Review Article ISSN 2639-944X

Journal of Medical - Clinical Research & Reviews

Analysis of The Application in Oncological Practice of New, Non-Invasive Rna Markers in Non-Squamous Cell Lung Carcinoma - Dissertation Project with Literature Review

Nikolay Evgeniev[1]*, Lena Marinova[2] and Nikolay Conev[3]

[1]*Department of Chemotherapy; Complex Oncology Center-Ruse, Bulgaria.*

[2]*Department of Radiotherapy; Complex Oncology Center-Ruse, Bulgaria.*

[3]*Department of Propedeutics of Internal Diseases; Medical University-Varna, Bulgaria.*

***Correspondence:**
Nikolay Evgeniev, Department of Chemotherapy, Complex Oncology Center-Ruse, Bulgaria.

Received: 02 November 2020; **Accepted:** 18 November 2020

Citation: Evgeniev N, Marinova L, Conev N. Analysis of The Application in Oncological Practice of New, Non-Invasive Rna Markers in Non-Squamous Cell Lung Carcinoma - Dissertation Project with Literature Review. J Med - Clin Res & Rev. 2020; 4(10): 1-6.

ABSTRACT

Based on the literature review of the use of miRNAs as a biomarker in oncological practice in NSCLC, in the forthcoming project we set a main goal - optimizing the early diagnosis and prognosis of this extremely socially significant, aggressive and resistant to chemotherapy and radiation neoplasm. To achieve this goal, two study groups of 40 individuals will be randomized - volunteers and patients with histologically proven NSCLC. In the group of patients with proven NSCLC, an attempt will be made to determine the degree of tumor malignancy in order to individualize drug treatment. The expected contributions from this research project are: 1) For the first time in Bulgaria a non-invasive genetic analysis is applied for the significance of miRNAs as a biomarker in the oncological practice of NSCLC.; 2) Optimization of early diagnosis of NSCLC.; 3) Reduction of NSCLC mortality through genetic risk determination and individualized drug treatment.

Keywords

Lung cancer, Non-squamous cell lung carcinoma (NSCLC), miRNAs, Biomarkers, Early diagnosis, Prognosis.

Introduction

Lung cancer (LC) is the most common cause of cancer deaths in males, accounting for 13% (1.6 million) of the total cancer cases and 18% (1.4 million) of the cancer deaths in 2008 [1]. LC is the third most common cancer for both sexes [2,3]. Over 70% of LC patients are diagnosed with an advanced clinical stage [1]. The majority of those diagnosed with an early clinical stage were found by chance in imaging studies on another occasion [4].

Non-small cell lung cancer is the leading cause of death from malignant neoplasms in the world, diagnosed in 85-90% of all malignant lung tumors. NSCLC is a heterogeneous disease - due to molecular heterogeneity at the same stage and the same histology have a different prognosis. Systemic chemotherapy in advanced NSCLC (stage IIB-IV) is based on histology (squamous versus non-squamous cell) and tumor genomics / proteomics.

Non-squamous cell (NSC) histological subtypes are divided into adenocarcinoma (acinar, papillary, adenocarcinoma with lepidic growth, solid mucosal and micro papillary carcinoma) and large cell carcinoma (clear cell and giant cell carcinoma).

Predictive factors

Adenocarcinomas have extensive expression of the enzyme thymidylate synthetase (TS) and are treated with Pemetrexed, which is a TS inhibitor. The presence of activating EGFR mutations (10% in Caucasian and 50% in Asian) requires treatment with TK inhibitors. The presence of ALK translocation (found in 2-7% of patients) requires treatment with ALK inhibitors. KRAS mutations are a predictive factor for the lack of effect of Platinum-based HT and therapy with TK inhibitors. KRAS mutations, which normally interact with EGFR mutations and ALK translocation, occur in smokers over 26%. These mutations are normally interacting with EGFR mutations and ALK translocation [5].

Prognostic factors in non-squamous cell carcinoma lung cancer (NSCLC)

- 1) Stage of the disease, 2) ECOG status or general condition of the patient; 3) Mutations in the K-RAS oncogene that occur in more than 30% of lung adenocarcinomas. They represent an unfavorable prognostic factor, directly related to reduced survival, 4) The expression of HER-2 receptors, which is found in 25% of NSCLC and is associated with a poor prognosis [6].

In the **TNM staging of the NSCLC** / VII revision of the TNM classification (TNM7), the stage grouping of several tumours has already been complemented with non-anatomic parameters (age, mitotic rate, histopathologic grade and location, among others) [7,8].

The clinical classification with TNM is based on evidence obtained prior to treatment based on: 1) Physical examination; 2) chest CT, including ribs and vertebrae; abdominal organs - liver, adrenal glands, 3) PET / CT when discussing surgical treatment or definitive radiation; Previous studies showed that the sensitivity and specificity of CT and PET for predicting malignant involvement of mediastinal lymph nodes were 60% and 81%, and 84% and 89%, respectively [9]. 4) Endoscopic examinations with biopsy: Endobronchial ultrasound-guided transbronchial needle biopsy (EBUS-TBNA) is reported to have a sensitivity of 85% and a negative predictive value of 90% [10]. A combined EBUS and oesophageal endoscopic ultrasound (EUS) allows better access to the mediastinal and hilar lymph nodes than is usually accessible by mediastinoscopy [11]; 5) Biopsies via mediastinoscopy, mediastinotomy and VATS: Mediastinoscopy has been regarded as the "gold standard" for staging of the mediastinum, but it is invasive and has limitations in accessing to the posterior and inferior mediastinal nodes. Furthermore, the sensitivity for mediastinoscopy is still only 80%-90%, and, in 10%-15% of cases, the technique returns a false-negative diagnosis [12,13]; 6) Surgical exploration; 7) Pleural / pericardial aspiration for cytology.

The NSCLC is often diagnosed at an advanced stage, and due to chemo and radiation resistance has an unfavorable prognosis (Figure 1).

The main reason for late diagnosis is the asymptomatic course of LC in the initial / I-II / clinical stages. Screening diagnostic methods such as chest radiography, high-tech computed tomography (CT) and sputum cytology are not sensitive and specific enough to diagnose BC at an early clinical stage. There are no biomarkers available to facilitate early diagnosis or to discriminate between benign and malignant nodules. MicroRNAs (miRNAs) are stable molecules that can be found and measured in peripheral blood, thus representing potential diagnostic biomarkers [14].

Microribonucleic acids (miRNAs) are RNA molecules made up of about 21-25 nucleotides, that do not encode proteins but perform an important function in regulating gene expression. Pre-miR-149 and pre-miR-196a were found to be significantly associated with OS and DFS in surgically resected patients with early NSCLC. There is currently a wealth of evidence for the involvement of

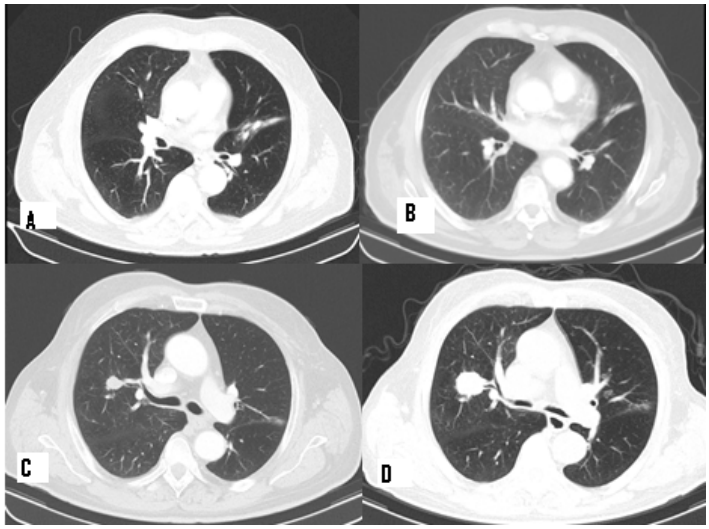

Figure 1: CT image of central adenocarcinoma of the right lung A / Before treatment; B / After 6 courses of systemic chemotherapy (SCh); C / After radiotherapy up to TD 60 Gy; D / Tumor progression 3 months after combination treatment / 6 courses of SCh and radiotherapy up to TD 60 Gy.

miRNAs in a variety of biological processes, such as regulators of cell proliferation, differentiation, apoptosis, and other processes associated with oncogenesis of lung cancer. A number of studies have shown different levels of miRNA expression in tissues, serum and other body fluids [15-17]. There is accumulated evidence for effects on the Wnt / β-catenin and phosphatidyl-inositol 3-kinase (PI3K) signaling system members (KRAS, p53, extracellular matrix regulators). On the other hand, numerous studies have recently shown significant changes in the expression levels of various miRNAs in a number of cancers, and these differences are the basis for the development of new non-protein biological markers (including prognostic and predictive) [18-23].

Literature review

Use of miRNAs as a biomarker in non-squamous cell lung cancer (NSCLC)

For diagnostic purposes: The expression profile of 10 miRNAs (miR-20a, miR-24, miR-25, miR-145, miR-152, miR-199a-5p, miR-221, miR-222, miR-223 and miR-320) have found to have significantly different expression levels in NSCLC serum samples compared with the control serum samples. In conclusion, the profiling of 10-serum miRNAs provides a novel noninvasive biomarker for NSCLC diagnosis [24]. Elevated expression levels of miR-25 and miR-223 in serum are the blood-based biomarkers of NSCLC, which can be easily detected by qRT-PCR [25]. The expression profile of miR-328 represents a potential diagnostic biomarker of NSCLC, especially for the identification of early-stage tumors [14]. Microarray profiling has showed that miR-198 was significantly downregulated in lung adenocarcinoma-associated malignant pleural effusion (LA-MPE) compared with benign pleural effusion (BPE). The present study suggests that cell-free miR-198 from patients with pleural effusion might have diagnostic potential for differentiating LA-MPE from BPE [26]. On the sputum samples of the case–control cohort, 4 (miR-21,

miR-486, miR-375 and miR-200b) of the 7 miRNAs have been selected, which in combination produced the best prediction in distinguishing lung adenocarcinoma patients from normal subjects with 80.6% sensitivity and 91.7% specificity. The marker panel in the independent populations have confirmed the sensitivity and specificity that provided a significant improvement over any single one alone. The sputum markers demonstrated the potential of translation to laboratory settings for improving the early detection of lung adenocarcinoma [27].

For prognostic purposes: The expressions of miR-146b, miR-221, let-7a, miR-155, miR-17-5p, miR-27a and miR-106a are significantly reduced in the serum of NSCLC cases, while miR-29c is significantly increased. There is a significant difference in miR expression when comparing cases and controls and find evidence that expression of let-7b is associated with prognosis in NSCLC [28]. MiR-147 is significantly down-regulated in NSCLC tissues than in paired adjacent normal tissues, and in sera of NSCLC patients than in sera of control patients. In addition, serum miR-147 is markedly down-regulated in advanced NSCLC patients and the patients with lymph node metastasis (LNM). Statistical analysis showed that patients with low serum miR-147 had much worse overall survival, and low serum miR-147 expression level was an independent prognostic factor for poor prognosis for NSCLC [15]. The overexpression of mature miR-21 in 25 (52.0%) of the 48 NSCLC paired specimens and overexpression of miR-205 in 31 (64.6%) is detected. Mature miR-21 overexpression correlates with overall survival (OS) of the patients (P =0.027), whereas overexpression of mature miR-205 do not. In conclusion, overexpression of mature miR-21 is an independent negative prognostic factor for OS in NSCLC patients [29]. Epigenetic inactivation of miR-34b/c by DNA methylation has independent prognostic value in patients with early-stage lung adenocarcinoma. Re-expression of miR-34b/c leads to a less aggressive phenotype in lung adenocarcinoma cell lines [30]. For the studies evaluating miR-21's association with clinical outcomes, the pooled HR suggested that high expression of miR-21 has a negative impact on overall survival (OS) in non-small cell lung cancer (NSCLC) (HR = 2.32[1.17–4.62], P < 0.05) and recurrence-free survival (RFS)/cancer-specific survival (CSS) in lung adenocarcinoma (HR = 2.43[1.67–3.54], P < 0.001). These results indicate that microRNAs show promising associations with prognosis in lung cancer; moreover, specific microRNAs such as miR-21 and miR-155 can predict recurrence and poor survival in NSCLC [31]. Low miR-145 expression (p=0.049), the combination of unfavourable microRNA levels (p50.02) and the combination of low miR-145 with p53 mutations (p=0.011) were independent markers of shorter time to relapse. In conclusion, miR-145 and miR-367 expression could be novel markers for relapse in surgically treated NSCLC. P53 may play a role in modulating miR-145 expression in NSCLC [32]. Increased miR-21 expression is associated with disease progression and survival in stage I lung cancer. This suggests that expression of miR-21 may contribute to lung carcinogenesis and serve as a therapeutic target or early-stage prognostic biomarker for lung adenocarcinoma. More advanced stage tumours have expressed significantly higher levels of miR-21 compared with TNM stage I tumours [33]. miR-328 has a role in conferring migratory potential to NSCLC cells working in part through PRKCA and with further corroboration in additional independent cohorts, these miRNAs may be incorporated into clinical treatment decision making to stratify NSCLC patients at higher risk for developing brain metastases (BM) [34]. Pre-miR-149 and pre-miR-196a were found to be significantly associated with OS and DFS in surgically resected patients with early NSCLC. MiR-149 and miR-196a may be involved in the pathogenesis of NSCLC, and can be used as prognostic markers for patients with surgically resected early-stage NSCLC [35]. MiR-31 was then validated as a marker for lymph node metastasis in an external validation cohort of 233 lung adenocarcinoma cases of the TCGA (P = 0.031, t test). Notably, miR-31 was a significant predictor of survival in a multivariate cox regression model even when controlling for cancer staging. Exploratory in analysis showed that low expression of miR-31 is associated with excellent survival for T2N0 patients. miRNA predicting the presence of lymph node metastasis and survival outcomes in patients of lung adenocarcinoma [36]. miRNA expression profiles correlated with survival of lung adenocarcinomas, including those classified as disease stage I. High hsa-mir-155 and low hsa-let-7a-2 expression correlated with poor survival by univariate analysis as well as multivariate analysis for hsa-mir-155. The miRNA expression signature on outcome was confirmed by real-time RT-PCR analysis of precursor miRNAs and cross validated with an independent set of adenocarcinomas. These results indicate that miRNA expression profiles are diagnostic and prognostic markers of lung cancer [37].

In order to determine the differential diagnosis between adenocarcinomas and squamous cell lung carcinomas and level of risk

Hsa-miR-205 is a highly accurate marker for lung cancer of squamous histology. The standardized diagnostic assay presented here can provide highly accurate subclassification of NSCLC patients. A microRNA-based qRT-PCR assay that measures expression of hsa-miR-205 has reached sensitivity of 96% and specificity of 90% in the identification of squamous cell lung carcinomas in an independent blinded validation set [38]. The expression profile of miRNAs may be included in the clinical treatment of high-risk NSCLC [34]. Profiling the hsa-let-7 family and hsa-miR-205 is a promising method for differentiating AD from SCC, even in such small specimens as transthoracic aspirates. Subject to the validation of these findings in further, larger studies, this could prove to be a reliable, standardizable tool for the subclassification of NSCLC [39].

BRCA1, HIF1A, DLC1, and XPO1 were each significantly associated with prognosis in early-stage lung cancer. The four coding gene classifier, alone or with miR-21 expression, may provide a clinically useful tool to identify high-risk patients and guide recommendations regarding adjuvant therapy and postoperative surveillance of patients with stage I lung adenocarcinoma [40] . Among the let-7 miRs, let-7g showed the largest fold change together with miR 26a, a hypoxia induced miR known to decrease

proapoptotic signaling. Other miRs strongly differentiating the two histology groups included miR-29a, which affects apoptosis and epigenetic normalization of NSCLC, and miR-21, which acts as an oncogene or "oncomiR" in many tumour types and plays an important role in tumour metastasis. Interestingly, in our study, miR-21 strongly differentiated the histology groups with high levels in adenocarcinoma in stage II but not in stage I tumours, suggesting that miR-21 may be a marker of tumour progression in adenocarcinoma, identifying tumours on the verge of acquiring metastatic potential [41]. Serum miRNA-34-miRNA has the potential for risk stratification in early and advanced NSCLC. This assessment is part of the COSMOS study [42] . Plasma miRNA - 24-miRNA is used to detect NSCLC with low, intermediate and high risk stratification is part of the MILD study [43]. A 14-miRNA panel has been used to differentiate early-stage lung cancer patients from individuals without lung cancer. The findings of the study suggest that the identified patterns of miRNAs may be used as a component of a minimally invasive lung cancer test, complementing imaging, sputum cytology, and biopsy tests [44] .

To predict the effect of the administered chemotherapeutic

Functionally, ectopic expression of miR- 301b enhances cell population growth, reduced apoptosis and reduced sensitivity of cells to chemotherapy. In the xenograft model, overexpression of miR- 301b promoted tumour growth. Additionally, miR- 301b and Bim expression were inversely correlated in clinical lung cancer samples. This provides new insights into the function of miRNA-301b in lung cancer and suggests that miRNA- 301b could be a potential molecular target for chemotherapy [45]. Increased expression of miR-200c, miR-203, miR-885-5p, miR-195 and miR-25 in gemcitabine resistant cell line of NSCLC are reported [46]. The correlation between high expression of miR-22 in whole blood and the lack of response in pemetrexed treated NSCLC patients indicates that miR-22 could represent a novel predictive biomarker for pemetrexed-based treatment [47].

Prognosis and determination of the degree of malignancy in lung cancer

Transformation from epithelial to mesenchymal malignant cells in lung carcinomas

The microenvironment of tumor cells induces TGFb1 and stimulates a miRNA gene expression program that induces resistance to anti-EGFR therapy and pushes lung tumor cells to transition from epithelial to mesenchymal transformation, local invasion, and distant metastases [45]. MiRNA-708 acts as an oncogene contributing to tumour growth and disease progression by directly downregulating TMEM88, a negative regulator of the Wnt signalling pathway in lung cancer [48]. Our study is the first report to connect miR-182 to lung cancer. Our results also show that restoration of tumour suppressor hsa-miR-145 inhibits cancer cell growth in EGFR mutant lung adenocarcinoma. Further study on these specific differentially expressed miRNAs may provide important information on peculiar tumourigenetic pathways and may identify useful biomarkers [49]. Expression of 13 miRNA genes predicts response to EGFR inhibition in cancer cell lines and tumours, and discriminates primary from metastatic tumours. Signature genes target proteins that are enriched for epithelial-to-mesenchymal transition (EMT) genes. Epithelial-to-mesenchymal transition predicts EGFR inhibitor resistance and metastatic behaviour. The EMT transcription factor, ZEB1, shows altered expression in erlotinib-sensitive NSCLC and PDAC, where many signature miRNA genes are upregulated. Ectopic expression of mir-200c alters expression of EMT proteins, sensitivity to erlotinib, and migration in lung cells [17]. The DNA methylation of mir-34b has not been associated with c-Met expression determined by immunohistochemistry, but both mir-34b methylation ($p = 0.007$) and c-Met expression ($p = 0.005$) has been significantly associated with lymphatic invasion in a multivariate analysis. The DNA methylation of mir-34b can be used as a biomarker for an invasive phenotype of lung cancer [50]. Interestingly, our study revealed that the altered proliferation in lung cancer cells is not accompanied by changes in apoptosis. Our findings provided new insight into the complex regulating pathway comprising of miR-145, EGFR, NUDT1 and other unknown factors which function in cell proliferation but not in apoptosis. Understanding miR-145's targets and its regulating pathways may lead to new therapeutic strategies for lung adenocarcinoma [51]. Conversely, RNA interference–mediated silencing miR-186 expression promoted cell-cycle progression and accelerated the proliferation of NSCLC cells. Cyclin D1 (CCND1), cyclin-dependent kinase (CDK)2, and CDK6 were each directly targeted for inhibition by miR-186 and restoring their expression reversed miR-186–mediated inhibition of cell-cycle progression. The inverse relationship between expression of miR-186 and its targets was confirmed in NSCLC xenografts and clinical specimens. Taken together, our findings established a tumour suppressive role for miR-186 in the progression of NSCLC [52].

Conclusion

Based on the above literature review of the use of miRNAs as a biomarker in oncological practice in NSCLC, in the forthcoming project we set a main goal - optimizing the early diagnosis and prognosis of this extremely socially significant, aggressive and resistant to chemotherapy and radiation neoplasm**.** To achieve this goal, two study groups of 40 individuals will be randomized - volunteers and patients with imaginatively and histologically proven NSCLC. In the group of patients with proven NSCLC, an attempt will be made to determine the degree of tumor malignancy in order to individualize drug treatment. The expected contributions from this research project are: 1 / For the first time in Bulgaria a non-invasive genetic analysis is applied for the significance of miRNAs as a biomarker in the oncological practice of NSCLC.; 2 / Optimization of early diagnosis of NSCLC.; 3 / Reduction of NSCLC mortality through genetic risk determination and individualized drug treatment.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69-90.

2. Stewart BW, Wild C. International Agency for Research on Cancer. World Health Organization. World Cancer Report 2014. Lyon, France and Geneva. Switzerland International Agency for Research on Cancer WHO Press. 2014.
3. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018 GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68: 394-424.
4. Raz DJ, Glidden DV, Odisho AY, et al. Clinical characteristics and survival of patients with surgically resected, incidentally detected lung cancer. J Thorac Oncol. 2007; 2: 125-130.
5. Luka Brcic, Marko Jakopovic, Marija Misic, et al. Analysis of the frequency of EGFR KRAS and ALK mutations in patients with lung adenocarcinoma in Croatia. Diagn Pathol. 2016; 11: 90.
6. Eun Kyung Kim, Kyung A. Kim, Chang Young Lee, et al. The frequency and clinical impact of HER2 alterations in lung adenocarcinoma. PLoS One. 2017; 12: e0171280.
7. Rami-Porta R, Giroux DJ, Goldstraw P. The new TNM classification of lung cancer in practice. Breathe. 2011; 7: 348-360.
8. Saeed Mirsadraee, Dilip Oswal, Yalda Alizadeh, et al. The 7th lung cancer TNM classification and staging system Review of the changes and implications. World J Radiol. 2012; 4: 128-134.
9. Toloza EM, Harpole L, Detterbeck F, et al. Invasive staging of non-small cell lung cancer a review of the current evidence. Chest. 2003; 123: 157S-166S.
10. Omark Petersen H, Eckardt J, Hakami A, et al. The value of mediastinal staging with endobronchial ultrasound-guided transbronchial needle aspiration in patients with lung cancer. Eur J Cardiothorac Surg. 2009; 36: 465-468.
11. Rintoul RC, Skwarski KM, Murchison JT, et al. Endobronchial and endoscopic ultrasound-guided real-time fine-needle aspiration for mediastinal staging. Eur Respir J. 2005; 25: 416-421.
12. Luke WP, Pearson FG, Todd TR, et al. Prospective evaluation of mediastinoscopy for assessment of carcinoma of the lung. J Thorac Cardiovasc Surg. 1986; 91: 53-56.
13. Coughlin M, Deslauriers J, Beaulieu M, et al. Role of mediastinoscopy in pretreatment staging of patients with primary lung cancer. Ann Thorac Surg. 1985; 40: 556-560.
14. Paola Ulivi, Giovanni Foschi, Marta Mengozzi. Peripheral Blood miR-328 Expression as a Potential Biomarker for the Early Diagnosis of NSCLC. Int. J. Mol. Sci. 2013; 14: 10332-10342.
15. Guangmin Chu, Jianbo Zhang, Xiaobing Chen, et al. Serum level of microRNA-147 as diagnostic biomarker in human nonsmall cell lung cancer. Journal of Drug Targeting. 2016; 7: 613-617.
16. Xiaogang Tan, Wenyan Qin, Liang Zhang, et al. A 5-MicroRNA Signature for Lung Squamous Cell Carcinoma Diagnosis and hsa-miR-31 for Prognosis. Clin Cancer Res. 2011; 17: 6802-6811.
17. Bryant JL, Britson J, Balko JM, et al. A microRNA gene expression signature predicts response to erlotinib in epithelial cancer cell lines and targets EMT British Journal of Cancer. 2012; 106: 148-156.
18. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990; 61: 759-767.
19. Aslam MI, Taylor K, Pringle JH, et al. MicroRNAs are novel biomarkers of colorectal cancer. Br J Surg. 2009; 96: 702-710.
20. Faber C, Kirchner T, Hlubek F. The impact of microRNAs on colorectal cancer. Virchows Arch. 2009; 454: 359-367.
21. Cho WC. MicroRNAs potential biomarkers for cancer diagnosis, prognosis and targets for therapy. Int J Biochem Cell Biol. 2010; 42: 1273-1281.
22. Slaby O, Marek Svoboda, Jaroslav Michalek, et al. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. Mol Cancer. 2009; 8: 102.
23. Webster RJ, Keith M Giles, Karina J Price, et al. Regulation of epidermal growth factor receptor signaling in human cancer cells by microRNA-7. J Biol Chem. 2009; 284: 5731-5741.
24. Xi Chen, Zhibin Hu, Wenjing Wang, et al. Identification of ten serum microRNAs from a genome-wide serum microRNA expression profile as novel non-invasive biomarkers for nonsmall cell lung cancer diagnosis. Int. J. Cancer. 2012; 130: 1620-1628.
25. Xi Chen, Yi Ba, Lijia Ma, et al. Characterization of microRNAs in serum a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Research 2008; 18: 997-1006.
26. Hye-Suk Han, Jieun Yun, Sung-nam Lim, et al. Downregulation of cell-free miR-198 as a diagnostic biomarker for lung adenocarcinoma-associated malignant pleural effusion. Int. J. Cancer. 2013; 133: 645-653.
27. Lei Yu, Nevins W. Todd, Lingxiao Xing, et al. Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers. Int. J. Cancer. 2010; 127: 2870-2878.
28. Niels HH, Heegaard, Aaron J. et al. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. Int. J. Cancer. 2012; 130: 1378-1386.
29. Athina Markou, Emily G. Tsaroucha, Loukas Kaklamanis, et al. Prognostic Value of Mature MicroRNA-21and MicroRNA-205 Overexpression in Non-Small Cell Lung Cancer by Quantitative Real-Time RT-PCR. Clinical Chemistry. 2008; 54: 1696-1704.
30. Miaomiao Yang, Hongchang Shen, Chen Qiu, et al. High expression of miR-21 and miR-155 predicts recurrence and unfavourable survival in non-small cell lung cancer. European Journal of Cancer. 2013; 49: 604-615.
31. Ernest Nadal, Guoan Chen, Marc Gallegos, et al. Epigenetic Inactivation of microRNA-34b/c Predicts Poor Disease-Free Survival in Early-Stage Lung Adenocarcinoma. Clin Cancer Res. 2013; 19: 6842-6852.
32. Marc Campayo, Alfons Navarro, Nuria Vinolas, et al. Low miR-145 and high miR-367 are associated with unfavourable prognosis in resected nonsmall cell lung cancer. Eur Respir J. 2013; 41: 1172-1178.

33. Motonobu Saito, Aaron J. Schetter, Steen Mollerup, et al. The Association of MicroRNA Expression with Prognosis and Progression in Early-Stage Non–Small Cell Lung Adenocarcinoma A Retrospective Analysis of Three Cohorts. Clin Cancer Res; 2011; 17: 1875-1882.

34. Shilpi Arora, Aarati R. Ranade, Nhan L. Tran, et al. MicroRNA-328 is associated with non-small cell lung cancer NSCLC brain metastasis and mediates NSCLC migration. Int J Cancer. 2011; 129: 2621-2631.

35. Jeong Hong MI, Young Choi Yi, Ji-Ae Jang, et al. Association between genetic variants in pre-microRNAs and survival of early-stage NSCLC. J Thorac Oncol. 2013; 8: 703-710.

36. Wei Meng, Zhenqing Ye, Ri Cui, et al. MicroRNA-31 Predicts the Presence of Lymph Node Metastases and Survival in Patients with Lung Adenocarcinoma. Clin Cancer Res. 2013; 19: 5423-5433.

37. Nozomu Yanaihara, Natasha Caplen, Elise Bowman, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. CANCER CELL. 2006; 9: 189-198.

38. Danit Lebanony, Hila Benjamin, Shlomit Gilad, et al. Diagnostic Assay Based on hsa-miR-205 Expression Distinguishes Squamous From Nonsquamous Non–Small-Cell Lung Carcinoma. J Clin Oncol. 2009; 27: 2030-2037.

39. Ambrogio Fassina, Rocco Cappellesso, Matteo Fassan. Classification of Non-small Cell Lung Carcinoma in Transthoracic Needle Specimens Using MicroRNA Expression Profiling. CHEST. 2011; 140: 1305-1311.

40. Ichiro Akagi, Hirokazu Okayama, Aaron J. Schetter, et al. Combination of Protein Coding and Noncoding Gene Expression as a Robust Prognostic Classi!er in Stage I Lung Adenocarcinoma. Cancer Res. 2013; 73: 3821-3832.

41. Maria Teresa Landi, Yingdong Zhao, Melissa Rotunno, et al. MicroRNA Expression Differentiates Histology and Predicts Survival of Lung Cancer. Clin Cancer Res. 2010; 16: 430-441.

42. Tammemagi MC, Lam SC, McWilliams AM, et al. Sin Incremental value of pulmonary function and sputum DNA image cytometry in lung cancer risk prediction. Cancer Prev Res. 2011; 4: 552-561.

43. de-Torres JP, Wilson DO, Sanchez-Salcedo P, et al. Lung cancer in patients with chronic obstructive pulmonary disease. Development and validation of the COPD Lung Cancer Screening Score. Am J Respir Crit Care Med. 2015; 191: 285-291.

44. Tobias Fehlmann, Mustafa Kahraman, Nicole Ludwig. Evaluating the Use of Circulating MicroRNA Profiles for Lung Cancer Detection in Symptomatic Patients. JAMA Oncol. 2020; 6: 714-723.

45. Duoguang Wu, Baishen Chen, Fei Cui, et al. Hypoxia- induced microRNA- 301b regulates apoptosis by targeting Bim in lung cancer. Cell Proliferation. 2016; 49: 476-483.

46. Hai-Hong Zhang, Zhi-Yi Zhang, Chun-Li Che, et al. Array analysis for potential biomarker of gemcitabine identification in non-small cell lung cancer cell lines. Int J Clin Exp Pathol. 2013; 6: 1734-1746.

47. Tindara Franchina, Valeria Amodeo, Giuseppe Bronte, et al. Circulating miR-22, miR-24 and miR-34a as Novel Predictive Biomarkers to Pemetrexed-Based Chemotherapy in Advanced Non-Small Cell Lung Cancer. J Cell Physiol. 2014; 229: 97-99.

48. Jin Sung Jang, Hyo-Sung Jeon, Zhifu Sun, et al. Increased miR-708 Expression in NSCLC and Its Association with Poor Survival in Lung Adenocarcinoma from Never Smokers. Clin Cancer Res. 2012; 18: 3658-3667.

49. William CS. Cho, Andrew SC. Chow, Joseph SK. Restoration of tumour suppressor hsa-miR-145 inhibits cancer cell growth in lung adenocarcinoma patients with epidermal growth factor receptor mutation. European Journal of Cancer. 2009; 5: 2197-2206.

50. Kousuke Watanabe, Noriko Emoto, Emi Hamano, et al. Genome structure-based screening identified epigenetically silenced microRNA associated with invasiveness in non-small-cell lung cancer. Int J Cancer. 2012; 130: 2580-2590.

51. William CS Cho, Andrew SC Chow, Joseph SK. MiR-145 inhibits cell proliferation of human lung adenocarcinoma by targeting EGFR and NUDT1. RNA Biology. 2011; 8: 125-131.

52. Junchao Cai, Jueheng Wu, Huizhong Zhang, et al. miR-186 Downregulation Correlates with Poor Survival in Lung Adenocarcinoma. Where It Interferes with Cell-Cycle Regulation. Cancer Res. 2012; 73: 756-766.