Review Article

Biomarker: A potential novel therapeutic target for early detection of Lung cancer

Abstract:

Cancer is a deadly disease which can affect any part of our body. Many cancers like Lung cancer, Breast cancer are present but, in our article, we mainly focused on Lung Cancer which have a high mortality rate. There are 2 types of Lung cancers mainly NSCLC (non-small cell lung cancer) and SCLC (small cell lung cancer) and although treatments are available but the survival rate is still very low due to late detection of these cancers. Biomarkers are biological genes which show changes when a tumor formation takes place. Our article provides an overview on Lung cancer, treatments and mainly focuses on potential biomarkers and also suggest some futuristic ideas that can help detect Lung cancer early.

Keywords: Lung cancer, NSCLC, SCLC, Biomarkers.

1. Introduction:

One of the most common cancers prevailing in the human world is Lung Cancer. This cancer severely damages the lungs in the human body which makes the person unable to breathe while simultaneously damaging other organs of the body due to metastasis. According to a data from World Health Organization [1], In 2020 alone, around 2.21 million cases of Lung cancers were diagnosed worldwide in which 1.80 million deaths occurred at a staggering death rate of 81.44% which meant in every 5 people diagnosed, 4 people died. Lungs are nothing but a sponge like organs filled with loads of air which helps in dispersing the inhaled air from the trachea to the blood while helping in exhaling the carbon rich air from the lungs with the help of certain tubules or branches called alveoli and bronchioles. [2].

2. Tumor generation in Lungs:

Cancer in Lungs happen when nearby cells, mainly the respiratory epithelium [3] grow and divide abnormally which causes disruption to the basic function of lungs. Cells have ability to regenerate themselves which means stem cells around the lungs can be a major location for the target of cancers [4].

3. Types of Lung Cancers:

The types of Lung Cancers depend, whether it starts in Lungs (Primary Lung Cancer) or it moves to the other parts of the body (Secondary Lung Cancer). On the basis of Primary Lung cancer, there are two types i.e., non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) [5].

3.1. Non-small Lung cancer (NSCLC): This is the most common type of primary Lung Cancer which happens to about 82% of patients diagnosed with Lung cancer [6]. All epithelial Lung cancers which are not small celled comes under this section. These are further divided into 3 types i.e., Adenocarcinoma, Squamous cell carcinoma, large-cell carcinoma.

3.1.1 Adenocarcinoma: Most common out of all Lung cancers. These arise from cells of small tubules of lungs called alveoli (type II) which help in producing surfactant like mucus and also helps in repairing damaged lung epithelium [7]. It usually occurs at the sides of the lungs mainly due to smoking [8] and also possibly due to excess of inflammation. It is a malignant cancer which can metastasize to various other organs like breast, stomach etc.

3.1.2. Squamous cell carcinoma: This cancer comprises about 30% of all Lung cancers [9]. It is also a type of NSCLC which happens towards the centre of the lungs or in left or right bronchioles [10]. Smoking contributes most to the occurrence of this cancer and is malignant as well.

3.1.3. Large-cell carcinoma: These are malignant tumors with no proven relation with other NSCLC cancers. As the name suggest, these are large cells compared to other tumors due to presence of bigger cytoplasm and nuclear matrix [11].

3.2. Small-cell lung cancer (SCLC): These are less common than NSCLC and occur in about 15% of diagnosed Lung cancer patients [12]. Also known as oat cell carcinoma due to cells being very small as compared to NSCLC. According to World Health Organization (WHO), these are small due to small cytoplasm and granular nuclear protein. These cells arise from epithelial cells of lungs and are known to disrupt DNA repair mechanisms [13].

4. Metastasis:

Cancers are not always confined to one region. They have a tendency to continuously divide and grow. This gives rise to malignancy in which the tumor gets matured from a benign to a malignant tumor which has the power to invade nearby tissues and travel to other parts of the body. This translocation of the tumors from primary site to secondary site is known as metastasis. Lung cancer is no different and some neoplasms tend to metastasize to different organs such as colon, breast, uterus, brain, bone [14] to ensure rapid growth of tumors.

4.1. Metastasis of Lung Cancer to Bones: One of the common sites of lung cancer to travel and reside are the bones. The reason for travel is unknown but one hypothesis suggest that this might be because of the presence of bone marrow which act as a resource for tumors. According to Asuka Tsuya *et al* [15], patients diagnosed with bone cancer had maximum metastasis in the spine followed by

ribs, ilium and least metastatic tumor were found in the sternum and humerus at around 2.9% of total bone metastasis. One study showed that tumor cells produced many proteins such as parathyroid hormone-related protein and macrophage inflammatory protein-1-α which increased osteoclast activities. These osteoclasts secreted growth factors mainly IGF-1 and TGF-β which stimulate tumor growth [16].

4.2. Metastasis of Lung Cancer to Brain: This is another popular site for the tumors to spread in the human body. Cancers invade the blood vessels and enter the hemisphere of the brain. According to study from J. Lee Villano *et al* [17], 15-30% of patients diagnosed with Lung cancer had metastasis of the brain. Possible reasons for brain metastasis could be due to cell signaling and nerve control. As brain is considered as the command centre of our body, having a control of it can help the tumor to grow and spread to even more different organs.

5. Treatments for Lung Cancer:

Survival possibility is very less in Lung Cancer because by the time tumors are detected, cancer is already grown bigger and, in most cases, metastasized to different organs. As lungs are a vital and sensitive organ of our body, treatments can only increase the survival rate of patients diagnosed by Lung Cancer by a mere few years.
Surgery is done in case of benign tumor in which either the cancer is removed or a part of lungs.
Commonly used treatments include Chemotherapy, Immunotherapy, Targeted therapy etc.

5.1. Chemotherapy: These are nothing by chemical drugs or agents that target cancer cells and destroy them. They are made in such a way to target rapid dividing cells and since cancer cells have the tendency to grow quickly, chemotherapy act as a good treatment. Chemo drugs usually do this by either interfering with the DNA replication of cancer cells or by disrupting the process of mitosis. Drugs like cisplatin, Etoposide, Vinorelbine have been constantly used for treating NSCLC [18] but according to the study conducted by Henning Willers *et al* [19], cisplatin-based chemo drugs were more effective towards Lung cancer than any other drugs under consideration. Longer survival rates were still lower but patients with improved life span were still higher as compared to other drugs.

5.2. Immunotherapy: These act as an assister to the immune system to kill cancer cells. During carcinogenesis, our immunity which were supposed to destroy any foreign substances entering our body gets suppressed due to inactivity of T-cells. Immune drugs such as Ipilimumab, Nivolumab, Interleukin-2 [20] help in reviving these suppressed T-cells. Ipilimumab drugs usually block PD-L1 proteins [21] which are known to guide the immune system to not kill non-harmful cells. In NSCLC and SCLC, the number of PD-L1 proteins are very high due to which their cells are not harmed by T-lymphocytes while drugs like Interleukin-2 produced by CD+4 cells are cytokines that increase the number of other T-cells like NK cells (Natural Killer) and B-lymphocytes which suppress cancer [22]. According to a study conducted by Gardiner R.E *et al* [23], checkpoint inhibitors have shown good results in treating NSCLC. In combination with Electrochemotherapy or ECT, these provide enhanced survival rates in clinical trials.

5.3. Targeted therapy: A new evolved treatment under precision medicine that targets specific proteins in cancers that aid them in growing, spreading etc. These can either be small-molecule

drugs or therapeutic antibodies [24]. Targeted therapy usually attacks the signaling pathways of the tumor mainly EGFR (Epidermal growth factor receptor). EGFR are known to control proliferation and cell growth [25]. In Lung cancers mainly NSCLC and SCLC, expression of EGFR is very high due to presence of excess mutations. Mutations were likely associated with exon 19 deletions. Targeted drugs like erlotinib, and monoclonal antibodies have shown to inhibit EGFR mutations which help in destroying the cancer [26]. Crizotinib is a drug that targets fusion of ALK-EML 4 genes and MET genes [27].
These are the treatments usually used for treating NSCLC and SCLC but as we mentioned, these can only increase the survival rate by 3-4 years. Hence early detection is the key for survival. This article focuses more on biomarkers as a key tool to detect lung cancers.

6. Biomarkers:

These are bio-molecules present in our body which show signs on the onset of tumor formation. Some of known biomarkers studied by researchers are Epigenetics, CTCs, MIR4435-2HG, PD-L1 proteins etc.

6.1. Epigenetics: This is quite new and less studied concept which has the potential to detect lung cancer effectively. Our body contains genes and epigenetics help control their expressions. During lung tumor formation, certain genes either get activated or suppressed and epigenetically changing gene expression can act as a biomarker for early detection of cancer. Methods like DNA methylation (addition of methyl group), Acetylation (addition of acetyl group), Micro-RNA have been extensively studied to change the expression of genes without altering the sequence of DNA.

6.2. CTCs: Circulating tumor cells have been studied for their role in cancer progression and resistance towards anti-cancerous drugs [28]. Liquid biopsy has been used to isolate CTCs and study tumor behavior [29].

6.3. MIR4435-2HG: High expression of MIR4435-2HG in blood or serum have been closely associated with tumor growth and progression. Drugs like cisplatin, carboplatin follow these MIE4435-2HG and act as a potential biomarker [30].

6.4. PD-L1 proteins: These are commonly targeted by immune-drugs due to their high expression in cancer genes. Drugs like Atezolizumab, Avelumab acts a PD-L1 blockers [31] and act as biomarkers for detecting tumors.

LINC00665 genes have been studied as biomarkers and drugs like cisplatin in NSCLC target these genes for detecting Lung cancer [32]. Blood protein biomarkers such as autoantibodies, exosomes have shown changes during tumor formation [33]. CLDN18.2 proteins express less in normal cells but abnormally in tumor cells and act as a potential biomarker in NSCLC [34]. APJ system serve as biomarkers during onset of NSCLC and SCLC and can affect tumor microenvironment as well [35]. NNMT have effects on EGFR and anti-NNMT drugs can stop tumor progression [36]. Volatile organic compounds (VOCs) like isoprene, hexanal have shown to act as biomarkers for Lung cancer [37]. Heat shock proteins (HSPs) such Hsp60, Hsp90 can also act as biomarkers for Lung cancer patients [38].

7. Experiments by researchers on potential Biomarkers:

YongKui Zhang *et al* [39] from Zhoushan Hospital, China experimented on three miRNAs miR-29c, miR-93, and miR-429 and checked whether these can act as biomarkers for detecting Lung cancer. Resected NSCLC samples from 70 patients were stored at -80□ without any prior chemotherapy or other treatments and compared them with non-tumor cells. RNA was isolated using miRNAs isolation kit. This was followed by q-PCR for 40 cycles and statistical analysis was done using one-way ANNOVA. Result showed that expression of miR-29c, miR-93 were higher compared to non-tumor cells while expression of miR-429 were quite similar to non-cancer cells. This showed that miRNAs like miR-29c and miRNA-93 can potentially act as biomarkers for detecting NSCLC.

Another experiment was conducted by Shan Lu *et al* [40] from Cincinnati, USA on Plasma secretory phospholipase A2-IIa. Plasma samples were collected from patients of Cincinnati Hoxworth Blood Center. ELISA test was used to determine the levels in the plasma of patients. This was followed by (IHC) staining in which the slides were treated with citric-acid based antigen retrieval buffer followed by incubation in blocking buffer which contained primary antibody. Slides were washed and again incubated with biotinylated secondary antibodies. Statistical analysis was done using geometric means, standard deviations and t-tests. Results from unpaired t-test showed that sPLA2-IIa levels were higher in patients with lung cancer as compared to non-tumor cells in control. This proved sPLA2-IIa can also serve as a potential biomarker for detecting Lung tumors.

8. Conclusion and Discussion:

Lung cancer is a life-threatening disease and although many treatments have been continuously utilized, survival rate is still poor due to invasiveness and late symptoms for Lung cancer. Hence early detection is key. Biomarkers are biological molecules that are present throughout our body

that can help detect the onset of cancers. Biomarkers like circulating tumor cells (CTCs), PD-L1 proteins and many others have been extensively studied but we feel that a much more research is required to be done on Epigenetics. Methods like methylation, micro-RNAs, Phosphorylation, Ribosylation can have an effect on lung cancer genes without altering the DNA sequence and hence can potentially be a novel therapeutic target for early detection of lung cancers. Also, extensive research could be done on biomarkers to specifically detect SCLC as well.

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