

P020 Aggresome formation in response to Ala-Ala-Phe-chloromethylketone is independent of the inhibition of tripeptidyl peptidase II

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We have tried to establish the role of Tripeptidylpeptidase II (TPPII) in proteasome-independent proteolysis. We found that treatment of cells AAF-cmk, a TPPII inhibitor, resulted in the accumulation of ubiquitylated proteins around the centrosome, in a response that resembles that obtained with proteasome inhibitors. Similarly, this accumulation in aggresome-like structures was also dependent on protein synthesis and microtubules, although the kinetics was faster for AAF-cmk than for proteasome inhibitors, as determined in time-lapse experiments using cells stably transfected with EGFP-Ubiquitin. The dependency on protein synthesis is significant, as it seems that a majority of the proteasome substrates are derived from newly synthesized proteins. These accumulations recruited proteasomes and the chaperone Hsp90. Interestingly, treating cells simultaneously with AAF-cmk and epoxomicin resulted in ubiquitin+ accumulations that appeared even in the presence of cycloheximide and therefore were independent of protein synthesis. This was also evident by Western Blot with anti-Ub. However butabindide, a more specific TPPII inhibitor, did not induce significant changes in the distribution of ubiquitylated proteins. Silencing of TPPII with siRNA did not affect the sensitivity of the cells to AAF-cmk with respect to untransfected cells and cells transfected with an irrelevant siRNA. The sensitivity of the cells to epoxomicin did not change either. We believe that the target of AAF-cmk is and activity upstream of the proteasome, dependent on its activity, and different from TPPII.