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Autophagy substrate SQSTM1/p62 regulates chromatin ubiquitination during the DNA damage response

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ABSTRACT
The importance of autophagy in the DNA damage repair process is clear; however, the detailed molecular mechanism is still largely unknown. Here we found that DNA damage-induced histone H2A ubiquitination is suppressed in autophagy-deficient cells in a SQSTM1/p62 dependent manner. SQSTM1 binds and inhibits E3 ligase RNF168s activity, which is essential for H2A ubiquitination. As a result, several important factors for DNA repair cannot be recruited to the sites of DNA double-strand breaks (DSBs) in autophagy-deficient cells, leading to diminished DNA repair and increased sensitivity of cells to radiation.

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Recently, the importance of autophagy in the DNA damage repair process was demonstrated. Autophagy-defective tumor cells are associated with genomic instability, such as increased DNA damage, gene amplification, and aneuploidy. Persistence of DNA damage in autophagy-deficient cells may be critically driven by a defect in DNA repair. It has been reported that loss of autophagy critically impairs homologous recombination, an important DNA repair mechanism, resulting from enhanced degradation of CHEK1/Chk1 (checkpoint kinase 1).

SQSTM1, a ubiquitin and LC3 binding protein, is a selective autophagy substrate and cargo receptor for degradation of ubiquitinated substrates by autophagy. Genetic ablation of *Sqstm1* in mice and drosophila reveals that SQSTM1/Ref(2)P is required for the aggregation of cytoplasmic ubiquitinated proteins and thus plays essential roles for their autophagic clearance.

SQSTM1 inhibits DNA damage-induced chromatin/histone ubiquitination

In our study, by using the FK2 anti-ubiquitin conjugate antibody, we found that transient transfection of *Sqstm1* siRNA induces abundant foci, which are mostly localized in the nucleus as visualized by confocal microscopy. After SQSTM1 is overexpressed, we observed that chromatin ubiquitination is significantly reduced in the presence of irradiation. However, other autophagic cargo receptors, for example, NBR1 or WDFY3/ALFY, are not able to affect the formation of the chromatin poly-ubiquitin chain, suggesting that SQSTM1 downregulated chromatin ubiquitination may be independent of its function as an autophagic cargo receptor.

Considering that SQSTM1 is one of the best-known autophagic substrates, it was not surprising that a higher SQSTM1 level is observed in autophagy-deficient cells. Irradiation fails to induce chromatin ubiquitination in the absence of autophagy; however, the chromatin ubiquitination is recovered by knocking down SQSTM1, indicating that loss of autophagy leads to a deficiency of chromatin ubiquitination in a SQSTM1-dependent manner. In addition, SQSTM1^{K7A,D69A} and SQSTM1^{K7A,D69A,I314E}, which cannot localize at autophagosome formation sites, also inhibit chromatin ubiquitination, further supporting the autophagy-receptor-independent role of SQSTM1.

SQSTM1 directly interacts with RNF168 and inhibits its E3 ligase activity

Chromatin ubiquitination plays an important role in the cellular response to DNA damage. Several reports have indicated that DNA-damage induced formation of poly-ubiquitin chains is catalyzed by the E3 ligase RNF168. Therefore, we tested SQSTM1s role in RNF168-induced ubiquitination. Our results indicated that SQSTM1 suppresses RNF168-induced histone poly-ubiquitination, by directly binding to RNF168. Furthermore, we showed that the LIM-binding (LB) domain (amino acids 170–220) of SQSTM1 is necessary for its binding with RNF168, which in turn is critical for the

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repression of RNF168s activity, tested by both in vivo and in vitro ubiquitination assays. Subsequently, we examined how SQSTM1 impairs the ubiquitin ligase activity of RNF168. The discharge assay showed that RNF168 efficiently promotes hydrolysis of the UBE2D3/UbcH5c C85S–Ub conjugate. In addition, SQSTM1, but not SQSTM1ΔLB, decreases the rate of discharging ubiquitin from the E2, suggesting that SQSTM1 inhibits RNF168s ability to transfer ubiquitin.

SQSTM1 accumulation by loss of autophagy inhibits DSB repair

Because chromatin ubiquitination plays an important role in DNA repair, next, we tested whether SQSTM1 could regulate the response to DSBs. In the DNA repair process, one important step is the recruitment of BRCA1, UIMC1/RAP80 and RAD51 to the sites of DSBs, which depends on the histone ubiquitination induced by RNF168. We found that SQSTM1 impairs the recruitment of BRCA1, UIMC1/RAP80 and RAD51 dependent on its LB domain, which is essential for its interaction with RNF168. In addition, in the absence of autophagy, irradiation fails to induce RNF168, BRCA1, UIMC1/RAP80 and RAD51 foci formation; however, the foci are recovered in SQSTM1 knockdown cells. These observations indicate that loss of autophagy leads to a deficiency of DNA repair protein recruitment to DSBs sites in a SQSTM1-dependent manner.

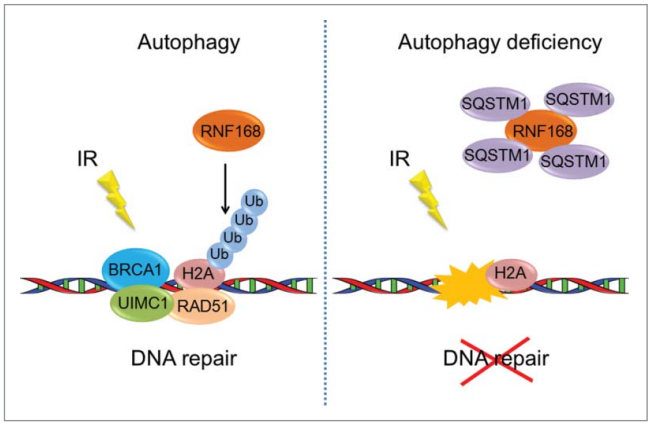

Figure 1. SQSTM1 is a physiological modulator of DNA repair. SQSTM1, which accumulates in autophagy-defective cells, directly binds to and inhibits nuclear RNF168, an E3 ligase essential for histone H2A ubiquitination and DNA damage responses. Consequently, cells that overexpress SQSTM1 or are deficient for autophagy fail to recruit repair factors such as UIMC1/RAP80, BRCA1 and RAD51 to DSB sites, leading to impaired DSB repair and cell survival.

To test whether SQSTM1 affects DNA damage, we generated stable HeLa cells expressing nucleus-localized SQSTM1 (K7A D69A I314E), SQSTM1 (K7A D69A I314E ΔLB) or an empty plasmid. Compared with the empty plasmid or $SQSTM1^{K7A,D69A,/I314E,\Delta LB}$, expression of $SQSTM1^{K7A,D69A,I314E}$ results in a persistence of γ-H2AFX/H2AX foci and impaired repair kinetics as measured by a comet assay, as evidence of persistent DSBs. Next, to investigate whether the persistent DSB has an effect on the growth of cancer cells, we tested the colony formation of HeLa cells after irradiation treatment. We found the colony formation rate is dramatically decreased in the $SQSTM1^{K7A,D69A,I314E}$ cell line, compared with control or $SQSTM1^{K7A,D69A,I314E,\Delta LB}$ cell lines. Furthermore, to examine whether nuclear SQSTM1 expression brought about regression of tumor growth ex vivo, cells were then injected subcutaneously into nude mice, which results in the formation of tumors. As expected, upon irradiation, $SQSTM1^{K7A,D69A,I314E}$-expressing tumors have retarded growth compared to control tumors, revealing increased sensitivity to ionizing radiation. Hence, our observations suggested that SQSTM1 sensitizes human cancers to radiation by limiting DNA repair (Fig. 1).