Supporting Information

Cloning the HER2 ECD coding region into pcoldI vector

The plasmid pCMV-Neo-her2 encoding a full length cDNA (4.4 kb) of human HER2 was kindly donated by Dr. Nejadmoghaddam. This plasmid was used as a template to amplify the HER2 Extra cellar domain 3 and 4 coding region with two primers (forward with *Nde*I restriction site 5′- AT*ACATATG*TGCTATGGTCTGGGCATGGAG-3′ and reverse with *Sal*I restriction site 5′ AAT*GTCGAC*TCACGTCAGAGGGCTGGCTCTCTG-3′) by polymerase chain reaction (PCR). The two primers were designed to the GenBank Accession No. NM_004448.

Overexpression and purification

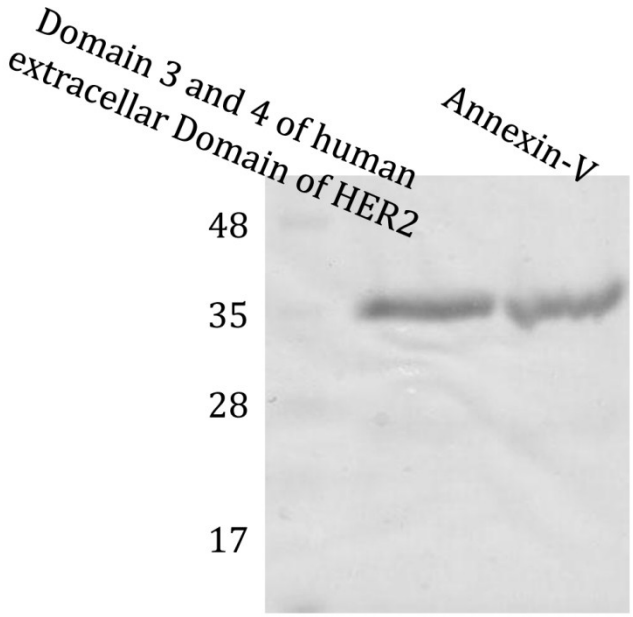
Five milliliters of Terrific Broth (TB) medium containing 50 mg ml^{-1} Ampicilin was inoculated with a fresh bacterial colony counting the expression plasmid, and grown at 37 °C overnight. Then 200 ml of medium was inoculated with 500 ml overnight cultures and grown at 37 °C with vigorous shaking until the OD_{600} reached 0.9. Subsequently, IPTG and lactose were added to the solution to a final concentration of 1 mM and 4 mM, respectively. The mixture was incubated at 15 °C overnight with vigorous shaking. The cells were harvested by centrifugation at 5000 g for 15 min. The cell pellet was resuspended in a lysis buffer [50 mM Tris– HCl, 300 mM NaCl, 10 mM imidazole, and 1 mM PMSF (added fresh) (pH 7.8)]. For purification of protein from inclusion body, different solubilization buffers for inclusion body aggregates were screened and purification protocol was optimized according to previous study (Nazari, Zarnani et al. 2017). In brief, cells harvested from 200 ml culture were lysed in a lysis buffer II (50 mM Tris-HCl, 5 mM EDTA, 1 mM PMSF, pH 8.5) and sonicated for 10 cycles of 1 min each (short pulses of 20 s followed by a gap of 40 s). Cell lysate was centrifuged at 20,000 g for 20 min at 4 °C. Then the pellet was suspended in wash buffer I (50 mM Tris-HCl, 5 mM EDTA, 1 mM PMSF, and 0.5% deoxycholic acid (DOC), pH 8.5 followed by sonication and centrifugation as mentioned above. Then, the pellet was aliquated and solubilized in different buffers finally buffer C: (50mMTris-HCl, 5mMEDTA, 1mM PMSF, and 2Murea, pH 12 was selected. Purification of His_6-tagged fusion protein was performed with the Ni-NTA spin column as described by the manufacturer (Qiagen). The Annexin V from previous study was also expressed as mentioned before (Nazari, Emamzadeh et al. 2012).

Results

Domain III and IV of extracellular of Human Erbb2 (HER2) protein

311aa 34.3kd

CYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQ
VFETLEEITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRS
LRELGSGLALIHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLACHQ
LCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQP
QNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQP
CPINCTHSCVDLDDKGCPAEQRASPLT



Nazari, M., et al. (2012). "Renilla luciferase-labeled Annexin V: a new probe for detection of apoptotic cells." Analyst **137**(21): 5062-5070.

Nazari, M., et al. (2017). "Optimized protocol for soluble prokaryotic expression, purification and structural analysis of human placenta specific-1 (PLAC1)." Protein expression and purification **133**: 139-151.