

## **Endocytic downregulation of ErbB2 is stimulated by cleavage of its C-terminus**

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### **Abstract**

High levels of the receptor tyrosine kinase ErbB2 are associated with cancer, and impaired endocytosis of ErbB2 could contribute to its overexpression. Therefore, knowledge about the mechanisms underlying endocytic downregulation of ErbB2 is warranted. The C-terminus of ErbB2 can be cleaved after various stimuli, and after inhibition of HSP90 with geldanamycin this cleavage is accompanied by proteasome-dependent endocytosis of ErbB2. However, it is unknown whether C-terminal cleavage is linked to endocytosis. To study ErbB2 cleavage in relation to endocytic trafficking using confocal microscopy, we made a fluorescent reporter construct consisting of YFP and CFP fused to the N- and C-terminus of ErbB2, respectively (YFP-ErbB2-CFP). This approach allowed us to make quantitative measurements of the ratios between the extracellular and intracellular parts of ErbB2 in various subcellular structures. After geldanamycin stimulation YFP-ErbB2-CFP became cleaved in non-apoptotic cells in a proteasome-dependent manner, and a markedly larger fraction of cleaved YFP-ErbB2-CFP was observed in early endosomes than in the plasma membrane. Furthermore, cleavage took place at the plasma membrane, and cleaved ErbB2 was internalized and degraded far more efficiently than full-length ErbB2. Concordantly, a C-terminally truncated ErbB2 was also readily endocytosed and degraded in lysosomes compared to full-length ErbB2. Altogether, we suggest that geldanamycin leads to C-terminal cleavage of ErbB2 which in turn releases the receptor from a retention mechanism and causes endocytosis and lysosomal degradation of ErbB2.