Published in final edited form as:

Chin J Nat Med. 2015 November ; 13(11): 801–807. doi:10.1016/S1875-5364(15)30083-2.

Cancer therapy using natural ligands that target estrogen receptor beta

Gangadhara R Sareddy¹ and Ratna K. Vadlamudi^{1,2,*}

¹Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

²Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

Abstract

Estrogen receptor beta (ER β) is one of the two key receptors (ER α , ER β) that facilitate biological actions of 17 β -estradiol (E2). ER β is widely expressed in many tissues, and its expression is reduced or lost during progression of many tumors. ER β facilitates estrogen signaling by both genomic (classical and non-classical) and extra-nuclear signaling. Emerging evidence suggests that ER β functions as a tissue-specific tumor suppressor with anti-proliferative actions. Recent studies have identified a number of naturally available selective ER β agonists. Targeting ER β using its naturally available ligands is an attractive approach for treating and preventing cancers. This review presents the beneficial actions of ER β signaling and clinical utility of several natural ER β ligands as potential cancer therapy.

Keywords

Estrogen receptor; Estrogen receptor beta; Tumor suppressor; ER beta agonists; Liquiritigenin; S-equol; Genistein

Introduction

The biological effects of 17 β -estradiol (E2) are mediated through their cognate receptors: estrogen receptor alpha (ER α) and estrogen receptor beta (ER β)^[1]. These receptors (ER α and ER β) have extensive similarities; however, ER β has quite a different function than ER α , and ER β functions as a tissue-specific tumor suppressor with anti-proliferative actions^[2]. Several studies showed that overexpression of ER β reduces cell proliferation and that knockdown of ER β enhances cell proliferation in cancer cells^[3–4]. ER β expression is down regulated or lost in several tumors including those of the breast, ovary, prostate, colon and brain^[5–11]. In this review, we summarize the recent evidence for the tumor suppressive role of ER β signaling in cancer progression and discuss the possibility of using natural ER β ligands as therapeutics for treating or preventing cancer.

*[Corresponding author] Tel: 2105674930, Fax: 2105674958; ; Email: Vadlamudi@uthscsa.edu

These authors have no any conflict of interest to declare.

ER β signaling leads to tumor suppression

ER β was discovered in 1996 as the second receptor of E2^[12]. This discovery of a second estrogen receptor advanced the estrogen field and suggested more complexity in hormonal signaling. The human ER β gene (ESR2) is located on chromosome 14q23.2, belongs to the nuclear receptor superfamily, contains three commonly conserved functional domains including an N-terminal activation function (AF1) domain, a central DNA binding domain (DBD) and a C-terminal ligand binding domain (LBD) that contains AF2^[13]. ER β shares extensive homology with ER α (97% similarity in DBD, 59% in LBD, and 16% AF1) (Fig. 1A). ER β functions as a transcription factor and is implicated in modulation of genes involved in multiple pathways. However, the molecular mechanism(s) through which ER β mediates growth inhibition of cancer cells remains elusive. Accumulating evidence suggests that ER β functions as either a homodimer (ER β : ER β) or heterodimer (ER α : ER β) depending on the status of the cellular expression of ERs^[1]. Expression of ER subtypes vary depending on tissue, some tissues such as uterus and mammary gland express more ER α than ER β , while some tissues such as lung and ovary uniquely express ER β , and tissues such as bone express both subtypes^[14]. The ratio of ER subtypes present in a cell dictates the complexity and magnitude of signaling depending on the status of mono or heterodimers (Fig. 2). Target gene studies using genome-wide approaches revealed that ER α and ER β share many genes; however, ER β has the potential to activate unique set of genes^[15]. Genome-wide ChIP-on-ChIP studies suggested that a dynamic interplay exists between ER α and ER β in their selection of chromatin binding sites and that the ligand subtype determines the spectrum of chromatin binding^[16]. Recent genomic studies also suggested that ER α and ER β have the potential to activate different sets of genes and that ER β effects can be non-classical via its interactions with other transcription factors such as AP1, SP1, NF- κ B and KLF5^[16-17]. Evidence suggests that ER β is expressed as multiple isoforms, (with variations in the C-terminal domain ER β 1–5); however, much of the published data is focused primarily on ER β 1^[1]. ER β isoforms 2 to 5 have weak ligand binding affinity due to deletions in C-terminal region and some isoforms such as ER β 4 and ER β 5 appear to respond to ligand signaling via hetero-dimerization with ER β 1 (Fig. 1B)^[18]. Global gene expression studies comparing E2 and natural ER β agonists revealed that natural ER β agonists notably reduce the stimulation of genes promoting proliferation and preferably induce genes that are more pro-apoptotic. Further, these studies showed that each ER β agonist has the ability to promote a unique set of genes and pathways in cancer cells. In addition, the gene expression pattern elicited by natural ER β ligands is dependent on the status and ratio of ER subtypes present in a cell^[19]. Collectively, these findings suggest that the overall action of ER β on the genome of hormone-responsive cells appears to depend on the relative concentrations of both ERs, the status of ER β isoforms, the repertoire of coregulators and the type of ligand in a given cell.

Natural ligands of ER β

Even though ER α and ER β are structurally similar, their ligand-binding domains differ enough to be selective for different ligands^[20]. Several studies have identified a number of naturally available selective ER β agonists that are currently being investigated for therapeutic use^[2]. Identification of unique ligands that act through ER β provides a unique

therapeutic opportunity to target ER β for tumor suppression. In this review, we focus on three naturally available ER β ligands (liquiritigenin, S-equol, and genistein) that have preferential agonist activity for ER β compared to ER α (Fig. 1C), and recent evidence suggests that these compounds may have potential utility in cancer prevention and/or treatment.

Liquiritigenin—The MF101 formulation that comprises 22 botanically derived active ingredients was initially found to function as a selective ER β agonist and was originally tested for reducing the frequency and severity of menopausal hot flashes^[21]. In a Phase II clinical trial, MF101 was found to be safe and well tolerated and was taken with high compliance^[22–23]. The quest to isolate pure compounds with potent ER β agonistic activity in the MF101 formulation led to the isolation of liquiritigenin from the plant extract of *Glycyrrhizauralensis*^[24]. Liquiritigenin has a 20-fold higher affinity for ER β than for ER α ; however, in biological assays, liquiritigenin functions as a more potent agent, suggesting mechanisms in addition to ER β binding contribute to its activity^[24]. Recent studies suggested that high potency of liquiritigenin is due to the ability of the ER β -liquiritigenin complex to recruit selective coactivators and its ability to bind unique regulatory chromatin sites of estrogen-responsive genes^[25].

Genistein—Genistein is one of the major isoflavones that is present in a number of plants such as soybean^[26]. Other isoflavones such as daidzein, and glycitein are also present in low abundance in soy foods^[27]. The intake of isoflavones in Asian countries is approximately 50 mg daily and only 3–5 mg in Western countries^[28]. Compared to Western women, Asian women have a lower incidence of breast cancer and their soy-rich diet is implicated in this beneficial effect^[29]. Genistein interacts with both ER α and ER β ; however, it has higher affinity for the ER β ^[30]. Genistein also has the potential to modulate ER isoform expression (ESR1, ESR2) and such actions may be involved in a self-limiting mechanism of estrogenic stimulation^[31].

S-Equol—S-equol is a compound that was isolated from the soy isoflavone daidzein via biotransformation^[32]. Metabolic conversion of daidzein occurs in the gut and depends on the gut flora present in an individual. Compared to Westerners, Asians in general are good equol producers^[33]. S-equol has preferential binding to ER β (K_i of 0.73 nmol·L⁻¹ for ER β compared to K_i of 6.41 nmol·L⁻¹ for ER α) and functions as an ER β agonist^[33]. S-equol is currently being tested to treat vasomotor hot flashes associated with menopause^[34] and for the treatment of benign prostate hyperplasia (BPH) (clinical trial NCT00962390). In a Phase II clinical trial, S-equol was found to be safe, and well tolerated and was taken with high compliance.

Utility of natural ER β ligands for cancer treatment

Breast cancer—Recent studies found a therapeutic efficacy using ER β agonists in combination with aromatase inhibitors, and this strategy may be useful in treating aromatase-inhibitor-(AI-) resistant breast cancer^[35]. Epidemiological studies observed an inverse correlation between the intake of genistein and the incidence of breast cancer^[36]. A subset of TNBC cells express ER β , and treatment with liquiritigenin reduced their ability to

invade and migrate. Mechanistic studies showed that treatment with liquiritigenin results in a significant decrease of CXCR4 protein expression, and the results from this study suggested that bone-directed invasion can be inhibited by ER β agonists^[37]. S-equol inhibited the invasion of ER β -expressing TNBC human breast cancer cells via the down-regulation of MMP-2 expression^[38]. Combination therapy of S-equol and tamoxifen activated intrinsic apoptotic pathway more efficiently than each compound alone, suggesting the utility of S-equol in enhancing antiestrogen therapy^[39]. However, some discrepancy exists in the literature regarding the effects of S-equol on breast cancer cells. Few reports showed no effect of S-equol on breast tumor growth^[40], while another study showed stimulatory effects of S-equol on metastasis^[41]. Further studies are needed to clear these discrepancies, and some of the differences in the outcomes may in part reflect the unique models used and the status of ER β .

Prostate cancer—Prostate cancer is the leading cause of cancer death in men, and castration-resistant prostate cancer (CRPC) represents a major problem in clinical management. Dietary consumption of genistein has been linked with lower incidence of prostate cancer^[42]. ER β agonist is shown to promote apoptosis in prostatic stromal, luminal and castration-resistant epithelial cells in mice models. Further, ER β agonist also has the potential to induce apoptosis in xenografted BPH specimens, including in the CD133⁺ enriched stem/progenitor cells^[43]. Since isoflavones have low toxicity, they may be useful in preventing relapse after primary treatments such as radical prostatectomy or radiation therapy^[44]. In interventional studies using purified S-equol, a concentration of >5–10 ng·mL⁻¹ has been shown to be associated with a positive outcome for vasomotor symptoms, osteoporosis, and prostate cancer^[33]. Genistein is shown to increase ER β expression by reducing ER β promoter hypermethylation and also increase its transcriptional activity leading to inhibition of prostate cancer cell proliferation^[45].

Glioma—Gliomas are the deadliest tumors of primary central nervous system. Epidemiological evidence suggests a tumor suppressive role for female sex hormones on brain tumors^[46–47]. The incidence of developing gliomas is greater in males than in females, and females of reproductive age have a survival advantage over males and menopausal females^[46, 48–51]. Several studies demonstrated that gliomas express ER β with low or weak expression of ER α . ER β is highly expressed in low-grade astrocytomas and non-neoplastic brain tissues. In contrast, most of the high-grade tumors express low or decreased ER β expression compared to low grade gliomas, and this lower ER β expression correlates with histological malignancy and poor survival of patients^[10, 52–53]. Hypoxic conditions increase the expression of ER β isoforms in glioma cells and overexpression of ER β 1 and 5 increases PTEN expression, leading to negative regulation on the PI3K/Akt/mTOR and MAPK signaling, supporting the tumor suppressive functions of ER β in gliomas^[54]. Recent studies from our laboratory using *in vitro* and *in vivo* preclinical models also demonstrated that ER β agonists such as liquiritigenin have the potential to inhibit glioma cell proliferation^[10]. Mechanistic studies showed ER β agonists reduce the growth by decreasing the proliferation of tumor cells and by inducing apoptosis. Another study showed that liquiritigenin significantly enhances ER β expression and sensitizes glioma cells to temozolomide by inhibiting the PI3K/AKT/mTOR pathway^[55]. Natural ER β agonists have good blood–brain

barrier permeability and less neuronal toxicity; hence, they are very suitable for therapeutic treatment of gliomas.

Colon cancer—Epidemiological and experimental evidence suggest that isoflavone intake plays an important role in preventing colorectal cancer^[56]. Lack of ER β expression is associated with colon cancer progression, and ER β status is an independent marker of poor survival^[57]. ER β is crucial in mediating the growth-suppressive effects of soy isoflavones against colon tumors, and soy isoflavones have potential for up regulation of ER β expression^[58]. Studies utilizing ER β KO mice revealed that ER β plays key roles in the anti-inflammatory pathways and tissue homeostasis in the colon. The results from this study further revealed that the protective actions of estrogen occur primarily during the initiation/promotion stages of disease development and established that hormonal signaling via ER β could serve as a chemo-preventive agent^[59]. ER β -mediated signaling is shown to inhibit proliferation as well as colon cancer xenograft growth^[60]. Genistein is demonstrated to have the potential to alter tissue homeostasis in the small intestine and colon by exerting antiproliferative and proapoptotic effects via ER β ^[61]. Genistein inhibited growth of colon cancer cells, and increased transcription of ER β and these changes depend on presence of ER β ^[62].

Ovarian Cancer (OC)—OC is the fifth leading cause of cancer death in women in the United States and the most lethal gynecological malignancy^[63–64]. OC cells express ER β and synthetic ER β agonists significantly reduced the OC growth *in vivo*^[65]. Recently, we tested the utility of natural agonists of ER β to inhibit OC progression by employing liquiritigenin and S-equol. Both ER β ligands significantly inhibited the proliferation of OC cells. Treatment with ER β agonists significantly upregulated the expression of ER β via autocrine signaling. Further, ER β agonists significantly reduced the growth of chemotherapy-resistant ovarian cancer cells (cisplatin-resistant ES2, and paclitaxel resistant SKOV-3TR cells) and sensitized them to apoptosis^[66]. Collectively, these findings showed that ER β agonists have the potential to significantly inhibit ovarian cancer cell growth and represent novel therapeutic agents for the management of ovarian cancer.

Other cancers—Naturally available ER β ligands have beneficial therapeutic effects on treating various other cancers. Liquiritigenin had an inhibitory effect on migration via downregulation proMMP-2 and PI3K/Akt signaling pathway in human lung adenocarcinoma A549 cells^[67]. Liquiritigenin is shown to induce apoptosis through MAPK-mediated pathway and liquiritigenin treatment significantly reduced growth of hepatocarcinoma in a xenograft model^[68–69]. Another study showed that S-equol induce apoptosis via activation of intrinsic and endoplasmic reticulum stress pathways in hepatocellular carcinoma cells^[70]. In B16F10 melanoma model, combination therapy of liquiritigenin with cis-diamine dichloroplatinum (CDDP) reduced cell viability, cell migration and metastasis and down regulated expression of MMP-2/9, PI3K, p-AKT and upregulated the PTEN^[71]. Liquiritigenin treatment reduced the cell viability, promoted apoptosis of pituitary adenoma cells and inhibited *in vivo* tumor growth^[72].

Summary

The discovery of naturally available ER β -specific agonists has opened new avenues for the development of novel therapeutics acting through ER β to prolong survival in cancer patients. During the progression of cancer to higher grades, selective pressure by various therapies (chemotherapy, hormonal therapy using anti-estrogens and aromatase inhibitors) modifies the expression, localization and functions of ER β and leads to decreased tumor suppression by ER β . Expression of ER β is down regulated in several tumors and over-expression of ER β is shown to reduce cell proliferation and knockdown of ER β enhances cell proliferation in several cancer model cells. Therefore, restoring ER β tumor expression has the potential for the development as a novel therapies for treating cancer. Recent findings implicate ER β -specific agonists with having growth inhibitory effects in several cancer models. The concentration of ligand, ratio of ER α : ER β and status of coregulators in a tissue influence the beneficial effects of ER β ligands. Since ER β can hamper the signaling of ER α and inhibit proliferation, testing of ER β agonist in combination with other endocrine therapies will have utility in treating and preventing development of resistance to hormonal therapies. Future studies examining the molecular mechanism of ER β signaling in cancer progression will be useful to maximize treatment opportunities using ER β natural ligands. Since some ER β agonists are currently in clinical trials and are well tolerated, they can be readily transferred to clinical use with current chemotherapies, thereby providing an additional tool for enhancing survival in cancer patients with limited toxicity.

Acknowledgments

[Research funding] This work was supported by the NIH/NCI grant CA178499-01 (RKV); CPRIT Training grant RP140105 (GRS) and 2014-15 ABTA Discovery grant (GRS).

References

1. Thomas C, Gustafsson JA. The different roles of ER subtypes in cancer biology and therapy [J]. *Nat Rev Cancer*. 2011; 11(8):597–608. [PubMed: 21779010]
2. Nilsson S, Gustafsson JA. Estrogen receptors: therapies targeted to receptor subtypes [J]. *Clin Pharmacol Ther*. 2011; 89(1):44–55. [PubMed: 21124311]
3. Hartman J, Edvardsson K, Lindberg K, et al. Tumor repressive functions of estrogen receptor beta in SW480 colon cancer cells [J]. *Cancer Res*. 2009; 69(15):6100–6106. [PubMed: 19602591]
4. Strom A, Hartman J, Foster JS, et al. Estrogen receptor beta inhibits 17beta- estradiol-stimulated proliferation of the breast cancer cell line T47D [J]. *Proc Natl Acad Sci U S A*. 2004; 101(6):1566–1571. [PubMed: 14745018]
5. Konstantinopoulos PA, Kominea A, Vondoros G, et al. Oestrogen receptor beta (ERbeta) is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumour's dedifferentiation [J]. *Eur J Cancer*. 2003; 39(9):1251–1258. [PubMed: 12763213]
6. Palmieri C, Cheng GJ, Saji S, et al. Estrogen receptor beta in breast cancer [J]. *Endocr Relat Cancer*. 2002; 9(1):1–13. [PubMed: 11914179]
7. Fox EM, Davis RJ, Shupnik MA. ERbeta in breast cancer-onlooker, passive player, or active protector? [J]. *Steroids*. 2008; 73(11):1039–1051. [PubMed: 18501937]
8. Treeck O, Pfeiler G, Mitter D, et al. Estrogen receptor {beta}1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells [J]. *J Endocrinol*. 2007; 193(3):421–433. [PubMed: 17535880]
9. Haldosen LA, Zhao C, Dahlman-Wright K. Estrogen receptor beta in breast cancer [J]. *Mol Cell Endocrinol*. 2014; 382(1):665–672. [PubMed: 23954741]

10. Sareddy GR, Nair BC, Gonugunta VK, et al. Therapeutic significance of estrogen receptor beta agonists in gliomas [J]. *Mol Cancer Ther.* 2012; 11(5):1174–1182. [PubMed: 22442308]
11. Omoto Y, Iwase H. Clinical significance of estrogen receptor beta in breast and prostate cancer from biological aspects [J]. *Cancer Sci.* 2015; 106(4):337–343. [PubMed: 25611678]
12. Kuiper GG, Enmark E, Peltö-Huikko M, et al. Cloning of a novel receptor expressed in rat prostate and ovary [J]. *Proc Natl Acad Sci U S A.* 1996; 93(12):5925–5930. [PubMed: 8650195]
13. Pettersson K, Gustafsson JA. Role of estrogen receptor beta in estrogen action [J]. *Annu Rev Physiol.* 2001; 63:165–192. [PubMed: 11181953]
14. Couse JF, Lindzey J, Grandien K, et al. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse [J]. *Endocrinology.* 1997; 138(11):4613–4621. [PubMed: 9348186]
15. Grober OM, Mutarelli M, Giurato G, et al. Global analysis of estrogen receptor beta binding to breast cancer cell genome reveals an extensive interplay with estrogen receptor alpha for target gene regulation [J]. *BMC Genomics.* 2011; 12:36. [PubMed: 21235772]
16. Charn TH, Liu ET, Chang EC, et al. Genome-wide dynamics of chromatin binding of estrogen receptors alpha and beta: mutual restriction and competitive site selection [J]. *Mol Endocrinol.* 2010; 24(1):47–59. [PubMed: 19897598]
17. Nakajima Y, Akaogi K, Suzuki T, et al. Estrogen regulates tumor growth through a nonclassical pathway that includes the transcription factors ERbeta and KLF5 [J]. *Sci Signal.* 2011; 4(168):ra22. [PubMed: 21487105]
18. Leung YK, Mak P, Hassan S, et al. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling [J]. *Proc Natl Acad Sci U S A.* 2006; 103(35):13162–13167. [PubMed: 16938840]
19. Gong P, Madak-Erdogan Z, Li J, et al. Transcriptomic analysis identifies gene networks regulated by estrogen receptor alpha (ERalpha) and ERbeta that control distinct effects of different botanical estrogens [J]. *Nucl Recept Signal.* 2014; 12:e001. [PubMed: 25363786]
20. Lo R, Matthews J. A new class of estrogen receptor beta-selective activators [J]. *Mol Interv.* 2010; 10(3):133–136. [PubMed: 20539031]
21. Cvorovic A, Paruthiyil S, Jones JO, et al. Selective activation of estrogen receptor-beta transcriptional pathways by an herbal extract [J]. *Endocrinology.* 2007; 148(2):538–547. [PubMed: 17095596]
22. Grady D, Sawaya GF, Johnson KC, et al. MF101, a selective estrogen receptor beta modulator for the treatment of menopausal hot flashes: a phase II clinical trial [J]. *Menopause.* 2009; 16(3):458–465. [PubMed: 19182698]
23. Stovall DW, Pinkerton JV. MF-101, an estrogen receptor beta agonist for the treatment of vasomotor symptoms in peri- and postmenopausal women [J]. *Curr Opin Investig Drugs.* 2009; 10(4):365–371.
24. Mersereau JE, Levy N, Staub RE, et al. Liquiritigenin is a plant-derived highly selective estrogen receptor beta agonist [J]. *Mol Cell Endocrinol.* 2008; 283(1–2):49–57. [PubMed: 18177995]
25. Jiang Y, Gong P, Madak-Erdogan Z, et al. Mechanisms enforcing the estrogen receptor beta selectivity of botanical estrogens [J]. *FASEB J.* 2013; 27(11):4406–4418. [PubMed: 23882126]
26. Adlercreutz H. Phyto-oestrogens and cancer [J]. *Lancet Oncol.* 2002; 3(6):364–373. [PubMed: 12107024]
27. de Souza PL, Russell PJ, Kearsley JH, et al. Clinical pharmacology of isoflavones and its relevance for potential prevention of prostate cancer [J]. *Nutr Rev.* 2010; 68(9):542–555. [PubMed: 20796219]
28. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes [J]. *Nutr Cancer.* 2006; 55(1):1–12. [PubMed: 16965235]
29. Tham DM, Gardner CD, Haskell WL. Clinical review 97: Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence [J]. *J Clin Endocrinol Metab.* 1998; 83(7):2223–2235. [PubMed: 9661587]
30. Chang EC, Charn TH, Park SH, et al. Estrogen Receptors alpha and beta as determinants of gene expression: influence of ligand, dose, and chromatin binding [J]. *Mol Endocrinol.* 2008; 22(5):1032–1043. [PubMed: 18258689]

31. Pons DG, Nadal-Serrano M, Blanquer-Rossello MM, et al. Genistein modulates proliferation and mitochondrial functionality in breast cancer cells depending on ERalpha/ERbeta ratio [J]. *J Cell Biochem.* 2014; 115(5):949–958. [PubMed: 24375531]
32. Setchell KD, Clerici C, Lephart ED, et al. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora [J]. *Am J Clin Nutr.* 2005; 81(5):1072–1079. [PubMed: 15883431]
33. Jackson RL, Greiwe JS, Schwen RJ. Emerging evidence of the health benefits of S-equol, an estrogen receptor beta agonist [J]. *Nutr Rev.* 2011; 69(8):432–448. [PubMed: 21790611]
34. Aso T, Uchiyama S, Matsumura Y, et al. A natural S-equol supplement alleviates hot flushes and other menopausal symptoms in equol nonproducing postmenopausal Japanese women [J]. *J Womens Health (Larchmt).* 2012; 21(1):92–100. [PubMed: 21992596]
35. Nair HB, Kirma NB, Ganapathy M, et al. Estrogen receptor-beta activation in combination with letrozole blocks the growth of breast cancer tumors resistant to letrozole therapy [J]. *Steroids.* 2011; 76(8):792–796. [PubMed: 21477609]
36. Adlercreutz H. Phytoestrogens and breast cancer [J]. *J Steroid Biochem Mol Biol.* 2002; 83(1–5): 113–118. [PubMed: 12650707]
37. Hinsche O, Girgert R, Emons G, et al. Estrogen receptor beta selective agonists reduce invasiveness of triple-negative breast cancer cells [J]. *Int J Oncol.* 2015; 46(2):878–884. [PubMed: 25420519]
38. Magee PJ, Allsopp P, Samaletdin A, et al. Daidzein, R-(+)equol and S-(-)equol inhibit the invasion of MDA-MB-231 breast cancer cells potentially via the down-regulation of matrix metalloproteinase-2 [J]. *Eur J Nutr.* 2014; 53(1):345–350. [PubMed: 23568763]
39. Charalambous C, Pitta CA, Constantinou AI. Equol enhances tamoxifen's anti-tumor activity by induction of caspase-mediated apoptosis in MCF-7 breast cancer cells [J]. *BMC Cancer.* 2013; 13:238. [PubMed: 23675643]
40. Onoda A, Ueno T, Uchiyama S, et al. Effects of S-equol and natural S-equol supplement (SE5-OH) on the growth of MCF-7 *in vitro* and as tumors implanted into ovariectomized athymic mice [J]. *Food Chem Toxicol.* 2011; 49(9):2279–2284. [PubMed: 21703324]
41. Yang X, Belosay A, Hartman JA, et al. Dietary soy isoflavones increase metastasis to lungs in an experimental model of breast cancer with bone micro-tumors [J]. *Clin Exp Metastasis.* 2015; 32(4):323–333. [PubMed: 25749878]
42. Jarred RA, Keikha M, Dowling C, et al. Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones [J]. *Cancer Epidemiol Biomarkers Prev.* 2002; 11(12):1689–1696. [PubMed: 12496063]
43. McPherson SJ, Hussain S, Balanathan P, et al. Estrogen receptor-beta activated apoptosis in benign hyperplasia and cancer of the prostate is androgen independent and TNFalpha mediated [J]. *Proc Natl Acad Sci U S A.* 2010; 107(7):3123–3128. [PubMed: 20133657]
44. Thelen P, Wuttke W, Seidlova-Wuttke D. Phytoestrogens selective for the estrogen receptor beta exert anti-androgenic effects in castration resistant prostate cancer [J]. *J Steroid Biochem Mol Biol.* 2014; 139:290–293. [PubMed: 23872207]
45. Mahmoud AM, Al-Alem U, Ali MM, et al. Genistein increases estrogen receptor beta expression in prostate cancer via reducing its promoter methylation [J]. *J Steroid Biochem Mol Biol.* 2015; 152:62–75. [PubMed: 25931004]
46. Hatch EE, Linet MS, Zhang J, et al. Reproductive and hormonal factors and risk of brain tumors in adult females [J]. *Int J Cancer.* 2005; 114(5):797–805. [PubMed: 15609304]
47. Huang K, Whelan EA, Ruder AM, et al. Reproductive factors and risk of glioma in women [J]. *Cancer Epidemiol Biomarkers Prev.* 2004; 13(10):1583–1588. [PubMed: 15466973]
48. Kabat GC, Etgen AM, Rohan TE. Do steroid hormones play a role in the etiology of glioma? [J]. *Cancer Epidemiol Biomarkers Prev.* 2010; 19(10):2421–2427. [PubMed: 20841389]
49. Kabat GC, Park Y, Hollenbeck AR, et al. Reproductive factors and exogenous hormone use and risk of adult glioma in women in the NIH-AARP diet and health study [J]. *Int J Cancer.* 2011; 128(4):944–950. [PubMed: 20473903]
50. Michaud DS, Gallo V, Schlehofer B, et al. Reproductive factors and exogenous hormone use in relation to risk of glioma and meningioma in a large European cohort study [J]. *Cancer Epidemiol Biomarkers Prev.* 2010; 19(10):2562–2569. [PubMed: 20802020]

51. Carroll RS, Zhang J, Dashner K, et al. Steroid hormone receptors in astrocytic neoplasms [J]. *Neurosurgery*. 1995; 37(3):496–503. [PubMed: 7501116]
52. Batistatou A, Stefanou D, Goussia A, et al. Estrogen receptor beta (ERbeta) is expressed in brain astrocytic tumors and declines with dedifferentiation of the neoplasm [J]. *J Cancer Res Clin Oncol*. 2004; 130(7):405–410. [PubMed: 15141349]
53. Batistatou A, Kyzas PA, Goussia A, et al. Estrogen receptor beta (ERbeta) protein expression correlates with BAG-1 and prognosis in brain glial tumours [J]. *J Neurooncol*. 2006; 77(1):17–23. [PubMed: 16292491]
54. Li W, Winters A, Poteet E, et al. Involvement of estrogen receptor beta5 in the progression of glioma [J]. *Brain Res*. 2013; 1503:97–107. [PubMed: 23399685]
55. Liu X, Wang L, Chen J, et al. Estrogen receptor beta agonist enhances temozolomide sensitivity of glioma cells by inhibiting PI3K/AKT/mTOR pathway [J]. *Mol Med Rep*. 2015; 11(2):1516–1522. [PubMed: 25351348]
56. Akhter M, Inoue M, Kurahashi N, et al. Dietary soy and isoflavone intake and risk of colorectal cancer in the Japan public health center-based prospective study [J]. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(8):2128–2135. [PubMed: 18708407]
57. Rudolph A, Toth C, Hoffmeister M, et al. Expression of oestrogen receptor beta and prognosis of colorectal cancer [J]. *Br J Cancer*. 2012; 107(5):831–839. [PubMed: 22828608]
58. Bielecki A, Roberts J, Mehta R, et al. Estrogen receptor-beta mediates the inhibition of DLD-1 human colon adenocarcinoma cells by soy isoflavones [J]. *Nutr Cancer*. 2011; 63(1):139–150. [PubMed: 21161820]
59. Weige CC, Allred KF, Allred CD. Estradiol alters cell growth in nonmalignant colonocytes and reduces the formation of preneoplastic lesions in the colon [J]. *Cancer Res*. 2009; 69(23):9118–9124. [PubMed: 19903848]
60. Hartman J, Edvardsson K, Lindberg K, et al. Tumor repressive functions of estrogen receptor beta in SW480 colon cancer cells [J]. *Cancer Res*. 2009; 69(15):6100–6106. [PubMed: 19602591]
61. Schleipen B, Hertrampf T, Fritzemeier KH, et al. ERbeta-specific agonists and genistein inhibit proliferation and induce apoptosis in the large and small intestine [J]. *Carcinogenesis*. 2011; 32(11):1675–1683. [PubMed: 21856997]
62. Pampaloni B, Palmini G, Mavilia C, et al. *In vitro* effects of polyphenols on colorectal cancer cells [J]. *World J Gastrointest Oncol*. 2014; 6(8):289–300. [PubMed: 25132926]
63. Landen CN Jr, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer [J]. *J Clin Oncol*. 2008; 26(6):995–1005. [PubMed: 18195328]
64. Ozols RF, Bookman MA, Connolly DC, et al. Focus on epithelial ovarian cancer [J]. *Cancer Cell*. 2004; 5(1):19–24. [PubMed: 14749123]
65. Chan KK, Leung TH, Chan DW, et al. Targeting estrogen receptor subtypes (ERalpha and ERbeta) with selective ER modulators in ovarian cancer [J]. *J Endocrinol*. 2014; 221(2):325–336. [PubMed: 24819599]
66. Sareddy GR, Chavez JE, SHMMHJKSKETRVR. Therapeutic efficacy of ERβ agonists on ovarian cancer [J]. *Cancer Res*. 2014; 74(19 Suppl) Abstract nr 606. ed.
67. Wang Y, Xie S, Liu C, et al. Inhibitory effect of liquiritigenin on migration via downregulation proMMP-2 and PI3K/Akt signaling pathway in human lung adenocarcinoma A549 cells [J]. *Nutr Cancer*. 2012; 64(4):627–634. [PubMed: 22519916]
68. Wang D, Lu J, Liu Y, et al. Liquiritigenin induces tumor cell death through mitogen-activated protein kinase- (MPAKs-) mediated pathway in hepatocellular carcinoma cells [J]. *Biomed Res Int*. 2014; 2014:965316. [PubMed: 24738081]
69. Zhou M, Higo H, Cai Y. Inhibition of hepatoma 22 tumor by Liquiritigenin [J]. *Phytother Res*. 2010; 24(6):827–833. [PubMed: 19953525]
70. Liang XL, Li M, Li J, et al. Equol induces apoptosis in human hepatocellular carcinoma SMMC-7721 cells through the intrinsic pathway and the endoplasmic reticulum stress pathway [J]. *Anticancer Drugs*. 2014; 25(6):633–640. [PubMed: 24487643]
71. Shi H, Wu Y, Wang Y, et al. Liquiritigenin potentiates the inhibitory effects of cisplatin on invasion and metastasis via downregulation MMP-2/9 and PI3 K/AKT signaling pathway in B16F10 melanoma cells and mice model [J]. *Nutr Cancer*. 2015:1–10.

72. Wang D, Wong HK, Feng YB, et al. Liquiritigenin exhibits antitumour action in pituitary adenoma cells via Ras/ERKs and ROS-dependent mitochondrial signalling pathways [J]. J Pharm Pharmacol. 2014; 66(3):408–417. [PubMed: 24491032]

Fig. 1.

A. Schematic representation of ER α and ER β structural domains. The percentage of amino acid homologies between various domains of ER α and ER β are also shown. AF1, N terminal activation function domain 1 that facilitate ligand independent coregulator interactions; DBD, DNA- binding domain (DBD) that facilitate interaction with ERE elements; LBD, ligand-binding domain that also harbors activation function domain 2 (AF2) which facilitate ligand dependent coregulator interactions. B. Schematic representation of ER β beta isoforms. Isoforms 2 to 5 have variation in the C-terminal region. C. Schematic representation of structures of natural ER β ligands

Fig. 2. Schematic representation of ERβ signaling. ERβ mediated signaling involves classical genomic activation ERβ target genes. Further, ERβ signals cross talk with other transcriptional factors such as AP1, SP1, and KLF5 that can activate several additional genes involved in tumor suppression and promote extra-nuclear signaling by interacting with cytosolic kinases. ERβ signaling also has the potential to interfere with ERα signaling via heterodimer formation (ERβ: ERα). During tumor progression, ERβ expression is commonly reduced or down regulated. Natural ERβ agonists preferentially activate ERβ signaling and also upregulate ERβ expression. Restoration of ERβ mediated signaling will have implications in treating/preventing tumor progression.