Biochem. Cell. Arch. Vol. 20, No. 1, pp. 0000-000, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

DETECTION OF GENETIC POLYMORPHISM IN EGFR GENE IN TRIBLE NEGATIVE BREAST CANCER WOMEN FROM IRAQ

M. Sahar[1], Mohammed H. Wali[2], A. Marwa[3] and H. Bushra[4]

[1,2]College of Biotechnology, Al-Nahrain University, Iraq.
[3]Department of Biology, Al-Rasheed University College, Iraq.
[4]MadenatAl-Imamaen Hospital, Baghdad, Iraq
*e-mail : mohammedwali2009@gmail.com

(Revised 29 October 2019, Revised 30 December 2019, Accepted 5 January 2020)

ABSTRACT : Epidermal growth factor receptor (EGFR), which also known HER-1 or ErbB-1 is the member of prototype of the type I receptor tyrosine kinase (TK) family. EGFR was expressed in Triple negative breast cancers, (TNBC) in which it defined and clinically detected by the lack of estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (Her2/cerbB2/ EGFR2) expression. Somatic mutations that caused overexpression of EGFR and consistent activation of EGFR receptor was reported in a number ofmalignancies. The link between genetic polymorphisms in gene of EGFR and the risk of deferent diseases, containing breast cancer is the increasing interest issue. As EGFR mutations is poorly defined. In this study, part of exon 8,18 and 19 of EGFR gene analyzed for the presence of SNPs We, therefore, search for characterize EGFR mutations in triple negative breast cancers. Thirty samples were selectedfrom triple negative breast tumors for EGFR polymorphisms (mutation) analysis. DNA was extracted from these samples and polymerase chain reaction was performed to amplify exon regions 8, 18and 19 of the EGFR gene. Sequencing of the purified product of PCR was performed, followed by sequencing analysis, EGFR mutations were found in exon 8 of EGFR gene of 30 samples. The obtained results concluded that studying and analyzing SNPs in the EGFR gene would give us a well understanding of proliferation of breast cancer status and also would have a benefit in controlling progression and then treatment cancer of breast.

Key words : Breast cancer, SNP, EGFR gene, exon, TNBC.

INTRODUCTION

The epidermal growth factor receptor (EGFR) is a receptor for tyrosine kinase that is essential in transducing extracellular signals from the cell surface to the cell interior, mediating fundamental processes such as cell proliferation, differentiation, apoptosis and migration. Dys-regulated expression of this receptor causes aberration of homeostatic cellular processes, leading tocells malignant transformation. EGFR protein was expressed in 30% to 52% of triple negative breast cancer women (Rakha *et al*, 2007; Pintens *et al*, 2009; Thike *et al*, 2010).

Triple negative breast cancer (TNBC) was clinically diagnosed by the lackof estrogen receptor (ER) and progesterone receptor (PR) expression and lack the of amplification or overexpression of human epidermal growth factor receptor 2 (HER2) (Yvonne Hui-Fang *et al*, 2011).

EGFR family consists of four EGFRs: EGFR (ErbB2 or HER1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4) (Masuda *et al*, 2012). These are transmembrane glycoproteins consisted of :

A- An extracellular ligand binding area.

B- Intracellulararea with tyrosine kinase activity.

Mutations that found inEGFRgene have been established in TNBC, which somatic mutations leaded to EGFR overexpression, reliable EGFR receptor activation founded in a number of malignancies, counting lung cancer (Scagliotti *et al*, 2004), colorectal cancer (Chung *et al*, 2005) and glioblastoma multiforme (Fuller and Bigner, 1992).

The EGFR gene consists of 20 exons, which the exons from (1 to 14) encode for the extracellular domain, whereas exon 15 encodes the transmembrane region, also exons (16 to 20) encode for the intracellular end. EGFR goes to the ErbB family of tyrosine kinase receptors, which other members confined ErbB2 (HER2/neu), ErbB3 (HER3) and ErbB4 (HER4) (Olayioye *et al*, 2000).

Earlier studies have miscarried to discover actuating mutations of EGFR gene in TNBC samples from Australian, European and Japanese patients (Jacot *et al*,

2011; Nakajima *et al*, 2014), however study groups of American have recounted frequencies of 0% and 3.4% for an EGFR exon 21 mutation with TNBC (Papadopoulou *et al*, 2007; Aifa and Rebai, 2008).

In the present schoolwork, we intended to estimate the incidence mutations or polymorphisms in EGFR gene in TNBC patients to define theEGFR used as a biomarker for anti-EGFR therapy in TNBC. Therefore, we analyzed the single nucleotides polymorphisms in exons 8, 18, 19 in 30 breast cancer patients and compared them with NCBI as stander for healthy individuals and examined their link with breast cancer so as to estimate their potential factors (prognostic/predictive).

MATERIALS AND METHODS

Sample collection

Breast cancer samples were collected from 30 women from clinics in Baghdad with mean age 15 ± 60 years during 1st of July 2019 to 1st of September / 2019. All of them were diagnosed with this disease. The women are with history of breast cancer, but they are triple negative breast cancer that had estrogen receptor, progesterone receptor and Her2 receptor negative when it examined by immunohistochemistry to detect protein in tissue. Demographical as well as risk data factors were collectedas well by a short structured survey, containing information about age, family history of breast cancer, menopausal status, age at onset. The blood samples collected and then stored at (-4^{0}C) until they wereused to genetic analysis.

DNA extraction

The DNA was extracted from all samples of blood via QIA amp DNA blood mini kit (Qiagen, USA) as described in the manufacturer procedure. Purity and concentration of extracted DNA was determined for each specimen by using NanoDrop apparatus. In order to confirm the presence of intact DNA molecules, DNA samples were gel-electrophoresed by using the Agarose gel.

Polymerase Chain Reaction (PCR)

The DNA that extracted from samples was castoff as a template for (20µl) PCR reactions. The PCR process was performed using 10µl Go Taq® Green PCR Master Mix (Promega, USA), 1µl of 10µm from each forward primer: TGCTGTGACCCACTCTGTCT and reverse primer CCAGAAGGTTGCACTTGTCC for exon 8. For the exon 18 the forward primer CTGAGGTGACCCTTGTCTCTGTGTTCTT and reverse primer AGAGGCCTGTGCCAGGGACCTTA were employed. In addition, forward primer TCACTGGGCAGCATGTGGCA and revers primer CAGCTGCCAGACATGAGAAA were used for the exon 19 of EGFR gene as described in Qiuyin *et al* (2003). All mentioned primers are complement to a site on part of exon 8, exon 18 and exon 19 of the EGFR gene. 3µl of DNA template was added for each set of PCR reaction. The final volume of each mixture was accomplished to 20µl by addition free-nuclease water. The process of PCR accompanied through 30 cycles with the subsequent steps in Table 1.

Table 1 : Program of PCR used for amplification of EGFR gene.

Thermal cycler protocol	No. of cycle	Temperature-time
Initial denaturation	1 cycle	94^{0}C for 5 minutes
Denaturation	30 cycle	95^{0}C for 30 sec.
Annealing		57^{0}C for 30 sec.
Extension		72^{0}C for 40 sec.
Final extension	1 cycle	72^{0}C for 10 min.

DNA sequencing

With the aim of analyze the nucleotides sequences for all samples and to determine the presence of single nucleotide polymorphism (SNP), DNA sequencing was accomplished on cancer patients at the national instrumentation center or environmental management (NICEM) using the sequencer ABI prism 3100 xl genetic analyzer (Applied Biosystems, USA).

RESULTS AND DISCUSSION

Purity and concentration of DNA that extracted from women by breast cancer

The DNA was effectively extracted sinceblood samples. The purity of DNA extracted from samples of blood was extended from 1.8 to 2 and the concentration of DNA was extended from 70 to 120 µg/ml.

Amplification of part of exon 8, exon 18 and exon 19 in EGFR gene

The blood samples that showed lacking in estrogen receptor(ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (Her2) expressionswere selected for the molecular study.The 20 samples of bloodthat showed triple negative (epidermal growth factor receptor 2 (Her2) receptor, estrogen receptor, progesterone receptor) breast cancer, were selected.

The exon 8, 18 and 19 in EGFR receptor gene were identified by using PCR. Figs. 1, 2 and 3 indicated that exon 8, 18 and 19 in EGFR gene were performed as a band sized 155bp, 185bp and 240bp separately. The

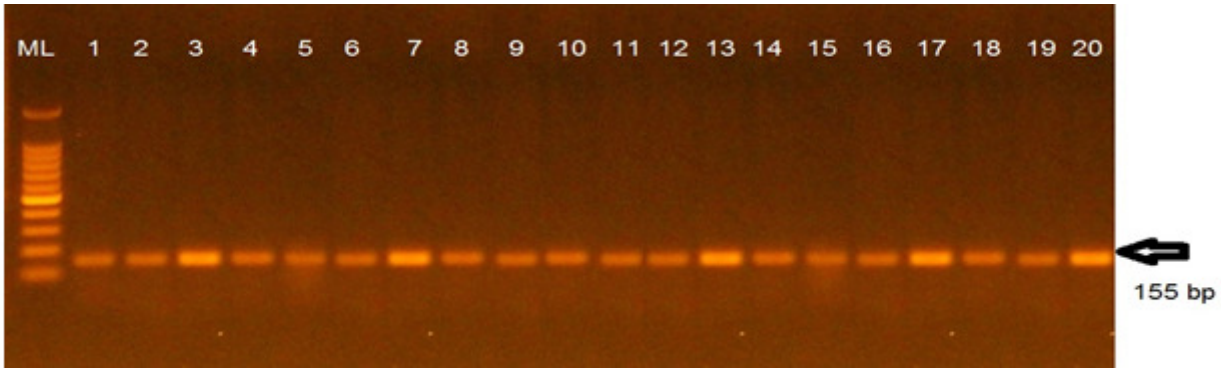

Fig. 1 : Amplification of exon8 in EGFR receptor of triple negative breast cancer blood sample with 157 bp lanes (1-20)characterize DNA from women with breast cancer. ML: molecular ladder (100-1000 bp).Electrophoresis using agarose 1.5%.

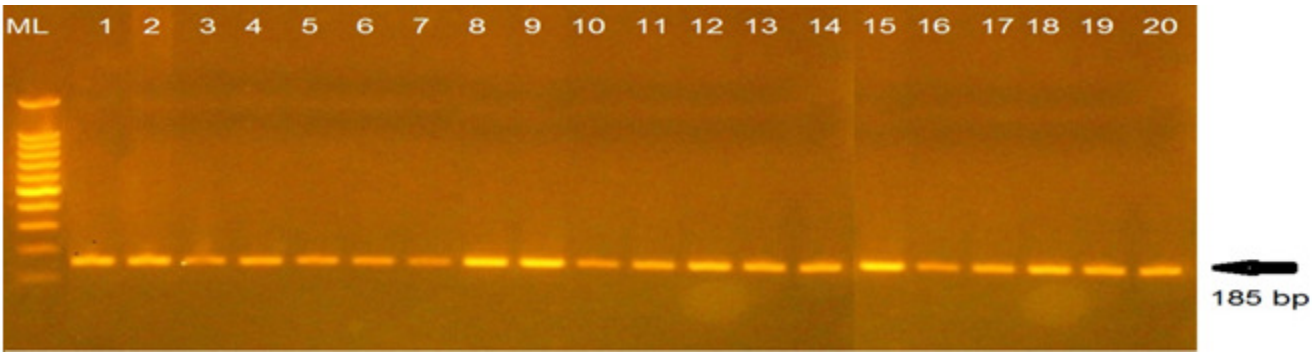

Fig. 2 : Amplification of exon18 in EGFR receptor of triple negative breast cancer blood sample with 185 bp. Lanes (1-20) symbolize DNA from women with breast cancer. ML: molecular ladder (100-1000 bp).Electrophoresis usingagarose 1.5%.

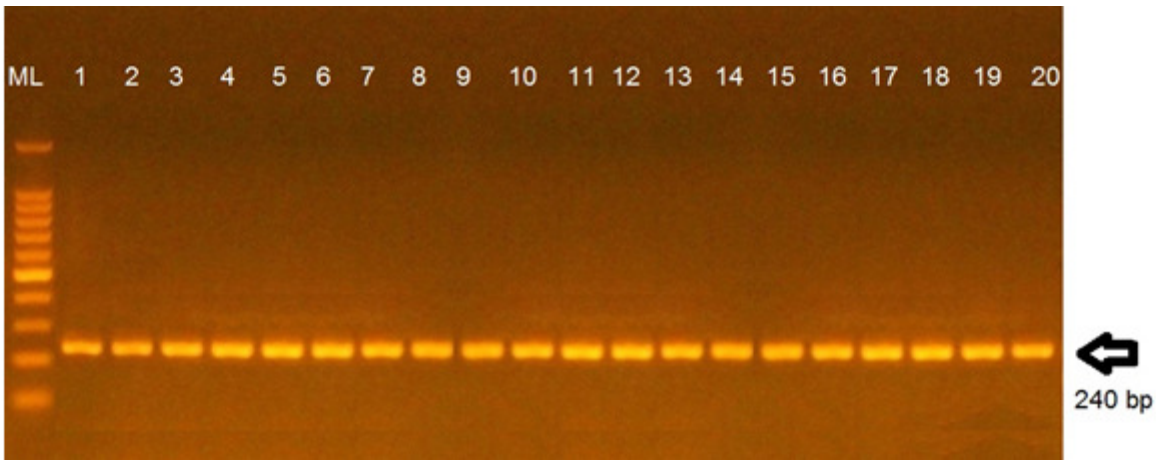

Fig. 3 : Amplification of exon19 in EGFR receptor of triple negative breast cancer blood sample with 240 bp. Lanes (1-20) signifies DNA from women with breast cancer. Electrophoresis usingagarose 1.5%. (ML: Molecular Ladder (100-1000 bp).

current data is similar to previous studies by Kallel *et al* (2009), Teng *et al* (2011) and Kallel *et al* (2009).

Sequencing

The goal of the current study was to scan three exons (8, 18, 19) in EGFR receptor gene in triple negative breast cancer women.The data for sequencing result of exon 8 in EGFR gene indicates the presence of one SNP G replaced by A (Figs. 4 and 5). The recognized SNP was a non-synonymous;it is substitution variability. The AGG codon was altered to AAG at position 55161562. The addressed point mutation altered the gene function because of altering in amino acid; the Arginine (R) changed to Lysine (K) at codon 521. This polymorphism was found in 3 (10%) samples.

On the other hand there is no polymorphism or mutations in exon 18 and 19 of EGFR gene that found in this study.

As cancer is a complex disease, so genetic variation (mutations, deletions or rearrangement) as well as gene polymorphisms of other genes could affect not only on the development of the cancer disease, but also on itsprogression and as a result,it can affect cancer

```
Features:
epidermal growth factor receptor isoform e precursorepidermal growth factor receptor isoform d precursor

Query  7         GGGCATGTCTG-CATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAAGGAC  65
                 || || ||||| ||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  55161507  GGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAGGGAC  55161566

Query  66        TGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCAACCTTCTG  125
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  55161567  TGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCAACCTTCTG  55161626

Query  126       GA  127
                  ||
Sbjct  55161627  GA  55161628
```

Fig. 4 : Alignment of exon8 in EGFR gene of breast cancer women using a sequencer that was analysed by BLAST tool. Query number represented the present data, whereas, the subject represented the reference gene sequence.

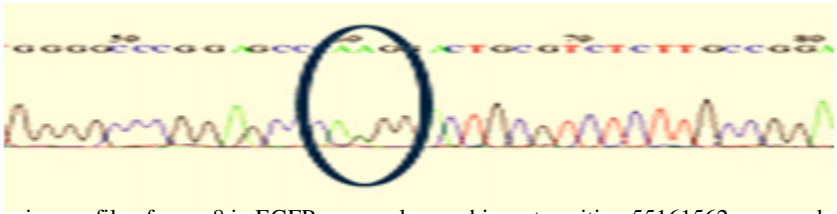

Fig. 5 : Nucleotide sequencing profile of exon 8 in EGFR gene polymorphism at position 55161562 as recorded by Finich TV software. A was substituted by G.

phenotypes (Teng *et al*, 2011). Furthermore, breast cancer has been considered a major illness that can be developed throughout the time due to single nucleotide polymorphism in certain genes. EGFR is one of the genes that can have a specific SNP affecting the progression of the breast cancer. It is recorded that the (R521K) SNP in the exon 8 of the EGFR gene has caused changing the amino acid sequence from Arginine to Lysine in the codon 521 position located in the extracellular subdomain IV of the EGFR gene sequence (Kallel *et al*, 2009).

In this study, we investigate the presence of one SNP polymorphism (2%) contained by the EGFR genes that were known to have a key role in breast cancer progression and onset. EGFR gene is overexpressed frequently in a wide range of solid tumours and it is a target for cancer drugs (Kallel *et al*, 2009). The opportunity that some genetic polymorphisms in the gene sequence of EGFR can change the regulation of, at least partially, EGFR gene activity and expression was an engaging suggestion. This may assist in identifying suspected patients with tumours that are more likely to react negatively against the EGFR therapy or radiotherapy (Papadopoulou *et al*, 2007). The genetic alterations in the EGFR gene would regulate its expression and ýactivity and that, interestingly, would have a benefit in antitumor drug management where cancer patients may or may not respond to EGFR-targeted drugs.

Single nucleotide polymorphisms (SNPs) were the most common form of variation in a certain gene that influences the manner a person responding to the environmental factor, which may alterthe disease risk. In the current study, non-synonymous SNPs in exon 8 of EGFR gene (AAG) were detected contained by the regulatory region of the genome coding region. Frankly, this non-synonymous SNP has changed an amino acid in EGFR proteins sequence and that would certainly affect to some extent the activity of the resulted protein. Generally speaking, if SNPs are within regulatory regions of acertain gene, which was shown in this study, it results dys-regulation transcription of the gene and may modify the structural folds of mRNA leading to an effect in gene expression (Brian *et al*, 2009).

Interestingly, the R521K SNP has been recorded in (2%) of samples (TNBC patients). There are several studies highlighted the influence of genetic mutations in exon 18 of EGFR gene and it is considered to be involved in the progression of breast tumour. Although, the effect of almost all mutations in the EGFR gene has not been proved to have a clinical significance, the occurrence of such single variation would obviously change the

extracellular subdomain IV of the EGFR gene. This alteration would have some impact on the prognosis of breast cancer since the EGFR gene is overexpressed in breast cancer patients. The result has clearly shown the existence of genetic alteration in the ORF area, which it can be employed to detect the disease in the individuals with breast cancer history. The EGFR gene variant has reduced function in ligand binding, growth stimulation and induction of the proto-oncogenes myc, fos, and jun, tyrosine kinase activation (Kallel *et al*, 2009; Kim *et al*, 2017).

In order to create relevant data regarding SNPs existence in the EGFR gene, more TNBC samples are required to be examined. It is really important to examine other regions of the EGFR gene for the existence of SNPs and that would potentially facilitate the way of dealing with the disease progression. Because most of the TNBC patients are diagnosed with stage 3 breast cancer, the respond to the chemotherapy is relatively low. Therefore, understanding the different genes that may influence the disease prognosis is essential in controlling the disease by selecting a suitable therapy.

REFERENCES

Aifa SRebai A (2008) ErbB antagonists patenting: playing chess with cancer. *Rec. Patents on Biotechnol.* **2**(3), 181–187.

Aurekha D, Sailaja K, NageswaraRao D, Raghunadharao D and Vishnupriya S (2009) Oestrogen receptor beta (ERβ) polymorphism and its influence on breast cancer risk. *J. Genet.* **88**(2), 101.

Bandres E, Barricarte R and Cantero C (2007) Epidermal growth factor receptor (EGFR) polymorphisms and survival in head and neck cancer patients. *Oral Oncology* **43**(7), 713–719.

Brian N C, Xuting W, Michelle R C, Gary S P, Maher A N and Douglas A B (2008) Discovery and verification of functional single nucleotide polymorphisms in regulatory genomic regions: Current and developing technologies. *Mutat. Res.* **659**(1-2), 147–157.

Chung K Y, Shia J, Kemeny N E, Shah M, Schwartz G K and Tse A (2005) Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J. Clin.Oncol.* **23**, 1803-10.

Fuller G N and Bigner S H (1992) Amplified cellular oncogenes in neoplasms of the human central nervous system. *Mutat. Res.* **276**, 299-306.

Jacot W, Lopez-Crapez E, Thezenas S, Senal R, Fina F and Bibeau F (2011) Lack of EGFR-activating mutations in European patients with triplenegative breast cancer could emphasise geographic and ethnic variations in breast cancer mutation profiles. *Breast Cancer Res.* **13**, 133.

Kallel M, MahaRebai M, Khabir A, Farid N and Reba A (2009) Genetic Polymorphisms in the EGFR (R521K) and Estrogen Receptor (T594T) Genes, EGFR and ErbB-2 Protein Expression and Breast Cancer Risk in Tunisia. *J. Biomed. Biotech.* 753683.

Kim A, Jang M H, Lee S J and Bae Y K (2017) Mutations of the Epidermal Growth Factor Receptor Gene in Triple-Negative Breast Cancer. *J. Breast Cancer* **20**(2), 150–159.

Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi G N and Ueno N T (2012) Role of epidermal growth factor receptor in breast cancer. *Breast Cancer Res. Treat.* **136**, 331-345.

Nakajima H, Ishikawa Y, Furuya M, Sano T, Ohno Y and Horiguchi J (2014) Protein expression, gene amplification and mutational analysis of EGFR in triple-negative breast cancer. *Breast Cancer* **21**, 66-74.

Neve R M and Lane H A (2000) The ErbBsignallingýnetwork: receptor heterodimerization in development and cancer. *The EMBO J.* **19**, 3159–3167.

Papadopoulou E, Simopoulos K and Tripsianis G (2007) Allelic imbalance of HER-2 codon 655 polymorphism among different religious/ethnic populations of northern Greece and its association with the development and the malignant phenotype of breast cancer. *Neoplasma* **54**(5), 365–373.

Pintens S, Neven P, Drijkoningen M, Van Belle V, Moerman P, Christiaens M R, Smeets A, Wildiers H and VandenBempt I (2009) Triple negative breast cancer: a study from the point of view of basal CK5/6 and HER-1. *J. Clin. Pathol.* **62**, 624-628.

Rakha E A, El-Sayed M E, Green A R, Lee A H, Robertson J F and Ellis I O (2007) Prognostic markers in triple-negative breast cancer. *Cancer* **109**, 25-32.

Reis-Filho J S, Pinheiro C, Lambros M B, Milanezi F, Carvalho S and Savage K (2006) EGFR amplification and lack of activating mutations in metaplastic breast carcinomas. *J Pathol.* **209**, 445-453.

Santarpia L, Qi Y, Stemke-Hale K, Wang B, Young E J and Booser D J (2012) Mutation profiling identifies numerous rare drug targets and distinct ýmutation patterns in different clinical subtypes of breast cancers. *Breast Cancer Res. Treat*. **134**, 333-43.

Scagliotti G V, Selvaggi G, Novello S and Hirsch F R (2004) The biology of epidermal growth factor receptor in lung cancer. *Clin Cancer Res*. **10**(12), 4227s-32s.

Teng Y, Tan W, Thike A , Cheok P, Tse G , Wong N, Yip G, Bay B and Tan P (2011) Mutations in the epidermal growth factor receptor (EGFR) gene in triple negative breast cancer: possible implications for targeted therapy. *Breast Cancer Res.* **13**, R35.

Thike A A, Cheok P Y, Jara-LazaroA R, Tan B, Tan P and Tan P H (2010) Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. *Mod. Pathol*. **23**, 123-133.

Yvonne H T, Wai J T, Aye A T, Poh Y C, Gary M K T and Nan-Soon W (2011) Mutations in the epidermal growth factor receptor (EGFR) gene in triple negative breast cancer: possible implications for targeted therapy. *Breast Cancer Res.* **13**.