Mutant ubiquitin as an endogenous reporter for proteasome failure: the threshold hypothesis

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UBB^{+1} is a mutant ubiquitin that accumulates in the neuropathological hallmarks of Alzheimer's disease (AD). UBB^{+1} is a potent inhibitor of the proteasome, however it paradoxically also is an efficient substrate. In AD brain, proteasome activity is diminished, and although there are many candidates, it remains unclear what are the causes of this inhibition. Because UBB^{+1} normally is a substrate for the proteasome, it is unlikely that UBB^{+1} forms the initial trigger for proteasome inhibition. We hypothesized that other AD-related mechanisms precede UBB^{+1} accumulation in inhibiting the proteasome and that UBB^{+1} will contribute to this inhibition after a certain threshold of accumulation is reached.
In this study we verified this threshold hypothesis by using the reversible proteasome inhibitor MG132 in several systems. Organotypical cortex slice cultures of rats were successfully transduced with lentiviral vectors encoding UBB^{+1} and control proteins, including a GFP based proteasome substrate that only accumulates after proteasome inhibition. Transduced slices were treated with drops of medium containing 10 µM MG132. The inhibitor was left on for six hours and subsequently either left on, or washed out. When MG132 was left on overnight, both UBB^{+1} and the GFP reporter protein accumulated. After washout of the inhibitor, proteasome activity was restored as demonstrated by the regained capacity to degrade the GFP reporter protein. UBB^{+1} however was not degraded in all cells, but remained accumulated in more cells than in UBB^{+1} transduced slices without inhibitor treatment. These results show that proteasome inhibition by UBB^{+1} indeed is subject to a threshold level for UBB^{+1}. Accumulated UBB^{+1} is capable of sustaining proteasome inhibition by itself, in cells that were able to degrade UBB^{+1} before treatment with the reversible inhibitor. This effect was quantified in neuroblastoma cell lines and found to be significant. Our results indicate that UBB^{+1} can be involved in neurodegeneration in AD by contributing to proteasome inhibition once a threshold for UBB^{+1} accumulation is reached.

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