NRluc-hER$_{281-549}$-CRluc

hERNCRluc

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Chemical name:	NRluc-hER$_{281-549}$-CRluc	
Abbreviated name:	hERNCRluc	
Synonym:		
Agent category:	Polypeptide	
Target:	Other	
Target category:	Other - protein folding	
Method of detection:	Optical imaging	
Source of signal/contrast:	Luciferin	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Estrogen receptors (ERs) are ligand-inducible nuclear transcription factors belonging to the superfamily of steroid hormone receptors (1). The endogenous ligands of ERs such as estradiol (E2) (2), an important estrogen in humans, are secreted by endocrine cells and distributed to their targets *via* blood circulation (3). Upon the binding of estrogens to their inactive apoprotein forms in the cytoplasm or nucleus, ERs are transformed into active receptors that can effectively bind to a DNA element (hormone response element, HRE), leading to transcription of genes (3). ERs consist of six functional domains (A–F): the A/B region contains transactivation functions, region C contains DNA-binding domain (DBD), and region E contains ligand-binding domain (LBD) with an activation function (AF-2) (4). The LBD is an antiparallel helical sandwich of three layers formed by

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twelve α-helices (H1–H12) and one β-turn (5). The helix H12 serves as a lid in the ligand-binding cavity, thus, behaves as a "molecular switch" to prevent or enhance ER from binding to an array of co-activator proteins (6). The binding of ligands induces a reposition of the helices, especially the reorientation of H12, in the LBD, and can generate different functions. For instance, the binding of ER agonists such as E2 or diethylstilbestrol (DES) leads to H12 aligned over the LBD to yield a specific binding site for the consensus LXXLL motif in AF-2; the binding of selective ER modulators (SERMs) such as raloxifene or tamoxifen sterically hinders the H12 positioning to prevent the formation of a binding surface for AF-2 (4). ERs are responsible for the growth, development, and maintenance of the reproductive skeletal, neuronal, and immune systems (6). The deficiency or excess of estrogens can lead to various diseases including osteoporosis and breast carcinoma. A variety of therapeutic strategies have been developed on the basis of the anti-ER mechanism, including the use of SERMs to provide optimal agonist or antagonist activities in ER-expressed tissues.

Renilla luciferase (Rluc) is a 36-kDa enzyme protein extracted from a bioluminescent soft coral (sea pansy (*Renilla reniformis*)) (7). Rluc can catalyze emission of light from substrates; i.e., the oxidation of coelenterazine to coelenteramide generates a green fluorescence (535–550 nm) (8). Rluc can be split into two domains: a large N-terminal domain (amino acids 1–221) and a C-terminal domain (amino acids 230–311) (9). Splitting Rluc into N- and C-terminal fragments destroys its enzymatic activity, resulting in a complete loss of bioluminescence. The enzymatic activity or bioluminescence can be restored if the N- and C-terminal fragments are in close proximity. This led to the development of the split reporter, a novel labeling strategy that has been used in imaging protein–protein interactions (10) and/or protein activity (11) *in vivo*. For instance, protein A is connected with the N-terminal fragment of Rluc, and protein B is connected with the C-terminal fragment of Rluc. Interaction between proteins A and B recovers the enzymatic activity of Rluc by bringing the two fragments of Rluc close together, which allows for recovery of bioluminescence (10).

NRluc-hER$_{281\text{-}549}$-CRluc (hERNCRluc) is an optical agent used to image the intramolecular folding of human ER (hER) *in vivo* (6). hERNCRluc consists of four sequentially linked components: an N-terminal Rluc (N-Rluc) fragment, a fragment of hER α-isoform (hERα) containing the LBD (amino acids 281–549, hER$_{281\text{-}549}$), a spacer linker ((GGGGS)$_2$), and a C-terminal Rluc (C-Rluc). The included hER fragment (hER$_{281\text{-}549}$) provides a binding cavity for various ligands, and its conformation can be altered upon binding of the ligands. Depending on the produced protein-folding pattern, partial to full complementation of the N-Rluc and C-Rluc occurs, which is measurable with bioluminescence imaging. hERNCRluc is suitable for distinguishing the functionalities in various ER ligands (i.e., agonists, antagonist, or SERMs) with *in vivo* imaging.

Synthesis

[PubMed]

Paulmurugan et al. briefly described the preparation of hERNCRluc *via* complementation strategy (6, 10). The N-terminal portion (amino acids 1–229) was amplified with polymerase chain reaction (PCR) using *Nhe*I forward primer with start codon and *Bam*HI reverse primer with the peptide linker sequence $(GGGGS)_2$ with phRL-CMV as a template. The C-terminal portion (amino acids 230–311) of the Rluc gene was also amplified with PCR using *Bam*HI forward primer and *Xho*I reverse primer with stop codon. The fragments were cloned into the *Nhe*I and *Xho*I restriction sites pcDNA3.1(+) to generate the plasmid vector pcDNA-NRluc-CRluc. Then the fragment (amino acids 281–549) of hERα gene was amplified with PCR using *Bam*HI forward primer and *Bam*HI reverse primer followed by subsequent insertion into the plasmid vector pcDNA-NRluc-CRluc. The produced pcDNA-NRluc-$hER_{281\text{-}549}$-CRluc plasmids were used to transfect 293T cells (ER-negative) using lipofectamine, where the fusion protein, NRluc-$hER_{281\text{-}549}$-Rluc, was expressed.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Paulmurugan et al. used hERNCRluc to examine the ligand-induced hER intramolecular folding *in vitro* (6). 293T cells were transiently transfected with pcDNA-NRluc-$hER_{281\text{-}549}$-CRluc. Before and after treatment with 1 μM of various ER ligands, Rluc protein activity was assayed to measure its complementation level, and the complemented Rluc protein level was evaluated with Western blot analysis. Compared with the control (DMSO), the complementation level was found to increase 80-fold ± 15-fold with ER SERMs (tamoxifen, raloxifene, 4-hydroxytamoxifen (4-OHT)), 15-fold ± 5 fold with ER agonists (E2, DES), and no apparent complementation with a very low affinity ER agonist genistein. The treatment with E2, DES, genistein, and raloxifene caused little change in the Rluc level, but a significant reduction was found in the cells treated with the SERM tamoxifen. These results demonstrated that the variations in Rluc activity were not mediated through the Rluc protein level but were more likely to be caused by different complementation patterns within the receptor induced by the ligands. To reduce the binding of endogenous agonist E2, a mutant hERα segment with a glycine-to-threonine (G521T) transition ($hER_{281\text{-}549/G521T}$) was studied under the similar conditions. 293T cells were transiently transfected with pcDNA-NRluc-$hER_{281\text{-}549(G521T)}$-CRluc followed by treatment with 1 μM of the agonists or SERMs. The threonine replacement of Gly521 led to a 94% reduction in the E2-induced Rluc activity, a 12–22% reduction in Rluc activity induced by DES, 4-OHT, or raloxifene, and no significant change in Rluc activity after genistein treatment. The results suggested that the mutant hER retained much higher sensitivity to DES and the SERMs compared with the endogenous agonist E2, which allows the *in vivo* evaluation of the effects of various ligands. Paulmurugan et al. also studied the kinetics of ligand-induced Rluc complementation *in vitro* (6). 293T cells were transiently transfected with pcDNA-NRluc-$hER_{281\text{-}549}$-CRluc and subsequently treated with 1 μM of agonists (E2, DES) or SERM (4-OHT). The cells were assayed for the Rluc activity at 6, 12, 18, and 24 h after treatment. All treated cells exhibited increased Rluc

activity compared with the control (DMSO), and the activity in 4-OHT–treated cells was significantly higher than that in cells treated with E2 or DES at all time points.

Animal Studies

Rodents

[PubMed]

Paulmurugan et al. used hERNCRluc to examine the ligand-induced hER intramolecular folding *in vivo* (6). 293T cells that were stably transfected with pcDNA-NRluc-$hER_{281\text{-}549}$-CRluc (expressing wild-type hER, the wild-type hER sensor) or pcDNA-NRluc-$hER_{281\text{-}549(G521T)}$-CRluc (expressing mutant hER, the mutant hER sensor) were implanted in the lower backs of nude female mice ($n = 3$). Bioluminescence imaging of Rluc activity was performed with a cold charged-coupled device (CCD) camera immediately after cell implantation and 18 h after intraperitoneal injection of raloxifene (0.5 mg). In each imaging session, 100 μl coelenterazine (50 μg) in PBS was injected intravenously into mice at 5 s before imaging. A significantly higher Rluc activity was observed at the site with the mutant hER sensor ($9.7 \pm 1.2 \times 10^3$ photons per second per square centimeter per steradian (p/s/cm^2/sr)) than at the site with the wild-type hER sensor ($2.16 \pm 0.52 \times 10^3$ p/s/cm^2/sr). The low Rluc activity in the wild-type hER sensor was most likely related to the competitive binding of endogenous agonist E2 in the animal. In a similar manner, the mutant hER sensor was used to differentiate ER ligands with *in vivo* imaging. Bioluminescence images were collected 24 h after the implantation of transfected cells (before ligand administration) and every 24 h after administration of 24 μg of ER agonist (DES) or SERM (4-OHT) in sesame oil or 50 μl sesame oil as a control. A significantly higher Rluc activity was observed in the animal that received the SERM (4-OHT) (~2.2×10^3 p/s/cm^2/sr) than in the animal that received the agonist (DES) or that served as the control (~1×10^3 p/s/cm^2/sr) at 48 h, demonstrating the feasibility to distinguish ER ligand in living animals.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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