Investigation of key miRNAs and potential mechanisms in non-small cell lung cancer development from chronic obstructive pulmonary disease

Tuba Denkçeken¹ and Elif Pala²

¹ Department of Biophysics, Faculty of Medicine, SANKO University, Gaziantep, Turkey
² Department of Medical Biology, Faculty of Medicine, SANKO University, Gaziantep, Turkey

Abstract. Lung cancer (LC) is the prominent cause of cancer-related death worldwide, and non-small cell lung cancer (NSCLC) represents approximately 85% of all diagnosed LC cases. It is stated that LC and chronic obstructive pulmonary disease (COPD) are directly linked at a molecular genetics level. Early diagnosis of LC is important for individuals affected by COPD. This study aims to construct a molecular network to discover molecules in NSCLC development from COPD. We downloaded the expression profiles of COPD patients from Gene Expression Omnibus database. The Database Annotation for Visualization and Integrated Discovery tool was utilized for enrichment analysis; STRING and Cytoscape were used for network construction. 15 hub genes were detected among 1517 differentially expressed genes (DEGs). Additionally, 20 differentially expressed miRNAs were identified from five datasets. We constructed miRNA-mRNA regulatory network between the groups of overlapping predicted target genes/DEGs and miRNAs that contained miRNA-mRNA pairs. UALCAN and OncomiR web-portals were used to validate hub genes and miRNAs in NSCLC. JUN, IL6, CD4 and hsa-miR-497-5p, hsa-miR-130b-5p were verified in both lung adenocarcinomas and lung squamous cell carcinomas. This study presents potential biomarkers and mechanisms underlying NSCLC development from COPD that would be targeted for early intervention.

Key words: COPD — NSCLC — Functional enrichment analysis — Protein-protein interaction — miRNA-mRNA regulatory network

Abbreviations: BP, biological processes; COPD, chronic obstructive pulmonary disease; DAVID, Database annotation for visualization and integrated discovery; DEGs, differentially expressed genes; DEMs, differentially expressed miRNAs; GEO, Gene Expression Omnibus; GO, gene ontology; IRGs, immune-response related genes; KEGG, Kyoto encyclopedia of genes and genomes; LC, lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinomas; MCODE, molecular complex detection; NCBI, National center for biotechnology information; NSCLC, non-small cell lung cancer; PPI, protein-protein interaction; TCGA, The cancer genome atlas.

Introduction

Lung cancer (LC) is one of the leading causes of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all diagnosed LCs and there are two main histological subtypes: lung adenocarcinoma (LUAD) and lung squamous cell carcinomas (LUSC) (Team NLSTR 2011). It is provided that LC and chronic obstructive pulmonary disease (COPD) are directly associated with molecular genetics level (Young and Hopkins 2011) and it was shown that 40–70% of patients with LC have COPD (Anthonisen et al. 2005). Therefore, early diagnosis of LC is important for COPD patients and there is a need for clinical biomarkers that reveal the risk of increased cancer development.

MicroRNAs (miRNAs) are small non-coding oligonucleotides capable of negatively regulating expression of mRNAs by inhibiting protein translation (Ma and Weinberg...
miRNAs participate in various biological processes and depending on their miRNA profiles, tumor cells can be distinguished from normal cells (Calin and Croce 2006). In addition, tumor cells can release the miRNAs in circulation in such a way that they can be detected in body fluids (Mitchell et al. 2008). Increased studies confirm the potential role of miRNAs as disease-specific biomarkers, which is promising for diagnostic, preventive, or therapeutic targets (Arroyo et al. 2011; Chan et al. 2013). Due to miRNAs high stability, strong specificity, high sensitivity, and detection easily in blood, they have been implicated in a variety of lung diseases (Tzortzaki et al. 2013).

Numerous public resources have been installed such as Gene Expression Omnibus (GEO) of National Center for Biotechnology Information (NCBI) with the improvement of high-throughput microarray and sequencing technology. Bioinformatics analyses based on the GEO present valuable data for searching biomarkers in several diseases (Wang et al. 2016; Manchia et al. 2017). However, to the best of our knowledge, there is no study available that have been reported on the bioinformatics-based identification of potential biomarkers concerning NSCLC development from COPD.

In our study, we aimed to find key genes and miRNAs from GEO datasets that could play an important role in the development of NSCLC from COPD patients by establishing gene ontology (GO), pathway enrichment, protein-protein interaction (PPI) network and miRNA-gene network. UALCAN and OncomiR web-portals were utilized to validate the determined hub genes and miRNAs in NSCLC.

Selection and inclusion criteria of studies

We examined the GEO database (https://www.ncbi.nlm.nih.gov/geo/) by using the following keywords: "chronic obstructive pulmonary disease OR COPD" (study keyword), "Homo sapiens" (organism), "Expression profiling by array" (study type). Besides, available datasets for related miRNAs were searched using the following keywords: "chronic obstructive pulmonary disease OR COPD", "miRNA", "Homo sapiens". The inclusion criteria were peripheral blood samples of COPD patients compared with control, and sufficient information to perform the analysis. Then, six datasets were collected for analysis. The bioinformatics workflow with the followed steps is depicted in Fig. 1.

Microarray data and data processing

One mRNA and five miRNA expression profiles were downloaded from the GEO database. The included miRNA expression profiles were GSE31568, GSE61741, GSE70080, GSE24709 and GSE102915, which consist of 24 COPD/70 control, 47 COPD/94 control, 16 COPD/16 control, 24 COPD/19 control, and 6 COPD/6 control samples respectively and one included mRNA expression profile GSE94916 dataset consists of six COPD and six control samples. We compared two groups of samples in every dataset to determine differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs). The comparison was performed by limma Rpackage based online program, GEO2R (http://
www.ncbi.nlm.nih.gov/geo/geo2r/) according to the cut-off criteria \( p < 0.05 \) and fold change > 2. According to these criteria, DEMs detected in two or more of the five datasets were considered as significant.

**Functional enrichment analysis**

The Database Annotation for Visualization and Integrated Discovery (DAVID) is a program that exhibits functional annotation of the huge amount of genes obtained from several genomic resources (Huang et al. 2008). We used the DAVID database to implement GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on significant DEGs. The species was limited to “Homo sapiens” and the \( p < 0.05 \) cut-off was considered as significant.

**PPI network construction and analysis of modules**

The STRING database (http://string-db.org/) is online software that aims to present a crucial estimation and combination of protein-protein interactions, including physical and functional relationships (Szklarczyk et al. 2019). Cytoscape is open-source software, used for the visual investigation of biomedical networks comprised of protein, gene, and other types of interactions (Shannon et al. 2003). The DEGs were plotted to STRING with a confidence score > 0.7 as a cut-off criterion to estimate the PPI information, and then interactions were visualized with Cytoscape. The genes with a node degree ≥ 25 were considered as hub genes. Next, the Molecular Complex Detection (MCODE) plug-in was used to screen modules of hub genes (Bader and Hogue 2003). Modules with MCODE scores > 5 and number of nodes > 10 were selected as significant. Moreover, the functional and pathway enrichment analyses of DEGs in mostly significant module were conducted by DAVID.

**miRNA-gene network construction**

All miRNA names were standardized according to miRBase v22 by using miRNAme Converter available in Bioconductor R package (Haunsberger et al. 2016). Then, MultiMiR package (http://multimir.ucdenver.edu/) includes 14 databases which were used to predict targets of miRNAs with the criterion of primary score listed in top 35 (Ru et al. 2014). Genes obtained by minimum three predicted algorithms were chosen for the following analysis. We subsequently selected the overlapping genes of significant DEMs-mRNA and the DEGs data. The miRNA-mRNA networks were visualized by Cytoscape. The combination of miRNAs and genes with degree ≥ 3 in miRNA-gene network and hub genes detected by PPI&CytoScape were considered as potential key genes.

**Validation analysis**

The identified potential key genes from GEO datasets were searched and verified in LUSC and LUAD based on The Cancer Genome Atlas (TCGA) datasets. UALCAN is an interactive web resource used for analyzing cancer transcriptome data which allows users to define biomarkers and provides publication-quality graphs and plots illustrating gene expression (Chandrashekar et al. 2017). \( p < 0.05 \) cut-off was considered as significance criterion. Also, OncomiR WashU Pan-Cancer miRNome Atlas was used for miRNA validation which is freely available to all users in which aligned and normalized miRNA-seq and RNA-seq data were obtained from TCGA. It enables the statistical analysis of DEMs for each cancer type (Wong et al. 2018).

**Results**

**Identification of DEGs and DEMs**

Gene and miRNA expression data were obtained from the GEO database. Following the GEO2R analysis, 1517 DEGs were extracted from the expression profile dataset GSE94916 of which were 31 upregulated and 1486 downregulated (\( p < 0.05 \) and \(|\log\text{FC}| > 2.0\)). Besides, the miRNA profile datasets were analyzed to screen DEMs in COPD using the GEO2R tool. Totally 20 DEMs (seven upregulated and 13 downregulated) were identified which appeared in at least two datasets and matched the cut-off criteria (\( p < 0.05 \) and \(|\log\text{FC}| > 2.0\)). They all showed consistent expression patterns in different datasets.

**Functional enrichment analysis**

For the DEGs, we listed top five statistically significant enriched GO terms on biological processes (BP), and KEGG pathways (\( p < 0.05 \)) (Fig. 2).

**PPI network and identification of hub genes**

The DEGs were used to set the PPI network by STRING, which composed of 1158 nodes and 1789 edges. Subsequently, we analyzed the STRING results using Cytoscape and 15 genes in the PPI network were identified as hub genes (degree ≥ 25). These hub genes included TP53, JUN, IL6, LCK, PLCG1, CD3G, CD3D, IL4, CD4, CCR7, CD3E, ZAP70, CTLA4, GNB5, and CD28. To further understand the interaction of 15 hub genes, the PPI network of them was constructed by STRING, which composed of 15 nodes and 48 edges (Fig. 3). We identified four clusters from the PPI network using MCODE. According to their degree of importance, the most important cluster that consists of
41 nodes and 269 edges was selected for further analysis. KEGG pathway enrichment analysis of the genes involved in this cluster was performed by DAVID (Fig.4). The pathway enrichment analysis showed that the genes were mostly enriched in T cell receptor signaling pathway, primary immunodeficiency, intestinal immune network for IgA production, Staphylococcus aureus infection and HTLV-I infection.

**Construction of the miRNA-gene regulatory network**

The overlapping mRNAs of the miRNA-target gene predictions and DEGs in GSE94916 were determined and these overlapped 255 DEGs were used to construct the regulatory network. The miRNAs with no targets were excluded and inversely correlated miRNA-target gene regulatory network was constructed. The remaining nine

---

**Figure 2.** Enriched gene ontology terms of top five differentially expressed genes obtained from the DAVID of biological processes (A) and KEGG pathway (B).

**Figure 3.** Protein-protein interaction network of 15 hub genes.
miRNA and 82 mRNA made 93 miRNA-mRNA pairs. The relationship between miRNAs and mRNAs is shown in Fig. 5. miRNAs; hsa-miR-1299, hsa-miR-556-3p, hsa-miR-1246, hsa-miR-1258, hsa-miR-130b-5p, hsa-miR-497-5p, and FLT3 showed degree ≥ 3 in the miRNA-gene network. These results were combined with the hub genes and considered to be potential key genes in developing NSCLC from COPD.

**TCGA verification of potential key genes**

TCGA data of LUAD and LUSC patients were used via the UALCAN data portal and OncomiR WashU Pan-Cancer miRNome Atlas to demonstrate the aberrant expression of potential key genes. Considering the cut-off criterion of $p < 0.05$ and the fact that our genes and miRNAs show the same expression pattern in all GEO, UALCAN and OncomiR WashU Pan-Cancer miRNome Atlas datasets; *JUN*, *IL6*, *CD4* genes (Fig. 6) and hsa-miR-497-5p, hsa-miR-130b-5p (Table 1) miRNAs were found to be significant in LUAD and LUSC.

**Table 1.** The $p$-values of the detected miRNAs in both cancer type LUAD and LUSC obtained from OncomiR web-portal

| miRNA            | Cancer type | $p$-value     |
|------------------|-------------|---------------|
| hsa-miR-497-5p   | LUAD        | $1.33 \times 10^{-2}$ |
|                  | LUSC        | $8.79 \times 10^{-10}$ |
| hsa-miR-130b-5p  | LUAD        | $8.71 \times 10^{-10}$ |
|                  | LUSC        | $2.54 \times 10^{-17}$ |

**Discussion**

The morbidity and mortality of LC are both relatively high among the cancers (Shen et al. 2016) and various epidemiological studies, including LC screening trials, have determined 2–4 fold increase in LC risk in COPD patients when compared to control (Gonzalez et al. 2016). With well-developed microarray technology, it is easier to identify the genetic changes underlying the development of NSCLC from COPD patients. Also, by using bioinformatics tools, it is possible to identify new biomarkers and establish networks.

![Figure 4. A. The most important module generated by MCODE. B. KEGG pathway in the module of A.](image-url)
that could be helpful to determine the relationship between these two diseases.

In our study, pathway enrichment analysis revealed that the hematopoietic cell lineage pathway was mostly enriched. It was demonstrated that this pathway is one of the key pathways in occurrence and migration in NSCLC (Li et al. 2016) also in COPD (Bi et al. 2015). Additionally, four clusters were acquired from the PPI network using MCODE. T cell receptor signaling pathway is the most enriched pathway in the highest significant cluster. It was also associated with COPD (Cruickshank-Quinn et al. 2018) and NSCLC (Chen et al. 2017) in some other studies. By constructing PPI, among 1517 of DEGs, 15 genes were identified as hub genes in COPD according to their high degrees in the network. JUN (degree = 41), IL-6 (degree = 41) and CD4 (degree = 29) were validated in LUAD and LUSC by using UALCAN. Jun proto-oncogene, activator protein-1 transcription factor subunit (JUN), is important for cell proliferation, survival, and apoptosis, and was reported to be a crucial contributing factor for tumorigenesis due to its downregulation in numerous types of human cancer (Fan and Ye 2018). IL-6 is one of the most important regulators of the cytokine-related tumor biology (Łukaszewicz et al. 2007). CD4 is a membrane glycoprotein and associated with the T-cell receptor signaling pathway (Kohm et al. 2002). CD4 T cells and macrophages are the crucial immune cells that mediate senescence surveillance of pre-malignant cells. Cells become malignant when they escape from senescence surveillance and progress further during tumor development, and then go through cancer surveillance. CD4 and CD8 T cell responses play a pivotal role in mediating the elimination of malignant cells (Ostroumov et al. 2018). Chen et al. (2017) searched the roles of immune-response related genes (IRGs) in lung cancer progression and found different expression profiles of IRGs in LUAD and LUSC but it is still unclear the precision mechanism of development of cancer in COPD. Evolving evidence has shown that the dysregulation of miRNAs is an important component of the pathogenesis of different cancers, including NSCLC. miRNAs regulate the expression of most genes and create a complex expression regulation network that interacts tightly with known gene regulatory networks. In this study, 20 DEMs were identified from five microarray datasets due to our cut-off criterion, of which seven were upregulated and 13 were downregulated. miRNA-gene regulatory network was constructed between targets of these miRNAs that overlap to DEGs which made 93 miRNA-mRNA pairs. hsa-miR-1299, hsa-miR-556-3p, hsa-miR-1246, hsa-miR-1258, hsa-miR-130b-5p, hsa-miR-497-5p and FLT3 were considered to be significant (degree ≥ 3) and hsa-miR-497-5p (degree = 3) and hsa-miR-130b-5p (degree = 3) were statistically significant according to the OncomiR WashU Pan-Cancer miRNome Atlas in both
miRNAs on NSCLC development from COPD

Figure 6. TCGA dataset analysis of JUN, IL6, and CD4 expression in LUAD (lung adenocarcinoma; A) and LUSC (lung squamous cell carcinoma; B).
LUAD and LUSC. Abnormal expression and function of miR-497 have been presented in different types of cancer (Hu et al. 2016; Pengcheng et al. 2017). Besides, there are some reports concerning NSCLC and miR-497-5p (Huang et al. 2019; Li et al. 2019). These two studies concluded that miR-497-5p is a tumor suppressor miRNA and exhibit its potential use in the treatment of human NSCLC in the future. miR-130b was downregulated in cancer tissues, and they acted as anti-tumor miRNA in different types of cancer (Wang et al. 2014; Ramalho-Carvalho et al. 2017). Furthermore, the importance of miR-130b was also determined in NSCLC (Mitra et al. 2014).

In this study, we applied bioinformatics analysis to identify key genes and miRNAs that may be used as prognostic biomarkers in COPD patients. In conclusion; JUN, IIL6, and CD4 hub genes and additionally hsa-miR-497-5p and hsa-miR-130b-5p were determined in COPD were validated in both LUAD and LUSC. This bioinformatics analysis contributed a comprehensive view to understand the mechanism underlying NSCLC development from COPD patients.

Disclosures. There is no a potential conflict of interest between the authors.

Author contributions. Tuba Denkçeken and Elif Pala designed the study, extracted corresponding data, prepared and approved the manuscript for submission.

References

Anthonisen NR, Skeans MA, Wise RA, Manfreda J, Kanner RE, Connnett JE. Lung Health Study Research G (2005): The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. Ann. Intern. Med. 142, 233-239 https://doi.org/10.7326/0003-4819-142-4-200502150-00005

Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL et al. (2011): Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA 108, 5003-5008 https://doi.org/10.1073/pnas.1019055108

Bader GD, Hogue CW (2003): An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 4, 2 https://doi.org/10.1186/1471-2105-4-2

Bi H, Zhou J, Wu D, Gao W, Li L, Yu L, Liu F, Huang M, Adcock IM, Barnes PJ (2015): Microarray analysis of long non-coding RNAs in COPD lung tissue. Inflamm. Res. 64, 119-126 https://doi.org/10.1007/s00011-014-0790-9

Calin GA, Croce CM (2006): MicroRNA signatures in human cancers. Nat. Rev. Cancer 6, 857-866 https://doi.org/10.1038/nrc1997

Chan M, Liaw CS, Ji SM, Tan HH, Wong CY, Thike AA, Tan PH, Ho GH, Lee AS (2013): Identification of circulating microRNA signatures for breast cancer detection. Clin. Cancer Res. 19, 4477-4487 https://doi.org/10.1158/1078-0432.CCR-12-3401

Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S (2017): UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19, 649-658 https://doi.org/10.1016/j.neo.2017.05.002

Chen M, Liu X, Du J, Wang XJ, Xia L (2017): Differentiated regulation of immune-response related genes between LUAD and LUSC subtypes of lung cancers. Oncotarget 8, 133-144 https://doi.org/10.18632/oncotarget.13346

Cruickshank-Quinn CI, Jacobson S, Hughes G, Powell RL, Petrache I, Kechris K, Bowler R, Reisdorph N (2018): Metabolomics and transcriptomics pathway approach reveals outcome-specific perturbations in COPD. Sci. Rep. 8, 17132 https://doi.org/10.1038/s41598-018-35372-w

Fan W, Ye G (2018): Microarray analysis for the identification of specific proteins and functional modules involved in the process of hepatocellular carcinoma originating from cirrhotic liver. Mol. Med. Rep. 17, 5619-5626 https://doi.org/10.3892/mmr.2018.8555

Gonzalez J, Marin M, Sanchez-Salcedo P, Zuñeta JJ (2016): Lung cancer screening in patients with chronic obstructive pulmonary disease. Ann. Transl. Med. 4, 160 https://doi.org/10.21037/atm.2016.03.57

Haunsberger SJ, Connolly NM, Prehn JH (2016): miRNAme-Converter: an R/bioconductor package for translating mature miRNA names to different miRBase versions. Bioinformatics 33, 592-593 https://doi.org/10.1093/bioinformatics/btw660

Hu J, Xu JF, Ge WL (2016): MiR-497 enhances metastasis of oral squamous cell carcinoma through SMAD7 suppression. Am. J. Transl. Res. 8, 3023-3031

Huang DW, Sherman BT, Lempicki RA (2008): Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44-57 https://doi.org/10.1038/nprot.2008.211

Huang X, Wang L, Liu W, Li F (2019): MicroRNA-497-5p inhibits proliferation and invasion of non-small cell lung cancer by regulating FGF2. Oncol. Lett. 17, 3425-3431 https://doi.org/10.3892/ol.2019.9954

Kohm AP, Carpenter PA, Anger HA, Miller SD (2002): Cutting edge: CD4+ CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. J. Immunol. 169, 4712-4716 https://doi.org/10.4049/jimmunol.169.9.4712

Li G, Gao Y, Cui Y, Zhang T, Cui R, Jiang Y, Shi J (2016): Overexpression of CD44 is associated with the occurrence and migration of non-small cell lung cancer. Mol. Med. Rep. 14, 3159-3167 https://doi.org/10.3892/mmr.2016.5636

Li G, Wang K, Wang J, Qin S, Sun X, Ren H (2019): miR-497-5p inhibits tumor cell growth and invasion by targeting SOX5 in non-small-cell lung cancer. J. Cell Biochem. 120, 10587-10595 https://doi.org/10.1002/jcb.28345

Denkçeken and Pala
Łukaszewicz M, Mroczko B, Szmitkowski M (2007): Clinical significance of interleukin-6 (IL-6) as a prognostic factor of cancer disease. Pol. Arch. Med. Wewn. 117, 247-251
https://doi.org/10.20452/pamw.144

Ma L, Weinberg RA (2008): MicroRNAs in malignant progression. Cell Cycle 7, 570-572
https://doi.org/10.4161/cc.7.5.5547

Manchia M, Piras IS, Huemeltal MJ, Pinna F, Zai CC, Kennedy JL, Carpinello B (2017): Pattern of gene expression in different stages of schizophrenia: Down-regulation of NPTX2 gene revealed by a meta-analysis of microarray datasets. Eur. Neuropsychopharmacol. 27, 1054-1063
https://doi.org/10.1016/j.euroneuro.2017.07.002

Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A et al. (2008): Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl. Acad. Sci. USA 105, 10513-10518
https://doi.org/10.1073/pnas.0804549105

Mitra R, Edmonds MD, Sun J, Zhao M, Yu H, Eischen CM, Zhao Z (2014): Reproducible combinatorial regulatory networks elucidate novel oncogenic microRNAs in non-small cell lung cancer. RNA 20, 1356-1368
https://doi.org/10.1021/rna.402754.113

Ostroumov D, Fekete-Drumus N, Saborowski M, Kühnel F, Woller N (2018): CD4 and CD8 T lymphocyte interplay in controlling tumor growth. Cell Mol. Life Sci. 75, 689-713
https://doi.org/10.1007/s00018-017-2868-7

Pengcheng S, Ziqi W, Luyao Y, Xiangwei Z, Liang L, Yuwei L, Lechen L, Wanhai X (2017): MicroRNA-497 suppresses renal cell carcinoma by targeting VEGFR-2 in ACHN cells. Bio.Rep. 37, BSR20170270
https://doi.org/10.1042/BSR20170270

Ramalho-Carvalho J, Graca I, Gomez A, Oliveira J, Henrique R, Esteller M, Jeronimo C (2017): Downregulation of miR-130b-301b cluster is mediated by aberrant promoter methylation and impairs cellular senescence in prostate cancer. J. Hematol. Oncol. 10, 43
https://doi.org/10.1186/s13045-017-0415-1

Ru Y, Kechriss KJ, Tabakoff B, Hoffman P, Radcliffe RA, Bowler R, Mahaffey S, Rossi S, Calin GA, Bemis L (2014): The multiMiR R package and database: integration of microRNA-target interactions along with their disease and drug associations. Nucleic Acids Res. 42, e133

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003): Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498-2504
https://doi.org/10.1101/gr.1239303

Shen Y, Xie Z, Yue A, Wei Q, Zhao H, Yin H, Mai W, Zhong X, Huang S (2016): Expression level of microRNA-195 in the serum of patients with gastric cancer and its relationship with the clinicopathological staging of the cancer. Eur. Rev. Med. Pharmacol. Sci. 20, 1283-1287

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P et al. (2019): STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47, D607-D613
https://doi.org/10.1093/nar/gky1131

Team NLSTR (2011): The national lung screening trial: overview and study design. Radiology 258, 243-253
https://doi.org/10.1148/radiol.10091808

Tzortzaki EG, Papí A, Neofytou E, Soulitzi N, Siafakas NM (2013): Immune and genetic mechanisms in COPD: possible targets for therapeutic interventions. Curr. Drug Targets 14, 141-148
https://doi.org/10.2174/1389450113140200002

Wang L, Zhu MJ, Ren AM, Wu HF, Han WM, Tan RY, Tu RQ (2014): A ten-microRNA signature identified from a genome-wide microRNA expression profiling in human epithelial ovarian cancer. PLoS One 9, e96472
https://doi.org/10.1371/journal.pone.0096472

Wang Y, Xue D, Li Y, Pan X, Zhang X, Kuang B, Zhou M, Li X, Xiong W, Li G, et al. (2016): The long noncoding RNA MALAT-1 is a novel biomarker in various cancers: A Meta-analysis based on the GEO database and literature. J. Cancer 7, 991-1001
https://doi.org/10.7150/jca.14663

Wong NW, Chen Y, Chen S, Wang X (2018): OncomiR: an online resource for exploring pan-cancer microRNA dysregulation. Bioinformatics 34, 713-715
https://doi.org/10.1093/bioinformatics/btx627

Young RP, Hopkins RJ (2011): COPD and lung cancer linked at a molecular genetic level. Chest 140, 266-267
https://doi.org/10.1378/chest.11-0220

Received: July 8, 2019
Final version accepted: September 24, 2019