Chapter 1

Introduction

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1.1 Lung cancer

Lung cancer is the most common cause of cancer-related deaths worldwide and accounting for ~18 % of all cancer deaths [1]. It is the highest diagnosed cancer sub-type in males across worldwide [2]. In the UK, lung cancer causes approximately 35,000 deaths each year with 10 % of survival rate [3]. The occurrence of the lungs and mouth cancers (tobacco-related cancers) is highest in Indian subcontinent, such as Bangladesh, Bhutan, Maldives, Nepal, Pakistan and Sri Lanka [4,5]. Smoking and tobacco consumption are the major risk factors for lung carcinoma [6]. About 66 % of lung cancer deaths are contributed by smoking [2]. The relationship between smoking and lung cancer was discovered in the 1950s. Cigarettes contain multiple carcinogens (>60) that have been shown to induce cancers in laboratories [7]. Besides active smoking, passive smoking also causes lung cancer to a lesser extent [8]. The IARC (International Agency for Research on Cancer) has identified around 50 different carcinogens in cigarette smoke, affecting both central and peripheral alveolar regions [9]. These carcinogens can cause mutation in the genes responsible for cell division, growth, and apoptosis. Consequently, tumor suppressor gene (TSG) inactivation, telomerase activation, overexpression of the epidermal growth factor receptor (EGFR), angiogenesis and invasion of apoptosis are significant alterations that contribute the lung carcinoma [10]. The development of lung cancer is initiated with the rapid increase in cell number, a condition known as hyperplasia. After hyperplasia, the cells lose the ability to differentiation and this stage is called dysplasia [11]. Carcinoma in-situ is the later stage of dysplasia, here the cells are cancerous and non-invasive [12]. The net rate of five-year survival in the patients of lung cancer is about 17.8% [13]. Its prevalence and mortality rate are exorbitant in the developed countries and constantly rising in

developing countries [14]. Several genetic alterations are responsible, including overexpression of EGFR, inactivation of the tumour suppressor gene, telomerase activation, etc. These are recognized as "key attributes" of lung cancer [15].

1.2. Types of lung cancer

Depending on the histology, diagnosis and clinical implications, WHO has classified lung cancer into two major categories [16].

1.2.1 Non-small cell lung cancer (NSCLC)

NSCLC develops from lung epithelial cells in the central bronchi to terminal alveoli. The histological type of NSCLC correlates with the location of origin, due to differences in respiratory tract epithelium from the bronchi to the alveoli. NSCLC is further divided into three sub-categories [17].

1.2.1.1. Adenocarcinoma

Adenocarcinoma accounts for 40% of all NSCLC patients. It generally occurs in type II alveolar cells, which are secretory in nature. It is the most common type of lung cancer in both smokers and nonsmokers of all ages. It primarily occurs at the lung periphery, and a likely reason is the use of filters in cigarettes, which prevent big particles from entering the deeper areas of the lungs. Adenocarcinoma is not aggressive, and it is more likely to be detected before it spreads to distant organs from the lungs [18].

1.2.1.2. Squamous-cell carcinoma

It accounts about 25-30% of NSCLC. It begins with bronchial epithelial cells, namely premature squamous cells in the airways. Cigarette smoking is strongly connected to squamous cell cancer [19].

1.2.2. Large-cell carcinoma

Large cell carcinoma is a rare subtype of non-small cell lung cancer. It is known as undifferentiated and/or large cell lung cancer because of the appearance of the cells under microscope. It accounts about 10-15% of NSCLC cases [20].

1.2.2.1. Small-cell lung cancer

Small-cell lung cancer (SCLC) accounts for 20% of all lung cancers. It spreads much more quickly than non-small cell lung cancer. Therefore, it is very difficult to treat. Tobacco carcinogen exposure is highly linked to SCLC. The majority of patients are diagnosed with metastatic disease, with just one-third having earlier-stage cancer responsive to possibly curative diagnostic treatment [21]. **Table 1.1** lists the characteristics of lung cancer subtypes based on the location they originate in the lungs [22].

Table 1.1. Location, occurrence rate and the descriptions of the lung cancer sub-types

Lung cancer Subtype	Location	Description
Adenocarcinoma (40%)	Peripheral	It develops in small airway epithelial and type 2 alveolar cells and is the most common type of lung cancer in non-smokers.
Squamous-cell carcinoma (20%)	Central-peripheral	It is strongly linked to cigarette smoking and mainly develops in proximal airway epithelial cells.
Large-cell carcinoma (3-5%)	Peripheral	It appears similar to adenocarcinoma, however the lesion developed is typically larger.
Small–cell lung cancer (15%)	Central	It is extremely associated with smoking and develops from neuroendocrine cells, which produce neurotransmitters, growth factors, and vasoactive chemicals. It is associated with paraneoplastic syndrome and also characterized by early distant metastases and fast progression.

1.2. Risk factors for lung cancer

1.2.1. Smoking

Cigarette smoking is predominantly an important factor in the development of lung cancer. The vast majority (80%) of cases of lung cancer are due to continuous exposure to tobacco smoke. A meta-analysis by Gandini and colleague had shown that tobacco smokers have a higher relative risk (RR) of lung cancer [23]. A study by Fleiss and Gross also showed that nonsmokers who were exposed to tobacco smoke were at higher risk of lung cancer than never smokers [24]. Report by Peto and colleagues showed that the risk of lung cancer gets reduced with the increase in the duration of smoking cessation [25]. A further study by Parsons and colleagues showed that smoking cessation in individuals diagnosed with early-stage lung cancer had beneficial prognostic outcomes [26].

1.2.3. Genetic factors

The occurrence of lung cancer in non-smokers and having a familial history of lung cancer supports the hypothesis that hereditary component is associated with the risk for lung cancer. An approach based on familial association studies have been used to discover lung cancer associated genes with high penetrance and low-frequency. The report has shown that via meta-analysis which involves the result from 32 studies showed that there is a 2-fold increase in the risk for lung cancer in persons with a family history of lung cancer mostly in non-smokers [27]. Lung cancer genetic studies are focused on identification of mutations and single nucleotide polymorphisms (SNP) which increase the risk of lung cancer. Various genes involved in carcinogen metabolism, nucleotide excision repair, base excision repair and cell cycle control appears to have a role in developing lung cancer [28,29]

1.2.4. Previous respiratory disease

The results from a pooled analysis by Brenner et al., suggested that the prevalence of previous lung diseases independently influences the development of lung cancer in non-smokers [30]. Individuals who suffered from respiratory diseases like Chronic Obstructive Pulmonary Disease COPD, asthma, pneumonia and tuberculosis previously were at higher risk of lung cancer. A case-control study in Southern Chinese men by Wang et al., concluded that previous COPD could increase the risk of lung cancer and can be considered as a significant risk factor for lung cancer [31]. Commonly chronic bronchitis and emphysema are grouped together as COPD characterized by coughing, inflammation of bronchioles and production of mucus with the 8 symptoms getting worse with time. Studies have shown that individuals who smoke and suffer from COPD are five times more prone to lung cancer than smokers without COPD [32]. Data collected by Liang et al., shows an increased risk of lung cancer in the individuals with preexisting tuberculosis [33]. Further, patients with upper lobe pneumonia were found to be at higher risk of acquiring lung cancer. These studies suggest that COPD can be considered as an independent risk factor for lung cancer without shared smoking history.

1.2.5. Viral infections

Robinson and colleague reported that Retroviral DNA and Human Papillomavirus DNA were found in most of the cases of squamous cell carcinoma (85% and 69% respectively). Retroviral DNA was also found in 47% cases of adenocarcinoma [34]. According to an international pooled analysis by Ragin et al., human papillomavirus (HPV) was found to more prevalent in lung cancer than the normal lung cells [35]. Various studies have shown the presence of HPV type 18 and 16 DNA in lung tumor tissues [36]. Some studies have also detected the presence of HPV E6-E7 mRNA in lung cancer cells [37]. Zhai K et al.,

suggested that there is a strong association between HPV 16/18 genotypes and lung cancer leading to an increased risk of lung squamous cell carcinoma [38].

1.2.6. Occupational exposure to carcinogens

Several workplace substances such as arsenic, asbestos, beryllium, cadmium, chloromethyl ethers, chromium, nickel, radon, silica, and vinyl chloride have been classified as carcinogens. Asbestos is the most widely known and most common occupational cause of lung cancer [39]. It is a class of naturally occurring fibrous minerals consisting primarily of 2 types: serpentine (chrysotile) and amphibole (amosite, crocidolite, and tremolites) [40]. Toxicologic studies in experimental animals have shown that all form of asbestos can cause cancer [41]. It is known to cause carcinogenesis by inducing production of reactive oxygen species (ROS) and reactive nitrogen species (NOS); chronic inflammatory response, DNA damage, and mutations [42]. The phagocytosis of persistent asbestos fibers by alveolar macrophages triggers chronic inflammatory response as well as the production of ROS and RNS [43]. Asbestos fibers are also reported to interfere with mitotic machinery, cause single strand breaks and alter p53 expression [44].

1.2.7. Diet and obesity

Various reports suggest that obesity and dietary factors are associated with increased risk of lung cancers. Low serum concentrations of beta-carotene, vitamin C, and alpha-tocopherol have been associated with the development of lung cancer [45] [46]. In a case-control study of 242 lung cancer patients and their 484 matched controls on age, sex, and place of residence have shown that consuming bread, rice, beef, liver, dairy products, vegetable ghee, and animal ghee were possible risk factors for the development of lung cancer in Iran [47]. A case-control study of 21,022 incident cases of 19 different types of cancer and 5,039 controls aged 20–76 years in Canada showed obesity accounted for 7.7%

of all cancers [48]. Another case-control study of 256 cases of lung cancer and 284 controls in Uruguay showed an increase in the risk of lung cancer due to consumption of red meat, beef and fried meat [49]. Previous studies have indicated that alcohol consumption, particularly consumption of beer, had been associated with the increased risk of lung cancer if tobacco smoking is controlled [50].

1.2.8. Environmental air pollution

Vehicle emissions are a major source of outdoor air pollution, producing gaseous and particulate pollutants including carbon monoxide, ozone, particulate matter, nitrogen dioxide aldehydes, benzene, 1,3–butadiene, polycyclic aromatic hydrocarbons, benzo[a]pyrene, and metals. Pollution from vehicles causes a broad range of acute and chronic diseases, including lung cancer [51, 52]. The International Agency for Research on Cancer has classified particulate matter in the polluted air as class 1 carcinogen [53].

1.2.9. Family history of lung cancer

Studies have established that familial history of lung cancer is also a major risk factor [54,55]. A study by International Lung Cancer Consortium showed that there is 1.5 times more risk of lung cancer in individuals with a first-degree relative (mother, father, sibling) suffering from lung cancer. This study also showed that the risk of lung cancer is higher when a sibling is suffering from lung cancer [56].

1.3. Symptoms of lung cancer

Most lung cancers do not cause any symptoms until they have spread, but some people with early lung cancer do have symptoms. Most of these symptoms are more likely to be caused by something other than lung cancer. Kretch et al. summarized the symptoms experienced in patients in lung cancer (100 consecutive patients). Pain, dyspnea, and anorexia were the most prevalent and severe symptoms, occurred in 86, 70, and 68 patients, respectively.

There were no differences between males and females. Males aged 64 and under had higher incidences of easy fatigue, taste changes and sleep problems. Men over 64 had higher incidences of cough and >10% weight loss [57].

1.4. Stages of lung cancer

Lung cancer staging is an integral part in knowing what your treatment options are. Typically, cancers that are an earlier stage are easier to treat but many people are able to live a long time with advanced-stage disease [58].

1.4.1. Non-small cell lung cancer has four main stages:

Stage 1: Cancer is found in the lung, but it has not spread outside the lung.

Stage 2: Cancer is found in the lung and nearby lymph nodes.

Stage 3: Cancer is in the lung and lymph nodes in the middle of the chest.

Stage 3A: Cancer is found in lymph nodes, but only on the same side of the chest where cancer first started growing.

Stage 3B: Cancer has spread to lymph nodes on the opposite side of the chest or to lymph nodes above the collarbone.

Stage 4: Cancer has spread to both lungs, into the area around the lungs, or to distant organs.

Small-cell lung cancer (SCLC) has two main stages. In the limited stage, cancer is found in only one lung or nearby lymph nodes on the same side of the chest [59,60].

1.5. Diagnosis of lung cancer

A variety of techniques is available to assist the clinician in achieving a definitive diagnosis of lung cancer. Selection of the most appropriate test is best done in a multidisciplinary fashion with input from a pulmonologist, chest radiologist, and thoracic surgeon. Furthermore, the most appropriate test is usually determined by the type of lung cancer i.e., SCLC or NSCLC), the size and location of the tumor, and the presumed stage of the cancer.

A diagnosis should be obtained by whatever method is easiest in patients who are presumed to have SCLC or who have very clear evidence of advanced NSCLC (i.e., Large pleural effusions or metastatic disease). Sputum cytology is a reasonable first step in patients with central lesions, but diagnostic accuracy depends of the rigorous acquisition, handling, and interpretation of samples. Flexible bronchoscopy is the most useful test for central lesions, whereas in the case of peripheral lesions, the sensitivity of transthoracic needle aspiration is higher than that of bronchoscopy [61,62].

1.5.1. Sputum Cytology

Sputum cytology is the least invasive means of obtaining a diagnosis in a patient who is suspected of having lung cancer. The diagnostic accuracy of sputum cytology is dependent on rigorous specimen sampling (at least three specimens) and preservation techniques, as well as on the location and size of the tumor (ie, central vs peripheral). Sputum cytology is particularly useful in patients who present with centrally located tumors (i.e., SCLC or squamous cell carcinoma) and in those who present with hemoptysis [63,64].

1.5.2. Flexible Bronchoscopy

Flexible bronchoscopy with its attendant procedures is a valuable diagnostic procedure in the workup of a patient who is suspected of having lung cancer. A comprehensive literature search conducted by the Duke University Center for Clinical Health Policy Research revealed 44 studies, each with 50 patients, that reported on the sensitivity of flexible bronchoscopy for the diagnosis of lung cancer. The decision about whether to perform a diagnostic bronchoscopy for a lesion that is suspicious for lung cancer largely depends on the location of the lesion [65,66].

1.5.3. Transthoracic needle aspiration (TTNA)

A meta-analysis of 47 studies and an additional 19 studies focusing on the performance characteristics of biopsy for the diagnosis of localized pulmonary lesions were analyzed by the Duke University Center for Clinical Health Policy Research. Five studies with 50 patients included in the meta-analysis were excluded from the final analysis. Among 11,279 patients, the overall sensitivity and specificity of TTNA for diagnosing peripheral lung cancers were 0.90 and 0.97, respectively [67,68].

1.6. Problems with current treatments

The existing chemotherapeutic drugs are toxic to all cells including cancer and normal cells. So the administration of these toxic agents kill the rapidly proliferating cancer cells as well as the normal cells which may lead to some serious side effects and may sometimes cause the death of patients [69].

1.7. Nanomedicine

Nanomedicine, comprises the manipulation and manufacture of materials and devices that are roughly 1 to 100 nanometres (nm; 1 nm = 0.0000001 cm) in size, to diagnosis, monitoring, prevention, treatment, repair, and regeneration of biological systems [70]. Nanomedicines have unique properties such as nanoscale size, high surface-to-volume ratio, and favorable physico-chemical characteristics. They have the potential to modulate both the pharmacokinetic and pharmacodynamic profiles of drugs, thereby enhancing their therapeutic index. Loading of drugs into nanocarriers can increase *in-vivo* stability, extend a compound's blood circulation time, and allow for controlled drug release [71]. Thus, nanomedicine compounds can alter the biodistribution of drugs by allowing them to accumulate preferably at the tumor site. A wide range of nanomaterials based on organic, inorganic, lipid, protein, or glycan compounds as well as on synthetic polymers have been

employed for the development of new cancer therapeutics [72]. Most clinical trials focus on marketed products, such as liposomal doxorubicin or albumin-bound paclitaxel. Either new indications or therapies in combination with other anti-cancer agents are investigated [73,74].

1.8. Targeted drug delivery approaches

Currently, nanomedicine-based formulations such as Abraxane and Doxil are available in the market and are being used clinically [75]. The nanotechnology-based formulations such as nanoparticles, liposomes, micelles, solid-lipid nanoparticles, etc. can be used by both such strategies of drug delivery as active and passive targeting [76,77]. The extravasation into tumour endothelium is the most prominent pathway for the size-based passive targeting through EPR (enhanced permeability and retention) effect [78]. Moreover, the specific types of receptors overexpressed in lung cancer are the key features for the active targeting through the interaction between corresponding receptors and ligand-decorated nanomedicines followed by the promoted endocytosis with consequent drug release [79]. A huge number of targeting ligands for active targeting in lung cancer have been identified. The nanomedicines, decorated with such targeting ligands, can specifically target the lung cancer and improve the therapeutic efficacy of anticancer drugs. The active targeting of the nanomedicines can be facilitated by various targeting ligands such as folate, transferrin, aptamers, and monoclonal antibodies etc. [71,80,81]. Generally, the nanomedicines can be conjugated with targeting ligands either by pre-conjugation or post-conjugation techniques [82].