

## **The Expression of C-erb B2, Neu Oncogene in the Breast Tissues of a Group of Saudi Female Patients**

Sahirah Ahmad Lary

*Biochemistry Department, Faculty of Science,  
King Abdulaziz University, Jeddah, Saudi Arabia*

*Abstract.* Breast cancer is the most common cause of death among women. The exact etiological mechanism still remains unclear. However, breast cancer varies with age and nationality. For example, in Saudi Arabia according to the annual report of tumor registry of 1991 breast cancer is 8.2-12% of the total distribution of common malignancy. In United States, according to the American cancer society, one in nine women will get breast cancer before the age of eighty-five. In the United Kingdom it has the highest mortality of any malignant disease. In the current work the expression of C-erb B2 oncogene has been investigated in breast tissues of some Saudi female patients. Clinical investigation on the patients has shown that there was a high risk of breast cancer in relation to heredity and genetic constituents as well as age of first birth, obesity, early menarche, drug medication and hormone replacement therapy. No significant difference was observed in DNA content of non-malignant and malignant tissues. The employment of southern blotting technique have demonstrated that tumour DNA exhibits two EcoR I fragments of approximately 6.6 Kb and 6.0 Kb and with Hind III fragments of 4.72 Kb (Lane 3, Fig. 1), an EcoR I fragments of 3.2 Kb and Hind III fragments of 3.3 Kb and 2.20 Kb. In non-malignant cell C-erb B2 hybridized with EcoR I fragments at 20.29 Kb and 2 Hind III at 6.0 Kb and 4.4 Kb and at 2.20 Kb. Clinical investigated parameters for Saudi women were in agreement with what has been reviewed in the literature in which the integration and the expression of C-erb B2 oncogene was the cause of malignancy in most of the cases investigated either in phenotypically, non-malignant or malignant tissues. The development of mammary tumour in all cases investigated may reflect the overproduction of the estrogen receptor in the investigated tissues.

## Introduction

Numerous genetic abnormalities have been found to occur in breast cancer. These aberrations may affect both oncogenes and tumour suppressor genes<sup>[1, 2]</sup>. Different members of each class of proto-oncogenes have been found to be over expressed in subsets of the diseases. On the other hand various studies seemed to demonstrate that oncogenic induction of breast cancer may result from appropriate hormonal stimulation.

Among all associated with ovarian activity, the estrogens have been most extensively studied because of their significant effect on the growth of breast epithelial<sup>[3]</sup>.

The possible effects of progesterone, ovarian and adrenal androgen, and prolactin have also been investigated<sup>[4]</sup>. Breast cancer however arises multifocally from malignant and non-malignant lesions occurring in affected women. The pre-malignant lesions are transformed into malignant with over-exposure to estrogen<sup>[3]</sup>. Estrogenic hormones may play numerous roles in neoplastic progression of breast carcinoma and as permissive promotional and tumour inducing agents. The DNA binding domain of estrogen receptor has a strong homology with one of the transforming protein of avian erythroblastosis virus (V-erb A)<sup>[5]</sup>.

Cellular homology of this oncogene is C-erb A and is a receptor for thyroid hormones<sup>[5]</sup>. The epidermal growth factor receptors (EGFR) were demonstrated to be regulated by estrogen in estrogen receptor positive breast cancer cells<sup>[3]</sup>. The gene for EGF(C-erb B) is one of the most interesting proto-oncogenes. It is typically amplified in estrogen receptor negative tumours with a poor prognosis and high degree of invasiveness, it causes an increase in tumour growth rate and invasion. However, in the presence of growth factor, C-erb B may also becomes oncogenic<sup>[3]</sup>. The proto-oncogene, C-erb B2 may also be involved in breast cancer progression and the C-erb B has also been associated with poor prognosis<sup>[6]</sup>.

A novel transforming genes involved in carcinogen induce rat neuroglioblastoma or the neu gene is related but distinct from C-erb B. This new transforming gene was first described by Dawkins *et al.* (1993)<sup>[7]</sup> in ethylnitrosourea-induced neuroblastoma, which arose in a neonate rat.

The C-erb B2/neu gene was found amplified and over expressed in mammary tumours<sup>[8-16]</sup>.

In normal cells the proto-oncogene C-erb B2 is present as a single copy gene and has been mapped to human chromosome 17q.12-12. 32. It encodes a 185 KDa trans-membrane protein or P185, which has an intracellular component with structural similarities to a growth factor receptor. The extra cellular

domain of P185 oncoprotein is 85% or 40% homologous with the epidermal growth factor receptor<sup>[7]</sup>. Chromosomal anomalies observed in breast cancer involves chromosomes 1, 3, 6, 7, 9 and 11<sup>[1]</sup>. In addition the loss of variable length of one allele on chromosome 11 has accompanied tumours lacking both estrogen and progesterone receptors, however estrogen receptor negativity and increased rates of tumour proliferation may possibly be related to aneuploidy<sup>[3]</sup>, such genetic anomalies may undoubtedly lead to proto-oncogene activation.

Rat neu proto-oncogene may be activated by a single point mutation in the coding sequence for the receptors trans-membrane region. In human gene however rearrangements are rare<sup>[7]</sup>. Cancer cells with C-erb B2 amplification are estrogen and progesterone receptor negative<sup>[17]</sup>. In human breast cancer cells the expression of C-erb B2 is inhibited by estrogen leading to a decrease of both C-erb B2 mRNA and P185 levels.

This protein is encoded by a gene located on chromosome 17. The gene is homologous with the C-erb B1 gene that encodes for (EGFR)<sup>[18]</sup>. It has been documented in the literature that there is a great homology between the estrogen receptor cDNA and the oncogene C-erb oncogenes<sup>[19]</sup>, and any increase in the activity of this oncogene is paralleled by an increase in the estrogen level.

## Materials and Methods

### *Subjects*

Seven women with breast cancer were chosen with ages ranging between 35-45 years. Patients were fully clinically examined. Full history data were taken such as family history, marital status, number of pregnancies, whether had breast fed babies or not, early menarche, late menopause, drug therapy and radio-therapy. Tumour tissues as well as non-malignant tissues were taken from the same breast immediately after mastectomy or lumpectomy.

### *Specimens*

The work was carried out under sterile conditions. One gram of each tissue was collected in sterile tight-capped tube. Previously immersed in liquid nitrogen until frozen or processed.

Samples were collected from King Abdulaziz University Hospital, Bakhsh Hospital, King Abdulaziz Hospital and Erfan Hospital.

### *Extraction of Patients' DNA*

Genomic DNA from breast tissues was extracted by using phenol, chloroform-isoemyl alcohol method according to the procedure of Maniatis *et al.*<sup>[20]</sup>.

The extracted DNA was subjected to restriction enzyme digestion (Hind III and EcoR I) and analyzed together with Hind III DNA marker on 1% agarose (w/v) in TAE buffer (40 mM Tris) acetate, 1 mM EDTA, pH 8).

### ***Transfer of DNA***

Following electrophoresis, DNA fragments were transferred from gels to the nylon membrane (gene screen plus, Dupont), according to the methods of Mainiatis *et al.*<sup>[20]</sup> and southern<sup>[21]</sup>.

### ***Oligonucleotide Tabelling of V-erb B2 Oncogene***

Twenty microliter reaction volume containing 2µl of oligonucleotide probe (0.5 pmol), 2µl of 10 × kinase buffer consisting of 0.5 M Tris HCl pH 9.5 and 50 mM MgCl<sub>2</sub>, 8µl (γ-<sup>32</sup>p) ATP 40 µCi > 3 × 10<sup>7</sup> cpm, 2µl of polynucleotide kinase and 6µl ddd H<sub>2</sub>O. The whole mixture was incubated at 37°C for 30 min. The reaction was then stopped by adding 40 µl of 0.1M EDTA. Labelled DNA was separated from the unincorporated radioactive nucleotide by using Sephadex G-50 column chromatography (NAP<sup>TM</sup>-5 column, Pharmacia). This was carried out by equilibrating the column with 10 ml of TNT buffer (10mM Tris-HCl pH 7.5, 1 mM EDTA and 100 mM NaCl). Fifty microliter of the reaction mixture was added to the column. Fraction was counted by Cerenkov scintillation counter using a Beckman L8 scintillation counter. The appropriate fraction of DNA were pooled and used directly in the hybridization reaction.

### ***Hybridization of Cellular DNA with the Oligonucleotide Labeled V-erb B2 Probe***

Cellular DNA from breast tissues attached to nylon membrane were hybridized with <sup>32</sup>p-labelled probe V-erb B2 oncogene at 65°C for 16 h according to the methods of<sup>[20,22,23]</sup> in (2 M NaCl, 1.5 ml of 50 mM Tris pH 7.5, 6ml of 50% dextran sulphate, 3 ml of 10% SDS and 1mg/ml denatured DNA, Salmon Sperm DNA, and 2 × 10<sup>7</sup> cpm end labeled probe. Nylon membrane was then rinsed briefly 4 times in 2 × SSC and 0.1 % SDS at room temperature and then 2 times in 2 × SSC and 0.1 SSC at 40°C for 30 min each time the membrane was rinsed in 2 × SSC and 0.1% SDS at room temperature for five min, and rinsed briefly with 2 × SSC and 0.1% SDS for 1 time.

### ***Autoradiography***

Autoradiography was carried out on the damp filter wrapped with Sarana wrap by exposing the nylon membrane to Kodak X-omat film at -70°C (using Dupont lightening plus intensifying screen), for 1-14 days. Fragments of DNA on autorads were identified.

## **Results and Discussion**

The present study was carried out on 7 normal subjects and 7 breast cancer patients. Malignant and non-malignant tissues were taken from the same breast. The investigated parameters were carried out on physical examination, morphological, biopsy and laboratory finding. All patients were at diagnosis and post-mastectomy when samples were taken for DNA preparation and oncogene studies.

### ***General Description of the Subjects***

This study includes 7 breast cancer patients. The general description of clinical parameters of the patients is represented in Table 1. The mean age of the patients was  $42.29 \pm 5.02$ . Four patients of seven (57%) had a family history of breast cancer. Two patients out of seven (28.5%) had early menarche, which started at the age of 9 and 10. Five patients out of seven (71.4%) had their menstrual period at the age of 13 and 14. One patient out of seven (14.2%) was unmarried. Four patients out of seven (57%) had their first child after the age of 25. Three patients out of seven (42.8%) had their first child before the age of 25. Four patients out of seven (57%) had breast fed babies and three patients out of seven (42.8%) had no breast fed babies. Three patients out of seven (42.8%) were obese before onset of the disease. As for medication, three patients out of seven (42.8%) were taking contraceptive pills and three patients out of seven (42.8%) were not on any medication and only one patient out of seven (14.28%) had hormonal therapy for the activation of the ovaries.

### ***DNA Content***

DNA content in non-malignant breast tissue was  $1.66 \pm 0.73$  mg/ml, while in the tumour cells from the same breast was  $3.13 \pm 2.36$  mg/ml. The amount of DNA in tumour cell increased non-significantly.

### ***The Expression of C-Erb B2 / Neu Oncogene in Breast Tissues***

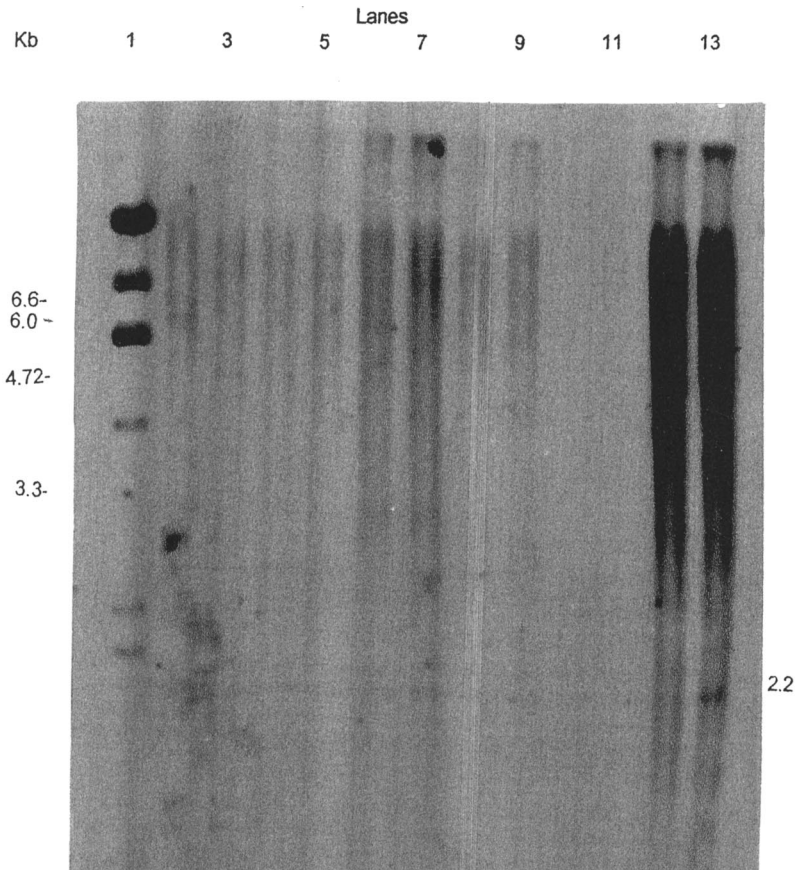
In the present work C-erb B2/neu oligonucleotide probe has been employed. It is a human C-erb B2/neu oligonucleotide probe supplied by the Oncogene Science Inc. It is a 40 base single stranded synthetic oligonucleotide consisting of 60% GC content. This sequence is the antisense orientation and derived from the translated exon 4<sup>[24]</sup>.

High stringency hybridization and wash procedures are recommended for long exposure DNA probes. In our present work, however, some alteration has been made in washing procedure of the radioactive filters, which was carried out at 40°C instead of 60°C (Materials and methods).

**Table 1. General description of the clinical parameters.**

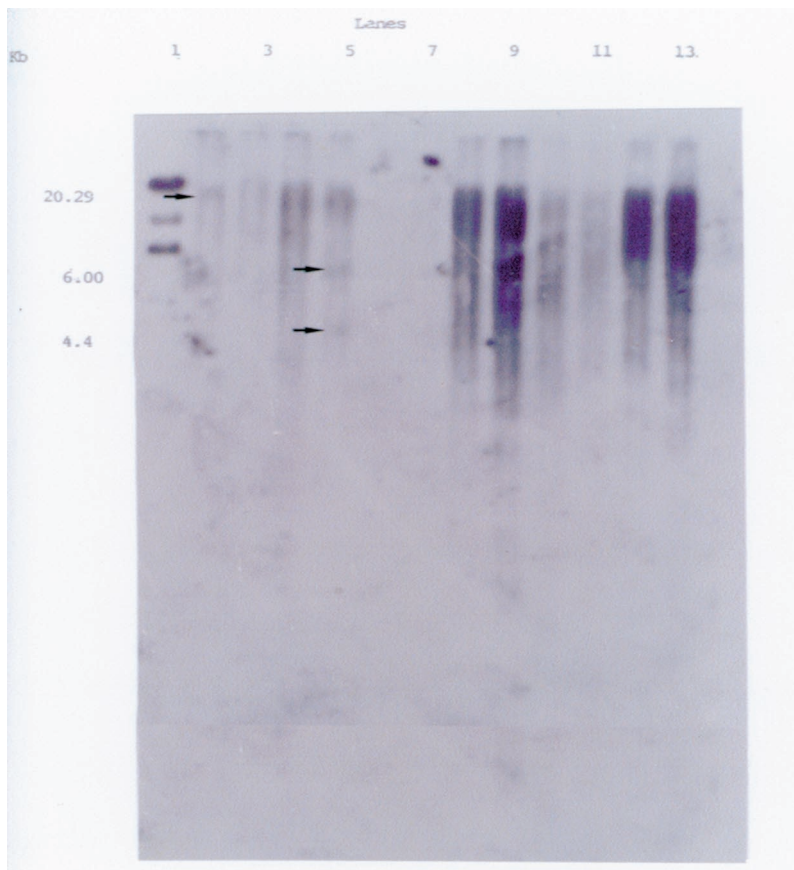
Patients	Family history	Early mernarche	Marital status	Number of pregnancies	First child at age of	Breast feeding	Weight (kg)	Medication before onset of the disease
1	-ve	9 years	Single	-ve	None	-ve	64	-ve
2	+ve Sister of her mother	10 years	+ve	4	29	+ve	90	Contraceptive pills
3	+ve Her mother	14 years	+ve	3	32	+ve	90	Activation of ovary by hormone
4	-ve	14 years	+ve	2	26	-ve	55	Contraceptive pills
5	+ve	14 years	+ve	3	22	+ve	45	-ve
6	+ve Grand mother	14 years	+ve	2	28	-ve	47	Contraceptive pills
7	-ve	13 years	+ve	6	20	+ve	85	-ve

Tumour cell DNA displayed two EcoR I fragments of approximately 6.6 Kb and 6.0 Kb which were hybridized with C-erb B2, (Lane 2, Fig. 1). C-erb B2 was also hybridized with a Hind III fragment of 4.72 Kb (Lane 3, Fig. 1), an EcoR I fragment of 3.20 Kb, (Lane 6, Fig. 1) and Hind III fragments of 3.30 Kb and 2.20 Kb, (Lane 7, Fig. 1). In non-malignant cell DNA C-erb B2 probe hybridized with an EcoR I fragment at 20.29 Kb (Lane 2, Fig. 2), the malignant tissue from the same breast did not show any expression of C-erb B2 oncogene. In non-malignant cell DNA C-erb B2 was also hybridized with two Hind III fragments at 6.0 Kb and 4.4 Kb, (Lane 5, Fig. 2). A third Hind III fragment from normal tissue was also identified of 2.20 Kb, (Lane 13, Fig. 1). Tumour cell DNA displayed two EcoR I fragments of approximately 6.6 Kb and 6.0 Kb which hybridized with C-erb B2, (Lane 2, Fig. 1). C-erb B2 was also hybridized with a Hind III fragment of 4.72 Kb (Lane 3, Fig. 1), an EcoR I fragment of 3.20 Kb (Lane 6, Fig.1) and Hind III fragments of 3.30 Kb and 2.20 Kb (Lane 7, Fig. 1). In non-malignant cell DNA C-erb B2 probe hybridized with an EcoR I fragment at 20.29 Kb (Lane 2, Fig. 2), the malignant tissue from the same breast did not show any expression of C-erb B2 oncogene.



**Fig. 1.** The expression of C-erb B2/neu oncogene in non-malignant and malignant tissues from breast cancer patients. DNA was extracted from non-malignant and tumour cells, cut with the restriction enzymes Ecor I and Hind III, ran on 1.10 Agarose gel +Epithedium bromide (1mg/ml) at 30V overnight and transferred onto nylon membrane. Nylon membrane was hybridized with v-erbB2 oncogene and was taken for radiography.

- Lane 1** : Hind III DNA size marker.
- Lanes 2, 4, 6** : DNA extracted from breast tumour, cut with Ecor I and hybridized with v-erbB2/neu oncogene.
- Lanes 3, 5, 7** : DNA extracted from breast tumour, cleaved with Hind III and hybridized with v-erbB2/neu oncogene.
- Lanes 8, 10, 12** : DNA extracted from non-malignant breast tissues, cleaved with Ecor I and hybridized with v-erbB2/neu oncogene.
- Lanes 9, 11, 13** : DNA extracted from non-malignant breast tissues cut with Hind III and hybridized with v-erbB2/neu oncogene.



**Fig. 2.** The expression of C-erb B2/neu oncogene in non-malignant and malignant tissues from breast cancer patients. DNA was extracted from non-malignant and tumour cells from the same breast, cut with the restriction enzymes EcoR I and Hind III, ran on 1% Agarose gel +Ethidium bromide at 30V overnight and transferred onto nylon membrane. Nylon membrane was hybridized with v-erbB2 oncogene and was taken for radiography.

- Lane 1** : Hind III DNA size marker as previously described.
- Lanes 2, 4, 6** : DNA extracted from non-malignant breast tissues, cut with EcoR I and hybridized with C-erbB2 neu oncogene.
- Lanes 3, 5, 7** : DNA extracted from non-malignant breast tissues, cut with Hind III and hybridized with C-erbB2/neu oncogene.
- Lanes 8, 10, 12** : DNA extracted from breast tumour, cut with EcoR I and hybridized with C-erbB2/neu oncogene.
- Lanes 9, 11, 13** : DNA extracted from breast tumour, cut with Hind III and hybridized with C-erbB2/neu oncogene.



In non-malignant cell DNA C-erb B2 was also hybridized with two Hind III fragments at 6.0 Kb and 4.4 Kb, (Lane 5, Fig. 2). A third Hind III fragment from normal tissue was also identified of 2.20 Kb (Lane 13, Fig. 1).

### ***General Investigation of the Patients***

According to the Annual Report of “The Tumour Registry” of 1991<sup>[25]</sup>, the incidence of breast cancer among Saudi women is very high, exceeding 8.2% or may be as 10% of the distribution of common malignancy. This high incidence of the disease has motivated us to study the role of oncogene in breast cancer in the western province of Saudi Arabia (Jeddah). In the current work the investigation of the patients showed that 57% of the cases had a family history of breast cancer. The etiology of this disease shows that the first causative factor associated with breast cancer is mainly hereditary and there is a strong genetic risk of breast cancer. Recent studies have shown that a women’s risk of developing breast cancer is doubled when she has a mother or sister with the disease and tripled when both her mother and her sister have breast cancer.

Some patients developing breast cancer have no family history of the disease, various cultural and environmental etiology factors have been postulated<sup>[26]</sup>.

About 28.5% of the cases investigated had early menarche, which started at the age of 13 and 14. Early menarche could increase the risk of breast cancer and women with early menarche have lower levels of circulating sex-hormone binding globulin and higher levels of estradiol, which may be a central agent in the development of breast cancer<sup>[27]</sup>. This could be controversial as 71.4% of the investigated cases had their menstrual period at the age of 13 and 14. Age of first birth was positively related to increase risk with women giving birth to their first child after the age of 35 are at greater risk and according to the National Health and Nutrition Examination Survey, (1989) greater risk was documented with age at first birth. In our investigation 57% of the patients had their first child after the age of 25 and 42.8% had their first child before the age of 25. However, the difference between the two cases is not significantly different and there is only a weak association between increases risk and age of giving birth. However, a four-fold risk associated with greater age was noted as abnormal parenchymal patterns on mammograms<sup>[28]</sup>.

Obesity was also studied in relation with breast cancer. In a review of 20341 women<sup>[29]</sup>, has shown a significant increased risk of breast cancer associated with obesity<sup>[29]</sup>.

In our study, 42.8% of our cases were obese before onset of the disease. Obese women may have an increased exposure of breast tissue to estrogen because of lower production of 2-OH estrogen compounds which may result in

a relatively hyperestrogenic state. Dietary fat affects enteric absorption of steroid hormones by influencing the intestinal flora and excess of fat in the diet is associated with low level of sex-hormone-binding globulin forming higher levels of free sex steroid hormones that would be available to target breast tissues<sup>[30]</sup>.

As for medication, Karamali<sup>[31]</sup> has demonstrated that hormone replacement therapy was associated with a 69% increase in breast cancer risk. In the current study, 42.8% of the patients were not on any medication and only 14.28% had hormonal therapy for the activation of the ovaries. Prolonged hormone replacement therapy may lead to increased risk in women with maternal history of breast cancer or women who had previously undergone hormone replacement therapy. Also, in women with prior benign breast disease or those who had menopause later than the age of 43. Also, the length of oral contraceptive use before the age of 25 years and a prolonged use before the first pregnancy were both associated with a significant increased risk for neoplastic breast disease. Forty two percent of women under investigation in this work were taking contraceptive pills and 42% were not under medication. However, it is worth mentioning that in general there was not any association between increased breast cancer risk and the use of oral contraceptive<sup>[25]</sup>. The breast, which has never been called upon for normal function, is more liable to become cancerous<sup>[25]</sup>. In the present study 57% of the patients had breast-fed babies and 43% had not breast-fed babies, so lactation may play a modest or indirect part in reducing the risk of breast cancer.

### ***DNA Content***

The amount of DNA in tumour cells was compared to that in non-malignant cells and there was a nonsignificant increase between non-malignant and tumour cells.

### ***The C-erb B2 / Neu Oncogene***

Two genes were identified, C-erb B2 as related to EGF receptor gene. The, C-erb B1 gene is the EGF receptor gene while, C-erb B2 is a different proto-oncogene which is the same as the neu gene.

The C-erb B2 has been identified in human genome<sup>[10, 24]</sup>. It maps to human chromosome 17 at q21, encodes a polypeptide with a kinase domain and highly homologous with, but distinct from, the epidermal growth factor (EGF) receptor. This gene is conserved in vertebrates and the neu gene detected in several rat neuro-oligoblastoma is itself the rat C-erb B2 gene<sup>[24, 32]</sup>. This gene was amplified in a salivary adenocarcinoma and gastric cancer cell line, MKN-7, suggesting that this over expressed gene is more likely to be involved in

neoplastic processes. Its sequence, however, demonstrated that the C-erb B2 gene encodes a protein similar to the EGF receptor protein and the neu gene product<sup>[33]</sup>.

In general, six v-erb-B related DNA clones were obtained from a human genomic library but among which one DNA probe prepared from lambda 107 clone was hybridized to EcoR I fragment of 6.4 and 13 kilo base human DNA. This result demonstrates that the clone lambda 107 represents a v-erb-B related gene, which is the C-erb B2 distinct from the EGF receptor gene, but there is a close similarity between C-erb B2 gene and the rat neu oncogene. This suggests that there is a definite involvement of C-erb B2 in human cancer. Gene amplification of C-erb B2 observed in human adenocarcinoma of the salivary glands was found to be 30 folds<sup>[24]</sup>.

More recent work has shown that C-erb B2/neu gene was found amplified and over expressed in mammary tumours<sup>[8-16]</sup>. Tumour cell DNA displayed three EcoR I fragments of approximately 6.6 Kb, 6.0 Kb and 3.30 Kb when hybridized with C-erb B2 probe and three Hind III fragments of 4.72 Kb and 2.20 Kb. Semba<sup>[24]</sup> showed similar result when DNA prepared from A431 vulva carcinoma cells exhibited amplification and produced 4 EcoR I fragments of 8.1 Kb, 5.9Kb, 5.4Kb and 3.5Kb. In the present work C-erb B2 hybridized to EcoR I fragments of 20.29 Kb, 6.0 Kb and 4.4 Kb of non-malignant breast tissue DNA.

The result obtained for non-malignant tissue DNA was as expected, non-malignant and malignant cells were taken from the same breast and were isogenic, *i.e.* they came from the same gene. The reason that some malignant cells DNA did not hybridize with C-erb B2 oncogene while they hybridize with non-malignant tissue DNA possibly because this oncogene is over expressed in non-malignant cells, more than in the malignant cells themselves and that the phenotype of those non-malignant cells was not compatible with its genotype.

In conclusion, it has been shown in the present work that the result obtained was reproducible for the diagnosis of breast cancer. Significant difference in DNA was found between malignant and non-malignant tissues. The clinical investigation on the patients and the result obtained for most of the investigated parameters for Saudi women were in agreement with what has been reviewed in the literature. The integration and the expression of c-erb B2 oncogene was the cause of malignancy in most of the cases investigated either in phenotypically non-malignant tissues or in the malignant tissues.

The development of mammary tumour in all cases investigated may reflect the overproduction of the estrogen receptor in the investigated tissue. Earlier it has been documented in the literature that there is a great homology between

the estrogen receptor cDNA and the oncogene c-erb oncogenes<sup>[19]</sup>, any increase in the activity of this oncogene is paralleled by any increase in the estrogen level. Several studies have also shown the expression of oncogenes and proto oncogenes in the breast tissues such as the expression of Bcl2 and Bax oncogenes<sup>[34]</sup>. bcl2 expression in ductal carcinomas<sup>[35]</sup> and the expression of Raf proto oncogene in Mcf-7 cell line<sup>[36]</sup>. C-erb B<sub>2</sub> mRNA was also detected in Mcf-7 cells.

Despite the extensive work for many years on breast cancer, the etio-pathogenesis of cancer still remain unclear, but for early detecting carcinoma of various origin, a number of biochemical markers might be useful not to ignore in order to study and evaluate the malignancy. Such markers are glutamyltransferase (GGTP) lactate dehydrogenase (LDH) and superoxide dismutase (SOD). They were proved to be the most sensitive biochemical markers in breast carcinoma for early detection of the disease<sup>[37]</sup>.

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## نشاط الجين الورمي C-erbB-2 في خلايا الصدر لمجموعة من الإناث السعوديات المصابات بسرطان الثدي

سهيره أحمد لاري

قسم الكيمياء الحيوية، كلية العلوم، جامعة الملك عبد العزيز

جدة - المملكة العربية السعودية

المستخلص. شملت آفاق البحث متابعة نشاط الجينين الورميين C-myc، erbB-2 في خلايا الثدي السليم والمريض في بعض النساء السعوديات. وقد أسفرت الاختبارات السريرية على المريضات أن هناك علاقة بين الإصابة بهذا المرض وكذلك العوامل الوراثية، والتركيب الوراثي، والبدانة، والبلوغ في سن مبكرة والتداوي بالعقاقير والهرمونات. ولم يلاحظ أي تغيير في محتوى الحمض النووي (DNA) بين الخلايا السليمة والخلايا المصابة، وباستخدام التقنية الحديثة بطريقة سذر Southern Blotting ثبت أن الخلايا المصابة قد أعطت ثلاثة أجزاء من الحمض النووي (DNA) المعامل بالإنزيم EcoR I بالأحجام ٦٠، ٦٠، ٦ كيلوبيز، ٦ كيلوبيز، و ٣٠، ٣٠ كيلوبيز. أما الحمض النووي (DNA) المعامل بالإنزيم Hind III فقد أعطى أجزاء بالأحجام التالية ٧، ٤ كيلوبيز، و ٣٠، ٣٠ كيلوبيز، و ٢١، ٢ كيلوبيز عند التهجين بالجين الورمي C-erb B-2. أما الخلايا السليمة فقد أدت عملية تهجين الجين الورمي بالحمض النووي لهذه الخلايا والمعامل بإنزيم EcoR I إنتاج ثلاثة أجزاء بالأحجام التالية ٢٩، ٢٠ كيلوبيز، و ٦٠، ٤ كيلوبيز. أما النتيجة السالبة التي تحصلنا عليها من تهجين الجين الورمي C-myc مع الحمض النووي (DNA) لكل من الخلايا المصابة والسليمة فقد يرجع ذلك إلى أن هذا الجين الورمي لا يوجد إلا في الأورام الخبيثة جداً، وقد تكون الأورام المدروسة ليست بهذه الشدة أو أن تحول هذه الخلايا ليس بفعل الجين الورمي C-myc.