Estrogen Receptor Beta

Subjects: Biochemistry & Molecular Biology

Contributor: Ana Božović

The discovery of the Estrogen Receptor Beta (ERβ) in 1996 opened new perspectives in the diagnostics and therapy of different types of cancer.

Keywords: Estrogen Receptor Beta ; endocrine-related cancers ; carcinogenesis ; biomarker ; metastases

1. The Molecular Pathways of Estrogen Receptor Alpha and Beta in Cancer

Decades ago, it became clear that estrogen has a carcinogenic effect. In the meantime, it has been discovered that it has a dual role in the proliferation and the cell cycle [1][2][3]. The discovery of ERβ means that the dual role of estrogen is possibly mediated through two receptors, ERα and ERβ, and probably through the complex profile of its isoforms [4][5][6][7].

The classical, direct genomic action of estrogen starts upon ligand binding to the cytosolic receptor, followed by nuclear translocation, dimerization, and binding to ERE sites in target genes. Other estrogen-dependent pathway signals originate from a pool of membrane ERs, and this pathway is triggered by ligand binding and mediated through signaling cascades (Akt, PKA, and ERK1/2) and usually activated STAT, CREB, NF-kB, and Jun transcription factors. Although both receptors, ERα and ERβ, share a similar domain structure and can bind estrogen and initiate transcription from ERE sites, in the case of ERβ, the activation function of the AF-1 domain is weaker than that of ERα, while the AF-2 function of ERβ is similar to ERα. In addition to the ligand activation, by tethering to the other transcription factors, such as AP1, SP1, or NF-κB, ERβ activates the expression of a unique sets of genes (ligand-independent action in the absence of estrogen), and this pathway is usually related to growth factor signaling via activated kinases. According to the study of Acconcia and coworkers, it seems that the main difference in the specific activation of survival pathways mediated by ERα and apoptotic pathways mediated by ERβ is through its non-genomic, membrane-initiated pathways. In a study on HeLa, HepG2, and DLD1 cell lines containing transfected or endogenous ERα and ERβ, the authors showed that, after estrogen stimulation, the ERα rapidly activates multiple signal transduction pathways (ERK/MAPK and PI3K/AKT) related to the cell cycle progression and prevention of the apoptotic cascade, while ERβ induces the rapid and persistent phosphorylation of p38/MAPK, which is involved in caspase-3 activation and cleavage of poly(ADP-ribose)polymerase, driving cells into the apoptotic cycle. They also showed that ERβ did not activate any of the ERα-mediated pathways of cell growth signaling [4]. Another important study was reported by Helguero and coworkers on HC11 mammary epithelial cells that express both receptors, treated with ERα- and ERβ-specific new developed agonists [5]. They showed that, after exposure to the ERα-selective agonist, 4,4′,4″-(4-propyl-(1H)-pyrazole-1,3,5-triyl)trisphenol (PPT), there was a 50% increase in cell number, but 2,3-bis(4-hydroxy-phenyl)-propionitrile (DPN)- Erβ-selective agonist decreased the cell number by 20–30%. After exposure to E2, the cell number was unchanged. They also showed that E2 and PPT treatment increased cyclin D1 expression, while DPN did not. The expression of proliferating cell nuclear antigen (PCNA) expression also decreased following DPN treatment, as well as the mitotic index and Ki67 expression. Their study demonstrates that estrogen signaling via ERα favors entry into the cell cycle, but ERβ transiently inhibits it, and the loss of ERβ expression favors cell transformation.

In line with in vitro studies are findings from association studies on clinical samples of prostate, breast, and thyroid carcinomas. The results of these studies showed that the ERα/ERβ ratio changes, with ERα being upregulated and ERβ being downregulated [8][9][10][11][12]. It has become clear that the antiproliferative activity of ERβ qualifies this receptor as an important therapeutic target, in cancers that involve estrogen signaling (breast, prostate, and colon) and in those that are gender-related (stomach, chronic lymphocytic leukemia, and thyroid) as reviewed in [13].

2. Epigenetic Regulation of ERβ

Epigenetic regulation is crucial for many biological processes: chromosome X inactivation, genomic imprinting, RNA interference, and reprogramming of the genome during differentiation and development [14]. The disruption of these processes manifests through aberrant gene methylation and/or histone acetylation, which leads to alterations in gene expression. The loss of cell adhesion proteins and the excessive stimulation of signaling pathways of estrogen receptors lead to abnormal growth and differentiation of tissue. Migration of cells increases; many intracellular pathways of apoptosis, DNA reparation, and detoxification are activated [15]. The disrupted epigenetic processes cause many diseases, including cancer.

One of the most important epigenetic mechanisms that lead to cancer initiation and progression is the methylation of CpG islands. CpG islands are located in the promoter regions and sometimes in the first exons of genes, spanning from 500 to several thousands of nucleotides. The frequency of CpG dinucleotides in these islands is higher than in other gene locations [16][17]. Non-methylated CpG islands are usually situated in the promoter regions of housekeeping genes, essential for the general functions of the cells, and in some tissue-specific genes [18]. Excessive activity of DNA methyltransferases (DNMT) leads to CpG islands hypermethylation in cancer DNMT catalyzes the transfer of the methyl group from S adenosyl L-methionine (SAM) to 5′-cytosines of CpGs [15]. The most important human DNMTs are DNMT1, DNMT3a, and DNMT3b. The most abundant is DNMT1, which sustains the methylation pattern. The other two mediate in de novo methylation [19]. The consequence of hypermethylation is the reversible inactivation of tumor suppressor genes, which is a heritable change that passes to the next generation of cells through mitosis [40]. In cancer cells, the global hypomethylation of internal exons and introns of genomic DNA and the hypermethylation of nonmethylated regions of gene promoters lead to methylation imbalance[20]. Methylation of CpG islands in promoter regions leads to long-term gene silencing and chromatin unavailability for transcription [21]. In hormone-dependent cancers, the expression of estrogen receptors is associated with clinical outcomes [22]. For example, in breast cancer, the methylation of specific gene clusters leads to the expression or absence of ERs and PR, causing metastases and relapse of the disease [23]. ERα is expressed in 75% of breast cancers, and ERα-positive patients better respond to therapy and therefore have a better prognosis [24]. ERα-negative patients have a worse prognosis. One of the assumed mechanisms of a lack of ERα is hypermethylation of the ERα gene promoter. There is no methylation in normal breast tissue in these regions. Hypermethylation of CpG islands occurs in all tumor stadiums with a higher frequency in the transition phase from the ductal carcinoma in situ to metastasis [25]. The expression of ERβ also changes during the progression of breast cancer. In the initial phases of the disease, the receptor level decreases, to be lost in the advanced stages of the disease [26][27][28]. On the other hand, there is a high level of ERβ in most prostate metastases, in the bones, and in lymph nodes [29]. The role of ERβ in metastases is unclear. It could be assumed that local factors lead to the re-expression of ERβ in metastases [30].

Li and coworkers cloned and characterized the promoter region of human ERβ in 2000 [31]. Soon afterwards, Hirata and al. described the two isoforms originating from the first two non-translational exons of the ERβ gene, exon OK and ON. These exons splice to exon 1 and form two isoforms, OK-1 and ON-1. Exon OK is located 50 Kb upstream of exon ON [32]. The scientists also described whole-length transcripts that have neither the exon ON nor the exon OK [33]. In 2001, Smith and coworkers discovered the third promoter region, which transcribes to the functional transcript [34]. The distribution of the OK and ON isoforms in the tissues is different, and it changes in cancer cells [32][35].

ERβ is a dominant form of ER in the prostate and the increased promoter methylation is primarily discovered in prostate cancer [30]. Nojima et al. showed that methylation in the 5′-untranslated region correlated inversely with ERβ expression in the prostate. They analyzed 19 CpG places between 376 and 117 bp upstream from ATG, in exon ON, in human prostate carcinoma, and in benign prostate hyperplasia (BPH). All 19 CpG places were methylated, and associated with the loss of ERβ expression. In BPH, CpGs were not methylated, and there was ERβ expression. Treatment of prostate cancer cells with 5-aza-2-deoxicytidine (5-AZAC) led to the reexpression of ERβ, which proves that the methylation is a reversible process [36]. Zhu and al. included the promoter region upstream of exon ON and compared different stages of prostate cancer and metastatic tissue. They concluded that the methylation level increased in both CpG islands in parallel with more advanced cancer stages. They identified three CpG clusters with a high level of methylation, while there was no transcription. These results have been confirmed in the cell lines, too [37]. ERβ expression is lower in breast and ovarian tumors than in normal tissue. Therefore, it is assumed that ERβ has a tumor suppressor role [38][9][39][40][41][42]. DNA methylation regulates the expression of ERβ in breast and ovary cancers. Zhao et al. measured the CpG methylation level in the ESR2 promoter region and the expression of ERβ in breast cancer cell lines and primary tumors. They showed that a lower level of ERβ expression is associated with ON promoter methylation, unlike the OK promoter, which was unmethylated in normal and tumor cells [43]. Rody et al. showed the increased level of methylation of the ON promoter. The treatment of cells with 5-AZAC was associated with an increased expression of ERβ [44]. In our study, we observed a significant association between ERβ promoter ON methylation and nodal metastasis in invasive breast cancer [45].

The other mechanism of epigenetic regulation of ERβ includes histone post-translational modifications. The important post-translational modifications of histones are acetylation, methylation, and phosphorylation. All of these modifications together result in transcription or an inhibition of transcription, depending on the aminoacid residue. Histone acetyltransferases (HATs) transfer acetyl groups to lysine residues in histones, leading to the uncoiling of chromatin and allowing for the accessibility of transcription factors and other regulators. Histone deacetylases (HDACs) remove the acetyl groups from histones. Histone methyltransferases (HMTs) add methyl groups to lysine or arginine residues in histones, while histone demethylases (HMDs) remove them [46]. There is not much information on the role of histone modifications in ERβ expression. Some studies showed that treatment with trichostatin, an HDAC inhibitor in breast, ovary, and prostate cancer cell lines, led to the re-expression of ERβ. The addition of 5′-aza-2-deoxycytidine (DNMT inhibitor) pronounced this effect even more, as reviewed in [47]. This means that histone deacetylases and DNA methylation supplement each other in inhibiting gene expression. Methylation can also act upon DNA-protein interactions and the activation of histone deacetylases, thus inducing chromatin condensation and the inactivation of gene expression [48][49]. In vitro studies show that DNA demethylation agents, especially combined with HDAC inhibitors, induce apoptosis, cell differentiation, and/or growth termination in lung, breast, prostate, and colon cells [50][51].

The small non-coding RNAs or microRNAs (miRNA) also participate in an important mechanism of epigenetic regulation of ERβ. Still, there is much to be learned about targeting ERβ by miRNAs. The most experimented with is miR-92, which targets the 3′-untranslated region of ERβ, thereby downregulating its expression. In MCF-7 cells, inhibiting miR-92 in a dose-dependent manner induces ERβ1expression. ERβ also regulates some miRNAs. It downregulates miR-145, miR-30a-5p, and miR-200a/b/429, which play a role in the inhibition of epithelial-mesenchymal transition. It upregulates miR-181a-5p, miR-10, and miR-375, which inhibit cholesterol biosynthesis in TNBC cells, regulate the composition of the extracellular matrix, and suppress proliferation, respectively. The studies so far show that miRNAs play an important role in regulating ERβ expression and cancer development (reviewed in [52]).

3. ERβ Role in the Metabolism of Cancer Cells

ERβ plays an important role in cancer cell metabolism. The studies on cell lines and animal models show that it participates in many metabolic pathways, as are lipid metabolism, oxidative phosphorylation, and glycolysis, as reviewed in [53]. In adipose tissue, ERβ, together with ERα, influences glucose and insulin metabolism, but estrogen predominantly binds to ERα [54]. Glycolysis is a very important process in tumor cells. Unlike normal cells, which metabolize glucose into lactate in anaerobic conditions, tumor cells do this in the presence of oxygen. Therefore, they, in some way, bypass the process of oxidative phosphorylation. In tumor cells, glucose intake increases, and the membrane glucose transporters (GLUTs) play a role in this process. Recent studies show that the key of glycolysis in tumors is the generation of the many intermediates they will use in the anabolic processes, which cancer cells need for their growth and energy sources [55]. In malignant mesothelioma cells, it was shown that ERβ represses succinate dehydrogenase B, a part of complex II of the electron transport chain of mitochondria. Therefore, ERβ lowers the activity of oxidative phosphorylation in mitochondria [56]. In ERα-negative breast cancer stem cells that expressed ERβ, adding its agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN), increased glycolysis and lactate secretion in the growth media. In addition, oxygen consumption rates decreased, but this was reversed by adding ERβ antagonist 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP). In ERβ-negative cells, the expression of glycolytic genes decreased, but the expression of genes participating in oxidative phosphorylation increased. This indicates that ERβ in tumor cells accelerates glycolysis and inhibits oxidative phosphorylation. The authors of the study argue that ERβ is a possible target for therapy with antagonists [57]. However, Song et al. found that the upregulation of mitochondrial ERβ in TNBC cells activated oxidative phosphorylation [58]. 27-Hydroxycholesterol (27-HC) is a metabolite of cholesterol, and many studies have shown it is associated with BC. 27-HC is also a SERM that binds to ERα and ERβ, but with a higher affinity to ERβ, causing its conformational change. However, the observed association of plasma 27-HCT and ERβ in tumors is weak [59].

There is still much work to be done in research on the role of ERβ in the metabolic pathways of cancer cells and its possible utilization in therapy.

4. ERβ as a Potential Target in Clinical Practice

The important role of ERβ in carcinogenesis leads to the conclusion that it could be used in clinical practice as a potential therapeutic target. Several molecules that can bind to ERβ are being examined. One of them is genistein, a powerful phytoestrogen that is a natural constituent of a soy bean and binds to ERβ. It is a selective estrogen receptor modulator (SERM). Many in vitro and in vivo studies have shown the anticancer effects of genistein. However, many studies have also shown that genistein promotes cancer cell growth, as reviewed in [60]. Its potential to be used as an effective cancer treatment has been examined in a number of clinical studies. One of them investigated the effect of genistein on

cytogenetic markers in postmenopausal women and showed a reduction of cytogenetic markers of cancer [61]. There was also a study where genistein combined with FOLFOX/FOLFOX-bevacizumab was proved safe for patients with colorectal cancer [62]. A clinical trial in phase 2, where genistein was used in prostate cancer patients, proved the safety and efficacy of genistein treatment [63]. The studies so far show that genistein could be a potential agent alone or in combination with other agents for the treatment of cancer. Several synthesized ERβ-selective agonists have been examined. Diarylproprionitryle, 8β-VE2, and SERBA-1 have all proved to prevent or reverse hyperplasia in prostate cancer animal models [64][65][66][67]. All these agonists could potentially be used in new therapies, but there is still much research to be conducted.

As we stated above, the main problem in hormone treatment is acquiring resistance to therapy in endocrine-related cancers. This has been seen in breast cancer (tamoxifen and aromatase inhibitors) and prostate cancer (anti-androgens). Other signaling pathways that bypass ER or AR signaling are probably being activated. One of the solutions to this problem is to target the cell growth PI3K/Akt/mTOR signaling pathway. This has been shown in advanced breast cancer, where inhibition of this pathway increased progression-free survival, as reviewed in [68].

References

1. Chang, M. Dual roles of estrogen metabolism in mammary carcinogenesis. BMB Rep. 2011, 44, 423–434, doi:10.5483/BMBRep.2011.44.7.423.
2. Cornil, C.A.; Ball, G.F.; Balthazart, J. The dual action of estrogen hypothesis. Trends Neurosci. 2015, 38, 408–416, doi:10.1016/j.tins.2015.05.004.
3. Ellem, S.J.; Risbridger, G.P. The dual, opposing roles of estrogen in the prostate. Ann. N. Y. Acad. Sci. 2009, 1155, 174–186, doi:10.1111/j.1749-6632.2009.04360.x.
4. Acconcia, F.; Totta, P.; Ogawa, S.; Cardillo, I.; Inoue, S.; Leone, S.; Trentalance, A.; Muramatsu, M.; Marino, M. Survival ver-sus apoptotic 17beta-estradiol effect: Role of ER alpha and ER beta activated non-genomic signaling. J. Cell. Physiol. 2005, 203, 193–201, doi:10.1002/jcp.20219.
5. Helguero, L.A.; Faulds, M.H.; Gustafsson, J.A.; Haldosen, L.A. Estrogen receptors alfa (ERalpha) and beta (ERbeta) differen-tially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11. Oncogene 2005, 24, 6605–6616, doi:10.1038/sj.onc.1208807.
6. Matthews, J.; Gustafsson, J.A. Estrogen signaling: A subtle balance between ER alpha and ER beta. Mol. Interv. 2003, 3, 281–292, doi:10.1124/mi.3.5.281.
7. Platet, N.; Cathiard, A.M.; Gleizes, M.; Garcia, M. Estrogens and their receptors in breast cancer progression: A dual role in cancer proliferation and invasion. Crit. Rev. Oncol./Hematol. 2004, 51, 55–67, doi:10.1016/j.critrevonc.2004.02.001.
8. Park, B.W.; Kim, K.S.; Heo, M.K.; Yang, W.I.; Kim, S.I.; Kim, J.H.; Kim, G.E.; Lee, K.S. The changes of estrogen receptor-beta variants expression in breast carcinogenesis: Decrease of estrogen receptor-beta2 expression is the key event in breast can-cer development. J. Surg. Oncol. 2006, 93, 504–510, doi:10.1002/jso.20336.
9. Bardin, A.; Boulle, N.; Lazennec, G.; Vignon, F.; Pujol, P. Loss of ERbeta expression as a common step in estrogen-dependent tumor progression. Endocr. Relat. Cancer 2004, 11, 537–551.
10. Kowalska, K.; Piastowska-Ciesielska, A.W. Oestrogens and oestrogen receptors in prostate cancer. SpringerPlus 2016, 5, 522, doi:10.1186/s40064-016-2185-6.
11. Slattery, M.L.; Sweeney, C.; Murtaugh, M.; Ma, K.N.; Wolff, R.K.; Potter, J.D.; Caan, B.J.; Samowitz, W. Associations between ERalpha, ERbeta, and AR genotypes and colon and rectal cancer. Cancer Epidemiology Biomarkers Prev. 2005, 14, 2936–2942, doi:10.1158/1055-9965.EPI-05-0514.
12. Qiu, Y.B.; Liao, L.Y.; Jiang, R.; Xu, M.; Xu, L.W.; Chen, G.G.; Liu, Z.M. PES1 promotes the occurrence and development of papillary thyroid cancer by upregulating the ERalpha/ERbeta protein ratio. Sci. Rep. 2019, 9, 1032, doi:10.1038/s41598-018-37648-7.
13. Warner, M.; Gustafsson, J.A. The role of estrogen receptor beta (ERbeta) in malignant diseases--a new potential target for antiproliferative drugs in prevention and treatment of cancer. Biochem. Biophys. Res. Commun. 2010, 396, 63–66, doi:10.1016/j.bbrc.2010.02.144.
14. Veeck, J.; Esteller, M. Breast cancer epigenetics: From DNA methylation to microRNAs. J. Mammary Gland. Biol. Neoplasia 2010, 15, 5–17, doi:10.1007/s10911-010-9165-1.

15. Vo, A.T.; Millis, R.M. Epigenetics and breast cancers. Obstet. Gynecol. Int. 2012, 2012, 602720, doi:10.1155/2012/602720.

16. Bird, A.P. CpG-rich islands and the function of DNA methylation. Nature 1986, 321, 209–213, doi:10.1038/321209a0.

17. Millis, R.M. Epigenetics and hypertension. Curr. Hypertens. Rep. 2011, 13, 21–28, doi:10.1007/s11906-010-0173-8.

18. Esteller, M. CpG island hypermethylation and tumor suppressor genes: A booming present, a brighter future. Oncogene 2002, 21, 5427–5440, doi:10.1038/sj.onc.1205600.

19. Meeran, S.M.; Ahmed, A.; Tollefsbol, T.O. Epigenetic targets of bioactive dietary components for cancer prevention and therapy. Clin. Epigenetics 2010, 1, 101–116, doi:10.1007/s13148-010-0011-5.

20. Baylin, S.B.; Herman, J.G.; Graff, J.R.; Vertino, P.M.; Issa, J.P. Alterations in DNA methylation: A fundamental aspect of neo-plasia. Adv. Cancer Res. 1998, 72, 141–196.

21. Cedar, H.; Bergman, Y. Epigenetic Silencing during Early Lineage Commitment; 2008, NBK27026 [bookaccession], doi:10.3824/stembook.1.42.1

22. Thomas, C.; Gustafsson, J.A. The different roles of ER subtypes in cancer biology and therapy. Nat. Rev. Cancer 2011, 11, 597–608, doi:10.1038/nrc3093.

23. Hill, V.K.; Ricketts, C.; Bieche, I.; Vacher, S.; Gentle, D.; Lewis, C.; Maher, E.R.; Latif, F. Genome-wide DNA methylation profiling of CpG islands in breast cancer identifies novel genes associated with tumorigenicity. Cancer Res 2011, 71, 2988–2999, doi:10.1158/0008-5472.can-10-4026.

24. Di Leva, G.; Gasparini, P.; Piovan, C.; Ngankeu, A.; Garofalo, M.; Taccioli, C.; Iorio, M.V.; Li, M.; Volinia, S.; Alder, H.; et al. MicroRNA cluster 221-222 and estrogen receptor alpha interactions in breast cancer. J. Natl. Cancer Inst. 2010, 102, 706–721, doi:10.1093/jnci/djq102.

25. Nass, S.J.; Herman, J.G.; Gabrielson, E.; Iversen, P.W.; Parl, F.F.; Davidson, N.E.; Graff, J.R. Aberrant methylation of the es-trogen receptor and E-cadherin 5' CpG islands increases with malignant progression in human breast cancer. Cancer Res. 2000, 60, 4346–4348.

26. Horvath, L.G.; Henshall, S.M.; Lee, C.S.; Head, D.R.; Quinn, D.I.; Makela, S.; Delprado, W.; Golovsky, D.; Brenner, P.C.; O'Neill, G.; et al. Frequent loss of estrogen receptor-beta expression in prostate cancer. Cancer Res. 2001, 61, 5331–5335.

27. Latil, A.; Bieche, I.; Vidaud, D.; Lidereau, R.; Berthon, P.; Cussenot, O.; Vidaud, M. Evaluation of androgen, estrogen (ER al-pha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse tran-scription-polymerase chain reaction assays. Cancer Res. 2001, 61, 1919–1926.

28. Pasquali, D.; Rossi, V.; Esposito, D.; Abbondanza, C.; Puca, G.A.; Bellastella, A.; Sinisi, A.A. Loss of estrogen receptor beta expression in malignant human prostate cells in primary cultures and in prostate cancer tissues. J. Clin. Endocrinol. Metab. 2001, 86, 2051–2055.

29. Leav, I.; Lau, K.M.; Adams, J.Y.; McNeal, J.E.; Taplin, M.E.; Wang, J.; Singh, H.; Ho, S.M. Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and meta-static carcinoma. Am. J. Pathol. 2001, 159, 79–92, doi:S0002-9440(10)61676-8 [pii].

30. Swedenborg, E.; Power, K.A.; Cai, W.; Pongratz, I.; Ruegg, J. Regulation of estrogen receptor beta activity and implications in health and disease. Cell Mol. Life Sci. 2009, 66, 3873–3894, doi:10.1007/s00018-009-0118-z.

31. Li, L.C.; Yeh, C.C.; Nojima, D.; Dahiya, R. Cloning and characterization of human estrogen receptor beta promoter. Biochem. Biophys. Res. Commun. 2000, 275, 682–689, doi:10.1006/bbrc.2000.3363.

32. Hirata, S.; Shoda, T.; Kato, J.; Hoshi, K. The multiple untranslated first exons system of the human estrogen receptor beta (ER beta) gene. J. Steroid Biochem. Mol. Biol. 2001, 78, 33–40, doi:10.1016/s0960-0760(01)00071-1.

33. Ogawa, S.; Inoue, S.; Watanabe, T.; Orimo, A.; Hosoi, T.; Ouchi, Y.; Muramatsu, M. Molecular cloning and characterization of human estrogen receptor betacx: A potential inhibitor ofestrogen action in human. Nucleic Acids Res. 1998, 26, 3505–3512, doi:gkb568 [pii].

34. Smith, L.; Coleman, L.J.; Cummings, M.; Satheesha, S.; Shaw, S.O.; Speirs, V.; Hughes, T.A. Expression of oestrogen receptor beta isoforms is regulated by transcriptional and post-transcriptional mechanisms. Biochem. J. 2010, 429, 283–290, doi:10.1042/bj20100373.

35. Suzuki, F.; Akahira, J.; Miura, I.; Suzuki, T.; Ito, K.; Hayashi, S.; Sasano, H.; Yaegashi, N. Loss of estrogen receptor beta iso-form expression and its correlation with aberrant DNA methylation of the 5'-untranslated region in human epithelial ovarian carcinoma. Cancer Sci. 2008, 99, 2365–2372, doi:10.1111/j.1349-7006.2008.00988.x.

36. Nojima, D.; Li, L.C.; Dharia, A.; Perinchery, G.; Ribeiro-Filho, L.; Yen, T.S.; Dahiya, R. CpG hypermethylation of the promoter region inactivates the estrogen receptor-beta gene in patients with prostate carcinoma. Cancer 2001, 92, 2076–2

083, doi:10.1002/1097-0142(20011015)92:8<2076::AID-CNCR1548>3.0.CO;2-A.

37. Zhu, X.; Leav, I.; Leung, Y.K.; Wu, M.; Liu, Q.; Gao, Y.; McNeal, J.E.; Ho, S.M. Dynamic regulation of estrogen receptor-beta expression by DNA methylation during prostate cancer development and metastasis. Am. J. Pathol. 2004, 164, 2003–2012.

38. Tong, D.; Schuster, E.; Seifert, M.; Czerwenka, K.; Leodolte, S.; Zeillinger, R. Expression of estrogen receptor beta isoforms in human breast cancer tissues and cell lines. Breast Cancer Res. Treat. 2002, 71, 249–255.

39. Brandenberger, A.W.; Tee, M.K.; Jaffe, R.B. Estrogen receptor alpha (ER-alpha) and beta (ER-beta) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: Down-regulation of ER-beta in neoplastic tissues. J. Clin. Endocrinol. Metab. 1998, 83, 1025–1028.

40. Pujol, P.; Rey, J.M.; Nirde, P.; Roger, P.; Gastaldi, M.; Laffargue, F.; Rochefort, H.; Maudelonde, T. Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. Cancer Res. 1998, 58, 5367–5373.

41. Rutherford, T.; Brown, W.D.; Sapi, E.; Aschkenazi, S.; Munoz, A.; Mor, G. Absence of estrogen receptor-beta expression in metastatic ovarian cancer. Obstet. Gynecol. 2000, 96, 417–421, doi: 10.1016/s0029-7844(00)00917-0.

42. Hartman, J.; Lindberg, K.; Morani, A.; Inzunza, J.; Strom, A.; Gustafsson, J.A. Estrogen receptor beta inhibits angiogenesis and growth of T47D breast cancer xenografts. Cancer Res. 2006, 66, 11207–11213, doi:10.1158/0008-5472.CAN-06-0017.

43. Zhao, C.; Lam, E.W.; Sunters, A.; Enmark, E.; De Bella, M.T.; Coombes, R.C.; Gustafsson, J.A.; Dahlman-Wright, K. Expres-sion of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: Regulation by methylation. On-cogene 2003, 22, 7600–7606, doi:10.1038/sj.onc.1207100.

44. Rody, A.; Holtrich, U.; Solbach, C.; Kourtis, K.; von Minckwitz, G.; Engels, K.; Kissler, S.; Gatje, R.; Karn, T.; Kaufmann, M. Methylation of estrogen receptor beta promoter correlates with loss of ER-beta expression in mammary carcinoma and is an early indication marker in premalignant lesions. Endocr. Relat. Cancer 2005, 12, 903–916, doi:10.1677/erc.1.01088.

45. Božović, A.; Markićević, M.; Dimitrijević, B.; Ćupić, S.J.; Krajnović, M.; Lukić, S.; Mandušić, V. Potential clinical significance of ERβ ON promoter methylation in sporadic breast cancer. Med Oncol. 2013, 30, 1–10.

46. Hervouet, E.; Cartron, P.F.; Jouvenot, M.; Delage-Mourroux, R. Epigenetic regulation of estrogen signaling in breast cancer. Epigenetics 2013, 8, 237–245, doi:10.4161/epi.23790.

47. Vrtacnik, P.; Ostanek, B.; Mencej-Bedrac, S.; Marc, J. The many faces of estrogen signaling. Biochem. Med. 2014, 24, 329–342, doi:10.11613/BM.2014.035.

48. Johnson, C.A.; Turner, B.M. Histone deacetylases: Complex transducers of nuclear signals. Semin Cell Dev Biol 1999, 10, 179–188, doi:10.1006/scdb.1999.0299.

49. Garinis, G.A.; Patrinos, G.P.; Spanakis, N.E.; Menounos, P.G. DNA hypermethylation: When tumour suppressor genes go silent. Hum. Genet. 2002, 111, 115–127, doi:10.1007/s00439-002-0783-6.

50. Zhu, W.G.; Otterson, G.A. The interaction of histone deacetylase inhibitors and DNA methyltransferase inhibitors in the treatment of human cancer cells abs. Curr. Med. Chem. Anticancer Agents 2003, 3, 187–199.

51. Walton, T.J.; Li, G.; Seth, R.; McArdle, S.E.; Bishop, M.C.; Rees, R.C. DNA demethylation and histone deacetylation inhibi-tion co-operate to re-express estrogen receptor beta and induce apoptosis in prostate cancer cell-lines. Prostate 2008, 68, 210–222, doi:10.1002/pros.20673.

52. Zhou, Y.; Liu, X. The role of estrogen receptor beta in breast cancer. Biomark. Res. 2020, 8, 39, doi:10.1186/s40364-020-00223-2.

53. Gandhi, N.; Das, G.M. Metabolic Reprogramming in Breast Cancer and Its Therapeutic Implications. Cells 2019, 8, 89, doi:10.3390/cells8020089.

54. Naaz, A.; Zakroczymski, M.; Heine, P.; Taylor, J.; Saunders, P.; Lubahn, D.; Cooke, P.S. Effect of ovariectomy on adipose tissue of mice in the absence of estrogen receptor alpha (ERalpha): A potential role for estrogen receptor beta (ERbeta). Horm. Metab. Res. 2002, 34, 758–763, doi:10.1055/s-2002-38259.

55. Kalyanaraman, B. Teaching the basics of cancer metabolism: Developing antitumor strategies by exploiting the differences between normal and cancer cell metabolism. Redox Biol. 2017, 12, 833–842, doi:10.1016/j.redox.2017.04.018.

56. Manente, A.G.; Valenti, D.; Pinton, G.; Jithesh, P.V.; Daga, A.; Rossi, L.; Gray, S.G.; O'Byrne, K.J.; Fennell, D.A.; Vacca, R.A.; et al. Estrogen receptor beta activation impairs mitochondrial oxidative metabolism and affects malignant mesothelioma cell growth in vitro and in vivo. Oncogenesis 2013, 2, e72, doi:10.1038/oncsis.2013.32.

57. Ma, R.; Karthik, G.M.; Lovrot, J.; Haglund, F.; Rosin, G.; Katchy, A.; Zhang, X.; Viberg, L.; Frisell, J.; Williams, C.; et al. Es-trogen Receptor beta as a Therapeutic Target in Breast Cancer Stem Cells. J. Natl. Cancer Inst. 2017, 109, 1–14, doi:10.1093/jnci/djw236.

58. Song, I.S.; Jeong, Y.J.; Jeong, S.H.; Kim, J.E.; Han, J.; Kim, T.H.; Jang, S.W. Modulation of Mitochondrial ERbeta Expression Inhibits Triple-Negative Breast Cancer Tumor Progression by Activating Mitochondrial Function. Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 2019, 52, 468–485, doi:10.33594/000000034.

59. Sawada, M.I.; GD, S.F.; Passarelli, M. Cholesterol derivatives and breast cancer: Oxysterols driving tumor growth and me-tastasis. Biomark. Med. 2020, 14, 1299–1302, doi:10.2217/bmm-2020-0460.

60. Tuli, H.S.; Tuorkey, M.J.; Thakral, F.; Sak, K.; Kumar, M.; Sharma, A.K.; Sharma, U.; Jain, A.; Aggarwal, V.; Bishayee, A. Mo-lecular Mechanisms of Action of Genistein in Cancer: Recent Advances. Front. Pharmacol. 2019, 10, 1336, doi:10.3389/fphar.2019.01336.

61. Atteritano, M.; Pernice, F.; Mazzaferro, S.; Mantuano, S.; Frisina, A.; D'Anna, R.; Cannata, M.L.; Bitto, A.; Squadrito, F.; Frisina, N.; et al. Effects of phytoestrogen genistein on cytogenetic biomarkers in postmenopausal women: 1 year random-ized, placebo-controlled study. Eur. J. Pharmacol. 2008, 589, 22–26, doi:10.1016/j.ejphar.2008.04.049.

62. Pintova, S.; Dharmupari, S.; Moshier, E.; Zubizarreta, N.; Ang, C.; Holcombe, R.F. Genistein combined with FOLFOX or FOLFOX-Bevacizumab for the treatment of metastatic colorectal cancer: Phase I/II pilot study. Cancer Chemother. Pharmacol. 2019, 84, 591–598, doi:10.1007/s00280-019-03886-3.

63. Lazarevic, B.; Boezelijn, G.; Diep, L.M.; Kvernrod, K.; Ogren, O.; Ramberg, H.; Moen, A.; Wessel, N.; Berg, R.E.; Egge-Jacobsen, W.; et al. Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: A randomized, placebo-controlled, double-blind Phase 2 clinical trial. Nutr. Cancer 2011, 63, 889–898, doi:10.1080/01635581.2011.582221.

64. Nilsson, S.; Koehler, K.F.; Gustafsson, J.A. Development of subtype-selective oestrogen receptor-based therapeutics. Nat. Reviews. Drug Discov. 2011, 10, 778–792, doi:10.1038/nrd3551.

65. Pravettoni, A.; Mornati, O.; Martini, P.G.; Marino, M.; Colciago, A.; Celotti, F.; Motta, M.; Negri-Cesi, P. Estrogen receptor beta (ERbeta) and inhibition of prostate cancer cell proliferation: Studies on the possible mechanism of action in DU145 cells. Mol. Cell. Endocrinol. 2007, 263, 46–54, doi:10.1016/j.mce.2006.08.008.

66. Savolainen, S.; Pakarainen, T.; Huhtaniemi, I.; Poutanen, M.; Makela, S. Delay of postnatal maturation sensitizes the mouse prostate to testosterone-induced pronounced hyperplasia: Protective role of estrogen receptor-beta. Am. J. Pathol. 2007, 171, 1013–1022, doi:10.2353/ajpath.2007.060979.

67. Dey, P.; Strom, A.; Gustafsson, J.A. Estrogen receptor beta upregulates FOXO3a and causes induction of apoptosis through PUMA in prostate cancer. Oncogene 2014, 33, 4213–4225, doi:10.1038/onc.2013.384.

68. Omoto, Y.; Iwase, H. Clinical significance of estrogen receptor beta in breast and prostate cancer from biological aspects. Cancer Sci. 2015, 106, 337–343, doi:10.1111/cas.12613.

69. Hervouet, E.; Cartron, P.F.; Jouvenot, M.; Delage-Mourroux, R. Epigenetic regulation of estrogen signaling in breast cancer. Epigenetics 2013, 8, 237–245, doi:10.4161/epi.23790.

70. Vrtacnik, P.; Ostanek, B.; Mencej-Bedrac, S.; Marc, J. The many faces of estrogen signaling. Biochem. Med. 2014, 24, 329–342, doi:10.11613/BM.2014.035.

71. Johnson, C.A.; Turner, B.M. Histone deacetylases: Complex transducers of nuclear signals. Semin Cell Dev Biol 1999, 10, 179–188, doi:10.1006/scdb.1999.0299.

72. Garinis, G.A.; Patrinos, G.P.; Spanakis, N.E.; Menounos, P.G. DNA hypermethylation: When tumour suppressor genes go silent. Hum. Genet. 2002, 111, 115–127, doi:10.1007/s00439-002-0783-6.

73. Zhu, W.G.; Otterson, G.A. The interaction of histone deacetylase inhibitors and DNA methyltransferase inhibitors in the treatment of human cancer cells abs. Curr. Med. Chem. Anticancer Agents 2003, 3, 187–199.

74. Walton, T.J.; Li, G.; Seth, R.; McArdle, S.E.; Bishop, M.C.; Rees, R.C. DNA demethylation and histone deacetylation inhibi-tion co-operate to re-express estrogen receptor beta and induce apoptosis in prostate cancer cell-lines. Prostate 2008, 68, 210–222, doi:10.1002/pros.20673.

75. Zhou, Y.; Liu, X. The role of estrogen receptor beta in breast cancer. Biomark. Res. 2020, 8, 39, doi:10.1186/s40364-020-00223-2.

76. Gandhi, N.; Das, G.M. Metabolic Reprogramming in Breast Cancer and Its Therapeutic Implications. Cells 2019, 8, 89, doi:10.3390/cells8020089.

77. Naaz, A.; Zakroczymski, M.; Heine, P.; Taylor, J.; Saunders, P.; Lubahn, D.; Cooke, P.S. Effect of ovariectomy on adipose tissue of mice in the absence of estrogen receptor alpha (ERalpha): A potential role for estrogen receptor beta (ERb

eta). Horm. Metab. Res. 2002, 34, 758–763, doi:10.1055/s-2002-38259.

78. Kalyanaraman, B. Teaching the basics of cancer metabolism: Developing antitumor strategies by exploiting the differen ces between normal and cancer cell metabolism. Redox Biol. 2017, 12, 833–842, doi:10.1016/j.redox.2017.04.018.
79. Manente, A.G.; Valenti, D.; Pinton, G.; Jithesh, P.V.; Daga, A.; Rossi, L.; Gray, S.G.; O'Byrne, K.J.; Fennell, D.A.; Vacc a, R.A.; et al. Estrogen receptor beta activation impairs mitochondrial oxidative metabolism and affects malignant meso thelioma cell growth in vitro and in vivo. Oncogenesis 2013, 2, e72, doi:10.1038/oncsis.2013.32.
80. Ma, R.; Karthik, G.M.; Lovrot, J.; Haglund, F.; Rosin, G.; Katchy, A.; Zhang, X.; Viberg, L.; Frisell, J.; Williams, C.; et al. Es-trogen Receptor beta as a Therapeutic Target in Breast Cancer Stem Cells. J. Natl. Cancer Inst. 2017, 109, 1–14, d oi:10.1093/jnci/djw236.
81. Song, I.S.; Jeong, Y.J.; Jeong, S.H.; Kim, J.E.; Han, J.; Kim, T.H.; Jang, S.W. Modulation of Mitochondrial ERbeta Expr ession Inhibits Triple-Negative Breast Cancer Tumor Progression by Activating Mitochondrial Function. Cell. Physiol. Bi ochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 2019, 52, 468–485, doi:10.33594/000000034.
82. Sawada, M.I.; GD, S.F.; Passarelli, M. Cholesterol derivatives and breast cancer: Oxysterols driving tumor growth and me-tastasis. Biomark. Med. 2020, 14, 1299–1302, doi:10.2217/bmm-2020-0460.
83. Tuli, H.S.; Tuorkey, M.J.; Thakral, F.; Sak, K.; Kumar, M.; Sharma, A.K.; Sharma, U.; Jain, A.; Aggarwal, V.; Bishayee, A. Mo-lecular Mechanisms of Action of Genistein in Cancer: Recent Advances. Front. Pharmacol. 2019, 10, 1336, doi:10.3 389/fphar.2019.01336.
84. Atteritano, M.; Pernice, F.; Mazzaferro, S.; Mantuano, S.; Frisina, A.; D'Anna, R.; Cannata, M.L.; Bitto, A.; Squadrito, F.; Frisina, N.; et al. Effects of phytoestrogen genistein on cytogenetic biomarkers in postmenopausal women: 1 year rand om-ized, placebo-controlled study. Eur. J. Pharmacol. 2008, 589, 22–26, doi:10.1016/j.ejphar.2008.04.049.
85. Pintova, S.; Dharmupari, S.; Moshier, E.; Zubizarreta, N.; Ang, C.; Holcombe, R.F. Genistein combined with FOLFOX or FOLFOX-Bevacizumab for the treatment of metastatic colorectal cancer: Phase I/II pilot study. Cancer Chemother. Pha rmacol. 2019, 84, 591–598, doi:10.1007/s00280-019-03886-3.
86. Lazarevic, B.; Boezelijn, G.; Diep, L.M.; Kvernrod, K.; Ogren, O.; Ramberg, H.; Moen, A.; Wessel, N.; Berg, R.E.; Egge-Jacobsen, W.; et al. Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer pri or to radical prostatectomy: A randomized, placebo-controlled, double-blind Phase 2 clinical trial. Nutr. Cancer 2011, 6 3, 889–898, doi:10.1080/01635581.2011.582221.
87. Nilsson, S.; Koehler, K.F.; Gustafsson, J.A. Development of subtype-selective oestrogen receptor-based therapeutics. Nat. Reviews. Drug Discov. 2011, 10, 778–792, doi:10.1038/nrd3551.
88. Pravettoni, A.; Mornati, O.; Martini, P.G.; Marino, M.; Colciago, A.; Celotti, F.; Motta, M.; Negri-Cesi, P. Estrogen recepto r beta (ERbeta) and inhibition of prostate cancer cell proliferation: Studies on the possible mechanism of action in DU1 45 cells. Mol. Cell. Endocrinol. 2007, 263, 46–54, doi:10.1016/j.mce.2006.08.008.
89. Savolainen, S.; Pakarainen, T.; Huhtaniemi, I.; Poutanen, M.; Makela, S. Delay of postnatal maturation sensitizes the m ouse prostate to testosterone-induced pronounced hyperplasia: Protective role of estrogen receptor-beta. Am. J. Patho l. 2007, 171, 1013–1022, doi:10.2353/ajpath.2007.060979.
90. Dey, P.; Strom, A.; Gustafsson, J.A. Estrogen receptor beta upregulates FOXO3a and causes induction of apoptosis thr ough PUMA in prostate cancer. Oncogene 2014, 33, 4213–4225, doi:10.1038/onc.2013.384.
91. Omoto, Y.; Iwase, H. Clinical significance of estrogen receptor beta in breast and prostate cancer from biological aspect s. Cancer Sci. 2015, 106, 337–343, doi:10.1111/cas.12613.
