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REVIEW

Tobacco Smoking and Lung Cancer

Perception-changing facts

Muhammad Furrukh

تدخين التبغ وسرطان الرئة

إدراك تغير الحقائق

محمد فروخ

الملخص: على مدى السنوات كان تدخين التبغ السبب الأكثر رسوخا لعمليات التسرطن الرئوي وغيرها من الأمراض الصدرية. وعلى مدى الخمسين عاما الماضية ، حدث تطور في عملية صقل التبغ وترشيحه مما تسبب بتغير في نوع السرطان الرئوي ، حيث أصبح السرطان الغدي هو النوع الأكثر انتشارا. وقد برز التدخين باعتباره نذير متنبئ قوي لخصائص المرض جنبا إلى جنب مع غيرها من المتغيرات. وفي هذا المقال نستعرض بإيجاز الحقائق العلمية حول التبغ وكيفية حدوث سرطان الرئة في المدخنين وغير المدخنين ، كما سنستعرض أيضا نتائج علاج سرطان الرئة في التجارب السريرية المختلفة.

مفتاح الكلمات: تدخين التبغ؛ السجائر؛ أورام الرئة؛ النيكوتين؛ البروتين الالتحامي؛ إنساني؛ الجين؛ المستقبل؛ عامل نمو البشرة؛ المسرطنات.

ABSTRACT: Tobacco smoking remains the most established cause of lung carcinogenesis and other disease processes. Over the last 50 years, tobacco refinement and the introduction of filters have brought a change in histology, and now adenocarcinoma has become the most prevalent subtype. Over the last decade, smoking also has emerged as a strong prognostic and predictive patient characteristic along with other variables. This article briefly reviews scientific facts about tobacco, and the process and molecular pathways involved in lung carcinogenesis in smokers and never-smokers. The evidence from randomised trials about tobacco smoking's impact on lung cancer outcomes is also reviewed.

Keywords: Tobacco Smoking; Lung Neoplasms; Nicotine; EML4 ALK fusion protein, human; K-Ras Gene; Receptor, Epidermal Growth Factor; Carcinogens.

It is estimated that one third of the world's adult population, and around 1.1 billion individuals, smokes tobacco, which makes every sixth human being a smoker.[1] Smoking-related illness is estimated to cause ~ 5 million deaths per annum around the globe, but is considered a leading preventable cause of death.[2] In developed countries, the rates of smoking have either leveled off or declined, but smoking-related deaths are on the rise in developing countries and are most common among the least-educated people. Initially, cigarette smoking prevalence was higher in males, but since the 1980s the gender gap has narrowed and plateaued.[3]

In 2003, in a school-based cross-sectional survey on water pipe-based tobacco smoking (*sheesha*) in Oman, 1,962 students were interviewed (26.6% were ever-smokers and 9.6% were current smokers). Among the current smokers, 15.5% were males and only 2.6% were females.[4] In the USA in 2009, approximately 20.6% of adults and nearly 20% of high school students were cigarette smokers. An estimated 9% of them were smokeless tobacco consumers. Smokeless tobacco products include products such as moist snuff, chewing tobacco, snus (moist powdered tobacco) and dissolvable nicotine products such as strips and sticks. Current evidence, however, does not support the opinion that the use of these products is safer than smoking. Additionally, there is substantial evidence that these products can be implicated in oral and pancreatic cancers, precancerous oral lesions, gingival recession, gingival bone loss around the teeth, tooth-staining, and nicotine addiction.[5,6]

In the USA, tobacco use is responsible for nearly 1 in 5 deaths.[7] In 2012, the estimated percentage of new lung cancers in males (116,470 cases) and females (109,690 cases) was 14% each. Among these

Department of Medicine, Sultan Qaboos University Hospital, Muscat, Oman
E-mail: furrukh_1@yahoo.com

lung cancers, 29% of male and 26% of female cases were estimated to be fatal.[3] Smoking accounts for at least 30% of all cancer deaths and 87% of lung cancer deaths.[8]

Cases of small-cell lung carcinoma (SCLC) cancer in never-smokers are exceptionally rare. Active smoking also increases the risk of numerous other cancers, including those of the nasal passages, sinuses, oral cavity, upper aerodigestive tract, pancreas, gynaecological system, kidney, bladder, stomach, colorectum and acute myeloid leukaemia.[9] The World Health Organization (WHO) has published guidelines to measure smoking, and classifies individuals as smokers, non-smokers, or ever-smokers, and then establishes further sub-categories.[10]

Passive smoking, or environmental tobacco smoke, is also classified as a known human carcinogen and is considered the cause of ~50,000 deaths annually. Passive smoking is a mixture of two forms of smoke from burning tobacco: sidestream smoke, which comes from the end of a lighted source (cigarette, pipe, or cigar), that contains smaller particles which easily make their way into the cells and is rich in carcinogens, and the main-stream smoke which is exhaled by a smoker.[11]

Methods

To find relevant information and articles, searches were made on PubMed, Google, Clinical-trials.gov, the Cochrane Library, abstracts of the World Conference for Lung Cancer, and the annual meetings of the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO). Online abstracts and full articles were also accessed from the National Library of Medicine (NLM) catalogue, including journals referenced in the National Center for Biotechnology Information (NCBI) database: Journal of Clinical Oncology, Lancet, Cancer, Lung Cancer, Clinical Lung Cancer, New England Journal of Medicine, Clinics, Cancer Research, Journal of Thoracic Oncology, Journal of the National Cancer Institute, Expert Review of Molecular Diagnostics, Cancer Epidemiology, Biomarkers & Prevention, Clinician Reviews, and CA: A Cancer Journal for Clinicians. The medical subjects heading (MeSH) terms were searched to confirm keywords. In addition to computer-based searches, the reference lists in reviews and original papers were scanned for further sources of information. Only English language articles were searched.

Tobacco

Tobacco is processed from the leaves of plants in the genus *Nicotiana*.[12] Besides its use as a drug, the tobacco plant is also used in bioengineering and as an ornamental plant. For many developed as well as developing countries, it remains a valuable cash crop.

Nicotiana tabacum and *rustica* are considered the main commercial species, with alkaloid nicotine as the addictive constituent of tobacco responsible for its tolerance and dependence; however, it is not a carcinogen.[13,14] After harvest, tobacco is cured over many days, allowing slow oxidation and degradation of the constituent carotenoids. This allows for the 'smoothness' of the smoke, giving cured tobacco its aromatic flavours.

After curing, tobacco is moved to a storage area for processing. For the intact plants, the leaves are removed from the tobacco stalks in a process called stripping, which makes the smoke milder and more inhalable. Tobacco is subsequently packed into various forms for consumption (i.e. smoking, chewing, snuffing, etc.) It is the cured tobacco which is easily inhalable and causes lung cancer and other disease processes.[15]

Patient Characteristics, Environmental Factors, and Lung Cancer

Certain patient characteristics have consistently shown an impact on lung cancer outcomes. For example, lung cancer is a disease of the elderly, although advancing age was not a prognostic factor for survival but high scores on the Charlson Comorbidity Index (CCI) were a factor. Taken together, toxicity, age and high CCI scores were significant predictors.[16] The incidence of lung cancer is higher among men (34%) as compared to women (13.5%). The age-standardised ratio for cancer incidence is 33.81%, and for mortality is 29.2% in men alone.[17]

In the past, the incidence was lower in females, but worldwide it is now the fourth most frequent

cancer in women (516,000 cases; 8.5% of all cancers) and the second most common cause of cancer deaths (427,000 deaths; 12.8% of the total).[18] The highest incidence rate in women is observed in North America, where lung cancer is now the second most frequent cancer in women. This is attributed to smoking. It is the lowest in central Africa, where it is the 15th most frequent cancer in women. As one in 5 women who develop lung cancer is a never-smoker, it remains a mystery as to what exactly causes their cancer.

Lung cancer in never-smokers is proposed to be due to multiple risk factors, including genetic predisposition—although this is exceedingly rare (1% with >3 affected relatives). Genetics mutations remain an underlying cause as we do encounter lung cancer at a relatively earlier age when it runs in families. Among the first studies revealing a genetic link was one conducted over 40 years ago by Tokuhata *et al*.[19] The study revealed that never-smokers with lung cancer were 40% more likely than never-smoking controls to report a first degree relative with lung cancer. Women were more likely to report such a family history and 10–15% had at least one first-degree relative with the disease.

In a landmark hormonal therapy study of 16,608 post-menopausal females, the risk of developing non-small-cell lung cancer (NSCLC) was not significant (*P* 0.21) in the experimental arm (treatment with oestrogen/medroxyprogesterone acetate) compared to the placebo group; however, after a follow-up of 5 years a divergence emerged, with more lung cancer diagnoses in the treatment arm. In addition, these females had poorly-differentiated tumours and a higher incidence of metastatic disease. There was a 30% increase in cardiovascular events, a 26% increase in breast cancer, and a 40% increase in cerebral vascular accidents (CVAs) compared to the placebo group. The hormonal treatment of postmenopausal women did not increase incidence of lung cancer, yet, it increased the lung cancer specific mortality, in particular deaths from NSCLC.[20]

Passive, or second-hand smoke from a spouse, friends, roommates, or childhood exposure from parents; vehicle or factory exhausts; cooking fumes in poorly ventilated kitchens; residence in mountainous areas (radon A, B, and C exposure), and occupational exposure or environmental toxins (asbestos and arsenic), have all been implicated in lung carcinogenesis.

Certain occupations are also associated with a higher risk of developing lung cancer (e.g. miners, asbestos workers, glass manufacturers, painters, printers and masonry workers). Many occupational substances carry a substantial risk, e.g. diesel and welding fumes, motor exhaust, natural fibres (asbestos, silica, wood, or coal dust), radon, reactive chemicals (mustard gas, vinyl chloride) and solvents (benzene, toluene). Adenocarcinoma subtypes are also associated with subpleural scars secondary to chronic inflammation (e.g. old infarcts, healed granuloma or pneumonitis and post-traumatic scars).[21] C-reactive protein (CRP) levels were documented to be higher in NSCLC in a study suggestive of an aetiologic role of chronic inflammation in NSCLC carcinogenesis.

Females with lung cancer tend to live longer compared to men because of diagnosis at a younger age, possibly diagnosis at an earlier stage, having adenocarcinoma more frequently, and perhaps due to inherent longevity. It is also possible that their superior survival in lung cancer is due to differences in nicotine metabolism, cytochrome P-450 enzymes and lifestyle.[22–24]

Tobacco Metabolism

Tobacco carcinogens are metabolised by cytochrome P-450 enzymes to make them readily excretable. Lipoxygenase, cyclooxygenase, myeloperoxidases, and monoamine oxidases may also be involved, although infrequently. The oxygenated intermediate metabolites undergo subsequent transformations (detoxification and secretion) by glutathiones, sufatases,or uridine-5'-diphosphate-glucuronosyltransferases (U5'DPGT).[25] A few of the metabolites generated during these processes react with the deoxyribonucleic acid (DNA) to form covalent binding products called DNA adducts in a process called metabolic activation. Carcinogens like polcyclic aromatic hydrocarbons (PAH) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) require metabolic activation to exert their carcinogenic effects. The carcinogenic metabolites of PAH-benzopyrenes (i.e. 7,8 diol 9,10 epioxides) and nicotine-derived nitrosamine ketone (NNK or NNAL) react with DNA to form adducts. Alpha-hydroxylase converts methyl adducts from

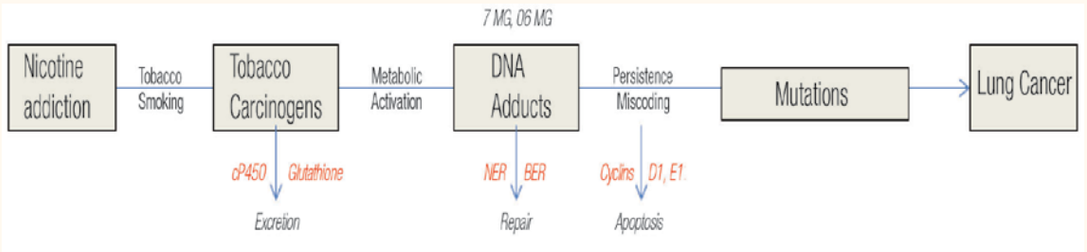

Figure 1: Link between nicotine addiction and lung cancer via tobacco smoke carcinogens and carcinogenesis.
Adapted from: Hecht SS. Tobacco smoke carcinogens and lung cancer.[25]
7-MG, 06-MG = 7-methylguanine, 06-methylguanine; DNA = dioribonucleic acid; NER = nucleoside excision repair; BER = base excision repair.

the former agent to form 7-methylguanine or O6 methylguanine. The damage may be repaired, or apoptosis may ensue. Miscoding may result in permanent mutations, including *K-Ras*, *p53, p16*, fragile histidine triad protein (F-HIT), or unknown mutations, which results in either the suppression of tumour suppressor genes or the activation of oncogenes. Not all smokers get lung cancer, but under 20% do. Susceptibility to the development of cancer depends on the balance between metabolic activation and detoxification of potential carcinogens in smokers [Figure 1].[25]

Poor patient performance status (PS) is also associated with poorer survival outcomes. The absolute benefit of chemotherapy in metastatic disease at one year varied according to the PS. In PS 0 and 1, the absolute benefit was 8%. In PS 2, the benefit was 5%, while in PS 3 it was 4%.[26] Median survival fell inversely with increasing PS in the Eastern Cooperative Oncology Group (ECOG) E1549 trial [Table 1].[27]

Race is also prognostic with lung cancer risk varying between different races and ethnicities. In the USA, age-adjusted Surveillance, Epidemiology and End Results (SEER) incidence rates for lung cancer in Afro-Americans and Caucasians are higher compared to Alaskans, Indians, Asians, Pacific Islanders and Hispanics.[28] Weight loss (hazard ratio [HR] 0.87; *P* <0.001) is considered to be associated with poorer survival.[29] In the E1594 trial, patients without weight loss (<10%) had superior survival.[27] Weight loss results from the release of cytokines (IL-6, IL-1β, interleukin-1RN, and tumour necrosis factor) with the contribution of anorexia, nausea, vomiting, diarrhoea, mediastinal lymphadenopathy compromising food passages, cytotoxic therapy and concurrent illness. The severity or burden of comorbidity has also been reported to have a clear relationship with poor survival in a variety of cancers, including lung cancer.[30]

Table 1: Survival difference by performance status and degree of weight loss in the E1594 trial[26]

ECOG PS	Median Survival	Weight Loss	Median Survival	*P* value
0	10.8 m	Nil	9.5 m	
1	7.1 m	>10%	4.9 m	0.0001
2	3.9 m			

ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Tobacco Smoking and Lung Cancer

Smoking is strongly linked with SCLC) and squamous-cell carcinoma (SCC). There has been a gradual change in the way cigarettes are manufactured which has resulted in a shift in the histology from SCC which was more frequent in the 1970s to adenocarcinoma subtypes which are currently more frequent. The impact of low tar cigarettes, introduced in the 1950s, on adenocarcinoma rates has been due to the introduction of filter vents in these cigarettes, making it easier for the smoker to draw in smoke, and allowing deeper inhalation than older, unfiltered cigarettes. Inhalation transports tobacco-specific carcinogens more distally toward the bronchoalveolar junction where adenocarcinoma often arises. Secondly, blended reconstituted tobacco releases a higher concentration of N-nitrosamines from tobacco stems.[31] A relatively older estimate of more than 26,000 cases from 17 published reports suggests that the adenocarcinoma to SCC ratio is approximately 0.4 in lung cancers in smokers as compared to 3.4 in never-smokers.[32] Lung cancer risk increases with the duration and

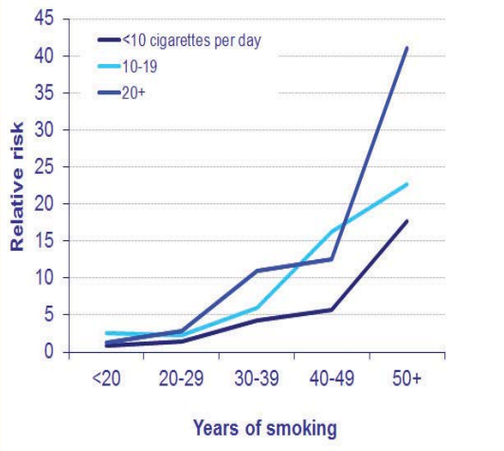

Figure 2: Relative risk of lung cancer, according to duration and intensity of smoking, men.

Adapted from Cancer Research UK. Tobacco and cancer risk statistics.[33]

intensity of tobacco consumption [Figure 2].[33] Table 2 illustrates the prevalence of various subtypes of lung carcinoma in smokers and never-smokers.

Approximately 20 potential carcinogens of ~3,500 chemicals have been detected in aburning cigarette. The most established are the polycyclic aromatic hydrocarbons (PAH) like benzo(a) pyrenes, and the tobacco-specific N-nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), while others include Asz-arenes, Dibenz(a,h)acridine, inorganic compounds like cadmium, chromium, nickel, arsenic, radioactive polonium (Po210) and organic compounds like butadiene.[32] Nitrates in the tobacco are reduced to $NH2^{-}$ and NH3 while smoking. Air-cured tobacco contains higher concentrations of aromatic amines as compared to flue-cured tobaccos (e.g. the urinary bladder carcinogens β2-naphthylamine and 4-aminobiphenyl).[34]

Cigarette smoke contains high levels of acrolein, which is toxic to the ciliated lining of the lungs, and other agents such as nitrogen oxides, acetaldehyde, phenols, and formaldehyde, which may contribute indirectly to pulmonary carcinogenicity in animals and humans.[35]

Cigarette smoke also contains free radicals (FR) (e.g. hydrogen peroxide [H2O2], hydroxyl ion [OH^{-}], sulfoxide anion) which induce oxidative damage in animal models as well as humans, while catechol and hydroquinone play their roles in single strand DNA breaks caused by the release of FR.[24] However, the evidence for the latter remains relatively low due to negative trials of anti-oxidant therapy in humans. Total NNAL and cotinine (nicotine metabolite) were measured in urine from smokers. The highest tertiles exhibited an 8.5-fold increased risk for lung cancer relative to those smokers with a comparable smoking history but possessing the lowest tertiles of these metabolites. These findings directly link NNK exposure to lung cancers in humans.[36]

Smoking has multidimensional effects on lung cancer [Figure 3]. Tobacco smoking remains the most consistent causative agent in lung carcinogenesis in animal and human models, yet, over the past decade or so, it has also emerged as a prognostic and predictive clinical characteristic.

Proto-oncogenes, Oncogenes, and Cellular Pathways in Malignant Transformation

Malignant transformation involves certain genetic and epigenetic changes such as hypomethylation, and methylation of the cytosine guanine promoter region (CpG) leading to the silencing of tumour suppressor genes. Generally, hereditary genetic defects lead to the relatively early onset of cancers,

Table 2: Prevalence of subtypes of lung carcinoma in smokers and never-smokers[83]

Histologic Subtype	Frequency (%)	
	Smokers	Never-smokers
SCC	42	33
Adenocarcinoma (includes NOS & BAC)	39	35
Carcinoids	07	16
Others	08	06
Prevalence of subtypes of lung cancer in smokers and never-smokers (52 patients) at SQUH		
SCC	91	9
Adenocarcinoma	61	39
Undifferentiated CA	33	-
SCLC	66.7	33.3

Source: Hecht SS. Tobacco carcinogens, their biomarkers and tobacco induced cancer.[83]

SCC = squamous-cell carcinoma; NOS = not otherwise specified; BAC = bronchioalveolar carcinoma; SQUH = Sultan Qaboos University Hospital; CA = cancer; SCLC = small-cell lung carcinoma.

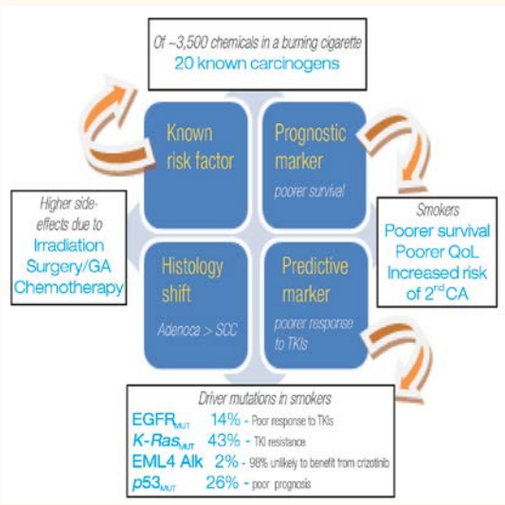

Figure 3: Impact of tobacco smoke on lung cancer.
GA = general anaesthesia; Adenoca = adenocarcinoma; SCC = squamous-cell carcinoma; TKI = tyrosine kinase inhibitor; QoL = quality of life; CA = cancer; $EGFR_{MUT}$ = epidermal growth factor mutation; $K\text{-}Ras_{MUT}$ = Kirsten rat sarcoma viral oncogene homolog mutation; EML4 Alk = echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase fusion.

such as with hereditary colon carcinoma. On the other hand, sporadic genetic defects which occur after exposure to environmental mutagens such as tobacco smoke, X-γ rays, chemical, hydrocarbons, viruses, etc., usually appear after a latent period of 5–20 years and therefore at older ages, such as in lung carcinoma.

A proto-oncogene is a normal gene that regulates cell growth and differentiation and is potentially capable of becoming an oncogene (mutation or increased expression) which initiates aberrant cell signal transduction pathways.

An oncogene is a modified gene which codes for a protein that induces a malignant transformation. A set of 21–25 nucleosides (miRNA) can control expression of these genes by downregulating them. Oncogenes arise as a result of a mutation in the proto-oncogene which increases the expression level or activity of the proto-oncogene, as is the case in point mutations, deletions or insertions. Gene amplification events lead to extra chromosomal copies of a proto-oncogene or translocation events that relocate a proto-oncogene to a new chromosomal site leading to higher expression of a cell surface protein receptor (e.g. epidermal growth factor receptor [EGFR] overexpression).[37] It can also lead to a fusion between a proto-oncogene and a second gene generating a fusion protein (e.g. echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase fusion gene [EML4 ALK] in lung cancer).[38] Proto-oncogenes are turned off once the embryogenesis and developmental processes they regulate are completed. However, in cancer, proto-oncogene activity remains high, or is inappropriately reactivated later in life.[39]

In order to grow and divide, cells respond to outside signals through the binding of extracellular ligands (growth factors) to the extracellular region of certain trans-membrane receptors, such as EGFR. When a ligand binds to a cell receptor, the receptor will frequently undergo a transformational change in its shape, which in turn leads to activation of tyrosine kinase (TK) activity in the intracellular domain and propagates the cell signal transduction pathways which regulate cell growth, proliferation, angiogenesis, apoptosis or cell death.[40] In cancer, these processes may become autonomous due to overexpression of these receptors by virtue of the genetic defects mentioned above.

Proto-oncogenes may also code for intracellular proteins that normally act downstream of cell surface receptor pathways to stimulate cell growth and division. An example of this would be the Kirsten rat sarcoma viral oncogene homolog (*K-Ras*) in lung cancer, and some proto-oncogenes like cyclin D1 and E1, which normally act to push cells through distinct stages of the cell cycle when the cells receive the appropriate signals [Figure 1].[39] Inactivation of apoptotic pathways may also be a step towards the accumulation of abnormal cells.

Molecular Signalling Pathways in Tobacco Smokers and Never-Smokers

CELL PATHWAY ACTIVATION IN SMOKERS—THE SMOKE PATHWAYS

Smokers have their own set of driver mutations which are distinct from lung cancer in never-smokers.[41] Common mutations in smokers include *p53* (>50%), *K-Ras* (~30%), *p16* (>70%), STK11 (11%), and others like *F-HIT* and T790M. In contrast, the incidence of EGFR (4%) and EML4 ALK mutations (2%) are relatively low in smokers with lung cancer. Some of these are successfully targeted while others are being explored as targets for new agents [Table 3].[42]

Table 3: Lung cancer; according to smoking status

	Never-smokers or Light ex-smokers	Current or ex-smokers
Aetiology	? Unknown factors Second-hand smoke Environmental Occupational Background radiation exposure Genetic predisposition Scar cancers	Tobacco smoke N – nitorsomines PAH – benzopyrenes Polonium 210 Inorganic compounds Organic compounds
Age	Relatively younger	Any
Gender	Usually females	Either gender
Histology	Usually adenocarcinoma	Any *Filter cigarettes - adenocarcinoma Unfiltered – SCC
Cell Pathways	EGFR; target for TKIs (erlotinib/ gefitinib) *p53* EML4 ALK - target for crizotinib (EML4 ALK resistance - target LDK 378) HER2 neu - ? Herceptin ROS 1 fusion - target for crizotinib	*K-Ras* - target MEK 1 & 2 inhibitors *p53* (G-T transversions at hot spots) T790M; target for Afatinib (TK_r) $BRaf_{(V600E)}$ - target for dabrafenib STK11/LKB1 F-HIT
Classical SCC[84]		TP63 over-expression Chromosomal instability Hyper-methylation 3q26 amplicon – SOX2 over-expression PI3K (PIK3CA gene), NRF2 pathways
Future	? Gene signature analyses	Genetic linkage studies established Candidate gene association studies

*SCC = squamous-cell carcinoma; EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor; EML4 Alk = echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase fusion gene; HER2-neu = human epidermal growth factor receptor 2; ROS 1 = member of subfamily of tyrosine kinase insulin receptor genes; K-Ras = Kirsten rat sarcoma viral oncogene homolog; TK_r = tyrosine kinase resistance; STK11 = serine/threonine kinase 11, also known as liver kinase B1 (LKB1); F-HIT = fragile histidine triad protein; * = usually; SOX2 = SRY (sex determining region Y)-box 2; TP63 = tumor protein 63; PI3K = phosphatidylinositide 3 kinase AKT signal transducer; NRF2 = nuclear factor erythroid related factor 2.*

The *p53* gene is a tumour suppressor gene which controls the apoptotic pathways and keeps a check and balance on cellular proliferation and death. The mutation occurs in a variety of human cancers, including lung cancer (>50%). Point mutations at guanine are common. In a sample of 550 *p53* mutations in lung tumours, 33% were guanine (G)→thymine (T) transversions, while 26% were G→adenine (A) transitions. *p53* mutations show a dose-dependent increase in G→T transversions at hotspots frequently after exposure to tobacco carcinogens. Lung cancers have a lot of overlap between the mutation spectrum of *p53* in smokers and never-smokers. As a result, *p53* genotyping cannot be used to preclude different tumours solely on its basis.[43,44] A trial at the Massachusetts General Hospital investigated the *p53* gene in surgically-resected lung cancers, and found 29% of patients (n = 85) harbouring *p53* mutations. The patients with *p53* mutations who were current smokers were significantly older and had smoked for significantly more years (P <0.01) than those without *p53* changes. A large number (40%) of G→cytosine (C) to T→A transversion mutations were observed due to increasing cumulative exposure to smoke. Interestingly, *p53* mutations were also seen in patients with a history of occupational exposure to asbestos—5% for patients without *versus* 20% with exposure (P <0.05).[45]

Genes in the wide *Ras* gene superfamily, including *H-Ras*, *N-Ras*, and *K-Ras* oncogenes, on chromosome 12p12.1 encodes a family of membrane-localised GTP-binding proteins that function for TK activation and subsequent downstream cell signal transduction in regulating cell growth, differentiation, and apoptosis. The *Ras* proteins interact with multiple effectors through the MAPK/STAT/PI3K signalling cascades.[46] Wild-type *K-Ras* has intrinsic GTPase activity, which catalyses the hydrolysis of bound GTP to GDP thereby inactivating the *Ras* growth-promoting signal, whereas oncogenic *K-Ras* is locked into the GTP-bound state, leading to constitutive *Ras* signalling. Mutations in *K-Ras* occurred in ~43% of NSCLC cases in one study.[42] It is common in mucinous adenocarcinoma, in elderly patients who are heavy smokers, and in earlier stages and grades, but not in large-cell lung cancers or bronchioloalveolar carcinoma (BAC). However, its frequency falls with stage and grade progression. The occurrence in never- smokers is low (~15%) and is more likely to be transition mutations.[47] In contrast, the majority of mutations from tobacco smoke exposure occur in codons 12 (G→T transversion) and 13.

A trial on Asian patients revealed that *K-Ras*

mutations were associated with ever-smoking status, male gender, and poor differentiation; however, Western studies have not been able to validate these findings.[48] In the National Cancer Institute of Canada (NCIC) BR.21 study, 28% of 731 patients had a *K-Ras* (wild) genotype that responded well to tyrosine kinase inhibitors (TKIs) while 15% had mutations that conferred primary resistance to targeted therapy.

Data from the TRIBUTE trial reveals that EGFR and *K-Ras* mutations rarely occur together, and that survival was inferior in the group of patients with the *K-Ras* mutation who were treated with the addition of TKIs to chemotherapy. The presence of a *K-Ras* mutation rules out an EGFR mutation, and is also a marker of TKI inactivity. Patients harbouring the *K-Ras* mutation are best treated with chemotherapy. In a recent trial, MEK 1 and 2 inhibitors (selumetinib), which are downstream of *K-Ras*, were given in combination with docetaxel and compared to docetaxel alone. The combination with the new agent enhanced response rates (RR) and progression-free survival (PFS), but overall survival (OS) remained only numerically superior in patients harbouring the *K-Ras* mutation.[49]

LKB1/STK11 (encodes a serine-threonine kinase) is a tumour suppressor which negatively regulates mammalian target of rapamycin (mTOR) signalling. It is inactivated in 5–15% of primary lung adenocarcinomas. Homozygous deletion or loss of heterozygosity (LOH) of chromosome 19p at the LKB1 locus occurred in 90% of the tested specimen in primary lung cancers.[50] The mutation is more frequent in lung cancers in smokers than never-smokers (*P* 0.007), and commonly occurs with *K-Ras* mutations (*P* 0.042) but infrequently with EGFR mutations (*P* 0.002). T790M (substitution of methionine for threonine at aa position 790) in tumours that progress on TKIs are more common in smokers and ex-smokers than in never-smokers.[51] This accounts for 50% of acquired resistance to TKIs. Afatinib (irreversible TKI) has been approved as a targeted therapy against T790M.[52]

The *p16* tumour suppressor gene is inactivated in >70% of human NSCLC via homozygous deletion or aberrant hypermethylation of the promoter region.[53] Smoke carcinogens may also cause LOH and chromosomal deletions in the F-HIT gene.[54] The downregulation of SIRT1 activity has also been found to be confined to lung tumours in smokers, whereas it remains upregulated in normal bronchial epithelial cells from active smokers.[55] There is substantial evidence that cigarette tar and nitric oxide (NO) act synergistically to cause single strand DNA breaks.[25] The lower incidence rates of the EGFR mutation and EML4 ALK mean that a smoker cannot undergo equally effective and possibly less toxic oral-targeted therapies.

Cell Signalling Pathway Activation in Never-Smokers

More than 20,000 people who do not smoke tobacco eventually develop lung cancer in the US each year. Cancer in never-smokers follows different cell signal transduction pathways, including EGFR mutations in 37% (exon 21 L858R or exon 19) enabling targeted therapy; *p53* mutations in 26%; human epidermal growth factor receptor 2 (HER2/neu) in 2%; over-expression and activation, or a higher frequency for EML4 ALK fusion in 12%; enabling oral crizotinib (targeted) therapy through other unknown mutations.[42,56] Never-smokers with higher EGFR frequency and gene copy numbers do well with TKI therapy, where it has shown to be associated with improvement in RR and PFS. There is a lower frequency of *p53* G→C to T→A mutations, and lower frequency of mutations at hot spots. As described earlier, never-smokers have a lower frequency of *K-Ras* mutation (~15%), the majority of which are transition mutations with a lower frequency of *K-Ras* transversions, and low serine/threonine kinase 11 (STK11) mutations (also known as liver kinase [LBK1] mutations).[47]

In a trial from East Asia of 152 never-smoking NSCLC patients, 75% harboured EGFR mutations, 6% had HER2 mutations, 5% had EML4 ALK fusions, 2% had *K-Ras* mutations, 1% harboured ROS1 fusions, 0% had b RAF mutations, and 10.9% had unknown mutations.[57] The odds of an EGFR mutation are 6.5 times higher in never-smokers (*P* <0.0001), 4.4 times higher in those with adenocarcinoma (*P* <0.0001), 1.7 times higher for females (*P* 0.039), and 4–6 times higher in East Asians.[58–63] Advanced age and acinar predominant subtypes were also independent predictors of EGFR mutations.

Impact of Tobacco Smoking on Lung Cancer Outcomes

Smokers are prone to frequent side-effects during therapeutic courses of chemotherapy and radiotherapy (i.e. mucositis), and while under general anaesthesia (GA), and to surgical complications. Their post-surgical survival is also poorer. The 10-year overall and disease-specific survival rate falls as the number of cigarette packs smoked increases in patients with surgically-resected, Stage I NSCLC.[64] Multivariate analysis from a retrospective study in Singapore, however, could not find a significant correlation between smoking and survival.[65] Smoking is also associated with poorer quality of life and predisposes patients to secondary cancers and chronic lung diseases, potentially making these patients unsuitable for or vulnerable to subsequent oncological interventions.

EGFR Mutations in Lung Cancer in Smokers

In a relatively older trial, most patients harbouring the EGFR mutation had adenocarcinomas and had smoked <100 cigarettes in his or her lifetime (never-smokers). Seven of the 15 adenocarcinomas resected from untreated never-smokers harboured the mutations, in contrast to 4 of 81 NSCLCs resected from untreated former or current smokers (*P* 0.0001).[66] In 2004, Lynch *et al*. initially described 9 patients with excellent responses to gefitinib, of whom 6 were never-smokers.[67] Cigarette-smoking history was used to estimate the likelihood of mutations in EGFR gene exons 19 and 21 in lung adenocarcinomas at the Memorial Sloan Kettering Cancer Center (MSKCC); the mutation was detected in 51% of 67 never-smokers, 19% of 151 former smokers and 4% of 47 current smokers. The number of packs smoked per year (more than 15 packs/year, *P* <0.001) and smoke-free years (smoking cessation less than 25 years ago, *P* <0.02) predicted the lower prevalence of EGFR mutations compared to smokers.[68]

A Japanese trial examined EGFR gene mutations within exons 18–21 and their correlations to clinico-pathological factors and other genetic alterations in 154 resected tumour specimens. EGFR mutations were observed in 39%, all of which were adenocarcinomas. Among the patients with adenocarcinoma, EGFR mutations were more frequently observed in non-smokers than former or current smokers (83%, 50% and 15.2%, respectively); in women than men (76.3% *versus* 34.0%); in tumours with a bronchioalveolar component than those without (78.9% *versus* 42.9%), and in well- to moderately-differentiated tumours compared to their poorly differentiated counterparts (72%, 64.4% and 34.2%, respectively). Tumours with EGFR mutations had no *K-Ras* codon 12 mutations, which remains a known tobacco carcinogen-induced mutation.[69]

Patients who smoke have a lower chance of having an EGFR mutation (14%) and the vast majority harbour wild-type EGFR, failing to qualify them for targeted TKI therapy, while current smokers who do harbour the mutations have poorer RR, PFS, and OS despite TKIs. However, all categories harbouring EGFR mutations benefit from the targeted TKIs, irrespective of their smoking status.

Tobacco Smoke—A Prognostic and Predictive Characteristic

In an exploratory subgroup analyses of a trial using salvage erlotinib (first or second line), OS was markedly increased in never-smokers (<20% of patients) on both univariate (*P* <0.001) and multivariate (*P* 0.048) analyses as compared to ex-smokers.[70] In a retrospective review of 139 NSCLC patients at MSKCC, a multivariable analysis revealed that the presence of adenocarcinoma with any bronchioalveolar features (*P* 0.004), and being a never-smoker (*P* 0.006) were independent predictors of response.[71]

Retrospective analyses of IDEAL 1 and 2 studies reveal that never-smokers derive greater benefit from TKI therapy compared to ever-smokers.[60,72] In a phase II Iressa Survival Evaluation in Lung Cancer (ISEL) study, a pre-planned subgroup analysis showed significantly longer survival in the gefitinib group than the placebo group for never-smokers (n = 375; median OS = 8.9 *versus* 6.1 months; *P* 0.012) in adenocarcinoma.[61] A survival advantage for erlotinib compared with a placebo was demonstrated in the NCIC BR.21, a randomised, double-blind study of patients (n = 731) with

advanced-stage NSCLC. A marginally significant interaction was observed between smoking history and treatment (*P* 0.054). The hazard ratios (HR) were 0.42 among never-smokers and 0.87 for smokers, indicating that erlotinib was beneficial irrespective of the smoking status, but the TKI was more useful in never-smokers. Patients with EGFR-positive tumours who had never smoked derived the greatest survival benefit from erlotinib relative to a placebo (HR 0.28; *P* 0.0007).[73]

In an unselected NSCLC population in the TALENT and INTACT 1 and 2 studies, there was no benefit when erlotinib was combined concurrently with chemotherapy when compared to chemotherapy alone. It should be noted, however, that the endpoints of these studies were not meant for evaluating variables like smoking status.[74,62,63] However, in the TRIBUTE trial, the addition of erlotinib to chemotherapy when compared to chemotherapy plus a placebo did reveal a doubling in survival in 10% of the never-smokers of the subgroups. When compared with former or current smokers, the never-smokers were relatively younger, predominantly female, and frequently harboured adenocarcinoma. While the median OS of never-smokers on a placebo was similar to that of current or former smokers on a placebo (~10 months), the never-smokers on erlotinib had a doubling of median survival (22.5 months), and an improved median time to progression (TTP) (6 *versus* 4.3 months).[75]

A trial by Mok *et al*. was carried out primarily on never-smokers (EGFR mutations in 61%) or on light ex-smokers (EGFR mutations in 47%). Their IPASS trial used oral gefitinib and compared it to paclitaxel carboplatin in East Asian chemo-naive, never- or light ex-smokers (n = 261). The RR was 71% with TKI and 43% with chemotherapy. The RR was 1% with TKI and halved with chemotherapy in wild-type EGFR. Patients with an EGFR mutation had a doubling in PFS with gefitinib, while in those having wild-type EGFR, the PFS almost tripled with chemotherapy.[76] For never-smokers in the Caucasian EURTAC study, the median PFS was 9.7 months in favour of erlotinib as compared to 5.1 months with chemotherapy.[77]

In a review of 4 randomised trials of gefitinib as first-line therapy in advanced NSCLC, the majority of patients were women (63–88%) and had the adenocarcinoma subtype (90–100%). In the various patient subgroups, the range for never-smokers was 65.8–100% and the EGFR mutation status was known in 33–100%. Activating somatic mutations were found in a high percentage in this subgroup (49–100%), and there was a consequent superior efficacy with TKIs.[78]

Erlotinib was compared alone or with carboplatin paclitaxel in never- or light former- smokers and showed similar efficacy, but TKI was less toxic in predominantly Caucasian never-smokers with advanced NSCLC.[79] In a Brazilian study comprising of NSCLC patients (n = 285), the majority were ever-smokers (76%). Among the never-smokers (n = 56), there were significantly more women (68%) and adenocarcinoma subtypes (70%). The OS at 5 years of never-smokers and ever-smokers were significantly different (*P* 0.049). The median survival time was 14.9 months for ever-smokers and 22.1 months for never-smokers. Multivariate analysis of factors related to OS, using the Cox regression for never-smokers, was highly significant (HR 0.58; *P* 0.047) without any influence of female gender or adenocarcinoma.[80] In a subgroup analysis of the Chinese OPTIMAL trial, the *P* value for the interaction test between various smoking statuses was 0.34, favouring the TKI in never-smokers.

On the contrary, a recent trial from western Japan (WTOG 3405) could not validate the effects of smoking status, age, sex, postoperative recurrence and mutation type on OS on univariate or multivariate analyses while comparing gefitinib to cisplatin and docetaxel chemotherapy.[81]

A recently published trial of 1,725 patients, cisplatin and pemetrexed *versus* cisplatin and gemcitabine showed an interesting result among enrolled never-smokers who comprised 14–15% of the overall patient population. This trial showed that never-smokers had a superior median OS in both chemotherapy arms compared to current or former smokers (15.9 *versus* 10.0 months in the cisplatin and pemetrexed arm; 15.3 *versus* 10.3 months for the cisplatin and gemcitabine arm).[82]

The superior survival in the preceding trials, are attributable to higher EGFR mutation rates in never-smokers. Therefore, tobacco smoking has indirectly emerged as a good predictor for response to TKI and is generally associated with enhanced survival outcomes.

Conclusion

Smoking has a multidimensional impact on lung cancer. It remains the most consistent causative agent for developing the disease and carries a definitive prognostic and predictive value. Adenocarcinoma is more common in never-smokers and females. The rates for EGFR and EML4 ALK mutations are higher in never-smokers providing these individuals a chance for targeted therapy. However, TKIs are ineffective in smokers with *K-Ras* mutations. Therapy optimisations should be integral while planning therapy. There is enormous room for molecular profiling of never-smokers where carcinogenesis stays presumptive. Smoking during a course of therapy remains detrimental, and patients should be advised to discontinue it as soon as possible. Strict regulations to control tobacco smoking can avert a number of human deaths globally, and lung cancer particularly. The fight between health authorities, the tobacco industry, and smokers continues to haunt humanity while the tobacco industry continues to pocket millions of USD in profit.

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