June 2007
Volume 6

CENTER FOR CANCER RESEARCH
frontiers IN SCIENCE

MOLECULAR BIOLOGY

Hsp90 Keeps the Activity of the Oncogenic ErbB2 Kinase at Bay

Xu W, Yuan X, Beebe K, Xiang Z, and Neckers L. Loss of Hsp90 association up-regulates Src-dependent ErbB2 activity. *Mol Cell Biol* 27: 220–8, 2007.

The oncogenic receptor tyrosine kinase ErbB2, also called HER2, has high kinase activity and is a preferred dimeric partner of other members of the family, which includes the epidermal growth factor receptor (EGFR or ErbB1), ErbB3, and ErbB4. Interaction of wild-type ErbB2 with the molecular chaperone Hsp90 is necessary for protein stability. In the current study, we demonstrated an additional function for Hsp90 association—namely to serve as a break on ErbB2 kinase activity.

Hsp90 inhibition by geldanamycin or its derivative 17AAG, which is currently in phase II clinical trials, induces rapid and profound ErbB2 degradation. We have previously shown that this requires a direct interaction between Hsp90 and the ErbB2 kinase domain. Point mutations within the kinase domain of ErbB2 that disrupt Hsp90 association (ErbB2-5M) confer resistance to Hsp90 inhibitors. Interestingly, ErbB2-5M displayed significantly elevated steady state kinase activity compared with the wild-type protein, and it was better able to transform NIH3T3 cells. These data suggested that Hsp90 association represses ErbB2 activity.

We sought to identify the molecular mechanism underlying the elevated activity of ErbB2-5M. One way to regulate the activity of a receptor tyrosine kinase is via phosphorylation of its activation loop (A-loop). Phosphorylation stabilizes the A-loop in a conformation that is permissive for substrate binding. Indeed, Western blotting with site-specific antibodies showed increased tyrosine (Y)877 phosphorylation in the A-loop of ErbB2-5M (compared with the wild-type protein). Phosphorylation of the A-loop is often mediated by intermolecular action between the protomers of a receptor dimer, but we showed that phosphorylation of Y877 on ErbB2 is carried out by Src kinase. There are ten members of the Src family. Although many of them are expressed primarily in hematopoietic cells, three of them, Src, Fyn, and Yes, are expressed in cells that also express ErbB2. We investigated the involvement of these three kinases in phosphorylating ErbB2 on Y877 by knocking down their expression with specific siRNAs and found that all three contribute to Y877 phosphorylation. Consistent with this finding, simultaneous knockdown of all three kinases reduced Y877 phosphorylation to a lower level than did individual knockdowns.

Further, Y877 on ErbB2-5M was not hyper-phosphorylated in SFY cells, which are deficient in these three kinases, but its phosphorylation was restored when Src expression was restored in these cells.

By using molecular and pharmacological techniques, we showed that Y877 phosphorylation markedly elevates ErbB2 kinase activity. In contrast, even though Src mediates phosphorylation of the analogous residue in the A-loop of EGFR (ErbB1), EGFR kinase activity is not affected. To explore why these highly homologous kinases respond differently to Src-mediated A-loop phosphorylation, we compared the sequences and 3-dimensional structures of EGFR and ErbB2 kinase domains. We observed some sequence differences in the A-loops (Figure 1, part A) and in the topology of surrounding regions. Energetic analysis indicated that, in EGFR, an unphosphorylated A-loop adopts an activated configuration stabilized by both intramolecular interactions and interactions with solvent. Phosphorylation of the loop further stabilizes the configuration but does not introduce additional conformational changes. In contrast, the unphosphorylated A-loop of ErbB2 cannot adopt an activated configuration due to lack of favorable intra-molecular and solvent interactions. It flips away from the ATP-binding cleft, and is incapable of aligning ATP and substrate. Upon Y877 phosphorylation, the phosphoryl group establishes strong salt bridges that induce a conformational change in the A-loop and enable it to attain the active conformation, as shown in Figure 1, part B.

Figure 1. The A-loop of the ErbB2 kinase domain adopts a different conformation from that of epidermal growth factor receptor (EGFR or ErbB1). *A)* Sequence alignment of the A-loops of EGFR and ErbB2. Identical residues are indicated with a vertical bar, similarity by a dot. Asterisks indicate the phosphorylated tyrosine residues. *B)* Superimposition of the modeled structures of the ErbB2 kinase domain with the A-loop in phosphorylated or unphosphorylated states. Red denotes the unphosphorylated and blue denotes the phosphorylated A-loop.

Our data thus uncovered a novel function of Hsp90 as a repressor of Src-dependent ErbB2 activation. These results also revealed the molecular mechanism by which Src kinases activate ErbB2, and they suggest that Src is a viable molecular target in tumors expressing elevated ErbB2 activity.

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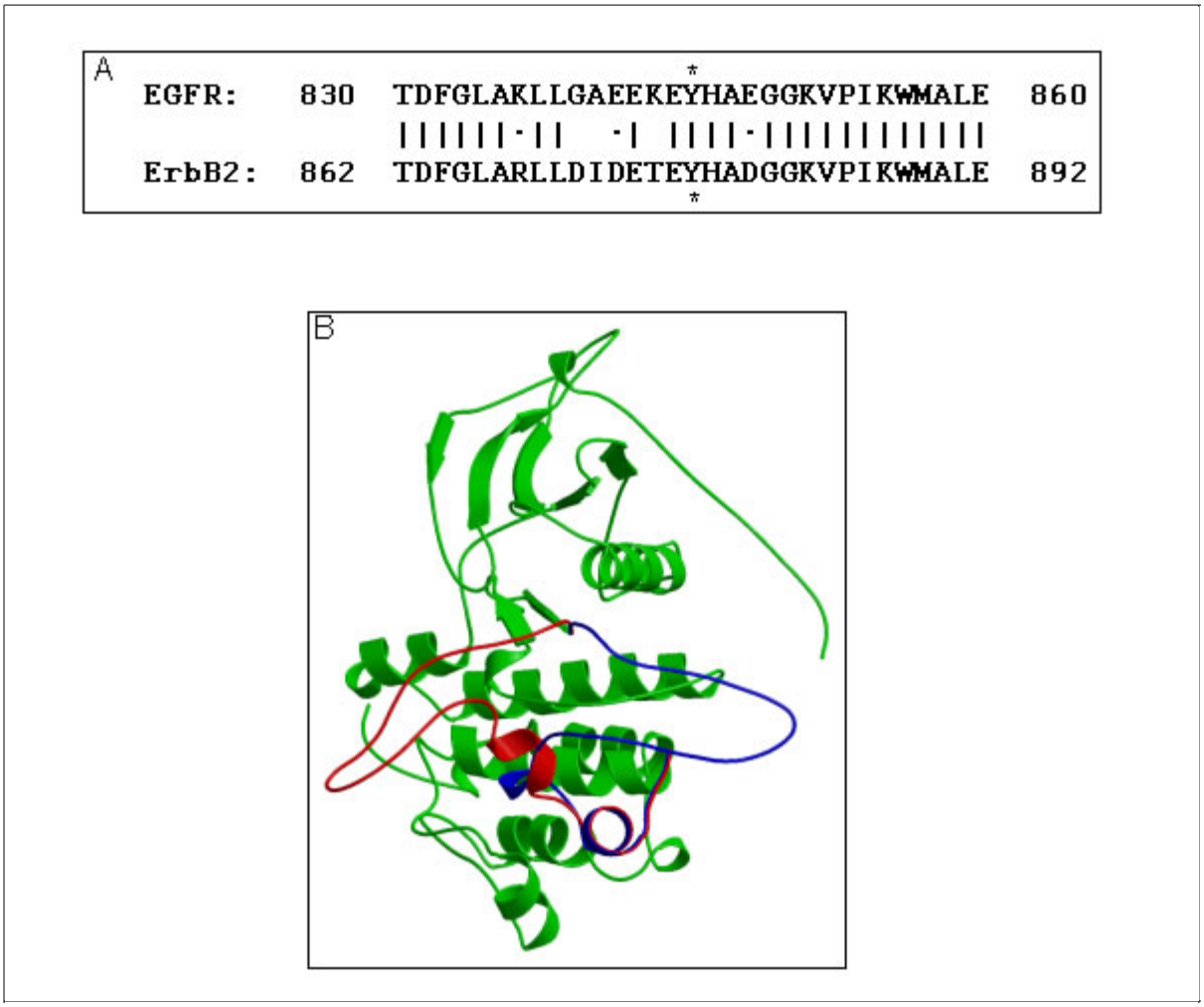

Figure 1. The A-loop of the ErbB2 kinase domain adopts a different conformation from that of epidermal growth factor receptor (EGFR or ErbB1). *A)* Sequence alignment of the A-loops of EGFR and ErbB2. Identical residues are indicated with a vertical bar, similarity by a dot. Asterisks indicate the phosphorylated tyrosine residues. *B)* Superimposition of the modeled structures of the ErbB2 kinase domain with the A-loop in phosphorylated or unphosphorylated states. Red denotes the unphosphorylated and blue denotes the

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