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The Level of Src, Her2, Bcl2, Vegf, Kras Genes Expression in the Cases of Lung Cancer Surgery

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ABSTRACT

Background: Lung cancer is the most common cancer in men and women, all over the world. More than 80% of patients with this cancer within 5 years of diagnosis lose their lives. In most of the cases, the cancer is usually diagnosed late. So it is necessary to have a reliable tool in order to diagnosis the disease in the early stages. In the present study for the diagnosis and prognosis as well as for therapeutic targets, 5-gene panel was applied for study on lung surgery samples.
Methods: Using open surgery method, 60 fresh samples of lung tissue were prepared from 30 patients with pulmonary disease, including 30 specimens of lung cancer tissue and 30 of normal tissue adjacent to the tumor. Total RNA was isolated from the specimens and after cDNA synthesis was used for qRT -PCR analysis.
Results: The results showed that the expression of 3 oncogenes; Bcl2, Src and Her2 in surgical specimens of lung cancer, significantly ($p < 0.05$) have a higher expression than healthy lung tissue.
Conclusion: According to overall results of present study, it can be concluded that lung tissue abnormalities, with the exception of cancer can lead to increasing in oncogenes overexpression. As well as lung cancer in both genders is almost equal spread.
KEYWORDS: Lung Cancer, Oncogene, Gene Expression Profile, RT-PCR

1. INTRODUCTION

Oncogene is a type of mutant gene that its function or expression cause abnormal stimulation of cell division and proliferation. In addition to oncogene that its activation causes the cancer, there are other genes that their mutations via different mechanism, i.e. the loss of function of both alleles of gene, have a significant role in cancer occurrence. These genes are called tumor suppressor genes which with planning the growth and activity of other cells cause cancer prevention. DNA repair genes are included the all genes that involved in the repair of different DNA damages. These genes Provides the conditions to repair the damaged DNA by secretion of different proteins. Whenever these genes are damaged, the cells loss their regeneration ability therefore genetic disorders and lack of DNA repair leads to the cancer s in men and women, all over the world. More than 80% of patients with this cancer within 5 years of diagnosis lose their lives. Lung cancer disease emerged in one or both of the lungs. (National Center for Health Statistics, 2012)

Some of the lung diseases at the beginning occur as simple inflammations. Based on the findings of investigations these inflammations may contain foci of premalignant that can develop to lung cancer later on. Lung diseases which make the risk of lung cancer is known by high and irregular inflammations. (Version3.5.2. Bethesda, 2011).

The genes which were used to determine gene profiling, in this project:

In the present study, it was attempted to confirm on the genes that play an important role in the cancer occurrence. As well as the level of gene expression was studied. In the following, some of these genes were represented and their effects in different types of cancers were investigated and described. These genes are included; Bcl2, Her-2.neu, src vegf kras that are appropriate targets for treatment. βActin is a gene that always is expressed in all the cells, in the present research was applied as a control gene. Proto-oncogene Bcl-2 was encoded by a gene with 230k- organic base which was used as a control gene and its product is a protein with 26KDa molecular weight. Genes of Bcl2 family product regulator proteins that regulate programmed cell death (Apoptosis).This gene is a member of a big family and all of them have at least one of the four main area of the BH (Bcl2 homology). Main members such as Bcl-2, Bcl-xl and Mcl1are Anti Apoptosis, and the rest of the members such as Bax, Bak, and Bok

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are Peru apoptosis. Prevention of apoptosis by these proteins was done via the activation of a group of Cysteine Protease known as Caspases inside the cells. Specifically the role of Bcl-2 gene in this process is preventing the release of Cytochrome from Mitochondria as a result avoid of its decomposition. Her2. neu gene is known as Neu, Ngl, Her2, Tkr1, CD340, Her-2, Mln 19,ErbB2 as well as is related to Epidermal Growth Factor 2 and a member of protein kinase family. The official name of Kras gene is v-Ki-ras2 that is the written abbreviation of Kirsten rat sarcoma viral oncogene homolog. Kras gene through a process known as signal transmission causes proteins transfer from outside of cell to cell nucleus. These messages guide the cells how to grow, divide, mature, and be committed to do special functions. SRC Gene is known as ASV; SRC1; c-SRC; p60-Src. Sarc is the written abbreviation of Sarcoma. *Proto-oncogene tyrosine-protein kinase Src* is encoded in human by the SRC gene. SRC protein family discovered the modern understanding of cancer as a disease in which the normal cell signaling interrupted. SRCs expression mechanism in human cancers, are unclear yet. Many studies on mechanisms which are related to cancer thorough SRCs showed that SRCs has a distinctive role in initiation, progression and metastasis of cancer through multiple changes in signaling pathways.(Wheeler et al. 2009). VEGF gene is also called FLT1. VEGF is one of the most important angiogenic factors that plays an important role in angiogenesis inducement. Studies have shown that both of promoter and the exons of gene have polymorphic sites, which can influence expression of this factor and its serum levels. The cancer that expresses VEGF will be able to grow and metastasize. (Browne & Crown, 2011)

REVIEW OF LITERATURE

Many studies have been done that show genetic profiling can play an effective role in providing accurate classification of cancer. Regarding this method difference between Acute lymphoblastic Leukaemia and Acute myeloid was identified. Diffuse Large B Lymphoma was also identified by determining the gene expression profiles. Many methods were applied so far, RNA is usually prepared from different cancer cells for DNA microarray and Obtained information is analyzed using the supervised analysis. There is another method is called GSEA (Gene Set Enrichment Analysis) beyond the sporadic gene expression analysis. In this system, the expression of a group of genes is studied, for example the profile of a group of genes involved in cell communication network. In the near future the use of these profiles will be more and widespread. (Bethesda, 2012)

RT - PCR

The original model in RT-PCR is single-chain RNA molecule. Because of that the DNA polymerase is not able to use RNA as a template; another stage has been added to the PCR. During this stage, using the Reverse Transcriptase enzyme and regarding template of RNA, its complementary (cDNA) is synthesized and amplified by PCR technique. (Pfaffl, 2010)

* After the RNA extraction from samples of lung surgery, using RT-PCR method cDNA was synthesized by thermal cycler device. A piece of tissue about 100 mg of the RNA later solution was transferred to the test tube that has been washed by DEPC and has already been treated with water as well as the tube contained 1 ml Tripure. If the sample weight was less than 20 mg, 600-300 ml of solution Tripure was added. The solution was homogenized by a homogenizer for 20 to 40 seconds with maximum speed on the ice, (Time depends on the tissue density). Homogenized solution was transferred to a microtube and was incubated for 5 min at room temperature. Solution was centrifuged 1300 rpm for 3 minutes therefore the deposition of cells was achived. (Animal cell culture protocols. Qiagen 2013). Using the RNeasy mini kit of Qiagen Company, RNA of all samples was extracted and kept at -70 ° C for the later stages. (RNeasy mini kit protocol Qiagen). After extracting the RNA from the sample, it is necessary to measure the concentration of obtained RNA. Using the ratio A260 / A280 the amount of impurities is calculated. The closer ratio to 1.8, means low level of impurities (General Guidelines for Working with RNA Kirsanov, KI et al. (2010)

Construction of cDNA:

Using kit fast transcription kit protocol of Qiagen Company according to the obtained RNA, cDNA was synthesized.

The *Thermal cycler device* that was used in study is manufactured by Biotech and the program to perform RT- PCR was as follows.

Real CDNA in a PCR thermal cycler:

26 0C for 10 min (primer anealing)
42 0C for 45 min (reverse transcription)
75 0C for 10 min (inactivated the enzyme)
75 0 C for 10 min (inactivated the enzyme)

After synthesizing cDNA, to ensure construction and the degree of its purity, cDNA concentration was determined by spectrophotometry as described. (Fast transcription kit protocol, Qiagene)

Real Time PCR:

The principles of this technique are as well as other ones; however, RNA was applied as a model of process instead of DNA. Process is that first, cDNA was synthesized and then was amplified with specific primers. In this study, Hot Taq EvaGreen qPCRMix kit and Step One Plus *Real-Time PCR* devices was applied.(Hot Taq EvaGreen qPCRMix protocol sinaclone). After gaining C T mean (Mean of the genes that was studied by Real time PCR). Collected Data was recorded in Bio Pronet software and graphs of the all studied genes were achieved and were compared with control gene the Betachitin.

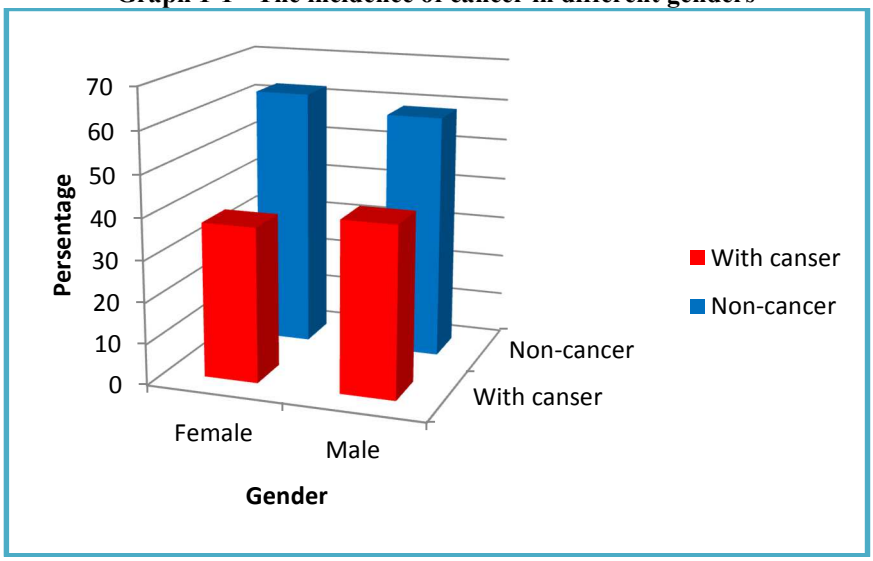
Designed primer sequences for studied genes (Table 1.1)

RESULTS

60 fresh samples of lung tissue were prepared from 30 patients with lung disease, including 30 specimens of lung cancer tissue and 30 of normal tissue adjacent to the tumor. Present study was performed on 60 samples of surgery with lung cancer. Among of them 30 cases was related to cancer tissue and 30 cases were healthy (normal) tissues. The average age of the 60 patients was 50.39, with standard deviation 17.4. The average age of non-cancer patients was 52.19 with a standard deviation of 21.6 and in patients with censer average were 61.9 with standard deviation10.1.

31.4% of the examined patients were female and 66.7% of them were male. The gender distribution between non-cancer cases and patients with cancer was approximately the same.62.5% of women and 58.8% of men were healthy and hadn't cancer. (Graph 1-1)

Graph 1-1 - The incidence of cancer in different genders



Statistical analysis of gene expression level in samples of lung surgery

In this study the lung cancer was considered as dependent variable and studied gens were considered as independent variables. The average expression of the studied groups was investigated by using SPSS statistics v.19 and independent t test. (Table 2.1)

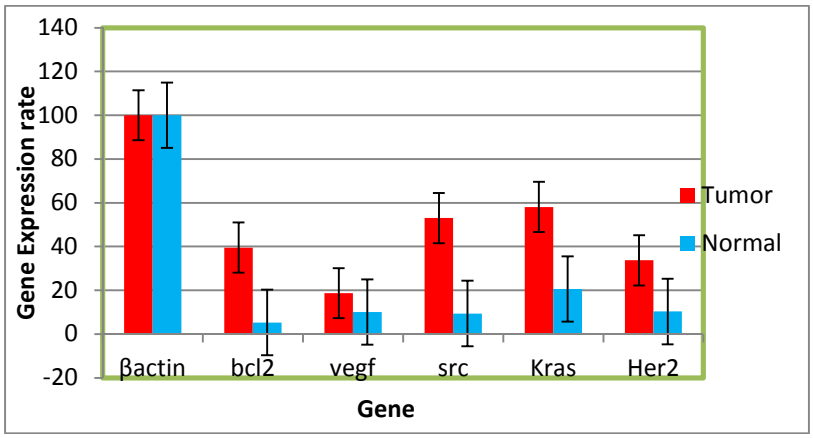
Table 1-2. Percentages of studied gens expression in comparison with reference gene (beta-actin) in term with samples were received from Tehran Shahid Masih Daneshvari Hospital in 2012.

* Statistically the difference was significant in level of $P < 0.05$.According to Table (2-1), there is a significant difference ($P \leq 0.05$) in gens expression between the surgery samples and healthy samples of adjacent tissues. Among 60 surgery samples that were obtained from patients with cancer, 30 samples were cancerous tissues and 30 were healthy tissues samples (adjacent to cancerous tissue).

Table 2-1: level of studied genes expression in normal and cancerous samples of lung surgery that were received from hospital Shahid Masih Daneshvari Hospital in 2012.

As table 3-3 shows, P value for some genes expression is close to 0.05 therefore the level of 3 following genes, Bcl-2, src, Her2 are in the significant range.

Graph1-2. Percentages of gene expression in comparison with beta-actin reference gene. (In samples of lung surgery)



As can be seen in graph 1-2, genes aspect of expression in cancerous and non-cancerous samples had different expression levels. Gene expression of Src, Her2, and Bcl2 in cancer surgery specimens was significantly higher than non-cancerous samples.

Conclusion

Generally, the reasons of cancer occurrence is interruption in the work of some genes therefore, identification of genes involved in cancer helps to finding more appropriate and effective methods of treatment. As a result gene therapy and purposeful treatment are the cases of study for many researchers that every day is added to the statistics. In spite of all the efforts, targeted non-toxic drugs are not available to be replaced with Chemotherapy. However, it is expected to achieve that by using molecular studies on genes involved in cancer, through different methods. As well as invention of tools for detection of patients with lung cancer in early stages of disease before the onset of clinical symptoms is necessary. Identification of mutant genes and genetic changes plays an important role in understanding of the pathogenicity of cancer, which subsequently leads to development of ways to cancer diagnosis and its treatment. Present study focused on fast and practical methods to diagnose the agent or genetic agents of cancer. The diagnosis will help the doctor to provide an appropriate and effective treatment plan for patient. If the cancer gene inhibitor drug be available, the patient will cure with targeted drugs. In this study the role of some genes, in incidence of lung cancer was demonstrated. In the present study, 5 genes were examined. 3 oncogenes of this group; K-ras, Bcl-2 and Her2 have been considered by pharmacists a long time ago and in order to inhibition of these gens many actions have been done. Real Time PCRmethod is the most effective and appropriate gene profiling method. In this way, small amount of specimen (2-3 mm3) is needed. Based on reports from ASCO University of United States, aging is the most important risk factor for developing cancer. More than 60% of cancers occur at the age of 65 years and above in the United States. Lung cancer is one of the most common cancers in this age group. As it was represented in Chapter III, the average age of the patients with lung cancer in this study was about 61 years that confirmed above statement. In many conducted investigations and in previous studies there wasn't observed a significant relationship between gender and lung cancer. However, some of the articles reported that the lung cancer occurrence in men is more than women. This study results was compatible with earlier studies. In other words there was not observed significant relationship between gender and lung cancer in this study.

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