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Update on Lung Cancer and Treatment Strategies

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Abstract

With the discovery of EGFR, it is now quite possible for the scientific world to treat patients with personalized medicine. Liquid biopsy is the invasive technique used to characterize human tumors by examining human body fluid. Different biomarkers are used to analyze tumor cells, but the most common of them is cell-free DNA. Liquid biopsy can identify tumor biomarkers to identify cancer of the lung at the start of the disease. In past studies, it was ascertained that plasma cfDNA concentration in patients with cancer is more in contrast to healthy persons. Numerous analytical ways have been synthesized to know molecular alteration through liquid biopsy. Molecular identification quantification assay as ddPCR make harmony in the detection of changes speed up against with a tumor biopsy. Different biomarkers that are used in liquid lung biopsy are Floating cfDNA and ctDNA, methylated ctDNA, CTCs in lung cancer, exosomes, TEP, and Circulating RNAs.

Key Words: cfRNA, ctDNA, Biomarkers, SCLC and NSCLC.

Introduction

Personalized medicine in lung cancer boosted with the discovery of (EGFR) mutation (Lynch et al., 2004). The successive study of the biological target that is effective than platinum doublet chemotherapy (Paez, 2004) Clears the way towards molecular chosen study with distinct tyrosine kinase inhibitors (TKIs) (McCusker et al., 2019) by this way, namouras target of oncogene are accessible in NSCLC (Lindeman et al., 2018) consequently, distinct genes are growing that is checked for right curative management of a patient. But they face only some degree of tissue that is found in the disease. Unluckily only 30 percent of the sample are insufficient for molecular diagnostics in clinical practice (Gandara et al., 2018). Instead of a lot of studies, the most common biomarkers for testing in the NLC patients, the most abundant targetable drivers, e.g., ALK and EGFR, have not always been assessed (Pennell, Arcila, Gandara and West, 2019). Liquid biopsy is the invasive technique used to characterize human tumors by examining human body fluid. Different biomarkers are used to analyze tumor cells, but the most common of them is cell-free DNA which is widely used for human lung cancer genotyping and entered in the clinical practice of resistant EGFR mutations (Rolfo et al., 2018)

As compared to tissue biopsy, liquid biopsy has more dominance than the later one. It is a minimum invasive technique; it can be done repeatedly without harming the patient and gives clear results. More ever, stage-wise results of tumors can be obtained. But the utilization of NGS (next-generation sequencing) gives detailed molecular characterization also (EGFR) variations tested +ve in Non-invasive Lung Evaluation study (Leighl et al., 2019)

Studies have correlated to the standard of tissue genotyping, explain the standard of advances in the numerated analysis of NSCLC, paving the way towards the "blood first" approach as it was "tissue first" before it. Clinical uses of liquid biopsy are increasing day by day and boosting the numerated analysis of numerous tumors, consisting of NSCLC.

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More ever, with the advancement in technology, the diagnostic techniques are improved where the detection of tumor cells was diagnosed at advance metastatic level are now detected at an early stage. Furthermore, new research is undergoing and broadening the utilization of SCLC, a violent disease in which the targeted therapy has failed.

Premature Detection of Lung Cancer by Liquid Biopsy

It is explained that liquid biopsy can identify tumor biomarkers to identify cancer of the lung at the start of the disease. Truly, various elements derived from tumors consist of protein, exosomes, CTC, cfRNA, and ctDNA detached from the body fluid to disclose molecular aspects of tumors. In past studies, it was ascertained that plasma cfDNA concentration in patients with cancer is more in contrast to healthy persons (Lipid Peroxidation Serum Levels in Breast Cancer Patients, 2016)

Furthermore, it was not only stated that the cfDNA concentration in plasma was high in non-small lung cancer patient in contrast to benign lung tumors but also stated that unity of cfDNA got from values of Ct, which is 100-bp qPCR product division by one in 400-bp, express excessive potential (68.2% specificity, 91% sensitivity) to differentiate benign lungs tumors and NSCLC (Szpechcinski et al., 2016)

Due to the less amount of detectable ctDNA in mutated ctDNA has only 50% sensitivity in early diagnosis of lung cancer, although, in the starting, protein in plasma is also advantageous to identify and recognize cancer in the lung at early stages. Hence, assessment of a mixture of sixteen leading genes mutation in ctDNA and eight rotating proteins, consisting of myeloperoxidase, osteopontin, pro-lectin, protein levels, tissue inhibitors of metalloproteinases 1, hepatocytes growth factor, cancer antigen 19-9, cancer antigen 125, and carcinoembryonic antigen called cancerSEEK, was organized to enhance early diagnose of various cancers. Cancer sought has not only the ability to diagnose early but also distinguish the original organ of cancer. It has a relatively higher rate of sensitivity which is 70% (Wang et al., 2018)

To detect cancer in the initial stage, noncoding RNAs in a fluid of the human body is also investigated. The more frequent cfRNA fragments in fluids of the samples are MiRNA, and different research stated that it is a very beneficial and non-invasive technique in case of cancer detection. Furthermore, to differentiate between benign and malignant, miRNA is used for tissue differentiation of cancer. Regardless if SCLC or NSCLC. Plasma panel A having 6 miRNAs (miR-375, miR-26b, miR-19b, miR-19a, miR-190b, and miR-17), stated great potential to differentiate the lungs cancer of a healthy donor, but plasma panel B having 3 miRNAs (miR-375, miR-190b, and miR-17) the later one defines high accuracy in differentiating among SCLC and NSCLC (Lu et al., 2018)

So that comply that non-invasive biomarkers play a significant character in identifying furthermore determine desirable treatment based on malignant and benign distinguishing and tissue discrimination. For early diagnosing of lung cancer, epigenetic biomarkers like cfDNA/RNA methylation are under consideration for non-invasive technique. So, the abnormal DNA methylation may be the result of cancer or on the other hand, cancer may be due to abnormal DNA methylation, hypo-methylation was discovered in proto-oncogenes, and hyper-methylation in the tumour-suppressor gene (Gai and Sun, 2019)

Studies show that methylation of DNA can occur at the early stage of lung cancer and can be utilized to recognize and screen lung cancer. PTGER4 and SHOX2 methylation are capable of differentiating lung cancer from the healthy person with 91 to 98% AUC (Weiss et al., 2017)

A high area under curve 0.971 was produced based on fragments from methylated cfDNA to distinguish between the start of lung cancer and healthy controls (Shen et al., 2018). Cancer cell migration and miRNA expression were mediated by cfRNA methylation (Yang et al., 2015). To diagnose malignant pleural mesothelioma, serum miR-34b/c methylation was used (Muraoka et al., 2013), and for the beginning stage diagnosis of lung cancer, cRNA methylation was used. Metabolites consist of tocopherols, carboxylic acid, and amino acid are downregulated in samples of cancer, which can be utilized to detect early-stage cancer. To differentiate the control sample with cancer cells with a specificity rate of 95% and a sensitivity of 100%, nine serum metabolites are used (Roś-Mazurczyk et al., 2017)

Experimental proof of phosphatidylethanolamines (PE) is there to differentiate malignant and benign with complete CT screening (Fahrmann et al., 2016). Using CT scans that give imaging examination is a useful technology to diagnose cancer in the beginning. But it was not used most often because of the higher rate of false-positive results and exposure to radiation. Using both

the techniques, like CT scan and non-invasive biomarkers combined, gives more accurate results than using CT scan alone. The panel of 3 miRNA from the TCGA database has a susceptibility of 81.2% to distinguish between healthy control and lung cancer, while using 2 miRNAs combined gives 89.9% sensitivity (Lin et al., 2017)

Moreover, autoantibody assays also ascertain to be able to complement CT scanning in diagnosing lung cancer as before the 5 years of lung cancer, it can be found in plasma (Ren et al., 2017). To sum up, those non-invasive techniques examined by liquid biopsy gives an easy and effective way to diagnose and screen lung cancer. Furthermore, when these techniques are combined with CT scan Screening, it will give more efficient and early-stage detection of lung cancer. So, all these have vast clinical applications.

Uses of Liquid Biopsy in the Management of Molecule Targeted Therapy

The leading innovation in non-small cell lung cancer till now is the targeting molecular remedy in disease. And the most vital therapy that is targeted is EFGR-TKIs (Epidermal Growth Factor Receptor tyrosine kinase inhibitors), and ALK receptor tyrosine kinase (ALK-TKIs), which enhanced NSCLC is compared with chemotherapy (Ricciardi et al., 2014)

Nevertheless, the resistance of drugs must not be considered unavoidable during the therapy of targeted drugs (Thress et al., 2015). The new drugs of the advanced generation can overcome drug resistance. As stated by NCCN recommendations testing deviation of the gene is necessary before selecting the appropriate treatment for drug-resistant NSCLC patients and newly diagnosed patients (Ettinger et al., 2017) But a lot of patients have not to approach to receive tumor biopsy because the method is invasive or to detect whether the gene is altered or not, the tissue of the tumor obtained is not sufficient (Coghlin et al., 2010)

About 20% of the patient, especially those who are resistant to drugs, have no means to access re-biopsy (Yoshida et al., 2016). Furthermore, heterogeneity of tumor the biopsy of the tumor in one site not capable of covering the wide outline of the genome which can be completed by genotyping of plasma as it is distributed evenly in the human body (Sundaresan et al., 2015)

Due to invasive techniques, it is difficult to monitor the treatment process dynamically. Early research also states that for testing variation of the gene, fluid samples like urine and plasma are also used and can monitor the resistance of drug during therapy process dynamically as the part of nucleic acid inside it is tumour-related and easy to get and handle (Chen, Zhao, Cui and Liu, 2016) Noted earlier ALK and EGFR are the main particles for targeting remedy.

Developments for Liquid Biopsy

Numerous analytical ways have been synthesized to know molecular alteration through liquid biopsy. The element to be considered is the susceptibility, from patient to patient; the %age of tumour-related to ctDNA differs and are mostly more restricted in most cases. Molecular identification quantification assay as ddPCR make harmony in the detection of changes speed up against with a tumor biopsy (Wang, Song and Zhang, 2016)

Normal PCR based assay is less precise than ddPCR because ddPCR use technology to divide the sample into drops with 1 or 0 DNA molecules to be developed. The results were proved by checking the signals from each part of the sample (Ma et al., 2017)

Further, the other key factor to be assessed is the specific range of the molecules. As known to all, normal PCR based assays can only mention the known driver mutations. More accurate analysis has been approved by current studies and breadth in every possible way to cover tumor-related gene mutations, in many genes from a specific part of exons or introns, up to complete genomes pattern. NGS permits absolute evaluation of precise treatment and, at the same time, can detect multiple gene mutations, targeted molecules therapy together with ALK and EGFR. (Blakely et al., 2017)

It is a special benefit of lowering the chances of mutation by using barcode technology; the sensitivity would be increased. (Ståhlberg et al., 2017). Many researchers nominate the application of NGS in clinical practices. (Rolfo et al., 2018) More work has been done to upgrade the potency of ctDNA buildup in liquid samples. Pan-cancer scale and genome-wide are used by Mouliere et al to disclose the size variance between mutant and non-mutant DNA. (Mouliere et al., 2018) ctDNA advancement subsists in parts sizes between 90 and 150 bp. Enhance the unveiled variation by selecting the parts between 90 and 150bp along with the actionable changes and number mutations. Done with the area under the curve (AUC) > 0.99 in comparison with (AUC) < 0.80 without parts selection.

Biomarkers used in Liquid Lung Biopsies

Floating cfDNA and ctDNA

In circumstances like inflammation or pregnancy, cell-free DNA is librated in the circulation system of blood by a process yet not perfectly understood (Kneip et al., 2011). Plasma DNA concentration in cancer patients is more than in healthy persons; the difference is known by DNA was taken tumor cells (ctDNA). The mechanism of transport of ctDNA is not well known, but earlier research work showed that its release mechanism might be due to rapture of a cell from apoptosis and necroses ctDNA is tumour-specific and imparts indicators of mutations and parts of DNAs of tumor cells. (Santarpia et al., 2018)

In the initial studies done by Sozzi et al. in it, a blood sample was of 7.5 ml was taken from 100 patients with treatment of non-small cell lung cancer and equated to specified controls. Lung cancer patients have plasma DNA concentration 8 times more than the one seen in match controls, so persons with greater concentrations of DNA in plasma are recognized as huge risk persons (Ponomaryova et al., 2013) ctDNA is mixed with non-tumour DNA and is mostly found highly fragmented. Ultrasensitive analytical assays are used to distinct ctDNA from cfDNA taken from the cells which are normal (Kneip et al., 2011)

In cancer cell genomes, the changes in ctDNA along with alteration in oncogenes or tumor supper genes and epigenetic alterations are present. The concentration of circulating tumor DNA corresponds to tumor life span, tumor outcomes, and tumor intensity. Novel sensible assays of blood to test circulating free DNA and circulating tumor DNA at low quantity for most gene-based deformity came to been synthesized. The increased concentration of cfDNA and ctDNA in patients with the start of lung cancer is proven by Novel studies (Newman et al., 2014). The presence of ctDNA and cfDNA acts as a base for treating patients with high-risk metastatic disease. This review is done to focus only on ctDNA in different test platforms as the main biomarker.

Methylated ctDNA

The potential tool for the recognition and protection of the ctDNA has emerged in the form of methylation of ctDNA recently. Specific tumor suppressor gene methylation in patients detected with lung cancer is mostly done nowadays. The DNA mutation is done by methylation as an advantage, but hypermethylation may initiate cancer formation. ctDNA is stable and diagnosed early in cancer initiation as correlated to other potential diagnostic biomarkers. The patients recognized with lung cancer are seen to have methylation in particular genes that are tumor control genes like CDKN2A, Ras association domain-containing protein1 (RASSF1A), Metyle Guanyl Transferase (MGMT), and Death-associated protein kinase 1(DAPK). Poor diagnosis is made if we do hypermethylation mentioned by a report (Ponomaryova et al., 2013)

In different reports, it is shown that the existence of methylated SHOX2 gene is a susceptible biomarker for lung cancer (Konecny et al., 2016). If hypermethylation is done with SHOX2 along with SCLC will cause squamous cell carcinoma. SHOX2 is also has been used as a biomarker in sputum and CSSF of the patient (Diaz-Lagares et al., 2016)

Tests that were done on bronchial fluids by a study showed that SHOX2 methylation would differentiate between benign & malignant lung cancer with a high specificity of 95% and sensitivity of 68%. The test, which is successfully proven & used as a lung cancer tool as compared to bronchial lavage specimen, is the related amount of methylated SHOX2 gene fragments detected in epigenomic assays based on TagMan technology (Kneip et al., 2011)

CTCs in Lung Cancer

In 1869 Thomas Ashworth explained CTC for the first time. He isolated blood cells from the periphery, and they resemble the primary cancer cells. It is shown that CTC comes from detachment from primary cancer cells. CTC showed an important role in spreading metastatic cancer cells (Revelo et al., 2019)

CTC is not only used as a target of various studies to treat cancer cells but also used as a prediction to observe the treatment of cancer cells (Hong, Fang & Zhang, 2016). Tumor cells in the primary stage will get some properties to invade and metastasize into far areas of tissue, and it is the first step of unrestricted cell division and angiogenesis and is a sign of cancer. (EMT) with a signalling series is a complicated process done by epithelial tumor cells make these cells enter the blood circulation and move to the peripheral target, in remote target they do reverse action called (MET) and once again do mesenchymal phenotype for more mutation and synthesis of metastases (Thiery, 2002). The diagnosis and recovery of cancers in tissues like the breast, prostate, and colon (Bredemeier et al., 2017) by segregation of CTC has been used for many years.

For DNA studies, including proteins and RNA molecules characterization, CTC will provide enough materials for this study. In the presence of CTCs, cancer-related symptoms may increase, while CTCs presence also helps in clinical diagnosis and treatment, especially in lung cancer (Mehlen & Puisieux, 2006).

These genes are present in large amounts in patients having the chemo-naïve metastatic disease; these genes ' presence is also compared with patients who are non-metastatic lung cancer (MO). The metastases and advancement of the disease and its burden correspond to the presence of CTC, so studies related to CTC have much importance in cancer disease (Hanssen et al., 2016).

If removal of primary tumor up to 45% is done instead of it, NSCLC tries to reoccur or metastasize due to the presence of CTCs. Even in the excessive reoccurrence of NSCLC, the separation of CTC from NSCLC (cancer) is much difficult work. Epithelial tumor markers and epithelial properties of tumor cells are decreased by initial EMT, and it is also its disadvantages. It also creates hurdles in diagnosing these markers by the present techniques. The anti-EpCAM ferromagnetic microbeads are used to separate CTC. The downregulation due to EpCAM during EMT decreases sensitivity and is not 100% (Craene & Berx, 2013)

The increased appearance of markers of mesenchymal in CTCs, like TWIST, vimentin, transcriptional factors Zeb-1 and N-cadherin, are due to EMT (Alix-Panabières, Mader & Pantel, 2016). In the advanced tumor stage, Mahmood et al. revealed the importance of the association between vimentin and N-cadherin (Mahmood, Ward, Muller, Sohal & Walters, 2017)

The advanced studies related to the presence of CTC in patients with breast cancer and prostate cancer done by other authors revealed that increased appearance of vimentin and N-cadherin are due to CTC. Nowadays, CTC assay is widely used instead of ctDNA for EGFR mutation recognition. The pattern of drug resistance, patient monitoring, and noticing EGFR mutations are easily made with CTC (Babayan et al., 2016). The early identification of lung cancer refers to the ability to isolation enough CTC and to point out different biomarkers from patient blood that will lead to cancer treatment at the right time.

Exosomes

The size of the exosome to be measured is 30-100nm and they are very small cellular membrane vesicles. They are released by all body cells and formed by the process called endocytosis (Zheng et al., 2018). Exosomes can be found in the fluids like blood, CSF, semen, and pleural effusions (van der Pol, Böing, Harrison, Sturk & Nieuwland, 2012)

The source of signals to be carried between cell to cell both in cancer and normal state is exosome, and it can also carry DNA and RNA between different cells (Valadi et al., 2007). Exosomes are at risk to extreme PH and prone to ribonucleases; therefore, they have a bilayer lipid membrane also to give protection to nucleic acids (mainly miRNA), so miRNA can survive for more time in contrast to cell-free miRNAs (Sourvinou, Markou & Lianidou, 2013)

The above features made exosomes important biomarkers in the premature identification of cancer. The exosomes can be separated physically by their size and densities with different centrifugation speeds. Exosomes can be separated mainly in the final stage as ultracentrifugation. In ultracentrifugation, exosomes are confined into pores of the microscopic cylindrical structure, so in this way, exosome separation is done easily. Another way to separate exosomes is density gradient. To get exosomes in pure form, we can use a nonphysical method which is immunoaffinity in polymeric-based precipitation, and in this method, beads coating of antibodies is used (Vanni, Alama, Grossi, Dal Bello & Coco, 2017)

Metastasis and cancer development are mainly related to exosomes and encourage tumorigenesis by relocating proteins, mRNA, and miRNA (Kharaziha, Ceder, Li & Panaretakis, 2012). The relocation of oncogenic factors to perform the malignant transformation in target cells is done by exosomes that have already come from cancer cells. For instance, the relocation of an estimated glomerular filtration rate to vascular endothelial cells where it can release VEGF (Abdouh et al., 2017). Two ways which undergo lung cancer promotion are (a) extinguishing propyl hydroxylase activity, (b) enhancing the agglomeration of hypoxia-inducible factor-1a (part of HIF-1), a two identical protein. These will enhance the continuity and spreading of cancer cells because of angiogenic properties. The ability of tight junction proteins will be lowered by exosomal miR-23a so the malignant cell can easily move through trans endothelial passages (Wu et al., 2016)

They enhance the ability of tumor cells to move to the periphery through blood by alerting the EMT

(Kahlert & Kalluri, 2013). Energizing EMT and enhancing its power to metastasize and appearance of vimentin in non-cancerous cells is due to exosomes in patient serum with metastatic lung cancer (Zomer et al., 2015). They have surface proteins that act as biomarkers for lung cancer, the staging of lung cancer can be done with exosomal proteins, and some exosomal tumor biomarkers are CD91, CD317, and EGFR (Siegfried, 2005)

If we compare the exosomal miRNA of a normal person with that of a lung cancer patient, then there will be large differences between these. So, miRNA will act as the early diagnostic tool in treating lung cancer. Separation of some miRNA is linked with enhanced capacity to metastasize (Liu et al., 2019)

For cancer treatment, exosomes can be used as a drug delivery system. In the body, exosomes are manufactured by all the cells and can penetrate the membrane, while they convey cellular materials like mRNA, miRNA DNA, and proteins are considered nontoxic.

TEP

The familiar function of platelets in hemostasis and they are the 2nd most common cells in the blood. Besides, its platelets also play other roles in disease processes. Platelets can alter so can stay indifferent environment, especially in lung cancer cells. Platelets can act as memory cells when they encounter tumor cells. This process is mainly guided by the movement of cancer-associated biomolecules into the platelets, so it will lead to TEP formation (McAllister & Weinberg, 2014)

In lung cancer, TEP has important clinical uses like its diagnosis and treatment monitoring. Platelets can protect CTCs and provide cancer favorable situations by doing angiogenesis and act as a source in tumor development (Joosse & Pantel, 2015). Joining of platelets RNA occurs when platelets meet the tumor cells; it permits platelets to interchange proteins and nucleic acid with tumor cells. Cancer cells can take advantage of these proteins in various stages of their life (Alhasan et al., 2016)

Platelets can move to peripheral tissues and persuade EMT and enhance the ability of tumor cells to go into the bloodstream. The cancer cells are protected by platelets in the bloodstream, so the metastasis will be decreased. Platelets in metastasis will deliver different proangiogenic and growth factors like PDGF, BFG, and VEGF, which will lead to the environment for the tumor cells to spread easily (Lakka Klement et al., 2009).

The cancer signals will be answered by platelets RNA machinery; specific mRNA will be produced in platelets by different tumors; in this way, various tumors can be diagnosed by only separating the moving platelets from distinct blood. It will lead to knowing the creator of the primary tumor with up to 70% accuracy, e.g., the study has been done to report the uptake of NSCLC biomarkers in platelets, the said biomarkers can be used for the identification of NSCLC (Best et al., 2015)

Platelets can interchange intracellular materials with tumor cells, this ability of platelets can be used as a therapeutic delivery vehicle, and some more research is needed for this ability of platelets.

Circulating RNAs

Besides exosomes, ctDNA, CTCs, and TEPs, the circulating RNAs act as essential biomarkers for liquid biopsy. Tyrosinase mRNA from peripheral blood of a patient having melanoma was first reported by Sevens et al. in 1996 and will give an idea about stages of melanoma. A clear sign of tumor genetic mutations can be taken from both microRNA (miRNA) and ctDNA, which are freely circulating. The minor administrative RNA is microRNA (miRNA) which can be deregulated in cancer; they are protected and are stable from endogenous RNAs activity. Further figures are using dispersing miRNA specifications with LDCT to enhance selectivity and to monitor disease frequency in LDCT recognize lung cancer patients.

Conclusion

The use of liquid biopsy for the examination, treatment and recommendation of non-small cell lung cancer patients is broadening day by day. In medical excellence, liquid biopsy is a convenient technique. Liquid biopsy is non-invasive as compared to tissue biopsy. Liquid biopsy is economical, and its affordability allows a repeatable analysis of the blood of a cancer patient. Liquid biopsy is with patient compliance, and the patient can be analyzed for cancer tumor in no time without disturbing his/her daily schedule. The biomarkers circulating in the bloodstream are cfDNA and ctDNA. These are the most important biomarker which is easily accessible for analysis of cancer tumor. Other biomarkers like exosomes, miRNA, TEPs, and CTCs are also present. In repercussion, many clinical trials can be done to find and analyze these biomarkers and also some other circulating biomarkers to recognize tumor biology. These biomarkers can be used to interpret the tumor protection properties against anti-tumour drugs.

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