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Epigenetic research in lung cancer diagnostics: A literature review

Relevance: According to the International Agency for Research on Cancer (IARC), lung cancer (LC) currently ranks first in cancer incidence and mortality worldwide. The gold standard of LC diagnostics is the histological verification, the determination of the degree of invasion and tumor phenotype. At first glance, epigenetic methods seem to be secondary after determining the patient's genetic profile. However, standard genetic analysis reveals only the DNA nucleotide sequence. Thus, epigenetic analysis is the only method that allows detecting potential abnormalities in cells. An important difference between genetic and epigenetic changes is that drugs are efficient against epigenetic changes but absolutely powerless against genetic mutations.

The purpose of the study was to review and analyze the available molecular genetic methods for DNA methylation profiling in lung cancer.

Results: All these observations support the hypothesis that methylation profiling in body fluids can help determine the people predisposed to or affected by LC. Circulating acellular DNA in the blood plasma contains tumor-specific mutations and disease-related DNA methylation patterns. Identifying new biomarkers-precursors of a potential cancer susceptibility or aggressiveness in such DNA would be a considerable advancement in prognostic medicine for patients at high risk of developing LC.

Conclusion: A low level of LC detection might limit the number of DNA samples of patients with LC included in the studies. This is also the reason why specific methylation biomarkers have not yet been confirmed for clinical use. Future research on a larger number of blood samples, combined with the entire epigenome studies, may contribute to finding a group of LC biomarkers to improve LC detection.

Keywords: molecular genetic methods, epigenetics, DNA methylation, lung cancer (LC), biomarker.

Introduction: Despite the recent achievement in prevention, diagnosis, and treatment, lung cancer (LC) remains the main cause of deaths from cancer. According to the International Agency for Research on Cancer (IARC), LC currently ranks first in incidence and mortality worldwide. In 2018, 2.094 million new cases of LC (11.6% of all malignant neoplasms) were registered; the mortality amounted to 1.8 million cases (18.4% of all deaths from cancer)[1]. 5-years survival amounts to 23% in non-small cell lung cancer (NSCLC) vs. only 6% in small-cell lung cancer [2]. In the Republic of Kazakhstan, in 2018, LC ranked second in the structure of cancer incidence and first place in cancer mortality (20.5 per 100,000 population). At that, the mortality from LC has increased significantly compared to 2017 (13.7). One-year mortality in LC is the highest among other cancers and has reached 49.4% in 2017[3].

The development of molecular biology and high-tech molecular genetics made possible a one-stage evaluation of the expression of thousands of genes that control cell growth, differentiation, and apoptosis to create a tumor's molecular profile. Progressive accumulation of genetic and epigenetic changes, including point mutations, amplifications, recombination of chromosome regions, and changes in their methylation, is one of the main known causes for LC development [4, 5].

Histological verification and the determination of the tumor phenotype and degree of invasion is the gold standard in LC diagnostics. These diagnostic methods utilize a biopsy sample of the tumor tissue.

At first glance, epigenetic methods seem to be secondary to the genetic profiling of the patient. However, standard genetic analysis reveals only the nucleotide sequence of DNA, which is never violated. In turn, if a cancer-protective gene is not methylated correctly, such a violation can cause gene malfunction. The gene may not "turn on" and fails to fulfill its protective function. In this view, epigenetic analysis is the only method of choice that allows detecting potential cell abnormalities. An important difference between genetic and epigenetic changes is that epigenetic changes are susceptible to drugs that are absolutely powerless against genetic mutations.

The purpose of the study was to review and analyze the available molecular genetic methods for DNA methylation profiling in lung cancer.

Materials and methods: A search for the available literary sources was conducted in the PubMed database for 2002-2019. The keywords included "molecular genetic methods," "epigenetics," "lung cancer." Out of 56 sources found, we selected 22 literature sources, which were original scientific articles or monographs, and contained an analysis of epigenetic studies in LC. 34 out of 56 sources found were excluded as not meeting the selection criteria.

Results and Discussion:

Immune checkpoint blockade immunotherapies have turned efficient in some types of cancer, including melanoma, NSCLC, renal cell carcinoma, bladder cancer, and Hodgkin's lymphoma. Correlative data from these clinical trials clearly point to CD8 + T-cell infiltration's

role in therapeutic efficacy in tumors. CD8⁺ T cells can exert effector function through their capacity to recognize and kill tumor targets [6]. Lately, there have been many studies on circulating biomarkers as minimally invasive diagnostics. Early last century, the Nobel Prize Winner Paul Ehrlich, one of the founders of immunology, suggested an important role of the immune system in carcinogenesis. In colorectal cancer, melanoma, esophageal, and ovarian cancer, the presence of CD8⁺ T cells correlates with a better prognosis [7]. The tumor subtype is associated with a specific immune signature in DNA methylation profiling and transcripts of tumors [8].

Circulating cell-free microRNAs. Circulating cell-free microRNAs (cf-miRNAs) represent the most promising and valuable class of non-invasive molecular biomarkers for detecting several cancers, including LC. MicroRNA are the most abundant cf-RNA molecules in the blood and showed remarkable long-term stability in plasma and serum samples, making them useful biomarkers for cancer detection. A single miRNA can target hundreds of mRNAs, thus regulating the expression of many genes. miRNAs are frequently altered in cancer and were shown to regulate cancer phenotypes, including proliferation, cell migration, and apoptosis [9].

In a large meta-analysis study by Yang, which involved 6919 patients with LC and 7064 controls, cf-miRNA profiles were studied in serum, plasma, and peripheral blood. The authors concluded that circulating miRNAs could serve as non-invasive diagnostic biomarkers for all stages of LC. miRNA combinations were found to be better indicators than individual miRNAs. Further subgroup analysis showed that serum might serve as an ideal specimen for miRNAs detection in LC. The authors identified a panel of miRNAs such as miR-21-5p, miR-223-3p, miR-155-5p, and miR-126-3p that might serve as potential biomarkers for LC. However, the clinical application of miRNA profiling for LC detection still needs further validation [10].

Plasma microRNAs hold the most promise in this regard. A retrospective study has suggested a fivefold reduction in false-positive rates when using plasma microRNAs in conjunction with low-dose CT scanning (LDCT). In contrast to antibody assays, plasma microRNAs also appear to have a reasonable sensitivity, but they were not tested in diagnosing aggressive cancers missed by LDCT. Thus, there is still a need for further tests, particularly aimed at the early detection of aggressive cancers. Given that the amount of circulating tumor cells correlates with a worse prognosis, this number could be used as a biomarker for the early detection of aggressive diseases [11].

DNA methylation in T-lymphocytes. Cancer-associated immunological changes lead to epigenetic alterations of DNA in peripheral T-lymphocytes [12, 13]. In the study of DNA methylation in T-lymphocytes of peripheral blood, their role as a prognostic marker for minimally invasive LC diagnostics was determined [14].

The presence of highly sensitive and specific biomarkers in the accessible body tissues or fluids makes the test more objective. Since changes in the DNA meth-

ylation pattern are one of the earliest modifications occurring during cancer development, DNA methylation profile as a biomarker may be useful for early detection of cancer [15].

The results of similar research in breast cancer (BC) can be of interest. A genomic analysis of peripheral blood in patients with BC and the control group revealed several promising epigenetic markers for early detection of BC. For example, Jan et al. found that the CpG islands of hyaluronoglucosaminidase 2 (HYAL2) were significantly hypomethylated in the peripheral blood in patients with BC compared to the control group [15]. This data convincingly indicated that HYAL2 hypomethylation in the blood could be an early non-invasive peripheral biomarker not originating from circulating tumor DNA. They concluded that the level of HYAL2 methylation in the blood acted as an early predictor of BC compared to the control group, with a sensitivity of 64% and a specificity of 90%. Another similar study conducted on the plasma of BC patients and healthy women demonstrated the status of hypermethylation of the promoter of a member of the kinesin family 1A (KIF1A) in BC cases. Thus, they concluded that an assessment of the methylation level of the KIF1A promoter in plasma could also be an early biomarker of BC [16].

Besides, the studies of DNA methylation in twins showed that the dock protein 7 (DOK7) promoter was hypermethylated in patients' blood compared to their twins. This hypermethylation was evident several years before the diagnosis was made, so the change in DOK7 is an early change in oncogenesis. Therefore, the methylation level of the DOK7 promoter could potentially be a biomarker for the early detection of BC [17]. DNA methylation status was studied inside and outside the CpG islets of a certain number of genes, and some of them were associated with a risk of developing BC. In a study by Kloten et al., the methylation status of the TSG group (secreted protein-bound protein (SFRP1, SFRP2, SFRP5), alpha-trypsin inhibitor heavy chain family, member 5 (ITIH5), WNT factor 1 inhibitor (WIF1), inhibitor Dickkopf WNT signaling pathway 3 (DKK3) and RASSF1A) were evaluated in circulating acellular DNA [18]. CpG islets DKK3 and ITIH5 were found unmethylated in women with benign breast pathologies and significantly hypermethylated in women with BC. They hypothesized that promoter methylation of DKK3 and ITIH5 in the blood could be used as a biomarker, mainly in patients with dense breast tissue. In contrast, methylation of RASSF1A CpG islets is not a good biomarker, given its low detection rate in healthy women. Brennan et al. studied the status of DNA methylation, localized outside the CpG island clusters of ATM serine/threonine kinase (ATM) and repeating elements of the long disseminated nuclear element 1 (LINE1) in a large group of women with BC and without it using leukocytes [19]. They observed higher methylation of the ATMmvp2a locus in patients with BC compared to the control group and concluded that this locus could be used as a biomarker for BC risk. They also found that the association of ATM methylation with the risk of developing BC was more reliable in young wom-

en and that this biomarker remained stable for at least six years [20].

Kuchiba et al., who also evaluated the global profile of leukocyte DNA methylation, found that the global level of peripheral blood leukocyte DNA methylation was low in BC patients. It could be a potential biomarker for the risk of BC [20].

Another large study conducted by Ponamareva showed a significant increase in the level of methylation of the RARB2 gene in blood cirDNA of patients with LC was detected compared to healthy donors ($p < 0.05$). In cirDNA associated with the cell surface, the level of methylation of the RARB2 gene increased in the series of healthy donors (1057 ± 211 copies/ml) – patients with chronic obstructive pulmonary disease (4853 ± 606 copies/ml) – patients with LC (7524 ± 939 copies/ml) ($p < 0.05$). The methylation index of RARB2 and RASSF1A genes in blood cirDNA increased significantly in patients with LC compared to patients with chronic obstructive pulmonary disease and healthy donors ($p < 0.05$). In patients with stage III LC, the content and methylation of the RARB2 gene in blood cirDNA was statistically significantly higher than in patients with stage I-II LC ($p < 0.05$). The author also noticed that the RARB2 gene methylation index in the blood cirDNA was statistically significantly lower after the tumor removal than before treatment. In patients with LC, an increase in the concentration of methylated alleles of one or two genes during follow-up monitoring was associated with the clinical signs of disease progression [21].

In a big meta-analysis, the Turkish colleagues proposed using the long noncoding RNAs as another a potential biomarker of NSCLC since their content is particularly increased in NSCLC. Since such RNAs arise mainly due to methylation at the DNA gene level, long noncoding RNAs seem to be good markers and targets for new approaches to the treatment of NSCLC, taking into account epigenetic mechanisms. Among all types of LC, the metastatic risk is high in adenocarcinomas. Budak et al. concluded that methylated PTPRF, HOXD3, HOXD13, and CACNA1A genes could be potential biomarkers for diagnosing and treating lung adenocarcinoma [22].

In the last decade, several gene expression signatures have been established to characterize and subtype lung tumors. However, methylation is currently considered the main “player” involved in gene expression regulation. Unlike RNA transcription profiles, which are snapshots of transcriptional activity at a specific time, the DNA methylation signature is a more stable and “long-term” marker of cell molecular state and predisposition for cancer.

Consequently, all these observations support the hypothesis that methylation profiling in body fluids can help determine the predisposition to or affection by LC. Circulating acellular DNA in the blood plasma contains tumor-specific mutations and disease-related DNA methylation patterns. The identification in such DNA of new biomarkers being the precursors of potential susceptibility to or aggressive course of cancer would improve the diagnostics of patients at high risk of lung cancer development.

Minimally invasive test methods such as blood screening are more convenient and objective compared to invasive research methods.

Conclusion: A low level of LC detection might limit the number of DNA samples of patients with LC included in the studies. This is also the reason why specific methylation biomarkers have not yet been confirmed for clinical use. Future research on a larger number of blood samples, combined with the entire epigenome studies, may contribute to finding a group of LC biomarkers to improve LC detection.

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