THE MOLECULAR ROLE OF HUMAN EPIDERMAL GROWTH FACTOR 2 (HER2) IN BREAST CANCER

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ABSTRACT: HER2 overexpression has been observed in 20-30% of breast cancer cases. In addition to main products of HER2 gene, there are other variations, of which, the most important one is Δ16HER2. In principle, although HER2 overexpression is the first step for carcinogenesis, it is not sufficient, and the main reason responsible for breast tumorigenesis is Δ16HER2. This onco-protein, which lacks exon 16, can form stable homodimers remaining active constitutively. Like Δ16HER2, 611-CTF, a specific HER2 carboxy-terminal fragment, expresses a constitutively active homodimers. Therefore, targeting of Δ16HER2 and 611-CTF is the most promising strategy for treating HER2+ breast cancer. Along with inhibitors of HER2 gene products, miRNAs are also novel candidates for treatment of breast malignancy. Several drugs, working against different domains of the HER2 receptor, have been presented for treatment of HER2 positive breast cancers and they can increase mortality in patients.

Keywords: Breast Cancer, HER2, Δ16HER2, Herstatin, P100, CTFs.

INTRODUCTION

Breast cancer is one of the most common malignancies among women in various countries. Taking advantage of gene expression profiling and microarray studies has been changing classification of cancer, from histological to molecular classification. One of the most important factors involving in breast carcinogenesis is Human Epidermal Growth Factor Receptor 2 (HER2) gene which is overexpressed in 20-30% of breast cancer cases (Yarden, 2001). The main product of HER2 gene is an integral membrane glycoprotein with tyrosine kinase activity. Activation of this protein induces pathways involved in cell survival and growth (Hung et al., 1986). There are other variations of HER2 gene, of which, the most important one is Δ16HER2, a major onco-protein in breast carcinogenesis. In fact, it has been claimed that HER2 overexpression is the first step of carcinogenesis, but not sufficient. The main reason responsible for breast malignancy is increased level of Δ16HER2 in mammary cells. This onco-protein constitutively keeps receptors active due to formation of stable homodimers, in other words, makes HER2 signal transduction pathway stable in the dearth of required ligands (Marchini et al., 2011). Diversity in HER2 protein causes different and sometimes unexpected responses to therapeutic procedures, including drug resistance. This fact makes it mandatory to design various therapeutic strategies for treatment of HER2+ breast cancers. Until now, there have been several drugs designed and presented to work against different domains of the HER2 and can inhibit extra- or intracellular regions of this receptor along with downstream proteins of various corresponded messenger pathways.
CLASSIFICATION OF BREAST CANCER

Criteria for breast cancer classification have been changed to molecular parameters with advances in gene expression profiling and microarray studies; consequently treatment strategies have become more precise and efficient. Breast carcinomas are divided into four groups based on molecular classification: 2.1. Basal-Like Breast Cancers which mostly correspond to Estrogen Receptor- (ER-), Progesterone Receptor- (PR-) and HER2-tumors (accordingly, they are also called the triple negative (ER-/PR-/HER2-) breast cancers); 2.2. Luminal-A Breast Cancers which are mostly low-grade ER+ tumors; 2.3. Luminal-B Breast Cancers which are also mostly ER+ tumors, but expressing low level of hormone receptors and are of a high-grade; and 2.4. HER2+ Breast Cancers which exhibit amplification and high level of HER2 expression and several other amplicons of this gene (Perou et al., 2000; Sørlie et al., 2001; Sørlie et al., 2003; Sotiriou et al., 2003; Hu et al., 2006b; Sotiriou and Pusztai, 2009).

HER2

Human Epidermal growth factor Receptor 2 (HER2), also known as HER2/neu and c-ErbB2, is an oncogene located on 17q21 and encodes a 185 kDa transmembrane glycoprotein with 1255 amino acids called HER2 or P185HER2 (Coussens et al., 1985; King et al., 1985; Gutierrez and Schiff, 2011; Sasso et al., 2011). HER2 along with other members of the HER family, i.e. HER1, HER3 and HER4, form active tyrosine kinase receptors on the surface of the cell (Hung et al., 1986). 25 years ago in basic studies on breast cancer cell lines it was determined that HER2 gene has undergone amplification and immediately after two years it was found that HER2 gene amplification is associated with important and essential features of the cell, such as proliferation and immortality; since then, drugs to deal with these abnormalities have been designed and produced (Gutierrez and Schiff, 2011).

All members of HER2 family have a similar structure: an extracellular domain constituted of ligand-binding site, a lipophilic transmembrane domain and an intracellular domain (carboxyl tail) with tyrosine kinase activity; however HER3 protein lacks the active intracellular domain. Activation of intracellular domains containing tyrosine kinase activity happened by binding of growth factors such as Epidermal Growth Factor 7 (EGF) to the extracellular domain; however there is no ligand available which binds directly to the HER2 protein (Beerli and Hynes, 1996) (Figure 1). HER2 extracellular domain is composed of four sub-domains with distinct responsibilities. Sub-domain I which forms N-terminus of HER2 protein is structurally highly homologous to subdomain III (each of them with approximately 170 amino acids)(Landgraf, 2007). Contrary to the other members of the HER family, sub-domain I on HER2 has permanent interactions with sub-domain III, keeping the receptor in an open conformation. It makes sub-domain II uncovered and ensures that HER2 is always ready to dimerize (Sliwkowski, 2003). As it is perceptible that sub-domain II is the dimerization domain. This region, which structurally is homologous to sub-domain IV (each of them approximately with 140 amino acids) (Landgraf, 2007), allows HER2 protein to bind to the other receptors of HER family to trigger downstream signaling (Sliwkowski, 2003). It is believed that sub-domain IV is responsible for stabilizing the HER2 protein and helps the receptor to be kept in an open conformation (Landgraf, 2007). Binding of ligands to the extracellular domain causes formation of active homo- or hetero-dimers and consecutively results in spontaneous tyrosine phosphorylation activity. These two activities (i.e. dimerization and tyrosine phosphorylation) are required for signal transduction. As there is no specific ligand initiating HER2 protein homodimerization, this receptor prefers to form heterodimers with other members of the HER family (Graus-Porta et al., 1997). Activating HER2 protein sparks a downstream signaling pathway with important consequences in cell proliferation, cell growth, differentiation, angiogenesis and cell survival (Yarden and Sliwkowski, 2001; Schlessinger, 2004; Rosen et al., 2010).

REGULATORY REGION OF HER2 GENE

As it has been observed in both tumor cells and in derived cell lines, overexpression of HER2 can be the result of amplification, in which case, expression will be 10 folds more than normal level. On the other hand, overexpression can be caused by only one copy of the HER2 gene. In addition, it has been shown in some HER2-amplified cell lines that level of HER2 mRNA is higher than expected rate, compared with the number of gene copies. Increased HER2 expression and its incompatibility with gene amplification status suggests a central
role of the regulatory regions in determining the level of HER2 expression (Hurst, 2001). Proximal promoter of the human HER2 gene consists of two typical regions: TATA box and CCAAT box which are located in positions -22 to -26 and -71 to -74 respectively (Figure 2). It is notable that TATA box in rodents’ HER2 gene is not conserved, making studies across the species rather difficult. Therefore, only human cells can be used to study promoter regions in the HER2 gene.

Two transcription initiation regions within the HER2 promoter have been mapped, the first region grouped around the major start site at +1 position, and the other one is at minor start site, centered at -69. Transcription initiation from these two points has two distinct molecular mechanisms. Initiation from the upstream position is in need of initiator-like elements; while the downstream sequence requires the TATA box. In HER2 overexpressing cells, -69 position undergoes an increase in expression (Bates and Hurst, 1997b; Scott et al., 2000). HER2 promoter activity was first examined in reporter constructs containing the -500 to +40 region (Ishii et al., 1987; Tal et al., 1987). But, the whole promoter area in these reporter constructs were designed in next steps and examined by different laboratories, so that more than 6 kb of the sequences in the 5’-flanking region of HER2 gene were studied (Bates and Hurst, 1997a). The results of these studies with high or low HER2 expression in mammalian cell lines were compared and it was found that the sequences up to -300 have a direct effect on gene expression levels; while, further 5’-flanking sequences have little positive effect on promoter activity. These findings are quite consistent with results determined by very precise maps created by DNase I digestion. One of these sites is located in the central region between the TATA and CAAT box and has been continued towards the upstream and downstream directions (Scott et al., 1994; Bates and Hurst, 1997a); however, it is still not possible to determine its exact boundaries. Lack of another hypersensitive site of up to 6 kb upstream of the +1 point suggests that these areas are not accessible in vivo for trans-acting elements. Another hypersensitive site in the upstream region of -6 kb has been observed, but not enough information is yet available regarding this site (Bates and Hurst, 1997a). Another promoter region which is located 12 kb upstream of +1 (Nezu et al., 1999) causes some changes in the 5’ exon, but experiments have shown that this promoter has no role in increasing or expressing the HER2 gene in cell lines derived from breast cancer tumors. A number of transcription factors corresponded to the HER2 promoter have been demonstrated to bind to the HER2 proximal promoter (Bates and Hurst, 1997b; Scott et al., 2000). However, only two of them named Activating Protein 2 (AP-2) and E-26 Transforming Specific (Ets) protein are required for promoter activity and HER2 overexpression in breast cancer. The AP-2 binding site has a GCTGCAAGGC consensus sequence and is located in -213 to -221 region (Figure 2). This region was identified as DNase I footprinted site in HER2 high-expressing cells, which AP-2 binding site did not undergo enzymatic digestion; however, in HER2 low-expressing cells this region was enzymatically digested. This evidence points to high activity of this binding site playing role in overexpression of HER2 in mammary tumor cells (Hurst, 2001). The mutation that prevents AP-2 from binding to the DNA can lead to reduced expression of HER2 resulting in decreased reporter activity in tumor cells with HER2 overexpression (Hollywood and Hurst, 1993). The AP-2 family consists of three highly homologous proteins named AP-2α, AP-2β and AP-2γ where all of them are capable of activating the proximal promoter (Bosher et al., 1996). However, studies in breast tumor-derived cell lines and breast primary tumors showed increased levels of AP-2β and AP-2γ; therefore, AP-2α plays no major role in overexpression of HER2 (Hollywood and Hurst, 1993; Turner et al., 1998).

Ets binding site (EBS) with GAGGAA consensus sequence located in the -28 to -33 region (Figure 2). This area is located upstream of a site which is highly sensitive to DNase I (Scott et al., 1994; Scott et al., 2000). Mutations in this region also cause weakness in reporter activity in tumor cells (Scott et al., 1994; Scott et al., 2000). Ets binding to EBS causes a severe bend in DNA (Scott et al., 2000). There if the Ets binding site is occupied, other TATA box binding proteins will not be able to bind to this box anymore. This finding is a comprehensive explanation for the fact that this is the -69 start point (and not the +1 start point) which is overly active in HER2+ tumor cells (Hurst, 2001). There have been at least 10 Ets proteins with different expression levels observed in breast cancer, of which, only the Polyoma Enhancer Activator 3 (PEA3) is highly associated with an increase of HER2 expression.

**BIOLOGICAL SIGNIFICANCE OF HER2**
ER and the HER2 signal transduction pathways are the major reasons of cell proliferation and immortalization in 85% of breast cancers; thus, in many therapeutic methods these two pathways have been targeted. Tamoxifen (an endocrine therapy agent) and Trastuzumab (an anti-HER2 monoclonal antibody), which are widely-applied in order to block ER and HER2 respectively, have had significant results both in treatment and improving the life expectancy of patients with 50% reduction in recurrence of the cancer (Piccart-Gebhart et al., 2005; Romond et al., 2005; Joensuu et al., 2009).

HER2 signaling pathway can be studied at three distinct levels: 1. The input layer to the membrane where the HER2 receptors are located. The signaling pathway was triggered from outside the cell due to binding of ligands to the other HER family proteins. 2. The core system processing layer which transduces signals to the nucleus and finally, the intracellular domains undergo trans-phosphorylation. Heterodimerization of HER2 with other members of HER family activates HER2 signaling pathway. HER2 can only form a substantial active homodimers with other HER2 receptors provided that the number of these receptors is increased due to the gene amplification or mRNA overexpression. Generally, HER family receptors are able to become a harbor for specific downstream proteins in a condition that they are trans-phosphorylated in their intracellular domains. Downstream proteins lead to the activation of secondary signaling factors responsible for interacting with other signaling pathways in the cell (Yarden, 2001; Barnes and Kumar, 2004; Bazley and Gullick, 2005; Citri and Yarden, 2006; Moasser, 2007). Transcription factors that are finally activated by this signaling system are involved in regulation of special genes responsible for various activities including cell proliferation, cell survival, differentiation, angiogenesis, invasion and metastasis (Graus-Porta et al., 1997; Citri and Yarden, 2006; Moasser, 2007). Comparing with other members of HER family, HER2 protein has the most kinase activity which is emanated of the fact that HER2 has an open conformational structure so that the region responsible for its dimerization forms dimers with other HER family members more efficiently. HER3 is activated as a result of Herregulin ligand; however, as stated before, this receptor lacks tyrosine kinase activity and like HER2 receptor, it must form heterodimers with other members of HER family. HER3/HER2 heterodimer has various anchoring sites for Phosphotidylinositol 3-kinases (PI3K) protein, resulting in activation of Protein Kinase B (also named Akt) as an anti-apoptotic factor (Morris et al., 1999; Schulze et al., 2005; Citri and Yarden, 2006; Moasser, 2007). HER2 can also be activated by formation of heterodimers with other receptor families such as insulin-like growth factor receptor (IGFR) (Nahta et al., 2005). Estrogen which activates estrogen receptors on the outer membrane of the nucleus can also trigger the HER2 signaling pathway (Shou et al., 2004).

The mutated form of HER2 protein lacking the extracellular region is called P95 and is seen in some cases of breast cancer (Molina et al., 2002; Scaltriti et al., 2007; Sasso et al., 2011). P95 is constitutively active due to the lacking of extracellular domain which is responsible for inhibiting the activity of HER2 in the absence of ligand; therefore, resistance to the trastuzumab therapy is observed in these tumors. More information regarding various kinds of HER2 transcripts will be discussed in the following sections.

**INCREASED EXPRESSION OF HER2 IN HUMAN CANCERS**

HER2 protein is expressed in limited level in normal tissues of the body. Increased expression of HER2 has been observed in 20-30% of breast cancers and some cases of ovarian and gastric tumors, as we recently reported the 23.3% rate of HER2 gene overexpression in 60 malignant breast samples (Tabatabaiean and Hojati, 2013). HER2 overexpression causes poor prognosis and aggravates the invasiveness of breast cancer tumors (Slamon et al., 1987; Yarden, 2001). Breast tumor cells can have more than 25 and in some cases up to 50 copies of HER2 genes; also an increase of 40-100 folds of HER2 protein comparing with its normal level has been observed in these cancers resulting in the presence of around two million HER2 proteins on the cell surface of tumors (Kallioniemi et al., 1992). The differences of HER2 expression levels between normal and tumor cells may contribute to the diagnosis and appropriate treatment of HER2+ breast tumors with trastuzumab. Trastuzumab is a well-tolerated monoclonal antibody in breast cancer patients because of its low-toxicity and its specificity to
HER2+ tumor cells (Gutiérrez et al., 2005). Amplification of HER2 gene has been observed in 20-25% of breast cancers and 50% of in situ carcinomas (Allred et al., 1992; Park et al., 2006; Ménard et al., 2000; Ménard et al., 2001). Breast cancers with HER2 gene amplification show unique biological and clinical features; they have increased sensitivity to various cytotoxic agents such as doxorubicin and relative resistance to endocrine therapy agents along with intrinsic tendency to brain and visceral metastasis (Ross et al., 2003; Gabos et al., 2006). The status of initially HER2-/ER+ breast tumors undergoing endocrine therapy may be changed into HER2+ during the treatment (Gutiérrez et al., 2005). This is due to the fact that ER protein is a negative regulator of HER2 expression; therefore, since ERs are blocked because of endocrine therapy their inhibitory effect is removed and HER2 can be overexpressed. (Newman et al., 2000; Guo and Sonenshein, 2004; Munzone et al., 2005; Xia et al., 2006; Lopez-Tarruella and Schiff, 2007).

HER2 VARIANTS
In addition to the two typical variants of HER2 transcripts (isoform a and b) that are produced due to the transcription initiation from two distinct transcriptional start points in the proximal promoter of HER2 gene, it has other variations generated by three different mechanisms: alternative splicing, alternative initiation in translation and post-translational enzymatic cleavage. These mechanisms generate three, two and one protein variants respectively. (Sasso et al., 2011).

1. Products of Alternative Splicing
1.1. P100: In 1993, the first variant of HER2, named P100, was described (Scott et al., 1993). P100 is a 100 kDa fragment which is secreted outside the cell. Its 2.3 kb mRNA is encodes 633 amino acids accounting for almost the extracellular domain of wild type HER2; therefore, P100 protein lacks the transmembrane and cytoplasmic domains. P100 interacts with HER2 by several mechanisms and it is demonstrated that this protein is an natural inhibitor of tumor cell growth (Scott et al., 1993; Doherty et al., 1999; Staverosky et al., 2005). In fact, P100 overexpression causes a procrastination and decreased level of HER2 phosphorylation in the presence of ligand and; hence, downstream pathways such as P42/44 MAP-Kinases are inhibited due to P100 activity. It is reported that the more progressed the cancer, the more P100 mRNA levels are decreased in breast carcinoma (Lennon et al., 2009), suggesting that it may be possible to overcome progression of tumorigenesis by means of increasing the level of P100 in breast tumors.

1.2. Herstatin: In 1999, Doherty et al. identified a 68 kDa protein named Herstatin (Christianson et al., 1998; Doherty et al., 1999). Herstatin is produced from alternative splicing mechanism and due to retention of 8th intron in HER2 mRNA. The first 340 amino acids of the Herstatin in N-terminus region are identical to wild type HER2, but from there on, there is a region with 77 new amino acids forming the C-terminal domain. As a natural inhibitor of HER2, Herstatin disrupts the formation of dimers, decreases phosphorylation of tyrosines and inhibits ligand-independent growth of HER2+ tumor cells (Sasso et al., 2011). In 2011, Azios et al. showed the importance of Herstatin in breast cancer patients as its ectopic expression disrupted the stable HER2, inhibiting the activity of HER2/HER3 heterodimerization and also the activity of epidermal growth factor receptors (EGFR) (Azios et al., 2001). The ability of Herstatin in suppressing colony formation in HER2+ or EGFR+ cells suggests a limiting potential role of Herstatin in tumor cells growth (Azios et al., 2001). In 2005, Hu et al. described the three-dimensional structure of Herstatin and determined the interacting binding of this protein with extracellular domain of HER2 (Hu et al., 2006a). The Interaction of Herstatin with HER2 on the cell surface caused an enhanced level of Herstatin and HER2 co-localization in cytoplasm, suggesting that formation of the Herstatin/HER2 complex may prevent transfer of HER2 from the endoplasmic reticulum to the cell surface. Suspension of HER2 in the cell by Herstatin is probably a natural intracellular mechanism that controls cell growth (Hu et al., 2005). Studies investigating the presence of Herstatin in normal and tumor cellular lines have indicated that Herstatin mRNA was expressed in normal fetal kidney and liver tissue, but it showed lower levels of expression in HER2-amplified breast carcinoma cells (Ross and Fletcher, 1999). An additional study showed that a Herstatin mRNA level does expressed in normal area adjacent to breast tumor. This expression pattern of Herstatin in normal breast tissues might contribute to prevent the formation of epithelial ducts caused by HER2 overexpression (Jackson-Fisher et al., 2008). A Further study revealed that Herstatin mRNA level in mammary carcinoma cells were similar to normal cells, but its protein expression pattern in tumor cells was 75% less than normal cells. In vitro studies showed that Herstatin inhibited the growth of human glioblastoma cell line U87MG.
in a dose-responsive manner, eventually preventing tumors formation (Barnes and Kumar, 2004). In addition, studies have shown that if glioblastoma cells have a truncated EGFR, Herstatin loses its inhibitory effect on cell growth; therefore, Herstatin is only useful in treating glioblastoma expressing the wild type EGFR (Sasso et al., 2011).

1.3. Δ16HER2: Interestingly, although the primary reason for tumorigenesis in HER2+ breast tumors is HER2 overexpression, it is not a sufficient factor. Indeed, one of the HER2 alternative splicing products called Δ16HER2 is the main factor leading tumorigenesis in breast tissue. This transcript encodes a receptor which lacks exon 16; a region immediately precedes the transmembrane domain of the wild type HER2 protein. Although Δ16HER2 constitutes 4-9% of total HER2, this relatively low level has a key role in HER2 transforming activity. In transgenic mice, tumors arose only when the extracellular region of HER2 had undergone small deletions resulting in formation of a new disulfide bond. Generally, HER2 overexpression could induce tumors that it is accompanied by in-frame cysteine amino acid deletion in the extracellular domain of HER2 protein (Ursini-Siegel et al., 2007). Lack of this important amino acid causes an extracellular conformational change in structure of HER2, so that disulfide bonds between molecules are increased. Consequently, stable homodimers of HER2/HER2 are created on the cell surface of tumors; initiating the constantly active HER2 signaling pathway (Marchini et al., 2011). In HER2 overexpressing primary human breast tumor, the level of Δ16HER2 variant is substantially propagated (Mrhalova and Kodet, 2003), so it is assumed that reduced efficiency of trastuzumab in cases with FISH ratio > 8 is due to the increased expression of Δ16HER2 (Dowsett et al., 2009). In addition to trastuzumab resistance, Δ16HER2 makes tumor cells resistance to endocrine therapy in HER2+/ER+ cases. HER2+/ER+ tumors, which account for half of the HER2+ tumors, are a new challenge in the treatment of breast cancer in a way that 70% of these tumors continue their growth in the body even after using Tamoxifen (Xia et al., 2008). In vitro and in vivo studies showed that Δ16HER2 caused estrogen-independent growth and resistance to Tamoxifen. To date, two mechanisms have been proposed for this phenomenon; in the first mechanism, in Δ16HER2 transfected cells which were resistant to Tamoxifen, levels of miR15-a and miR-16 were dramatically reduced. In normal cells, these two miRNAs bind to 3'UTR of Bcl-2 mRNA and result in inhibition of anti-apoptotic Bcl-2 protein synthesis. But in Δ16HER2 transfected cells which were resistant to Tamoxifen, expression of Bcl-2 protein was dramatically increased because of downregulation of miR15-a and miR-16 (Cimmino et al., 2005; Xia et al., 2008). The second mechanism is based on the reduced expression of miR-342 in breast carcinoma cells expressing Δ16HER2 and primary breast tumors that do not respond to Tamoxifen. miR-342 controls the expression of genes involved in the response to Tamoxifen. In fact, control of Δ16HER2 expression in MCF7 cell lines made these cells sensitive to apoptosis which is induced by Tamoxifen and, in turn, cell growth was dramatically reduced (Cittelly et al., 2010). A further study showed that Δ16HER2 was capable to arise tumors via activating of sarcoma (Src) kinase and Signal Transducer and Activator of Transcription 3 (STAT3) (Marchini et al., 2011). Final consequence of these activations is initiation of Akt and MAP-kinase pathways resulting in enhanced cell growth (Marchini et al., 2011; Silva 2004). Remarkably, only five copies of Δ16HER2 were found to be adequate to run neoplastic transformation of mammary epithelial cells in Δ16HER2-LUC mice, while in about 80% of MMTV-wild type HER2 transgenic mice, 30-50 copies of the wild type HER2 were required to induce breast cancer (Christianson et al., 1998). All these findings suggest that the oncogenic form of HER2 is Δ16HER2 (Sasso et al., 2011). The most efficient drug for treating trastuzumab-resistant patients with effective level of Δ16HER2 variant is dasatinib, a tyrosine kinase inhibitor, which coincidently targets Δ16HER2 and Src kinase. It deactivates Src and hinders the stability of Δ16HER2, thereby, inhibiting tumor growth and in parallel, obviates the resistance to trastuzumab. In addition to dasatinib, other tyrosine kinase inhibitors such as erlotinib, emodin, lapatinib and gefitinib are effective in treating trastuzumab-resistant breast tumors. (Marchini et al., 2011).

7.2. Products of Alternative Initiation in Translation and Enzymatic HER2 Cleavage

A series of Carboxyl Terminal Fragments (CTF) is expressed in a subgroup of HER2+ patients (Yuan et al., 2003). These CTFs are created by at least two different mechanisms including proteolytic cleavage and alternative initiation in translation. In the first mechanism, metalloproteases cleave extracellular domain of HER2, near to the transmembrane domain. The consequent protein is a 95-100 kDa fragment known as P95HER2. This fragment starts with alanine, 648th amino acid in the wild type of HER2 protein (Yuan et al., 2003; Liu et al., 2006).
In the second mechanism, translation starts from two internal methionines in positions 611 and 687. It results in two new P95HER2 fragments with 100-115 kDa and 90-95 kDa weight, called CTF-611 and CTF-687 respectively. The difference in these two proteins (76 amino acids) is related to transmembrane domain and the short cysteine-rich extracellular region (Christianson et al., 1998). CTF-611 easily passes the secretory pathway and is transported to the cytoplasmic membrane, but CTF-687 is only localized in the cytoplasm and nucleus (Pedersen et al., 2009). A subsequent study demonstrated that despite of bearing a domain containing kinase activity, soluble CTF-687 lacks enzymatic role. In contrast, two fragments containing a transmembrane domain at the carboxyl tail domain, i.e. CTF-611 and CTF-648, activate multiple signaling pathways; however, the scale of these activations by these two CTFs is quite different. Since CTF-611 forms the stable homodimers through disulfide bonds, it can activate protein kinases, stimulated by mitogen and Akt pathway, more strongly than CTF-648 which is more comparable to wild type HER2 protein (Pedersen et al., 2009). CTF-611 regulates many genes, of which, the most important include MMP1 (Matrix Metalloproteinase-1), ANGPTL4 (Angiopoietin-like 4), MET (Homosapiens met proto-oncogene, a hepatocyte growth factor receptor), CD44 (Cluster of Differentiation 44), PLAUR (Plasminogen activator receptor, urokinase type), EPHA2 (EPH receptor A2), ITGA2 (Integrin Alpha 2), TGFB (Transforming growth factor beta), TGFA (Transforming growth factor alpha), and IL-11 (Interleukin-11), which are mainly involved in the progression of metastatic tumors (Pedersen et al., 2009). Moreover, the cytoskeleton binding protein called Cortactin, which is involved in regulation of cell migration, is affected by CTF-611. In 2009, it was demonstrated that expression of CTF-611 caused an increase in phosphorylation of Cortactin and consequently it increases migration of carcinoma cells (García-Castillo et al., 2009). Based on available evidences, it is suggested that CTF-expressing breast cancer patients are more likely to experience metastasis and have a poorer prognosis compared with patients expressing wild type HER2 (Molina et al., 2002). In 1998, Christianson et al. showed that CTF-611/CTF-648 expressing breast tumors are associated with lymph node metastasis (Christianson et al., 1998), and resisting to trastuzumab, but respond to lapatinib (Scaltriti et al., 2010). Effectiveness of lapatinib on these tumors is due to its inhibitory role on tyrosine kinase activity which can also inhibit the activity of the CTFs (Pedersen et al., 2009). Many studies have been supported the idea that CTFs can be used as biomarkers for invasive breast cancer subtypes (Molina et al., 2002; Sáez et al., 2006).

MICRORNAS AND HER2
miRNAs are a set of rather small, intracellular and non-coding RNAs inhibiting gene expression by binding to their target mRNAs, resulting in inhibition of translation or mRNA degradation. (Winter et al., 2009). miRNAs are able to regulate various stages of the cell including proliferation, growth, development, differentiation, cell survival and apoptosis. Of these activities, regulation of HER family expression and their downstream activities can be considered. These molecules can, therefore, be used as biomarkers for diagnosis and detection of cancer and its various grades and stages (Heneghan et al., 2010).

MICRORNA AND CANCER
Microarray and protein assay techniques have shown that a single miRNA can handle regulation and expression of hundreds of genes (Lim et al., 2005; Muniyappa et al., 2009). Aberrant expression and activity of these miRNAs can result in diverse problems and abnormalities in the body, of which, cancer is one of the most important example. miRNAs can act as tumor suppressors or as oncogenes (OncomiRNA) (Shenouda and Alahari, 2009). Increased expression of oncomiRNAs lead to a dramatic reduction in tumor suppressor miRNAs, while reduced expression of tumor suppressor miRNAs results in enhanced stability and activity of oncogene miRNAs; combined, these two events may lead to increased proliferation and tumorigenesis (Volina et al., 2010).

1. HER2 Signaling Pathways Regulated by microRNA
miRNAs control the HER2 signaling pathway on different levels by regulating different target genes. miRNA binding site in HER2 3’UTR has no conserved region among different mammalian species. However, in silico analyses indicated the existence of poorly conserved regions in the HER2 3’UTR; therefore, regulation of HER2 expression can be conducted by miRNAs. miR-125a and miR-125b are important examples which can be pointed to play a role directly in the regulation of HER2 in breast cancer (Scott et al., 2007). Target sequences of
miR-125a and miR-125b in the HER2 3’UTR and HER3 are common, resulting that these two miRNAs can regulate expression of HER2/HER3 mRNAs cooperatively and can reduce phosphorylation of their downstream factors. miR-125b also has the ability to regulate Homo sapiens v-raf-1 murine leukemia viral oncogene homolog 1 (Raf1) expression in breast cancer cell lines (Hofmann et al., 2009). Therefore, it is concluded that HER2/HER3 signaling pathway can be controlled by miR-125a and miR125b as the potential therapeutic molecules. Expression of miR-205, which is capable to block Akt, is decreased in breast cancer. Using this miRNA causes inhibition of the Akt-dependent signaling pathways and increases the response to lapatinib and gefitinib (Iorio et al., 2009). miR-331-3p, a tumor suppressor miRNAs, directly controls HER2 through binding to two distinct target sites (Epis et al., 2009). Transfected tumor cells with miR-331-3p also reduced HER2 signaling pathway by reducing Akt activity. miR-21 is a type of oncomiRNA acts as a regulator of Extracellular Signal-Regulated Kinases 1 and 2 (ERK1/2) and causes formation of invasive breast tumors (Huang et al., 2009).

Figure 1. Structure of HER family receptors. Four members of HER family including HER1, HER2, HER3 and HER4 are illustrated by purple, green blue and yellow respectively. All members of this family have a similar structure: an extracellular domain consisting of ligand-binding site, lipophilic transmembrane domain and an intracellular domain with tyrosine kinase activity. HER2 and HER3 lack the ligand-binding site and active intracellular region respectively (http://www.biooncology.com/research-education/hdis/her2-dimerization/index.html)

Figure 2. Structure of proximal promoter of the human HER2 gene. Proximal promoter of the human HER2 gene consists of two typical regions: TATA box and CCAAT box, which are located in positions -22 to -26 and -71 to -74 respectively. Two regions of transcription initiation in HER2 promoter have been marked. The first group is around the major start site at +1 position, and the other group is at minor start at -69. Transcription initiation from these two
points has two distinct molecular mechanisms. Initiation from the upstream position is in need of initiator-like elements; while the downstream sequence requires the TATA box (Hurst, 2001)

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