Introduction

Breast cancer is the leading fatal cancer in women (1). Estradiol, with other growth factors like epidermal growth factor (EGF) or insulin-like growth factor-1 (IGF-1), features in formation of breast and other reproductive tissues in embryogenesis, pregnancy, puberty and lactation. However, excessive expression of these signal molecules results in uncontrolled proliferation (2). This is the underlying mechanism in growth of malignant tissue.

While the ovaries are the major estrogen source and primary aromatase expression zones in premenopausal women, in postmenopausal women, these are peripheral tissues. Especially adipose is the main aromatase expression tissue in postmenopausal women (3). With the theory that estrogens are inducing tumor proliferation, in conjunction with with the fact that postmenopausal women have decreased estrogen secretion, this provoked researchers to focus on estrogen and aromatase secretion in extraglandular tissues (4).

Therefore, inhibition of the aromatase enzyme which takes a role at the last step of estrogen biosynthesis is the aim of this therapy.

Aromatase expression in breast tissue

Breast stroma largely consists of adipose tissue. Adipose tissue contains different amounts of mature adipocytes and undifferentiated fibroblasts (5). Aromatase transcripts in breast adipose tissue are mainly located in fibroblasts which are the precursors of mature adipocytes. Furthermore, they are located in epithelial cells of normal and benign breast tissues. Here the role of aromatase is converting adrenal/ovary originated androgen to estron. Then the estrons are converted to estradiols by enzyme 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD type 1) (3).

The parenchymal interaction between malignant breast epithelial cells and both adipose fibroblasts and endothelial cells are responsible for estrogen biosynthesis and lack of adipogenic...
Aromatase inhibitors in breast cancer therapy

Aromatase inhibitors are essentially useful in postmenopausal patients because they inhibit extra-ovarian aromatase. Because ovaries in premenopausal women can raise estrogen levels by reflex increases in luteinizing hormone (LH) and follicle stimulating hormone (FSH), but in postmenopausal women these main estrogen sources and expression regions are peripheral tissues and estrogen synthesis may be suppressed by aromatase inhibitors.

Therapies of postmenopausal hormone-dependent breast cancer patients contain two different strategies: One of them is blocking the connection between estrogens and their receptors by anti-estrogens, other one is inhibition of estrogen synthesis by aromatase inhibitors (4). Nonsteroidal antiestrogen tamoxifen was synthesized in 1967 and found to be efficient in advanced or first-phase breast cancer therapy. At the same time tamoxifen is prohibitive against formation of cancer in the other breast and development of cancer among women classifying in high-risk group (6). However tamoxifen has both estrogen agonistic and antagonistic effects according to target tissue. While affecting chiefly as antagonist in breast, it affects as estrogen agonist in bone, liver and uterus (4). As a result of estrogenic activity, it has a protective effect on bones. On the other hand it raises endometrial cancer and thromboembolic event risks throughout long-term therapies (7). Moreover, tumors go on growing by developing resistance at the progressive phases of therapy. Thus it is proclaimed that tamoxifen therapy is efficacious for five years term (4). Other selective estrogen receptor modulators (SERM) such as droloxifene, idoxifene, toremifene, raloxifene are known to have agonistic effects and they have no advantages observed in clinical trials when compared with tamoxifen. Moreover they have deficient effects after using in tamoxifen resistant tissues (7). This case caused to look for new antiestrogens which have no agonistic effects and resulted with finding out antiestrogen fulvestrant. Fulvestrant attaches to estrogen receptor (ER) competitively like tamoxifen but differently from tamoxifen, attachment to ER results with degradation and decreasing in numbers of ER.

In order to abstain from agonistic effects of tamoxifen and raise the efficiency and safety, a different approach had been worked up in antiestrogen therapy in earlier 1970s. Aromatase inhibitors which are inhibiting the transformation of androgens to estrogens without agonistic effects had been identified (4). Aromatase inhibitors (anastrozole, letrozole and exemestane) were found to be effective when used in therapies of tamoxifen resistant and relapsed breast cancers (6). The reason of aromatase therapy success against tamoxynephine resistant patients is having different effect mechanisms. While tamoxifen is blocking estrogen’s effect on receptor, aromatase inhibitors block estrogen synthesis in breast and peripheral tissues. Because of being partial estrogen agonist in breast tissue, tamoxifen causes less effect when compared with optimum antitumor activity while aromatase inhibitors are being more efficacious first-step therapy alternative because of not having estrogenic effects (6). Aromatase inhibitors have such a different advantage as not having cross-resistance between steroidal and nonsteroidal types. Therefore steroidal aromatase inhibitors can be used in nonsteroidal aromatase inhibitor-resistant breast cancers. Inverse scenario is valid in this case too. In a clinical trial upon steroidal aromatase inhibitor formestane-resistant patients, giving nonsteroidal inhibitor anastrozole as secondary yields 62% clinical benefit (7).

In order to detect optimum therapy strategies for postmenopausal breast cancer patients, antiestrogen and aromatase inhibitor-resistant breast cancer model was developed in a mouse. In that model, via transfection of hormone-sensitive MCF-7 human breast cancer cells with human aromatase gene to timus and ovary lacking mouse, new MCF-7CA cells which are able to synthesize enough estrogen in order to form a tumor was generated. This model denotes clinical case of postmenopausal breast cancer patients having extra-ovarian tissues as major estrogen source (6). Aromatase inhibitors such as letrozole, anastrozole, formestane, exemestane, were experienced both alone and in a combination in that model. In first four weeks of letrozole therapy tumor size reduced greatly. However, there was no response observed in MCF-7CA tumors treated by formestane, anastrozole, tamoxifen and fulvestrant. Furthermore, in letrozole therapy tumor size doubled from the beginning in 37 weeks, however this period is 16 weeks in tamoxifen therapy. This shows advantage of letrozole because it has extended the illness progression time (6). Letrozole therapy shows letrozole decreases estradiol concentration 90% in tumors. This shows growth adaptation of MCF-7CA tumors in the medium deprived of estrogen which start growing after 37 weeks
of letrozole therapy. Another result from that study is the probability of a lack of response of the tumors growing throughout letrozole therapy to the adding therapy with any steroidal or nonsteroidal aromatase inhibitors. Because letrozole is a really strong aromatase inhibitor and therefore, letrozol resistant MCF-7ca tumors don’t response to less effective drugs according to letrozol in aromatase enzyme inhibition (6).

In MCF-7CA tumor model aromatase inhibitors were compared both alone and in combination with antiestrogens. Consequently, it was found that letrozole +tamoxifen and anastrozole+tamoxifen were less effective than only letrozole therapy. This may be a result of dramatic decrease in estrogen levels provided by letrozole or anastrozole and so dominance of tamoxifen’s estrogenic activity upon tumor because of being a partial agonist (8). The combination of letrozole and antiestrogen fulvestrant was found by far effective than any therapy of each agent and 29 weeks of therapy has prevented tumor growth (4). Because fulvestrant causes ER degredation additionally to its antiestrogen efficacy.

Various case studies indicate the fact that regular use of nonsteroidal antiinflammatory drugs such as aspirin or cyclooxygenase (COX) inhibitors may decrease cancer risk (1). Cyclooxygenase enzyme has minimum two isoforms: COX-1 and COX-2. COX-1 is always expressed in lower levels, however COX-2 expression occurs as an immediate response to various stimuliants (9). It is observed that COX-2 is excessively expressed in various tumors including breast cancer. PGE2, which is produced by COX-1 and COX-2 isoenzyme stimulates estrogen biosynthesis via increasing cytochrom P450 CYP19 gene (aromatase gene) in breast stromal cells. The ascent of COX-2 expression in breast cancer tumors is coordinat- ed with increased tumorgenic transformation, advanced phase tumors and decreased survival time. At the same time COX-2 expression is related with angiogenesis, increased proliferation, high expression levels of p53 and human epidermal growth receptor 2 (HER2) and existence of axillary node metastases too. HER2, as a marker of agressive disease and malign prognose, stimulates COX-2 transcription via Ras → Raf → mitogen activated protein kinase (MAPK) pathway. In human breast cancer cell culture, it has been observed that COX-2 inhibitors were decreasing aromatase expression and activation. This preclinical finding supports the vision indicating the fact that aromatase repressive effects of COX-2 inhibitors may cause a more effective repression on local estrogen biosynthesis in progressive breast cancer patients with accompanying clinical sores.

COX-2, in addition to being an aromatase activator, may cause the progress of breast cancer by aromatase-independent mechanisms such as decreasing apoptosis and activating angiogenesis (1). In fact COX-2 was determined in new blood vessels surrounding the tumor, therefore COX-2 inhibition targets not only tumor epithelial cells, but also targets endothelial cells.

In humans it was observed that COX-2 levels increased in both ductal carcinoma in situ (DCIS- noninvasive tumor of breast) and invasive breast cancer and high proliferation, low apoptosis and increased formation of new blood vessels accompanied this. Therefore it is thought that COX-2 inhibition may have three different anticarcinogenic mechanisms:

- inhibition of proliferation in epithelial cells
- increased apoptosis
- reducing angiogenesis

Observation of antiangiogenic and proapoptotic effects make us think that COX-2 inhibitors may be efficient in both ER-positive and negative tumors. Furthermore COX-2 inhibition made tumors significantly sensitive to chemotherapy and radiotherapy. Therefore, various combinations are being searched in clinical tri- als.(9)

In postmenopausal ER-positive patients, therapeutic efficiency of exemestane-celexocib combination had been compared with exemestane and letrozole therapy. Clinical responses of postmeno- pausal women treated with these therapies for 3 weeks alone are found in order of 62%, 60% and 55%. When clinical benefit periods are compared, it is 96.6 weeks for exemestane + celexocib and 49.1 weeks for exemestane alone therapies. Duration of repeating time for exemestane + celexocib treated breast cancer is longer (9).

In advanced breast cancer, the safety and tolerability of aromatase inhibitor and COX-2 inhibitor combination was compared to aromatase inhibitor alone. The major concern about COX-2 inhibitors safety was arterial thrombotic event risk which is common with nonsteroidal antiinflammatory drugs. Up to now exemestane + celexocib combination was tried on more than 180 patients and resulted with minimum cardiovascular events. More than %10 of patients had hot flushes/ night sweats (%25), nause (%13) and dyspepsia/ heartburn (%11). After 3 months of treatment exemestane + celexocib combination had been compared with exemestane alone and letrozole alone. Clinical responses of postmeno- pausal women treated with these therapies for 3 weeks alone are found in order of 62%, 60% and 55%. When clinical benefit periods are compared, it is 96.6 weeks for exemestane + celexocib and 49.1 weeks for exemestane alone therapies. Duration of repeating time for exemestane + celexocib treated breast cancer is longer (9).

Resistance to aromatase inhibitors
The suppression of tumor cell proliferation with aromatase inhibi- tor treatment disappeared in the course of time and tumor cells continue to proliferate in ER (+) breast cancer patients. When estrogen levels decrease, ER expression, function and intracellular signal increase and hypersensitivity to lower estrogen levels develop adaptatively (7). Thus tumor cells continue to proliferate with low estrogen levels. This situation is defined as resistance.
30-40% of primary breast cancer patients’ ER levels are very low or absent, this situation is defined as primary (full) resistance. The situation that ER (+) tumors response to endocrine therapy at the beginning, but a few times later some of them continue to proliferate defined as secondary resistance. Some tumors have ER but resistant to drug primarily from beginning of the treatment. Therefore explaining the hormonal resistance which just one mechanism in breast cancer patients is not possible (10). But these mechanisms must be clarified to select the appropriate therapy for the patient, increase the effectiveness of therapy and prevent the resistance development.

It is thought that resistances in ER (+) breast cancer is based on ER-mediated signal mechanism (1). Binding estradiol to ER causes some confirmational changes and activates receptor. Activated ER complexes regulate estrogen response elements’ transcription in the promoter regions of target genes (like progesterone receptor, type 1 insulin-like growth factor receptor (IGF1R) with binding their specific regions on DNA. Thus, estradiol can increase the expression of genes related with proliferation, inhibition of apoptosis, invasion and metastasis in ER expressing tissues. This effect is defined as ER’s genomic function. Interaction between ER and growth factors defined as ER’s nongenomic functions. ER provide tumor growth by epidermal growth factor receptor (EGFR)/ human epidermal growth factor receptor-2 (HER2) and IGF1R signal activation with nongenomic mechanisms. The signals through these receptors can activate 2 pathways: MAPK and phosphoinositide-3 kinase (PI3K)/ Akt pathways (12). These kinases activate ERs by phosphorylating them.

Estrogen causes IGF signaling increase by increasing IGF1R and insulin receptor substrat (IRS)-1 expression in breast cancer cells. Tumors have IRS-1 expression, phosphorylation and MAPK phosphorylation while growing with estrogen existence. Removing estrogen decrease IRS-1 expression and MAPK activity, stop the tumor growth. But tumors can maintain their functions via nongenomic pathway in estrogen deprived medium; this model demonstrates developing resistance to aromatase inhibitors (12). In resistant tumors while ER’s genomic functions are suppressed, EGFR/ HER2 and IGF1R signals activation by ER’s nongenomic mechanisms provide tumor growth (13).

It is understood that adaptor proteins (p-Shc and Grb-2) increase with MAPK cascade signaling proteins in long term letrozole treatment in tumors and then tumors don’t response to letrozole. These findings show that tumor cells adapt to estrogen deprivation by upregulating estrogen signaling pathway at the beginning and this become true with kinase signaling proteins activation to sustain transcription and cell proliferation. ERs significance in here is proved by combining letrozole and ER down-regulator fulvestrant. This treatment is more effective than letrozole alone treatment because of not activating kinase signaling pathway and tumor growth inhibition is maintained for an extended period (14).

Three approaches are recommended to overcome endocrine resistance according to these findings: maximal blockage of ER signaling, combining endocrine therapies with new therapies that target HER family and combination with drugs that target the downstream of signaling pathways.

• Maximum blockage of ER signal (11):

After it was recognised that functional ER pathway still exists in the cells which developed resistance against estrogen deficiency, providing maximum ER signal blockage is aimed during new endocrine treatment methods are being developed. ER antagonist fulvestrant causes cellular lack of ER via rapidly degrading fulvestrant-ER complex. However in the course of time, despite fulvestrant treatment, tumor cells start to grow again. Development of resistance was delayed when exemestane, was given additionally to fulvestrant. Resistance formation times were compared with various combination of fulvestrant and aromatase inhibitors. Consequently, it has been understood that decreasing of ligand level and ER density is providing maximum blockage and thus, efficent therapy and prevention of endocrine resistance may be possible.

• Co-targeting ER and HER signaling mechanisms: preventing acquired resistance (11)

In the clinical trials, HER2 monoclonal antibody trastuzumab or EGFR/ HER2 tirosine kinase inhibitors gefitinib, erlotinib or lapatinib with endocrine treatment were used for this purpose.

When a combination of anastrozole and gefitinib was compared to a single anastrozole treatment on ER (+) advanced breast cancer patients, it was found that resistance development was delayed in combination therapy. Targeting HER2 in ER (+) breast cancer may increase endocrine sensitiveness providing re-expression of silenced ERs. During treatment of ER (-)/ HER2 (+) breast cancer patients with trastuzumab, it was proved that trastuzumab can regulate both ER expression and endocrine response. It was seen that the metastatic breast cancer patients treated with anastrozole + trastuzumab live longer than anastrozole treated patients without recurrences.

The antiproliferative effect of oral tirosine kinase inhibitor lapatinib, which is also a strong inhibitor of EGFR and HER2, increases on breast cancer cells that have high levels of HER2 in the absence of estrogen. Furthermore, lapatinib can increase tamoxifen sensitivity of tamoxifen-resistant cells distinctly according to preclinical studies. Lapatinib and aromatase inhibitor combination is being tried on.

• Targeting downstream signaling:

The other intracellular pathways downstream from cell surface growth factor receptors may be responsible for endocrine resistance; therefore targeting these pathways in combination therapy strategies may be an appropriate approach. PI3K/ Akt/
mammalian target of rapamycin (mTOR) pathway is activated by several growth factors like insulin, IGF-1, fibroblast growth factor, EGF and vascular endothelial growth factor.

It was found that life extension in letrozole combination with mTOR inhibitor temsirolimus treated patients is longer than letrozole alone treated breast cancer patients (11). This can be explained by delayed resistance development in combination therapy.

There is a need for biological analysis to clarify resistance mechanisms individually on each patient, because lots of different mechanisms may be responsible of resistance. Targeting pathway, which takes part in resistance, may result more successfully in treatment.

Future strategies for aromatase inhibitors (15)

Combination of aromatase inhibitors with growth factor inhibitors

After the identification of growth factor receptor pathways’ effects on ER functions, it was thought that it can be useful to target these pathways with ER in treatment (12). Increasing activity of growth factor pathways during resistance development to aromatase inhibitors made researches think to use molecules which inhibit these pathways for delaying resistance development. Clinically aromatase inhibitors’ combinations with tirosine kinase inhibitors or growth factor receptor antagonists were started to be tested (15).

Inhibition of other molecules, which plays a role in various steps of signal transduction pathways for the resistance development, also can be targeted in new treatment strategies. For example; farnesyl transferase inhibitors (FTI), Raf-1 kinase inhibitors, MAP/ extracellular signal-regulated kinase (ERK) (MEK) inhibitors, PI3K inhibitors are new drug candidates which can target key molecules in breast cancer treatment. It was proved that FTI, MAPK inhibitors are effective in experimental breast cancer models. It is thought that if signal transduction inhibitors (STI) are combined with aromatase inhibitors, it can be more effective in both controlling tumor growth and prevent or overcome resistance (10).

Blockage of aromatase specifically in breast

Aromatase takes part in lots of tissues and therefore blocking enzime in these tissues by aromatase inhibitors cause some adverse effects like osteoporosis, hot flashes, vaginal atrophy (16). To prevent these effects, blocking the enzyme only in the specific tissue is aimed. It may be useful for specific therapy to block transcriptions via promoter II and I.3 which mediate aromatase expression in breast cancer tissue. As a result of this notion, the factors which take role in regulating aromatase expression via promoter II in breast tissue and can be targeted for specific therapy are began to investigated (15). Various proteins interact with promoter II/I.3 as a response to tumor originated factors like PGE₂, an important regulator of aromatase expression in breast tissue (16). It has been revealed that COX-2 inhibitors can block aromatase expression by preventing PGE₂ formation. But after detailed research about these drugs’ effects on cardiovascular diseases, they can be used (15).

It may be possible to develop a new generation of aromatase inhibitors by using antisense technology based on techniques that target tissue specific aromatase transcripts or drugs which prevent interaction between specific aromatase promoter activating proteins and those promoters (16).

Preventing Breast Cancer

Because of the increasing risk of breast cancer in women, the researches are adamant about preventing it.

It was shown that aromatase over expression has a role in carcinogenesis. One of the hypothesis for breast cancer in women is induction of mutations by estrogen metabolites. Estrogen can be changed into catechol-estrogens and then to estrogen-quiones enzymatically by cytochrome P450 1B1. Estrogen's these metabolites may cause releasing of nucleotide-estrogen conjugates by binding DNA covalently, this process is defined as depurination. Point mutations may happen during repairing these naked segments of DNA, this also contributes carcinogenic process. Reactive oxygen radicals may occur during redox cycle that catechol-estrogens change into quiones and these may also result in DNA damage and depurination. To prevent cancer, these factors must be targeted. Therefore aromatase inhibitors, which decrease both aromatase expression and estrogen synthesis, may be optimal agents to prevent breast cancer. Clinical trials are going on for this purpose (15).

In addition, one of the strategies about breast cancer therapy is researching these drugs’ efficacy with ER independent mechanisms on ER (-) tumors. Because in the studies it has been found that ER (+) tumors are more sensitive than the tumors that receptor status is not known (3).

Discussion and conclusion

Targeting the aromatase enzyme therapeutically in breast cancer therapy was first thought about in 1960s. Aminogluthetimide was the first aromatase inhibitor which was tried for this purpose. The first generation aromatase inhibitor aminogluthetimide was as effective as tamoxifen in breast cancer therapy, but its side effects limited its widespread use. Tamoxifen was presented in the 1970s and became the gold standard for breast cancer's hormonal therapy. Second generation aromatase inhibitors were tried in Europe in the 1980s and they were also found as effective as tamoxifen. In 1990s, the third generation aromatase inhibitors were developed in the U.S. for treating postmenopausal breast cancer and proved their superiority to tamoxifen. These new inhibitors suppress estrogen production in extraovarian tissues and in the breast cancer tissue (3).

Blocking effectively the estrogenic activity, reduces the recurrence risk and prolongs disease-free life in postmenopausal women with...
According to these findings, American Society of Clinical Oncology (ASCO) made a change in its guide for early stage postmenopausal breast cancer therapy. The society recommended that after the surgery for 5 years the adjuvant therapy, should include an aromatase inhibitor to reduce the recurrence risk of cancer (17,18). But as a result of aromatase existing in many other tissues, inhibiting all of them with aromatase inhibitors may cause some side effects like osteoporosis, hot flashes while inhibiting the growing of tumor tissue in breast. For this, it is recommended to use biphosphonates to prevent the decrease in bone mineral density and bone loss, especially for postmenopausal women who are using aromatase inhibitors. As a result, today aromatase inhibitors are the most effective endocrine therapy for ER-positive postmenopausal breast cancer (3).