAG also plays direct roles in preventing cells from accumulating abnormal chromosomes and hence the danger of developing oncogenic mutations, in addition to autophagy activation; since the maintenance of chromosomal integrity is a fundamental biological process to thwart tumor formation, this property of UVRAG explains why it is frequently mutated in cancers.

Given that defective autophagy itself induces insufficient clearance of aged or

damaged proteins and organelles, a potential source of oxidative stress leading to genomic damage, it is perhaps not surprising to note that cells with decreased UVRAG accumulate DNA damage and become sensitized to the cytotoxicity of DNA-damaging agents. Unexpectedly, however, UVRAG itself was found to accumulate around the sites of DNA damage soon after irradiation––a characteristic shared by many proteins that respond to DNA damage/repair, which had never been reported for any of the key factors in the autophagy pathway. Encouraged by these results, we depleted somatic cells and embryonic cells of UVRAG and found that these cells were unable to repair broken DNA regardless of their autophagy status. To gain further insight into the function of UVRAG, we purified the protein from irradiated cells and determined the interacting partners of UVRAG. Intriguingly, a predominant partner for UVRAG turned out to be the DNA-dependent protein kinase (PRKDC/DNA-PK) complex, which is a key enzyme involved in nonhomologous end joining (NHEJ), a process that seals DNA ends throughout the cell cycle without the need for a homologous template. We found that UVRAG binds the PRKDC complex directly inside the nucleus, and that this interaction is stronger in response to genotoxic insults. Along this line, removal of UVRAG or expression of a mutant form of the protein that cannot bind the PRKDC complex causes cells defective in performing NHEJ, whereas UVRAG overexpression lowers the bar for triggering NHEJ. A more detailed mechanistic analysis revealed that UVRAG itself is brought to the damaged sites by the PRKDC complex, which in turn facilitates the assembly of a stable PRKDC complex and the activation of PRKDC for NHEJ repair.

Much evidence implicates the linkage of DNA repair proteins with centrosomes: a significant number of proteins in the DNA damage/repair pathway also regulate centrosome integrity, and, similarly, centrosome-related factors are involved in DNA damage/repair. Some tumor suppressors, such as TP53/p53 and RB1/Rb, are also found to regulate both centrosome and DNA damage/repair pathways. However, the orchestration of the DNA damage/repair pathway and the centrosome dynamics is far from understood, despite the key roles of both processes in maintaining genomic stability and keeping tumors in check. We show that in addition to the cytoplasmic distribution of UVRAG, a distinct percentage of UVRAG localizes at centrosomes and this is achieved by its interaction with CEP63, a constitutive component of centrosomes. Depletion of UVRAG perturbs the centrosome cycle, leading to severe centrosome amplification with profound consequences on spindle malformation and chromosomal segregation errors. This defect can be corrected by normal UVRAG but not by its CEP63-binding inactive form. So it seems that centrosome-associated UVRAG is part of the machinery that guards centrosomes integrity and ensures correct segregation of genetic materials during cell division. Inevitably, deficiency of UVRAG is accompanied by centrosome abnormalities and increased levels of aneuploidy that potentially favors tumor formation.

In further exploring the multitasking nature of UVRAG, we discovered that the role of UVRAG in DNA repair, centrosomes, and autophagy are mutually independent. Remarkably, a mutant UVRAG defective in autophagy is as capable as the wild-type UVRAG of activating DNA repair and centrosome interaction. Likewise, a mutant version of UVRAG lacking the PRKDC or CEP63 binding activity maintains efficient BECN1 interaction. These findings clearly differentiate the autophagy regulatory function of UVRAG from its role in chromosomal segregation and stability, which also poses interesting questions about its mechanism of action. First, why would cells dedicate autophagy factors for centrosome or DNA repair in a manner that is completely independent of their autophagy function? Furthermore, how are different populations of UVRAG modified or primed to engage in spatiotemporally distinct activities? How are these complex processes coupled mechanistically? It is also of interest as to how much overlap exists between the mechanisms by which UVRAG executes these processes. Although it remains to be tested whether this regulation of genome stability we have discovered with UVRAG might also be seen with other autophagy factors, the antagonism between autophagy and genomic instability in some contexts highlights a molecular crosstalk between the autophagy machinery and the DNA surveillance/repair process. Regardless of these uncertainties, in describing an interaction between UVRAG, autophagy, and genomic instability, our work reveals an intriguing new route for tumor suppression.