RESEARCH COMMUNICATION

Binding Capacity of ER-α, Ligands and SERMs: Comparison of the Human, Dog and Cat

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Abstract

The estrogen molecule is the major risk factor related to mammary gland tumors, with estrogen receptor alpha (ER-α) as the important target stimulating growth. Therefore one alternative approach to treatment of breast cancer is to use selective estrogen receptor modulator (SERM), hormonal therapy. In this study, the structures of ER-α in humans, dogs and cats were predicted using the amino acid sequencing data bank and corrected for general protein structures, receptor sites and docking by adding 2,344 ligands with 15 SERMs into the database and calculating estimated inhibition constants (Ki). Thereby, ranking of best ligands of SERMs in humans, dogs and cats could be achieved. The results show that the shapes of ER-α differ between species but the major pocket sites are the same. Bazedoxifene, a new SERM proved to be the best estrogen antagonist and ER-α inhibitor in all species (human, dog, cat) with the lowest Ki. The other good ligands for dogs and cats are Neohesperidin, Dihydrochalcone, and Schreiber2. The differences in these protein structures may explain why there are only a few SERMs or other ligands which can be used as anti-cancer drugs.

Keywords: Estrogen antagonist, ER-α, mammary gland tumor, SERMs

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Introduction

Nowadays, the incidence of cancers including mammary gland tumor or breast cancer has increased more than in the past. Risk factors are genetics, patient age, hormone, toxin, food and life style. Not only the dogs but also the cats are found mammary gland tumor like a human (Schneider, 1970). Conventional treatments of breast cancer are chemotherapy and surgical removal. The adverse effects of chemical substances and postsurgical metastasis are resulting in shorter survival time. Alternative treatments are hormone therapy and/or directly control function of estrogen receptors. Cosman and Lindsay (1999) studied the effect of hormone therapy on patient survival time and they found that the patient life span had positive correlation to hormone therapy.

Estrogen plays important roles in mammary gland tumorigenesis especially early stage and most of receptors identified are ER-α. This hormone stimulates chondrocytes proliferation and bone growth; however, it may involve in chondrosarcoma development (Cleton et al., 2005). Estrogen receptors have been classified into two sub groups (ER-α and ER-β). Estrogen receptors alpha (ER-α) locate in uterus, vagina, mammary gland, liver, pituitary gland and estrogen receptors beta (ER-β) locate in ovary, prostate gland, lung, hypothalamus and urinary bladder (Mitlak and Cohen, 1997). Estrogen receptors play important roles in clinical diagnosis of mammary tumor in human and other mammals such as dogs and cats. Mulas et al. (2000) studied ER in feline benign and malignant mammary gland tumor by immunohistochemistry. They determined ER- α and PR expression as predictors of disease-free period in canine mammary tumor (2001). Several research groups identified ER-α and ER-β in benign and malignant mammary gland tumor by biochemistry and immunohistochemistry technique (Mulas et al., 2001; 2005; Illera et al., 2006).

The similarities of ligand binding domain of ER subtypes were studied by homology modeling (DeLisle et al., 2001). Nevertheless, ER-α and ER-β were ligand specific (DeLisle et al., 2001; Hillisch et al., 2004). ER-α agonists induced uterine cells proliferation, reduced bone lysis, reduced LH and FSH in plasma in spite of ER-β (Hillisch et al., 2004).

ER-α is the most important target in breast cancer over the past 30 years. Selective estrogen receptor modulators (SERMs) alter estrogen and ER-α binding capacity. For example, Tamoxifen, Raloxifene and Bazedoxifene. Tamoxifen is anti-estrogenic effect and can be used as therapeutic and preventive medicine. It has been changed

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therapeutic concepts of breast cancer (Ariazi et al, 2006). Although this drug increases survival time, its adverse effects on bone and uterus have been found (Mitlak and Cohen, 1997; Cosman and Lindsay, 1999). Raloxifene uses as prevention of osteoporosis in elderly; however, it alters lipid metabolism in liver cells (Mitlak and Cohen, 1997; Cosman and Lindsay, 1999).

This study simulated 3D structure of ER-α by homology modeling technique and calculated binding affinity of ligands to ER binding sites by molecular docking technique. We aimed to predict ER-α and specific ligands that act resemble the anti-breast cancer molecules.

Materials and Methods

Protein sequencing and structural comparisons

The ER-α Protein information could be collected from protein data bank (PDB), provided by several websites, http://www.rcsb.org, http://www.ncbi.nle.nih.gov, etc. Human ER-α, dogs ER-α, and cats ER-α sequences were downloaded from RCSB (http://www.rcsb.org) by sequence of 3ERT, NCBI (www.ncbi.nlm.nih.gov) by sequence of XP_533454.2, UniProt (http://www.uniprot.org) by sequence of Q53AD2, respectively. The similarities of ER-α sequences between human, dog and cat were compared by Clustalw (http://www.ebi.ac.uk/Tools/clustalw2/index.html).

Homology modeling

The collected amino acid sequences were performed protein structures by Modweb server. Firstly, Modweb (http://salilab.org/modweb) predicted the possibility of ER-α structure in pdb file type. Then ER-α structure were proved by Procheck (http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html). Result showed by Ramachandran's plot which favors area posed in red zone. This standard method confirmed the corrected protein structure by amino acids angle. The operation system e.g. Pymol, Rasmol was selected and used for studied protein structure.

Secondly, Q-site finder (http://www.modelling.leeds.ac.uk/qsitefinder) simulated the pocket sites of ER-α by hydrophobic probe clusters finding. The most favorable binding energy of pocket sites was marked.

Molecular docking

Ligand databases were collected from Chembank (Harvard University). Then 2,344 ligands and 15 SERMs were converted to pdb file type by Openbarbel (http://openbabel.org/wiki/install). After that electric charges were added and all pdb file type was converted to pdbqt by AutoDockTools (http://autodock.scripps.edu/downloads). This study converted all files by Linux operating system. Next step was settled grid box by AutoDockTools (ADT). The grid box was a 3-dimension box which was docking area on ER-α pocket sites. Ligands were allowed to freely dock to the pocket sites. Grid parameter file (gpf) was necessary for generating grid energy maps by AutoGrid4. Then allowed Docking Parameter File (DPF) to coordinate with AutoDock program and led program know "What is the map file requirement?", "Where is the ligand center on the receptor?", "How many loops of docking are setting?" etc. Finally, run shell script files.

Table 1. CLUSTAL 2.0.10 Multiple Sequence Alignment

ER-a	10	20	30	40	50	60
Human	MTMTLHTKAS	GMALLHQIQG	NELEPLNRPQ	LKIPLERPLG	EVYLDSSKPA	VYNYPEGAAY
Dog	MTLHTKASGM	ALLHQIQGPE	LDSLNRPQLK	IPLERPLGEV	YVDSSKPAVY	NYPEAGAYDF
Cat	MTMTLHTKAS	GMALLHQIQG	NELETLNRPQ	LKIPLERPLG	EVYVDGSKPA	VYNYPEGAAY
ER-a	**70**	**80**	**90**	**100**	**110**	**120**
Human	EFNAAAAANA	QVYGQTGLPY	GPGSEAAAFG	SNGLGGFPPL	NSVSPSPLML	LHPPPQLSPF
Dog	NAAPAAPAPL	YGQSGLGYGP	GSEAVAAAAF	GANGLGGFPP	LNSMSPSPPV	LLHPPPQLSS
Cat	DFNAAAAASA	PVYGQSGLAY	GSGSEAAAFG	ANGLGGFPPL	NSVSPSPLVL	LHPPPQLSPF
ER-a	**130**	**140**	**150**	**160**	**170**	**180**
Human	LQPHGQQVPY	YLENEPSGYT	VREAGPPAFY	RPNSDNRRQG	GRERLASTND	KGSMAMESAK
Dog	FLHPHGQQVP	YYLENEPSGY	AVRQAGPPAF	YRPNSDNRRQ	GGRERLASTS	DKGNMAMESA
Cat	LHPHGQQVPY	YLENEPSGYA	VREAGPPAFY	RPTSDNRRQS	GRERLASTGD	KGSMAMESAK
ER-a	**190**	**200**	**210**	**220**	**230**	**240**
Human	ETRYCAVCND	YASGYHYGVW	SCEGCKAFFK	RSIQGHNDYM	CPATNQCTID	KNRRKSCQAC
Dog	KETRYCAVCN	DYASGYHYGV	WSCEGCKAFF	KRSIQGHNDY	MCPATNQCTI	DKNRRKSCQA
Cat	ETRYCAVCND	YASGYHYGVW	SCEGCKAFFK	RSIQGHNDYM	CPATNQCTID	KNRRKSCQAC
ER-a	**250**	**260**	**270**	**280**	**290**	**300**
Human	RLRKCYEVGM	MKGGIRKDRR	GGRMLKHKRQ	RDDGEGRGEV	GSAGDMRAAN	LWPSPLMIKR
Dog	CRLRKCYEVG	MMKGGIRKDR	RGGRMLKHKR	QRDDGEGRNE	VGSSGDVRTS	SLWPSPLLIK
Cat	RLRKCYEVGM	MKGGIRKDRR	GGRMLKHKRQ	RDEGEGRNEV	GSSGDVRASN	LWPSPLLIKH
ER-a	**310**	**320**	**330**	**340**	**350**	**360**
Human	SKKNSLALSL	TADQMVSALL	DAEPPILYSE	YDPTRPFSEA	SMMGLLTNLA	DRELVHMINW
Dog	HTKKNSPALS	LTADQMVSAL	LEAEPPIIYS	DYDPSRPFSE	ASMMGLLTNL	ADRELVHMIN
Cat	TKKNSPALSL	TADQMVSALL	EAEPPIIYSD	YDPSRPFSEA	SMMGLLTNLA	DRELVHMINW
ER-a	**370**	**380**	**390**	**400**	**410**	**420**
Human	AKRVPGFVDL	TLHDQVHLLE	CAWLEILMIG	LVWRSMEHPG	KLLFAPNLLL	DRNQGKCVEG
Dog	WAKRVPGFVD	LSLHDQVHLL	ECAWLEILMI	GLVWRSMEHP	GKLFFAPNLL	LDRNQGKCVE
Cat	AKRVPGFVDL	SLHDQVHLLE	CAWLEILMIG	LVWRSMEHPG	KLLFAPNLLL	DRNQGKCVEG
ER-a	**430**	**440**	**450**	**460**	**470**	**480**
Human	MVEIFDMLLA	TSSRFRMMNL	QGEEFVCLKS	IILLNSGVYT	FLSSTLKSLE	EKDHIHRVLD
Dog	GIVEIFDMLL	ATSSRFRMMN	LQGEEFVCLK	SIILLNSGVY	TFLSSTLKSL	EEKDHIHRIL
Cat	MVEIFDMLLA	TSSRFRMMNL	QGEEFVCLKS	IILLNSGVYT	FLSSTLKSLE	EKDHIHRVLD
ER-a	**490**	**500**	**510**	**520**	**530**	**540**
Human	KITDTLIHLM	AKAGLTLQQQ	HQRLAQLLLI	LSHIRHMSNK	GMEHLYSMKC	KNVVPLYDLL
Dog	DKITDTLIHL	MAKAGLTLQQ	QHRRLAQLLL	ILSHIRHMSN	KGMEHLYNMK	CKNVVPLYDL
Cat	KITDTLIHLM	AKAGLSLQQQ	HRRLAQLLLI	LSHIRHMSNK	GMEHLYNMKC	KNVVPLYDLL
ER-a	**550**	**560**	**570**	**580**	**590**	**600**
Human	LEMLDAHRLH	APTSRGGASV	EETDQSHLAT	AGSTSSHSLQ	KYYITGEAEG	FPATV
Dog	LLEMLDAHRL	HAPASRGGVP	MEETNQSQLA	TTGPTSSHSL	QTYYITEEAG	NFPTTV
Cat	LEMLDAHRLH	APANRGGAPM	EEMNQSQLAT	TGSTSAHSLQ	AYYITEEAGA	FPTTV

Results

Similarities between human ER-α, dog ER-α and cat ER-α performed by Clustal alignment are shown in Table 1. Differences of amino acid sequences between human ER-α, dog ER-α and cat ER-α result in the variation of protein structures. This is one of reasons that why we can or cannot use same drugs trigger protein across the species. In this study, the same location of pocket sites, represented by the largest white mesh area in Figure 1 (B, D, F).

The results of simulation showed the different binding

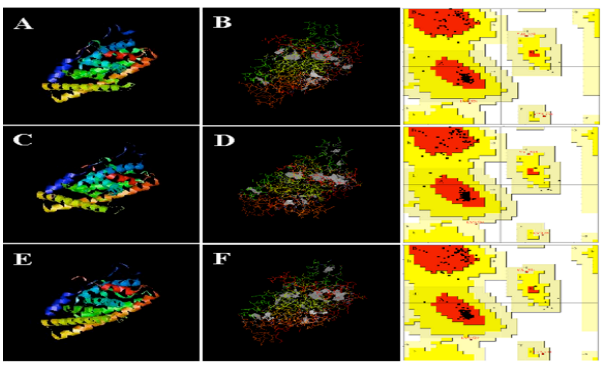

Figure 1. ER-α with Predicted Pocket Sites. A, B) Human ER-α; C, D) canine ER-α; E, F) feline ER-α. Right columns show Ramachandran's plots of human ER-α, dog ER-α, and cat ER-α, respectively

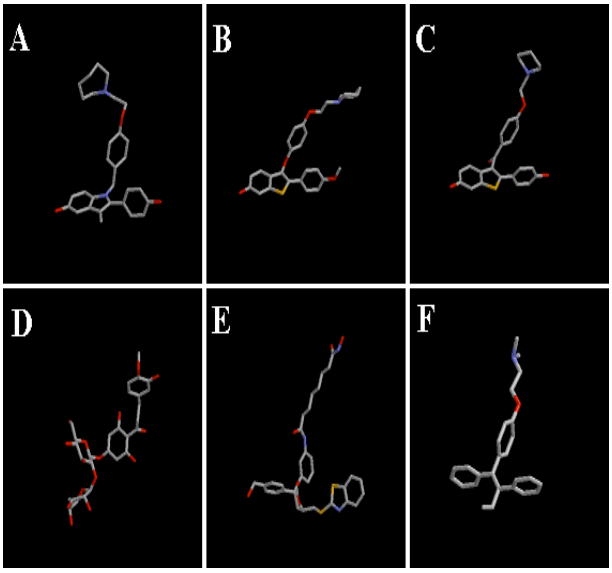

Figure 2. Docking Molecules. A) Bazedoxifene; B) Arzoxifene; C) Raloxifene; D) Neohesperidin dihydrochalcone; E) Schreiber2; F) Tamoxifen

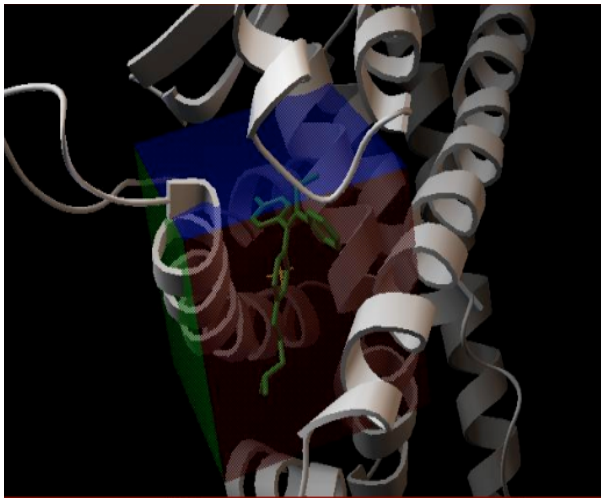

Figure 3. Area Studied. Human ER-α was docking by 4-hydroxytamoxifen (green molecule). Grid box (15x15x15 A°)

domain shapes between human ER-α, dog ER-α and cat ER-α but the major pocket sites are very similar. However, there is more similarity between human ER-α and cat ER-α binding domains. Ramachandran's plot was reported at 95.1% in human ER-α, 94.6% in dog ER-α and 95.5% in cat ER-α.

Fifteen SERMs and 2,344 ligands were collected from Chembank and prepared to dock with human ER-α, dog ER-α, and cat ER-α by Openbarbel. Docking molecules have structure quite similar to natural estrogen. Most of them composed of 4 rings (A, B, C, D); however, the newest SERMs e.g. Bazedoxifene, Raloxifene have one more ring (Figure 2).

AutoDockTools (ADT) settled grid boxes as area of interest. In this study, grid box was set to 15x15x15 A° (Figure 3). At the pocket sites of interest, SERMs and ligands allowed to move freely until the suitable docking position found.

The estimated inhibition constants (Ki) of ligands on ER-α were calculated by Cheng-Prusoff equation.

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_d}}$$

Where IC50 is molar concentration of ligands which produce 50% maximum possible inhibition, [L] is the concentration of the ligand and Kd is the dissociation constant of the ligand. The lower Ki is related the better inhibition properties. The best top ten ranking inhibitory ligands for human ER-α, dog ER-α and cat ER-α show in Table 2.

According to molecular docking results, most of the inhibitor should have Ki between 0.1-10 nM or 100-10,000 pM. Bazedoxifene is the best inhibitor of human ER-α whereas the other new generations of SERMs are in the top five ligands with picomolar Ki level (Table 2). Meanwhile, SERMs in the dogs have only Bazedoxifene and Raloxifene are in the top ten ranking and the first ranking is Neohesperidin dihydrochalcone. For cat's results, there are no SERMs in the top ten ranking.

Molecular dynamic simulation of ER-α docking with 15 SERMs and 2,344 ligands showed that only a few SERMs and ligands can be bounded to pocket sites of human, dog and cat. It may indicate that some SERMs and ligands used in human may not compatible to dog and cat.

Discussion

Table 3 shows compatible SERMs in the human, dog and cat from the best to the worst downward. Bazedoxifene is the best ranking in all species studied.

Table 2. Top Ten Ranking Inhibitory Ligands including SERMs Relevant to estimated Ki in Human, Dog and Cat ER-α Forms

Rank	Human		Dog		Cat	
	Ligands	Ki (pM)	Ligands	Ki (pM)	Ligands	Ki (pM)
1	Bazedoxifene	52.80	Neohesperidin Dihydrochalcone	151.82	Schreiber_2	25.79
2	Beta-carotene	143.54	Schreiber_2	168.05	Tinyatoxin	29.30
3	Arzoxifene	178.58	Beta-carotene	248.65	Beta-catotene	31.18
4	Raloxifene	188.35	Remiszewski_013	340.90	Leptomycin	31.87
5	Lasofoxifene	229.27	Zafirlukast	476.40	u-74389g	37.72
6	Ormeloxifene	312.73	Bisindolylmaleimide II	497.14	Diosmin	40.26
7	Chap16	363.97	Bisindolylmaleimide VI	514.06	Rutoside	48.74
8	Chap1	545.69	Bazedoxifene	689.49	Colletti_14	70.36
9	Fortovase	565.71	Raloxifene	747.21	Indinavir	108.70
10	Lovastatin	614.60	Homoharringtonine	1050.00	Calmidazolium chloride	110.18

Table 3. Comparison of Compatible SERMs with Human, Dog and Cat ER-α Forms Relevant to Estimated Inhibition Constants (Ki)

SERMs	Ki (pM)		
	Human	Dog	Cat
Bazedoxifene	53	689	244
Arzoxifene	179	30960	461
Raloxifene	188	747	1070
Lasofoxifene	229	17150	1670
Ormeloxifene	313	303940	1050
4-hydroxytamoxifen	3610	137400	49150
Toremifene	7140	174580	61310
Tamoxifen	9690	447920	126600
Clomifene	15300	113570	35890

Bazedoxifene is a new generation of SERMs and currently undergoing on phase III studies. It is approve for postmenopausal osteoporosis; however, it also has anti-estrogenic effect on breast and uterus. Bazedoxifene is binding with ER-α with high affinity (Miller et al, 2001). However, the selective effects of Bazedoxifene in cultured breast cancer cells (bMCF-7) were noted. Bazedoxifene did not stimulate ER-α mediated transcriptional activity and antagonist to estradiol (Miller et al., 2001).

Schreiber_2 molecules are in the second rank in dogs and first rank in cats. Schreiber_2 is a deacetylase inhibitor that prevent deacetyl group from lysine. It inhibits DNA transcription and use as novel anticancer agent (Remiszewski, 2002; Vigushin and Coombes, 2002). Neohesperidin dihydrochalcone (NHDC) an artificial sweetener derived from citrus. So far, NHDC has not showed anti-cancer properties but in this study it antagonist to dog ER-α.

Beta-carotene is good inhibitor in all species at picomolar Ki level. So, high dietary consumption of β-carotene may be protective effect. There was less occurrence of breast cancer among women who had high blood levels of beta-carotene than those who had low levels (Wald et al., 1984). Another choice for breast cancer chemotherapy is aromatase inhibitors such as letrozole, anastrozole. Aromatase is an enzyme involved in estrogen synthesis. Aromatase inhibitors block the synthesis of the estrogen and lower the estrogen levels. The less estrogen levels the slow growth of breast cancers (Grube et al, 2001; Howell et al, 2005; Ariazi et al, 2006).

Estrogen receptor alpha (ER-α) is one the most popular target in mammary gland tumor (Nieto et al., 2000; Mulas et al., 2000; Ariazi et al., 2006; Diaz and Sneige, 2005; Imanov et al., 2005; Iller et al., 2006). There are different structures of ER-αand ER-βcan be bounded to estrogen and also SERMs, depended on species(Garderen et al., 1999; Darawiroj et al., 2003; Fuqua et al., 2003; Illera et al., 2006; Gallardo et al., 2007). Therefore, SERMs areused as estrogenic agonist or antagonist depending on what is the required action on organs (Miller et al., 2001).

In human, anti-estrogen therapy is a new therapeutic conceptwhile the new drugs are ongoing invented and experimented continuously (Cosman and Linsay, 1999; Dutertre and Smith, 2000; Grube et al., 2001; Vigushin and Commbes, 2002; Wolohan and Reichert, 2003; Howell et al., 2005). The new generations of SERMs have more inhibitory properties than the past one (Mitlak and Cohen, 1997; Tong et al., 1997; Dutertre and Smith, 2000; Grube et al., 2001; Miller et al., 2001; Wolohan and Reichert, 2003; Hillisch et al., 2004). However, their side effectsand/or estrogenic effects on particular organs must be considered. For example, Bazedoxifene is mainly used to prevent osteoporosis andit is also effect on the prevention of breast cancer. Furthermore, the studies are focused only in osteoporosis and drug safety (Cleton et al., 2005; Chandrasekaran et al., 2009). There is study on the action of the breast cancer protective properties in humans and animals which is very interesting. Only a few studies of ER-α structures and SERMs perform in canine and feline (DeLisle et al., 2001).

The results show that the shapes of ER-α structure are different between species (human, dog, cat) but the major pocket sites are very similar. Bazedoxifene, is the best estrogen antagonist and ER-α inhibitor in all species with the lowest Ki. The other good ligands for dogs and cats are Neohesperidin,Dihydrochalcone, and Schreiber_2, respectively.The differences of ER-α structure may explain why there are only a few SERMs or a few ligands can be used as the anti-cancer drug. It may further studyofwhich SERMs and ligands arecompatiblefor companion animals.

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