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Identification of an HSP90 modulated multi-step process for ERBB2 degradation in breast cancer cells

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The receptor tyrosine kinase ERBB2 interacts with HSP90 and is overexpressed in aggressive breast cancers. Therapeutic HSP90 inhibitors, i.e. Geldanamycin (GA), target ERBB2 to degradation. We have previously shown that HSP90 is responsible for the missorting of recycling ERBB2 to degradation compartments. In this study, we used biochemical, immunofluorescence and electron microscopy techniques to demonstrate that in SKBR3 human breast cancer cells, GA strongly induces polyubiquitination and internalization of the full-length p185-ERBB2, and promotes its cleavage, with the formation of a p116-ERBB2 form in EEA1-positive endosomes (EE). p116-ERBB2 corresponds to a non-ubiquitinated, signaling-impaired, membrane-bound fragment, which is readily sorted to lysosomes and degraded. To define the sequence of events leading to p116-ERBB2 degradation, we first blocked the EE maturation/trafficking to late endosomes/lysosomes with wortmannin, and found an increase in GA-dependent formation of p116-ERBB2; we then inhibited the proteasome activity with MG-132 or lactacystin, and observed an efficient block of p185-ERBB2 cleavage, and its accumulation in EE, suggesting that p185-ERBB2 polyubiquitination is necessary for proteasome-dependent p116-ERBB2 generation occurring in EE. As polyubiquitination has also been implicated in autophagy-mediated degradation of ERBB2 under different experimental conditions, we exploited this possibility and demonstrate that GA strongly inhibits early autophagy, and reduces the levels of the autophagy markers atg5-12 and LC3-II, irrespective of GA-induced ERBB2 polyubiquitination, ruling out a GA-dependent autophagic degradation of ERBB2. In conclusion, we propose that HSP90 inhibition fosters ERBB2 polyubiquitination and proteasome-dependent generation of a non-ubiquitinated and inactive p116-ERBB2 form in EE, which is trafficked from altered EE to lysosomes.

Keywords
ERBB2, cleavage, geldanamycin (GA), polyubiquitin, proteasome