TRIM72-mediated degradation of the short form of p62/SQSTM1 rheostatically controls selective autophagy in human cells

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Dear Editor,

Autophagy is an evolutionarily conserved catabolic process that involves the sequestration and transport of organelles, macromolecules, or invading microorganisms to lysosomes for degradation [1]. Sequestosome 1 (p62/SQSTM1) was the first protein shown to bind target-associated ubiquitin (Ub) and LC3 conjugated to the phagophore membrane, thus, acting as an important autophagy receptor for ubiquitinated targets [2]. Human p62/SQSTM1 has alternative 5′donor sites during splicing of immature mRNA, which results in two isoforms of the p62/SQSTM1 transcripts, p62L and p62S. In contrast to p62L, differences in the 5′-untranslated region (UTR) and coding sequence (CDS) region between the two transcript variants resulted in a loss of 1–84 amino acids (included within the PB1 domain) in p62S (Fig. 1a, Additional file 1: Fig. S1a). In this study, two pairs of primers were designed to specifically amplify the cDNA fragments of p62 isoforms in different cell lines (Additional file 1: Table S1). Expression of p62L was detected in all 9 cell lines, whereas p62S expression was detected in most cell lines, with the exception of human skeletal muscle cells (HSkMC) and adult hepatocytes (Additional file 1: Fig. S1b). No p62S expression was found in various tissues of mice (Additional file 1: Fig. S1c), indicating a human cell-type-specific expression pattern in the biogenesis of p62S. An shRNA specifically targeting p62S but not p62L was designed (Additional file 1: Table S2), and validated by quantitative PCR and immunoblot analysis (Additional file 1: Fig. S1d), indicating the presence of p62S.

p62S and p62L protein levels were upregulated when treated with the proteasome inhibitor bortezomib (BTZ), but not with the autophagy inhibitor bafilomycin (BAF, Fig. 1b; Additional file 1: Fig. S2a, b), indicating that p62S and p62L were mainly degraded by the proteasome pathway rather than the autophagy pathway.

Using the yeast two-hybrid screening system, tripartite motif-containing 72 (TRIM72) was identified as interacting with p62S but not with p62L (Fig. 1c). The interaction of TRIM72 and p62S was subsequently verified by glutathione-S-transferase (GST)-pull-down (Fig. 1d) and co-immunoprecipitation assays (Additional file 1: Fig. S3a). TRIM72 promoted the degradation of p62S in a dose-dependent manner (Additional file 1: Fig. S3b), and ectopic expression of TRIM72 decreased p62S protein level and increased lipidated LC3 (LC3 II) level, while TRIM72 knockdown increased p62S level and decreased LC3 II level (Additional file 1: Fig. S3c). Pulse-chase experiments revealed that p62S, but not p62L protein,
was reduced upon TRIM72 overexpression, indicating a specific effect of TRIM72 on p62S proteasomal degradation (Fig. 1e). Furthermore, wild-type TRIM72, but not an E3 ligase death mutant (TRIM72ΔRING or TRIM72C14A), supported the ubiquitination of p62S (Fig. 1f). Together, these results suggest that TRIM72 is a p62S-specific E3 ligase.

Regarding the physiological function of the TRIM72-mediated degradation of p62S protein, PB1 lacking p62S bound to the poly-ubiquitinated cargo and was capable of binding to LC3 as efficiently as p62L (Additional file 1: Fig. S4a, b). Upon treatment with rapamycin, p62S reduced GFP-LC3 puncta formation in HeLa cells, indicating that p62S suppressed rapamycin-induced autophagy, which was reversed by wild-type TRIM72 but not its mutants (TRIM72ΔRING and TRIM72C14A, Fig. 1g). In addition, TRIM72 regulated autophagy, mainly through p62S, but not through p62L, as shown in p62−/− MEF cells (Additional file 1: Fig. S4c).

Because invading microorganisms are mainly degraded through autophagy, a Salmonella infection assay was used to measure cellular autophagic activities [2]. Introduction of rapamycin and p62L into HeLa cells reduced the number of Salmonella infection by increasing autophagy (LC3 II), while introduction of p62S increased the number of infected Salmonella by reducing autophagy. When p62S was down-regulated by wild-type TRIM72, the number of infected Salmonella were significantly reduced due to increased autophagy (Fig. 1h).

Another function of cellular autophagy is to remove proteins from oxidative damaged (carbonylation) [3]. The degradation of carbonylated proteins in HeLa cells after rapamycin treatment was accelerated by increasing autophagy (LC3 II). This process was reduced in the presence of p62S, and the introduction of wild-type TRIM72 almost abolished the inhibitory effect of p62S, which was abolished by BTZ (Fig. 1i).

Taken together, our results revealed a previously unexplored mechanism involving alternative splicing of p62/SQSTM1 mRNA with TRIM72-mediated Ub signaling, to rheostatically control the cellular autophagic flux (Fig. 1j). Further exploitation of this mechanism offers new opportunities to develop therapeutic interventions for these related pathophysiological processes.
Fig. 1 (See legend on previous page.)
Abbreviations
BTZ: Bortezomib; BAF: Bafilomycin; CDS: Coding sequence; GST: Glutathione-S-transferase; HSNC: Human skeletal muscle cell; LC3: Microtubule-associated protein 1A/1B-light chain 3; SQSTM1/p62: Sequestosome 1; TRIM72: Tripartite motif-containing 72; Ub: Ubiquitin; UTR: Untranslated region.

Supplementary Information
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Additional file 1: Materials and Methods. Fig. S1 Human p62 has a shorter isoform p62S lacking the PB1 domain at the N-terminus. Fig. S2 Human p62S is mainly degraded through the proteasome pathway. Fig. S3 Human E3 ligase TRIM72 mediates the ubiquitination and degradation of p62S-regulated cellular autophagy. Fig. S4 Human p62S antagonizes the autophagy receptor function of p62L. Table S1 Sequences of the primers used in RT-PCR and qPCR. Table S2 Sequences of the shRNAs for p62S and TRIM72.

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Author contributions
RGH, CYL, and WJG conceived, designed, and supervised the project. CCW, HP, CYL, and JY performed most of the experiments. RGH, CYL, and ZW wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All the data and materials generated or analyzed in this study are available for other researchers after the manuscript is published.

Declarations
Ethics approval and consent to participate
Animal experiments were performed in compliance with guidance for the care and use of laboratory animals, and were approved by the Institutional Animal Research Ethics Committee of Shanghai Institute of Biochemistry and Cell Biology (2019-012).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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