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Investigating gene expression profile of non-small cell lung cancer

Mini Review

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Abstract: Lung cancer is mainly a lifestyle-associated disease with poor prognosis and the lowest five year survival rate of all types of cancer. Lung cancers are divided into two main groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Surgical treatment is generally indicated in cases of early stage NSCLC, and those patients treated with radical and aggressive surgery have a somewhat better survival rate. The main problems with lung cancer treatment are due to late diagnosis, rapidly developing drug resistance and side effects of the treatment that are experienced by almost all patients. The next step for distinguishing histologically complicated lung cancers and determining optimal treatment strategies is gene expression analysis. Supported by gene expression data, it is possible to prognosticate the course of the disease.

Keywords: *Lung cancer • Non-small cell lung cancer • Gene expression • Microchip • Molecular markers*

1. Introduction

Lung cancer is a worldwide problem that has been a leading cause of mortality for decades [1,2]. The prognosis for lung cancer is generally poor. The five year survival rate from the date of diagnosis, being only 5-16%, is the lowest from all cancer diagnoses [3]. Surprisingly low is the survival rate even for the patients treated in stage I of the disease, staying between 55% and 63% [4]. Fortunately, lung cancer incidence in the Western world has stabilized or even declined [2,3]. The incidence, as well as expected incidence ratio between males and females, is different in the member states of the European Union compared to those in the United States, but it is moving towards equalisation [2,3]. Fortunately, lung cancer is one of the most easily preventable diseases [5].

Despite the specific histological and topic (TNM) diagnosis of lung cancer, the clinical development of the same stage and type of cancer for patients from different age groups can vary substantially. From the molecular biology point of view, it could be said that every patient has his or her very own disease with the common name of lung cancer. A similar phenomenon has been described for other cancers. This gives permission to claim that the traditional diagnosis, based on the histology and spread of cancer, is not informative enough regarding the development of the disease or for optimal treatment. Therefore, one of the next steps towards a more precise diagnosis is gene expression analysis of the cancer tissue - a method that is applied more frequently for investigation of the molecular characterization and genetic etiology of various tumours.

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2. Classification of lung cancers

Although the WHO classification system includes numerous types of malignant lung tumours, lung cancers can roughly be divided into two groups: small cell lung carcinoma (SCLC) and non small cell lung carcinoma (NSCLC). These constitute 20 and 80 percent of all cancer cases respectively [6].

SCLC and NSCLC can clearly be distinguished from each other based on their histology as well as clinical course. NSCLC is divided, based on the tissue of origin, mainly into adenocarcinoma, squamous cell carcinoma, large cell carcinoma and carcinoid [6].

Although the tissue of origin of a large cell carcinoma and a carcinoid is unknown, the up-regulated molecular markers of the neuroendocrine system give basis to believe that at least some part of those cancers originate from the cells of the diffused pulmonary neuroendocrine system [7-9]. Although in the case of small cell carcinoma a precancerous state has not been demonstrated, some studies refer to a possibility that in this case the cancer originates from molecularly strongly damaged bronchial epithelium [10]. On a molecular level, however, lung cancer, as well as any other type of cancer, is a biological process and a complex disease that even within its subcategories can have substantial differences.

3. Gene expression analysis and its application in oncology

Gene expression is a process where the heritable genetic information is translated and used for the synthesis of a functional product of the gene. There are two crucial steps of gene expression: transcription, whereby a copy of mRNA is produced using a DNA strand as a template, and protein synthesis or translation, which results in the synthesis of a functional protein. In cases of some non-coding genes, the final product of gene expression will be functional RNA. Gene expression is the most fundamental process for any type of cell where the genotype is translated into phenotype. By analysing gene expression profile it can be assessed what process a cell is occupied by, and to what extent. Changes in gene expression influence the function of all living cells. Modern methods of molecular biology allow investigating genes with different gene expression in genome-wide studies. As a result of such studies, the gene expression pattern is visualized, giving a complex overview of all the biological processes. The expression pattern usually highly correlates with the type of histology of the tumour [11]. The gene expression pattern of a tumour is also correlates and is very similar in different parts of the same tumour, and the signatures giving an indication for the treatment response are usually similar in both the site of origin as well as in the metastasis of a tumour [12,13]. Due to the wide selection of parameters, samples can be differentiated despite of low number of expressed characteristics. The bioinformatics analysis of the tumour expression data allows not only the investigation of the metastasis, spread and the pace of it, but also provides information on the possible treatment response. This includes identifying predictive markers, as well as the survival predicting prognostic markers. In some cases the same markers can be responsible for both of those roles. The genes *ERCC1* and *RRM1* would be an example of this, where the expression is both a prognostic as well as predictive marker for early stage untreated NSCLC [14].

The basis for gene expression profiling was established ten years ago with a paper describing the gene expression of B-cell lymphoma and the prognostic subtypes of it [15]. The Bhattacharjee research group published the first lung cancer gene expression profile with the subclasses of lung adenocarcinoma classification in the year 2001 [16]. The greatest success has been achieved in breast cancer research. In 2002, a breast cancer study was published where significant molecular differences were detected in the gene expression pattern between patients' cancers that were in the same stage but with different clinical courses [17].

Gene expression profiling has brought some understanding in designating the tissue of origin of lung cancers [18] and revealed significant differences of lung cancer in both genders [19]. Additionally, success has been achieved in the diagnostics of cancers with unclear site of origin [20,21]. Following successful treatment of lung cancer, gene expression profiling and the analysis of changes are crucial for differentiation between a new primary site and a metastasis [22,23].

The first clinical applications of gene expression profiling could be: differentiating between cancer subclasses, determining possible prognosis, predicting the tumour response for different treatment methods, early (non invasive) diagnosis of cancer through detection of suitable markers, and the discovery of new tumour suppressor- and/or oncogenes involved in the pathogenesis of cancer.

Gene expression profiling has three significant applications in scientific research: (1) gene expression profiles analysis of preserved tissue samples and their comparison with the data on disease progression to discover new prognosis predicting genetic markers, (2) investigating resistance or a response to a drug

Table 1. The number of NSCLC prognostic markers identified by studies performed between 2002 and 2007.

Year	Author and reference	Nr. of patients	Nr. of controls	Nr. of prognostic genes#
2002	Beer et al. [25]	86		50*
2004	Tomida et al. [29]	50	36	25
2006	Raponi et al.[13]	129		50
2006	Lu et al. [27]	metaanalysis	64	
2007	Bianchi et al. [26]	170	metaanalysis	10
2007	Chen et al. [30]	125	146	5
2007	Lau et al. [31]	147		3
2007	Zheng et al. [28]	187		2

*# prognostic genes of NSCLC; * prognostic genes of adenocarcinoma*

using gene expression patterns. This is in case of standard treatments as well as for personalized care for specific patients, (3) selection of new markers during gene expression profiling for studying and influencing signalling pathways as well as for possible mutation analysis for designing new drugs.

4. The association between gene expression and survival rate in cases of lung cancer

When analyzing gene expression and survival data for cancer and control tissues, it is possible to distinguish between the genes that set apart control and cancerous cells, to specify subclasses of cancer, and to find prognosis defining markers [24]. When using the cancer tissue expression profiles of patients who have had oncospecific treatment as a comparison, it is possible to pick out the genetic markers that are predicting treatment response. Markers that are correlating with patient survival rate have been investigated more in adenocarcinoma. In most studies, correlation has been found between markers for survival and metastasis. Some examples would include genes that affect adhesion (*APC, CDH8, DSP*), cell's mobility (*IL8RB, ENPP2, CCL2*), immune response (*CASP8, CASP10*), and apoptosis (*INHA, PSEN1, BCL2*). The possibility of differentiating between patients with high or low survival, through different number of markers, has been demonstrated repeatedly [25-27]. The first set of NSCLC prognosis predicting markers consisted of around 50 to 60 genes.

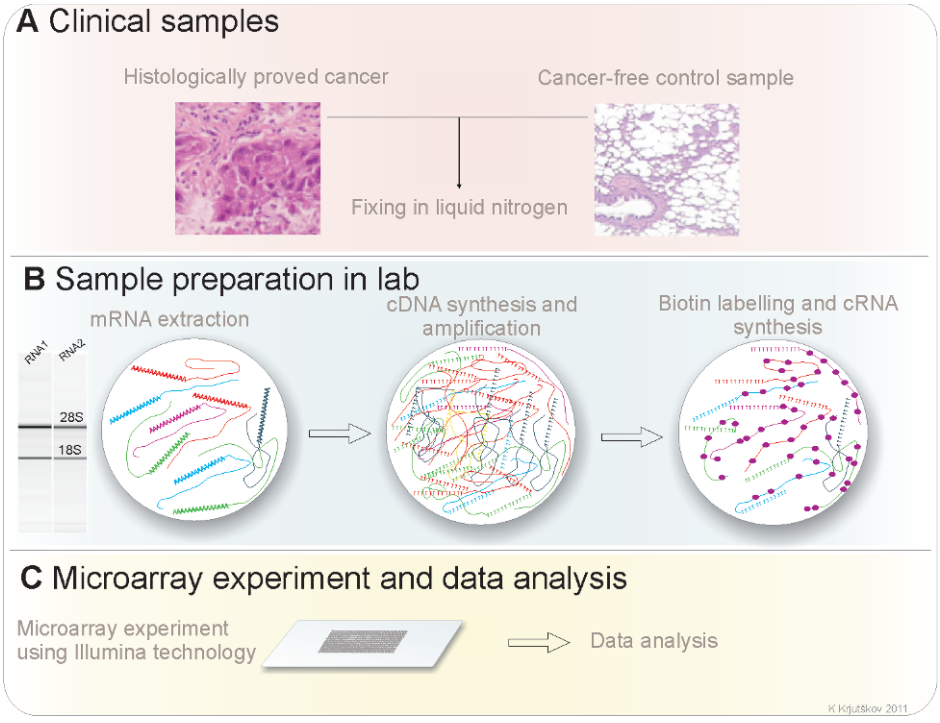
Through the increasing number of patients investigated and due to improving methodologies, the number of possible markers required for predicting disease progression has been decreasing (Table 1). In 2007, a study was published where the prognosis for survival of operated lung cancer patients was based on gene expression profiles of only two genes involved in DNA repair [28]. In everyday practice, however, it is not expedient to bring the number of markers used for prognosis to the very minimum because it is not possible to describe the whole biology of a cancerous cell based on a small number of genes. Similarly, the presence of an aberrantly expressed gene may not be the same for all cancer patients.

5. Smoking related gene expression differences in lung cancer

The main risk factor for lung cancer is smoking. Cigarette smoke includes over 60 different carcinogens, from which over 20 are strongly associated with lung cancer development [29]. 85-90% of the cases of lung cancer are associated with smoking, and only 10-15% of lung cancer cases occur in non-smokers [5]. At the same time, the cases of lung cancer that do affect non-smokers lead one to assume that the aetiology of lung cancer in smokers and non-smokers is at least partly different. About half of the lung cancer patients diagnosed in the USA are former smokers, which indicate that even after they quit smoking leaves them at risk for lung cancer. Epidemiological studies have found some dissimilarity in cancer progression of all stages between smoking and non-smoking women and men (Surveillance, Epidemiology and End Results (SEER) Program, http//seer.cancer.gov/).

A study comparing the adenocarcinoma gene expression profiles in smoking and non-smoking patients found that even though the gene expression pattern of lung cancer for the two groups is somewhat similar, the cancer of smokers has changes in genes involved in immunomodulating (*BTN2A1*), apoptosis (*SLC25A*) and signaling transfer (*ZYX*). Referring to the so called "scar cancer" theory, a notion is expressed that the

Figure 1. Gene expression profile schematic.



adenocarcinoma of non-smokers arises from relatively normal cells that have previously been damaged by infection [30].

6. Racial differences in lung cancer

It is known from epidemiological studies that several differences in histological classification in the location and progression of lung cancer exist between different races [31]. Studies in molecular biology on different races have mainly been conducted to assess known prognostic and predictive markers. The known differences have not been described in gene expression but rather in the genotype. Significant differences have been described in Asians, Europeans, African-Americans and Latin-Americans in *EGFR* and *CYP2E1, ADH3* and *GSTP1* mutations, all of which have a predictive value for treatment response [32].

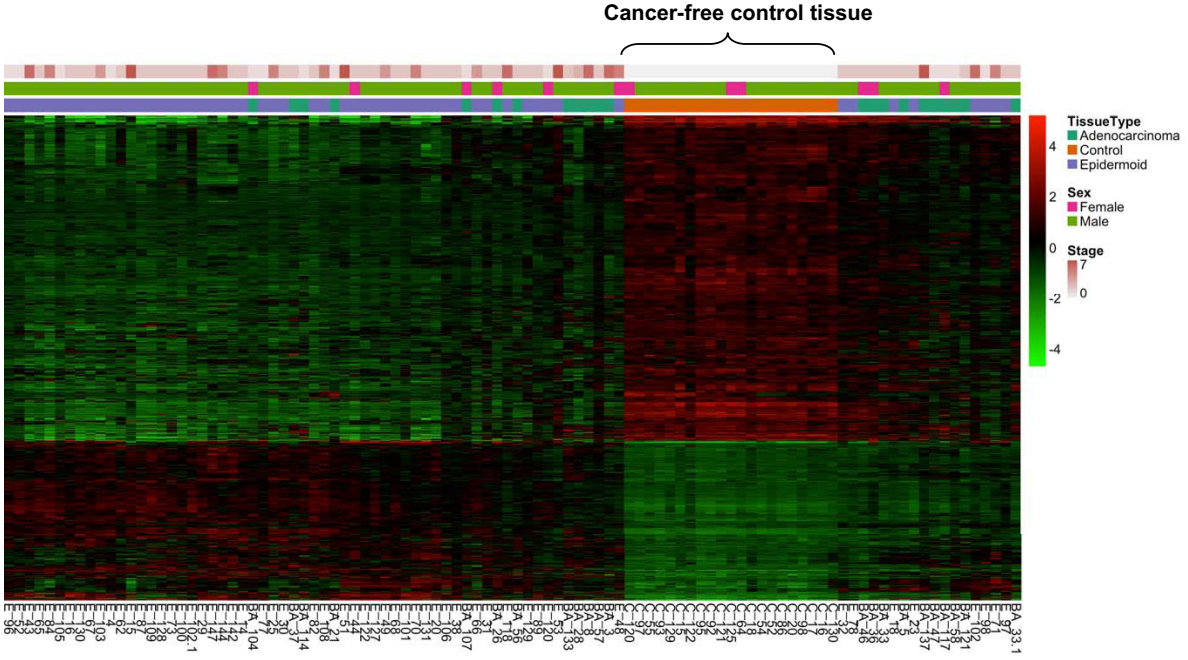
7. Sex differences of gene expression in lung cancer

The biology and progression of NSCLC is gender specific [19]. The survival rate of women with stage I to III lung cancer is better than the survival rate in men [33]. It has been demonstrated that the treatment response in women, despite the stage or type of cancer, is better that in men [19]. The treatment response is better in case of surgery, chemotherapy, as well as radiation therapy. From the histological types of cancer, the most prevalent one in women is adenocarcinoma. The gene expression profile studies have shown that only 7-8 genes have significantly different expression between the lung cancer in women and men. All of these genes are located on sex chromosomes (1 in X chromosome and 6 in Y chromosome), and most of these do not have a direct role in NSCLC [34]. It has been found that the DNA reparation ability is lower in women than men, which could partly explain the better treatment response to the standard drugs used for lung cancer treatment [35,36].

8. Selecting biological samples for gene expression profiles on cancer

In the case of lung cancer, cancerous tissue and blood are selected, from which RNA and/or DNA is extracted for gene expression analysis. Analysis on DNA is suitable for clinical practice due to its good storage life and stability. On the other hand, the main limitation for an analysis based on DNA is that information received based on the genome sequence is often too general.

Figure 2. A heatmap demonstrating the differences in gene expression between NSCLC and healthy control tissue. Green marks the down-regulated areas and red the up-regulated markers. Two cancer groups are clearly differentiated from the control group. On the left are mainly squamous cell carcinoma and bronchioalveolar cancers and on the right are adenocarcinomas and squamous cell carcinoma. Sex and stage of cancer do not differentiate in this experiment.



Studying RNA is more informative, but RNA application in everyday clinical practice is much more complicated, as RNA degrades much more rapidly. Also, the amount of tissue needed is relatively large (tissue sample of 5x5 mm) and studies based on RNA are still relatively time consuming (2 days).

There is existing evidence on the possibility to extract RNA in acceptable amounts and quality from relatively old tissue samples, which are formalin-fixed and paraffin-embedded [37]. However, the routine applicability of retrospective gene expression analysis of ordinarily preserved tissue samples remains a challenge.

9. Gene expression experiment

In order to study gene expression, microarrays are used with specific sequences attached for each gene. The schematic of the experiment is shown in Figure 1. First, a sample of the tissue removed during the operation and stored in liquid nitrogen is taken and the RNA is extracted from it. Based on protein coding mRNA, cDNA (complementary DNA) is synthesised and amplified, from which cRNA (complementary RNA) is synthesised. The cRNA is marked with biotin and attached on the array/chip. The labelled fragments pair up on the chip with complementary sequences, creating a stabile double helix. Thereafter, the unpaired cRNA molecules are washed off the array chip and the fluorescent signals linked to the biotin-streptavidin conjugate are measured. As the strength of the signal is directly dependent on the amount of the labelled cRNA, it is possible to estimate the level of gene expression based on the intensity of the signal. Differences in gene expression are quantified in fold changes relative to the control tissue (in common case non-cancerous tissue). The processed data can be visualized graphically on a so called *heatmap*, where the up- and down-regulated genes are coloured for example with red and green respectively (Figure 2). Analysing genes with differing gene expression allows differentiation between different types of cancer as well as the ability to determine dissimilarities between healthy control individuals.

Comparison of the survival rate and clinical data of the subclasses of cancer determined by expression profiling makes it possible to deduce the peculiarities of different prognoses and spread predicting markers. The freely available programs on the internet, such as g:profiler (HYPERLINK “http://biit.cs.ut.ee/gprofiler/index.cgi” http://biit.cs.ut.ee/gprofiler/index.cgi) or Genecodis 2 (HYPERLINK “http://genecodis.dacya.ucm.es/analysis/” http://genecodis.dacya.ucm.es/analysis/), can be applied for the analysis of the up- and down-regulated genes and processes.

For the diagnostic purpose of gene expression analysis it is not practical to use genome wide RNA

expression arrays that are prepared for scientific research purposes. This would result in a significant amount of irrelevant information due to the large number of genes on the array. Therefore, it is practical to select the minimal number of genes from which the gene expression analysis could still characterise the prognosis and possible treatment response to the expected extent. In recent studies, few genes and collections of genes have been determined for evaluation of the prognosis of lung cancer [28,38]. Additionally, research has been published on a gene collection that predicts treatment response to cisplatin, a commonly used drug for lung cancer treatment [14,39,40].

Although this article focuses on gene expression, it should be said that it is not a definitive but rather a selective study. As a result of gene expression profiles, a number of up- and down-regulated genes are determined. The epigenetic causes of these changes in activity (DNA methylation profile for instance) need to be investigated in further studies. The final proof for expression profile results would be assessment of the presence of the protein encoded for and/or verification of its function.

10. Discussion and conclusions

The reasons for gene expression profiling study are myriad. Foremost, the objective is to find markers, or a collection of them, suitable for specific molecular diagnostics in lung cancer. From a longer perspective, this will also apply for other cancerous tumours. At first, histologically complex types of cancer need to be differentiated and their tissue of origin must be determined based on molecular characteristics. It is possible that in the future a B, standing for the biological assessment of the tumour, will be added to the TNM system currently used. Treatment practice would be directly affected by a determined marker collection that allows for a predictive response to commonly used treatments and for an assessment of disease progression. Despite the fact that some markers can be both predictive as well as prognostic, the collection of markers should not contain 1 or 2 but rather 20 to 200 different markers. It is possible to develop several diagnostic microchips with different sets of markers for tumours with overlapping indications. A good example of such a chip already commercially available exists in case of breast cancer: Oncotype DX and MammaPrint, with 21 and 70 genetic markers respectively, and both are of prognostic value.

The use of a specific assessment of a cancer-free resection line needed in everyday onco-surgery using molecular markers can definitely not be excluded, but due to the time consuming nature of such a study and the amount of study material needed it will for the time being remain a possibility for the future.

Overall, it can be said that lung cancer is an aggressive disease with a poor prognosis in most cases. However, it is also a disease that to a great extent is preventable. Based on this review, it can be stated that for lung cancer diagnosis, in addition to classical, histological and topic diagnosis, a component describing the RNA expression of the cancer cell should be added. Information gained from genome wide expression profiles provides a good overview on the processes transpiring in a cancer cell and aids in predicting the prognosis as well as the possible treatment response for the disease. Gene expression for scientific purposes has been studied long enough to be fully implemented in a clinical setting for the diagnosis and treatment of real cancer patients.

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