論文の内容の要旨

論文題目　Differential roles of annexins and ESCRTs in lysosome repair
（リソソーム修復におけるアネキシンと ESCRT の異なる役割）

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Lysosomes are the mammalian cell's degradative organelles that maintain cellular metabolism and homeostasis. Their function is contingent on the integrity of the lysosomal membrane but as sites of degradation, lysosomes are likely to encounter membrane-destabilizing material. Endocytosed pathogens, protein aggregates, foreign particles and so on can cause lysosomal membrane permeabilization, which inactivates lysosomes and, in extreme cases when lysosomal hydrolases enter the cytosol, results in cell death. Lysosomal membrane permeabilization is thus frequently part of the pathogenesis of aging and diseases characterized by lysosomal accumulation of aberrant material, such as neurodegenerative diseases and lysosomal storage diseases. Understanding how lysosomal membrane permeabilization is resolved could lead to the development of more effective disease treatments.

Damaged lysosomes were initially thought to be directly eliminated by autophagy but later found to be repaired whenever possible by the ESCRT (Endosomal Sorting Complexes Required for Transport) machinery, which are also involved in plasma membrane repair. The first reports on lysosome repair also discovered another similarity with plasma membrane repair—both are triggered by calcium influx into the cytosol (from the lysosomal lumen and the extracellular space, respectively). The discovered similarities between lysosome repair and plasma membrane repair suggest that they might have even more in common.

Besides the ESCRT machinery, plasma membrane repair is carried out by another major group of repair proteins that resolve plasma membrane injuries are the annexins (ANXs). These mammalian proteins associate with membranes upon binding to calcium. Six out of seven of the ubiquitously expressed ANXs (ANXA1, A2, A4, A5, A6, and A7) have been found to be important for resolving plasma membrane injuries. Furthermore, ANXA7 was shown to be the upstream recruiting factor of ESCRTs, which

do not have calcium-binding domains. However, despite their prominence in plasma membrane repair, the involvement of ANXs in lysosome repair has not been fully explored. Hence, I began my study to investigate ANX activity during lysosome damage and whether there was any relationship between ANX and ESCRTs during lysosome repair.

First, I examined the localization of the ubiquitously expressed ANXs before and after inducing lysosome damage by treatment with LLOMe, a routinely used lysosomal membrane permeabilization-inducing agent. These ANXs were cytosolic before LLOMe treatment and localized to LAMP1-marked lysosomes after, indicating that ANXs can respond to lysosome damage.

Next, whether the ANXs were important for lysosome repair was determined with the previously established Magic Red recovery assay. Lysosomal protease-dependent Magic Red fluorescence is lost from lysosomes that are damaged and restored upon repair. Following a brief exposure to LLOMe, Magic Red fluorescence recovered in cells depleted of ANXA4, A5, A6, A7, or A11 but was suppressed in cells depleted of ANXA1 or ANXA2, like cells depleted of the ESCRTs, ALIX and TSG101. Furthermore, ANXA1-ANXA2 double depletion did not cause additive suppression of Magic Red recovery. Thus, ANXA1 and ANXA2 are important for lysosome repair and possibly mediating repair in the same pathway.

As ESCRT recruitment requires ANXA7 during plasma membrane repair, I speculated that ANXA1 and ANXA2 served a similar function to ANXA7 by recruiting ESCRTs during lysosome repair. However, ANXA1 and ANXA2 are not required for ESCRT recruitment to damaged lysosomes as ESCRT localization was unaffected in cells depleted of ANXA1, ANXA2, or ANXA1-ANXA2. ANXA1/A2-mediated lysosome repair. Hence, ANXA1 and ANXA2 might serve as an ESCRT-independent repair mechanism for lysosome like how they do during plasma membrane repair.

ANXA1 and ANXA2 activity during lysosome damage was then examined more closely with live-cell microscopy. They were observed to be recruited to LLOMe-damaged lysosomes at the same time, suggesting that they respond to the same signal. Their recruitment was also found to be independent of the other, as siRNA-mediated depletion of ANXA1 did not affect recruitment of ANXA2, and vice versa. These data indicate that

ANXA1 and ANXA2 are recruited independently.

Why there was a need for an additional lysosome repair mechanism involving ANXA1 and ANXA2 was then investigated. The recruitment dynamics of ANXA1/A2 and ESCRTs were examined by live-cell microscopy, which revealed that LLOMe treatment induced the recruitment of the ESCRT CHMP4A to most lysosomes but ANX¬¬¬A1 appeared only on a subset of lysosomes. ANXA1/A2 recruitment is thus more selective than ESCRT recruitment, suggesting that ANXA1 and ANXA2-mediated lysosome repair requires an additional or unique signal for activation.

I then searched for the reason for the ANXA1 and ANXA2's selective recruitment. A difference in recruitment signal between ANXA1/A2 and ESCRTs was ruled out as including the calcium chelator BAPTA-AM when inducing lysosome damage with LLOMe treatment suppressed the recruitment of both ANXA1 and the ESCRT CHMP4A. This led me to consider damage sensitivity as the reason for their differential recruitment: their recruitment might be induced by different levels of calcium, which would depend on membrane wound size (larger wounds would release more calcium). To test this hypothesis, I used the lysosomal retention of 10K-dextran (hydrodynamic radius = 2.3 nm), which can be introduced into lysosomes via endocytosis, to gauge lysosome damage. More heavily damaged lysosomes are more likely to release 10K-dextran.

Live-cell microscopy of ANXA1 and CHMP4A with 10K-dextran retention as a marker for lysosome damage revealed that ANXA1 was recruited to lysosomes that lost most 10K-dextan signal immediately after LLOMe treatment. On the other hand, CHMP4A could be observed on ANXA1-positive lysosomes and on other lysosomes that lost 10K-dextran at a more gradual rate, suggesting that while ESCRTs localized to all damaged lysosomes, ANXA1 and ANXA2 recruitment was limited to more heavily damaged lysosomes. This was confirmed by taking the reverse approach: lysosomes were first categorized as 10K-dextran positive or 10K-dextran negative, and then assessed for the presence of ANXA1 or CHMP4A. Indeed, ANXA1 signals were mostly found on 10K-dextran negative lysosomes whereas CHMP4A signals were found at high levels among 10K-dextran negative and positive lysosomes. These data indicate that ANXA1 and ANXA2 preferentially localize to lysosomes with membrane wounds permitting the release of 10K-dextran.

Next, I examined whether ANXA1/A2-mediated repair is important for resolving larger membrane wounds by monitoring the rate of 10K-dextran release from lysosomes. If lysosomes with wounds allowing the release of 10K-dextran were not repaired efficiently, 10K-dextran would leak out lysosomes at a faster rate. This was observed in cells depleted of ANXA1 or ANXA2 and to a lesser extent in cells depleted of the early ESCRT factors, ALIX and TSG101. Hence, membrane wounds exceeding the size of 10K-dextran may require the activity of ANXA1 and ANXA2 to be resolved efficiency without excessive loss of lysosomal contents.

The findings in this study suggest that lysosome repair is primarily mediated by ESCRTs but the repair of more heavily damaged lysosomes (that become unable to retain 10K-dextran) calls for an additional repair mechanism involving the established plasma membrane repair proteins, ANXA1 and ANXA2. Thus, the mechanism of lysosome repair might vary according to the level of damage, with more strategies being employed with increasing damage severity before crossing a 'critical threshold' when the lysosome is deemed irreparable and discarded. This insight could contribute to our understanding of the degree of lysosomal membrane permeabilization required to cause cell death and lysosome dysfunction in aging and diseased tissues.