UNIVERSITI PUTRA MALAYSIA

REVERSE TRANSCRIPTASE IN SITU EXPRESSION PATTERNS OF P53, CYCLIN E AND RB GENES AT DIFFERENT STAGES OF BREAST CANCER

MOHAMMADREZA ZAMANIAN

FPSK(M) 2007 7
REVERSE TRANSCRIPTASE IN SITU EXPRESSION PATTERNS OF P53, CYCLIN E AND RB GENES AT DIFFERENT STAGES OF BREAST CANCER

By

MOHAMMADREZA ZAMANIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

May 2007
In dedication to:

My beloved wife Zeinab, who has supported me in all of my life events, particularly in raising the decision to change our future

And

To my son Alisina, for giving soul to our life

I hope I can make up the lost time with you
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in partial fulfilment of the requirement for the degree of Master of Science

REVERSE TRANSCRIPTASE IN SITU EXPRESSION PATTERNS OF P53, CYCLIN E AND RB GENES AT DIFFERENT STAGES OF BREAST CANCER

By

MOHAMMADREZA ZAMANIAN

May 2007

Chairperson: Associate Professor Patimah Ismail, PhD
Faculty: Medicine and Health Sciences

Breast cancer is one of the most important health problems among females. One of the most important challenges regarding breast cancer diagnosis and treatment is the precise clinical staging of the disease. With regards to the selection of appropriate treatment method, identifying the lymph node involvement by cancerous cells is a major determinant. Until now, many attempts have been made to find stage specific molecular markers to help the clinicians make precise staging of the disease. Through this, evaluating the activity of some important genes in cancer evolution and progression seems to be the most sensible step in this direction.

p53, Cyclin E and Rb are genes that are mostly interactive in cell cycle regulation and cell division as well, has been shown to have an important role in cancer development particularly in breast cancer. Abnormalities in their inhibitory and or stimulatory roles in cell cycle progression can lead cells to enter hyperproliferative or neoplastic states. Therefore, assessments to determine their activity can lead to finding any differences in their expression levels between benign and malignant breast tissues, as well as different stages of breast cancer.
In the present study we have used Reversed Transcriptase in situ Polymerase Chain Reaction (RT in situ PCR) in order to determine p53, Cyclin E and Rb mRNA expressions in different human breast samples including benign and malignant tissues. This method allows detection of very low copies of mRNA at cellular level.

In the current study, the presence of p53, Cyclin E and Rb mRNA expressions were investigated in 17 cases of human breast tissues, which were donated as paraffin embedded materials by the pathology ward of Milad hospital, located in Tehran, Iran.

We divided the samples into four groups based on their pathology reports. Five samples in each first group as named; non-malignant human breast lesions or NM, lymph node negative human breast cancer (No regional lymph node involvement; LNN) and lymph node positive human breast cancer (Positive for regional lymph node involvement; LNP). There were just two samples available in the fourth group of our study as extra nodal metastatic human breast cancer (Positive for distant metastasis; MB).

Our data analysis was mostly based on qualitative assessment of the images which includes the presence of expression in tissue sections as well as the location of the signals throughout the tissue and inside the cells. In addition, we did statistical analysis to compare the abundance of expression among different categories of our samples. Analysis of the data showed that the closest results to significant level (<0.05) were those comparing benign and malignant groups especially for Rb mRNA. While, the most improbable results to significant level were those comparing among four study groups especially between LNP and MB.
Our findings demonstrated a dominant presence of p53 and Cyclin E mRNA expression in malignant breast tissues as compared to benign lesions. On the contrary, benign breast lesions showed a more dominant expression of Rb mRNA than malignant tissues.

A comparison between different breast cancer groups in our study showed slight differences in the proportions and intensities of p53, Cyclin E and RB mRNA expressions. These differences could be meaningful but the nature of our study, which was a qualitative method of research, does not allow definitive inference from the findings.

In conclusion, RT in situ PCR as a qualitative method is able to localize mRNA gene expression in human breast lesions. In addition, mRNA expression levels are obviously different in benign tissues compared to malignant tissues. However, it is not possible to rely on the slight differences between three malignant groups of our study. It is therefore necessary to do further investigations with quantitative research methods such as microarray analysis and or quantitative RT-PCR.
Abstrak tesis yang dikemuleakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PATEN EKSPRESI RT IN SITU PCR DALAM P53, CYCLIN E DAN RB GEN DALAM TAHP KANSER PAYUDARA YANG BERLAINAN

Oleh

MOHAMMADREZA ZAMANIAN

May 2007

Pengerusi : Profesor Madya Patimah Ismail, PhD
Fakulti : Perubatan dan Sains Kesihatan

Kanser payudara adalah masalah kesihatan yang paling utama yang dihadapi oleh golongan wanita. Salah satu cabaran utama dalam mendiagnosis dan pengubatan kanser payudara adalah dalam kepersisian dalam mengklasifikasikan peringkat-peringkat kanser. Dalam pemilihan cara pengubatan yang paling bersesuaian, pengenalpastian kalanjar limfa yang terlibat juga merupakan salah satu cara yang utama. Sehingga sekarang, pelbagai cubaan telah dijalankan untuk mencari marker molecular spesifik dengan tujuan untuk membantu para pakar surgen dan Perubatan kedal dan Qulcologia untuk membuat keputusan tepat mengenai peringkat-peringkat kanser payudara. Melalui ninya, penilaian aktiviti sesetengah gen penting di dalam evolusi dan perkembangan kanser didapati penting didalam pemilihan jenis rawatan kanser berkenaan.

p53, Cyclin E dan Rb adalah gen yang terlibat dalam kitaran dan pembahagian sel, telah dibuktikan memainkan peranan penting dalam pembentukan kanser, terutamanya kanser payudara. Ketidaknormalan peranan gen tersebut di dalam proses menyebabkan dan/atau mencetus perkembangan kitaran sel boleh menyebabkan sel memasuki peringkat hiperpoliferasi malahan neoplastik. Oleh itu, penilaian aktiviti gen tersebut
boleh membawa kepada penemuan sebarang perbezaan tahap ekpresi di antara benigna dan malignan, termasuk peringkat-peringkat kanser payudara.

Di dalam kajian ini, kami telah menggunakan Reversed Transcriptase in situ Polymerase Chain Reaction (RT in situ PCR) untuk menentukan ekpresi p53, Cyclin E dan Rb mRNA dalam sampel payudara manusia yang berbeza termasuklah tisu benigna dan malignan. Kaedah ini membolehkan jumlah mRNA yang sangat rendah pada peringkat sel dikenalpasti.

Kami telah mengkaji kehadiran ekpresi p53, Cyclin E dan Rb mRNA dalam 17 kes tisu payudara manusia yang tertanam dalam “paraffin” yang didermakan oleh wad patologi Hospital Milad yang terletak di Tehran, Iran.

Kami telah membahagikan sampel kepada empat kumpulan berdasarkan laporan patologi. Lima sampel di dalam setiap kumpulan; lesi keabnormalan payudara manusia benigna, kanser payudara manusia negatif nodus limfa dan kanser payudara manusia positif nodus limfa. Hanya terdapat dua sampel dalam kumpulan keempat kajian kami sebagai kanser payudara manusia metastatik.

Perbandingan di antara kumpulan-kumpulan kanser payudara dalam kajian kami menunjukan sedikit perbezaan nisbah dan kekuatan ekpresi p53, Cyclin E dan Rb mRNA. Walaupun perbezaan ini boleh digunakan, tetapi adalah berkemungkinan ianya tidak tepat kerana kami menggunakan kaedah kualitatif dan sampel yang terhad.
Kesimpulannya, RT *in situ* PCR adalah kaedah kualitatif yang boleh mengenalpasti lokasi ekresi gen mRNA dalam tisu payudara manusia. Tambahan pula, tahap ekresi mRNA adalah nyata berbeza di antara tisu benign berbanding dengan tisu malignan. Bagi membolehkan perbezaan yang sedikit di antara tiga kumpulan malignan dalam kajian kami diambil kira, adalah perlu untuk melakukan kajian yang lebih mendalam termasuk kaedah kajian kuantitatif seperti analisis microarray dan/atau quantitative RT-PCR.
ACKNOWLEDGEMENTS

“All the praise to Allah the Al-Mighty for his blessing and benevolence”

I wish to express my sincere gratitude and appreciation to the numerous individuals who have contributed towards the completion of this thesis:

At first, I wish to express my sincere appreciation and gratitude to Assoc. Prof. Dr. Patimah Ismail, the chairperson of my supervisory committee. Her paramount interest, effort and concern on my research project are much valued. I would also like to thank the other members of my supervisory committee: Dr. Cheah Yoke Kqueen for his insightful advice and interest, and Dr. Saadat Molanaii whose expertise has taught me many aspects of pathology of breast cancer. In addition, my cordial appreciation is extended to Dr. Molanaii for preparing me 17 samples included in current study.

I am also very grateful to the technical staff of the department of biomedical sciences, faculty of medicine and health sciences, UPM, especially to Ms. Juita bt. Chupri for her cooperation and friendly assistance during my research work at UPM. My deep gratitude and special thanks go to my friends and postgraduate students at UPM, especially Mr. Vasu Dewan for his encouragement and friendly cooperation.

Also my special love and gratitude go to my family members in Iran; my parents and parents in law, and especially to my brother Alireza, for their love and continuous support.

My love and appreciation also goes to my son, Alisina, who has been our motivation in life and particularly to my wonderful wife Zeinab, who has always been an anchor in my life. We have been together through hard and easy times but she has never failed to amaze me with her support, encouragement and love for me and our son, Alisina.
I certify that an Examination Committee has met on 25th May 2007 to conduct the final examination of Mohammadreza Zamanian on his Master of Science thesis entitled “Reverse Transcriptase In situ Expression Patterns of p53, Cycline and Rb Genes at Different Stages of Breast Cancer” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Abdul Manan Mat Jais, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Seow Heng Fong, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Sabariah Abdul Rahman, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Yasmin Anum Mohd Yusof, PhD**
Associate Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**
Professor, Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 3 August 2007
This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee are as follows:

**Patimah Ismail, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Cheah Yoke Kqueen, PhD**  
Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Salamat Molanai, MD, PhD**  
Lecturer  
Pathology Ward - Milad Hospital  
Tehran - Iran  
(Member)

---

**AINI IDERIS, PhD**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 9th August 2007
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

MOHAMMADREZA ZAMANIAN

Date: 2nd August 2007
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ix</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>x</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxii</td>
</tr>
</tbody>
</table>

## CHAPTORS

### 1 INTRODUCTION

- Problem Statement  
  - Page 1
- Research Objectives  
  - Page 4
- Hypothesis  
  - Page 6

### 2 LITERATURE REVIEW

- Normal Breast  
  - Page 8
- Breast Cancer  
  - Page 8
  - Epidemiology of Breast Cancer  
    - Page 8
  - Clinical Findings  
    - Page 9
  - Predisposing Factors  
    - Page 9
  - Diagnosis  
    - Page 11
  - Pathology  
    - Page 11
  - Breast Cancer Treatment  
    - Page 19
  - Prognosis and survival of Breast Cancer  
    - Page 21
  - Genetics of Breast Cancer  
    - Page 22
  - Hormone Receptor Status in Breast Cancer  
    - Page 27
- Cell Cycle  
  - Page 28
  - Definition  
    - Page 28
  - Cell Cycle Phases  
    - Page 29
  - Cell cycle regulations  
    - Page 29
  - Cell Cycle Check Points  
    - Page 31
  - G1/S Cyclin  
    - Page 35
  - p53  
    - Page 36
  - Retinoblastoma Protein  
    - Page 37
  - Breast Cancer and Cell Cycle  
    - Page 39
  - Breast Cancer and Cyclin E  
    - Page 39
  - Breast Cancer and p53  
    - Page 41
  - Breast Cancer and Rb  
    - Page 42
  - RT in situ PCR  
    - Page 44
  - Rt in situ PCR in Breast Cancer  
    - Page 44
  - Problem Statement  
    - Page 45
3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Human Breast Samples
3.1.2 Grouping of the Samples
3.1.3 Other Materials

3.2 Preparation of Tissue Sections

3.3 Processing and Pre-treatment of Tissue Sections

3.4 Protease Digestion

3.5 DNase Digestion

3.6 Probe Labeling

3.7 Optimization of reagents Performance

3.8 One step RT in situ PCR Assay

3.9 Immuno Detection of PCR Products

3.10 Preparing the Controls in RT in situ PCR

3.11 Data analysis

4 RESULTS

4.1 Histological analysis of Breast tissue sections

4.1.1 Normal Human Breast Tissues
4.1.2 Malignant Human Breast Tissues

4.2 RT in situ PCR Analysis

4.2.1 Optimization of the Reagents Performance
4.2.2 Optimization of the in situ Conditions
4.2.3 Controls

4.3 Localization of the p53, Cyclin E and Rb mRNA Expressions in Breast Tissues

4.3.1 Localization of p53 mRNA Expression in Human Breast
4.3.2 Localization of Cyclin E mRNA Expression in Human Breast
4.3.3 Localization of Rb mRNA Expression in Human Breast

4.4 Statistical Analysis

5 DISCUSSIONS

6 CONCLUSION

Future Work

REFERENCES

APPENDICES

BIODATA OF THE AUTHOR
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>TNM Staging system of breast cancer and related survival rates</td>
</tr>
<tr>
<td>2.2</td>
<td>Grading system of breast cancer and related survival rates</td>
</tr>
<tr>
<td>3.1</td>
<td>The sequences of oligonucleotide primers used in the study</td>
</tr>
<tr>
<td>3.2</td>
<td>Preparation of RT PCR solution in one step RT \textit{in situ} PCR assay</td>
</tr>
<tr>
<td>4.1</td>
<td>Summary of p53 mRNA expression analysis in four study groups</td>
</tr>
<tr>
<td>4.2</td>
<td>Comparison of p53 mRNA expression in NM versus MAL and Early versus Progressed categories</td>
</tr>
<tr>
<td>4.3 A</td>
<td>Comparison of p53 mRNA expression between NM, LNN, LNP and MB groups</td>
</tr>
<tr>
<td>4.3 B</td>
<td>Comparison of p53 mRNA expression between NM, LNN, LNP and MB groups</td>
</tr>
<tr>
<td>4.4</td>
<td>Summary of cyclin E mRNA expression analysis in four study groups</td>
</tr>
<tr>
<td>4.5</td>
<td>Comparison of cyclin E mRNA expression in NM versus MAL and Early versus Progressed categories</td>
</tr>
<tr>
<td>4.6 A</td>
<td>Comparison of Cyclin E mRNA expression between NM, LNN, LNP and MB groups</td>
</tr>
<tr>
<td>4.6 B</td>
<td>Comparison of Cyclin E mRNA expression between NM, LNN, LNP and MB groups (Cont.)</td>
</tr>
<tr>
<td>4.7</td>
<td>Summary of Rb mRNA expression analysis in four study groups</td>
</tr>
<tr>
<td>4.8</td>
<td>Comparison of Rb mRNA expression in NM versus MAL and Early versus Progressed categories</td>
</tr>
<tr>
<td>4.9 A</td>
<td>Comparison of Rb mRNA expression between NM, LNN, LNP and MB groups</td>
</tr>
<tr>
<td>4.9 B</td>
<td>Comparison of Rb mRNA expression between NM, LNN, LNP and MB groups (Cont.)</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.1 A</td>
<td>Non-malignant breast tissue, terminal duct-lobular unit (TDLU) structures in an adult female, magnification 10X, H&amp;E staining</td>
<td>60</td>
</tr>
<tr>
<td>4.1.1 B</td>
<td>Non-malignant breast tissue, terminal duct-lobular unit (TDLU) structures in an adult female, magnification 20X, H&amp;E staining</td>
<td>60</td>
</tr>
<tr>
<td>4.1.1 C</td>
<td>Non-malignant breast tissue, ductal-tubular structure of the breast covered by two types of cells; epithelium and myoepithelial cells, an adult female, magnification 20X, H&amp;E staining</td>
<td>61</td>
</tr>
<tr>
<td>4.1.1 D</td>
<td>Non-malignant breast tissue, ductal-tubular structure of the breast covered by two types of cells; epithelium and myoepithelial cells, an adult female, magnification 40X, H&amp;E staining</td>
<td>61</td>
</tr>
<tr>
<td>4.1.2 A</td>
<td>Invasive ductal carcinoma in a 39 years old female in stage II (N+, M-, T &lt; 5cm), tumor cells arranged in clusters, magnification 4X, H&amp;E staining (LNP2)</td>
<td>64</td>
</tr>
<tr>
<td>4.1.2 B</td>
<td>Invasive ductal carcinoma in a 52 years old female in stage IIIa (N+, M-, T &gt; 5cm), tumor cells arranged in cords and trabeculae, magnification 4X, H&amp;E staining (LNP7)</td>
<td>64</td>
</tr>
<tr>
<td>4.1.2 C</td>
<td>Invasive ductal carcinoma in a 59 years old female in stage IIIa (N+, M-, T &gt; 5cm), tumor cells arranged in clusters, magnification 10X, H&amp;E staining (LNP1)</td>
<td>65</td>
</tr>
<tr>
<td>4.1.2 D</td>
<td>Invasive ductal carcinoma in a 38 years old female in stage IIIa (N-, M+, T &gt; 5cm) tumor cells arranged in clusters, magnification 10X, H&amp;E staining (LNN2)</td>
<td>65</td>
</tr>
<tr>
<td>4.1.2 E</td>
<td>Invasive ductal carcinoma in a 62 years old female in stage II (N+, M+, T &lt; 5cm), tumor cells with prominent and often multiple nucleoli, magnification 40X, H&amp;E staining (LNN5)</td>
<td>66</td>
</tr>
<tr>
<td>4.1.2 F</td>
<td>Invasive ductal carcinoma in a 48 years old female in stage IIIa (N+, M-, T &gt; 5cm), tumor cells tumor cells with prominent and often multiple nucleoli, magnification 4X, H&amp;E staining (LNP6)</td>
<td>66</td>
</tr>
<tr>
<td>4.1.2 G</td>
<td>Invasive ductal carcinoma in a 64 years old female in stage IV (N+, M+, T &gt; 5cm), pleomorphic nuclei with high mitotic activity, magnification 20X, H&amp;E staining (MB1)</td>
<td>67</td>
</tr>
</tbody>
</table>
4.1.2 H Invasive ductal carcinoma in a 64 years old female in stage IV (N+, M+, T > 5cm), pleomorphic nuclei with high mitotic activity, magnification 40X, H&E staining (MB1)

4.2.1 Performing positive control to verify reagents performance of access RT-PCR system using Candida tropicalis ATCC 750 RNA

4.2.3 A RT in situ PCR – Negative control for p53 mRNA expression with omission of primers in a lymph node negative breast carcinoma sample (LNN3).

4.2.3 B RT in situ PCR - Negative control for p53 mRNA expression with omission of Digoxigenin in a non-malignant breast tissue sample (NM1).

4.2.3 C RT in situ PCR - Negative control for p53 mRNA expression with omission of Anti - Digoxigenin in a lymph node positive breast cancer sample (LNP5).

4.2.3 D RT in situ PCR - Negative control for cyclin E mRNA expression with omission of primers in a lymph node positive breast carcinoma sample (LNP4).

4.2.3 E RT in situ PCR - Negative control for cyclin E mRNA expression with omission of Digoxigenin in a breast carcinoma sample with distant metastasis (MB1).

4.2.3 F RT in situ PCR - Negative control for cyclin E mRNA expression with omission of Anti-Digoxigenin in a non-malignant breast tissue sample (NM2).

4.2.3 G RT in situ PCR - Negative control for Rb mRNA expression with omission of primers in a non-malignant breast tissue sample (NM3).

4.2.3 H RT in situ PCR - Negative control for Rb mRNA expression with omission of Digoxigenin in a lymph node negative breast carcinoma tissue sample (LNN2).

4.2.3 J RT in situ PCR - Negative control for Rb mRNA expression with omission of Anti-Digoxigenin in a lymph node positive breast carcinoma tissue sample (LNP5).

4.2.3 K RT in situ PCR - Positive control for cyclin E mRNA expression with omission of overnight DNase digestion in a lymph node positive breast carcinoma tissue sample (LNPl).

4.2.3 L RT in situ PCR - Positive control for Rb mRNA expression with omission of overnight DNase digestion in a lymph node negative breast carcinoma tissue sample (LNN4).
4.2.3 M RT in situ PCR - Positive control for p53 mRNA expression with omission of overnight DNase digestion in a lymph node negative breast carcinoma tissue sample (LNP6).

4.3.1.1 A RT in situ PCR - Evaluation of p53 mRNA expression in non-malignant breast tissue, sample NM3

4.3.1.1 B RT in situ PCR - Evaluation of p53 mRNA expression in non-malignant breast tissue, sample NM1

4.3.1.1 C RT in situ PCR - Evaluation of p53 mRNA expression in non-malignant breast tissue, sample NM2

4.3.1.1 D RT in situ PCR - Evaluation of p53 mRNA expression in non-malignant breast tissue, sample NM4

4.3.1.1 E RT in situ PCR - Evaluation of p53 mRNA expression in non-malignant breast tissue, sample NM5

4.3.1.2 A RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue without involvement of regional lymph nodes, sample LNN3

4.3.1.2 B RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue without involvement of regional lymph nodes, sample LNN4

4.3.1.2 C RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue without involvement of regional lymph nodes, sample LNN6

4.3.1.2 D RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue without involvement of regional lymph nodes, sample LNN1

4.3.1.2 E RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue without involvement of regional lymph nodes, sample LNN2

4.3.1.3 A RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue with involvement of regional lymph nodes, sample LNP6

4.3.1.3 B RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue with involvement of regional lymph nodes, sample LNP7

4.3.1.3 C RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue with involvement of regional lymph nodes, sample LNP4
4.3.1.3 D RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue with involvement of regional lymph nodes, sample LNP5

4.3.1.3 E RT in situ PCR - Evaluation of p53 mRNA expression in breast carcinoma tissue with involvement of regional lymph nodes, sample LNP1

4.3.1.4 A RT in situ PCR, Metastatic breast cancer tissue, sample MB1, Gene p53, magnification 40X

4.3.1.4 B RT in situ PCR, Metastatic breast cancer tissue, sample MB2, Gene p53, magnification 40X

4.3.2.1 A RT in situ PCR, Benign breast tissue, sample NB2, Gene Cyclin E, magnification 40X

4.3.2.1 B RT in situ PCR, Benign breast tissue, sample NB1, Gene Cyclin E, magnification 40X

4.3.2.1 C RT in situ PCR, Benign breast tissue, sample NB3, Gene Cyclin E, magnification 40X

4.3.2.1 D RT in situ PCR, Benign breast tissue, sample NB4, Gene Cyclin E, magnification 40X

4.3.2.1 E RT in situ PCR, Benign breast tissue, sample NB5, Gene Cyclin E, magnification 40X

4.3.2.2 A RT in situ PCR, Axillary lymph node negative breast cancer tissue, sample LNN4, Gene Cyclin E

4.3.2.2 B RT in situ PCR, Axillary lymph node negative breast cancer tissue, sample LNN3, Gene Cyclin E, magnification 40X

4.3.2.2 C RT in situ PCR, Axillary lymph node negative breast cancer tissue, sample LNN1, Gene Cyclin E, magnification 40X

4.3.2.2 D RT in situ PCR, Axillary lymph node negative breast cancer tissue, sample LNN2, Gene Cyclin E, magnification 40X

4.3.2.2 E RT in situ PCR, Axillary lymph node negative breast cancer tissue, sample LNN6, Gene Cyclin E, magnification 40X

4.3.2.3 A RT in situ PCR, Axillary lymph node positive breast cancer tissue, sample LNP5, Gene Cyclin E, magnification 40X

4.3.2.3 B RT in situ PCR, Axillary lymph node positive breast cancer tissue, sample LNP6, Gene Cyclin E, magnification 40X
4.3.2.3 C RT \textit{in situ} PCR, Axillary lymph node positive breast cancer tissue, sample LNP1, Gene Cyclin E, magnification 40X

4.3.2.3 D RT \textit{in situ} PCR, Axillary lymph node positive breast cancer tissue, sample LNP4, Gene Cyclin E, magnification 40X

4.3.2.3 E RT \textit{in situ} PCR, Axillary lymph node positive breast cancer tissue, sample LNP7, Gene Cyclin E, magnification 40X

4.3.2.4 A RT \textit{in situ} PCR, Metastatic breast cancer tissue, sample MB1, Gene Cyclin E, magnification 40X

4.3.2.4 B RT \textit{in situ} PCR, Metastatic breast cancer tissue, sample MB2, Gene Cyclin E, magnification 40X

4.3.3.1 A RT \textit{in situ} PCR, Benign breast tissue, sample NB2, Gene Rb, magnification 20X

4.3.3.1 B RT \textit{in situ} PCR, Benign breast tissue, sample NB5, Gene Rb, magnification 40X

4.3.3.1 C RT \textit{in situ} PCR, Benign breast tissue, sample NB4, Gene Rb, magnification 40X

4.3.3.1 D RT \textit{in situ} PCR, Benign breast tissue, sample NB1, Gene Rb, magnification 40X

4.3.3.1 E RT \textit{in situ} PCR, Benign breast tissue, sample NB3, Gene Rb, magnification 40X

4.3.3.2 A RT \textit{in situ} PCR, Axillary lymph node negative breast cancer tissue, sample LNN2, Gene Rb, magnification 40X

4.3.3.2 B RT \textit{in situ} PCR, Axillary lymph node negative breast cancer tissue, sample LNN6, Gene Rb, magnification 40X

4.3.3.2 C RT \textit{in situ} PCR, Axillary lymph node negative breast cancer tissue, sample LNN1, Gene Rb, magnification 40X

4.3.3.2 D RT \textit{in situ} PCR, Axillary lymph node negative breast cancer tissue, sample LNN3, Gene Rb, magnification 40X

4.3.3.2 E RT \textit{in situ} PCR, Axillary lymph node negative breast cancer tissue, sample LNN4, Gene Rb, magnification 40X

4.3.3.3 A RT \textit{in situ} PCR, Axillary lymph node positive breast cancer tissue, sample LNP7, Gene Rb, magnification 20X

4.3.3.3 B RT \textit{in situ} PCR, Axillary lymph node positive breast cancer tissue, sample LNP1, Gene Rb, magnification 40X
4.3.3.3 C  RT *in situ* PCR, Axillary lymph node positive breast cancer tissue, sample LNP4, Gene Rb, magnification 40X

4.3.3.3 D  RT *in situ* PCR, Axillary lymph node positive breast cancer tissue, sample LNP5, Gene Rb, magnification 40X

4.3.3.3 E  RT *in situ* PCR, Axillary lymph node positive breast cancer tissue, sample LNP6, Gene Rb, magnification 40X

4.3.3.4 A  RT *in situ* PCR, Metastatic breast cancer tissue, sample MB1, Gene Rb, magnification 40X

4.3.3.4 B  RT *in situ* PCR, Metastatic breast cancer tissue, sample MB2, Gene Rb, magnification 40X
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMV</td>
<td>Alfa Mosaic Virus</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>DAB</td>
<td>3,3'-diaminobenzidine</td>
</tr>
<tr>
<td>DEPC</td>
<td>Diethyl pyrocarbonate</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPX</td>
<td>DePex</td>
</tr>
<tr>
<td>dATP</td>
<td>Deoxyadenosine triphosphate</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxy nucleic triphosphate</td>
</tr>
<tr>
<td>dUTP</td>
<td>Deoxy-uridine-5' triphosphate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tertra acetate</td>
</tr>
<tr>
<td>et al.</td>
<td>et alii</td>
</tr>
<tr>
<td>ETOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>LNN</td>
<td>Lymph node negative (No lymph node involvement)</td>
</tr>
<tr>
<td>LNP</td>
<td>Lymph node positive (Presence of lymph node metastasis)</td>
</tr>
<tr>
<td>MAL</td>
<td>Malignant</td>
</tr>
<tr>
<td>MB</td>
<td>Metastatic breast tissue (Presence of distant metastasis)</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NM</td>
<td>Non-malignant</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase-polymerase chain reaction</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-boric acid disodium EDTA</td>
</tr>
<tr>
<td>TE</td>
<td>Tris EDTA</td>
</tr>
<tr>
<td>Tfl</td>
<td><em>Thermus flavus</em></td>
</tr>
<tr>
<td>Tris</td>
<td>Tris(hyromethyl) aminomethane</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

Breast cancer is a great problem of human’s and especially women’s health at the present time. It is diagnosed one million times every year worldwide (Berns et al. 2004). It is the second leading cause of cancer deaths in women today (after lung cancer) and the most common cancer among women, excluding nonmelanoma skin cancers. In Iran, breast cancer continues to increase in numbers yearly and remains an important health problem, although its statistics is very similar to that of other countries in the region (Harirchi et al. 2002).

One of the most important issues in improving health indices regarding breast cancer is using individualized treatment methods. Strategies for treatment in breast cancer depends on the extent of disease progression in the body that will be evaluated by certain criteria; size of the tumor mass, lymph node involvement and the presence of metastasis which is based on TNM system (T; tumor size, N; lymph node involvement and M; metastasis). In order to help the clinicians to decide on treatment modalities, it is necessary to have a standard method for determining disease progression. Breast cancer is usually divided into four stages based on above criteria. There are different prognoses, choices of treatment, response rates to therapy and survival in each of different stages of breast cancer. In order to use targeted therapy clinicians need to know the precise stage of a breast cancer, which determines the extent of disease.