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The Molecular Mechanisms of Tobacco in Cancer Pathogenesis

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Abstract

Context: Studies have shown that cancer is a multi-factorial disease in its pathogenesis, in addition to genetic disorders, the effect of environmental factors can also be pointed. Among all environmental factors, tobacco that is considered as the leading cause of respiratory and cardiovascular disease plays a key role in cancer pathogenesis and progression. More than 5,000 chemicals and 62 carcinogenes have been detected in tobacco, which could contribute to tumorgenesis through activating oncogenes, inhibition of tumor suppressor genes, genetic and epigenetic changes, alteration of growth pathways, angiogenesis and metastasis.

Evidence Acquisition: To access the articles, we used valid external and internal databases. In order to set the search formula with maximum collectivity, at the first step, the main keywords were characterized and then equivalent terms were identified using various sources. In order to retrieve the last research papers, searches were conducted constantly from 1970 until 2015. The obtained results were screened in terms of relevance and quality indicators such as proper research design, control groups, inclusion and exclusion criteria, and also the statistical analysis. Accordingly, 150 articles were obtained and finally 64 articles which were eligible and had high relevance to the topic were selected and reviewed.

Results: This review explains the association between tobacco smoking and the incidence of different human cancers; also it focuses on molecular mechanisms through which carcinogenic chemicals in tobacco smoke promote cancer progression. Among multiple components of tobacco smoke, three carcinogens, including polycyclic aromatic hydrocarbons (PAH), nictotine and nicotin-derived nitrosamine ketone (NNK) convincingly play major roles in the pathogenesis of a wide range of cancers. In fact, these toxic and carcinogenic agents alter the expression of oncogenes, tumor suppressors, DNA repair, and last but not least, apoptosis-related genes through several mechanisms, such as point mutations, deletions, translocations and gene recombination. Moreover, implication of different tumorgenic signal transduction pathways, such as PI3K/AKT, STAT3, ERK1/2 and COX-2 in tobacco-induced tumorgenesis should not be underestimated.

Conclusions: Although many facts about the carcinogenic character of tobacco are yet unknown, understanding the molecular mechanisms of cancer development associated with smoking could be promising for early detection, treatment, and reducing metastasis of tobacco-related cancers.

Keywords: Cancer, Molecular Mechanisms, Tobacco

1. Context

It is well-established that both environmental factors and genetic disorders play an important role in the pathogenesis of human cancers (1). Of particular interest, only 7% of cancers are influenced by genetic abnormalities and 93% are the result of environmental factors, through which 30% come from smoking, 35% from the diet, 25% are the result of infectious diseases and 10% of radiation (2, 3). With this regard, it is apparent that consumption and exposure to tobacco smoke is a major public health issue. The tendency to smoking is one of the problems that people of different human societies face, and unfortunately the number of consumers of these substances increases almost every day (4, 5). Smoking causes many diseases including hypertension, diabetes, stroke, cataract, osteoporosis, miscarriage, respiratory failure, cardiovascular diseases, and many of malignancies, such as cancers of the mouth, pharynx, larynx, esophagus, lung, stomach, colorectal, kidney, bladder, breast, prostate, blood, cervix, and pancreas (6, 7). According to studies conducted by the International agency for research on cancer (IARC), cigarette smoke contains more than 5,000 chemicals and 62 carcinogens that is destructive and has been recognized as a risk factor for cancer (8). Carcinogenic components include nitrogen oxide, isoprene, butadiene, benzene, formaldehyde, acetaldehyde, acrolein, arsenic, cadmium, ethyleneoxide, 2-naphthylamines, nitromethane, eruption, radioactive polonium, metals, nitrosamine, and polycyclic aromatic

hydrocarbons (PAH). Among all, PAH and nitrosamine are two most important components with carcinogenic properties (9, 10). Moreover, there is a compelling body of evidence which introduces arsenic as another important carcinogen in ciggerettes (11). Some researchers believe that smoking during pregnancy increases the risk of cancer in children (12). Sperm disorders are the other side effects of paternal smoking on genital cells that make children susceptible to cancer through chromosomal changes. Researchers believe that consumption of more than 5 packs of cigarettes over the years by the father puts children at higher risk for hematological malignancies (12). Tobacco use kills more than 5 million people every year (13) and accounts for 30% of cancer-related deaths in the developed countries (7). Epidemiological studies showed that smoking is the major risk factor involved in cancer development and smokers are three-times more likely to develop lung cancer than non-smokers (14, 15).

2. Evidence Acquisition

To access the articles for this study, Science direct and Google scholar were used as the search motors. We searched a number of external databases including Pubmed, Web of science, Scopus Citation Index, and also internal reliable websites such as ISC and Iran medex. In order to set the search formula with maximum collectivity, at the first step, the main keywords were characterized and then equivalent terms were identified using various sources, including the Mesh medical terminology. In order to retrieve the last research papers, searches were conducted constantly from 1970 to 2015. The obtained results were screened by a team of specialists in terms of relevance and quality indicators such as proper research design, sample size, control groups, inclusion and exclusion criteria, and also the statistical analysis. Accordingly, 150 articles were obtained and finally 64 articles which were eligible and had high relevance to the topic were selected and reviewed.

3. Results

3.1. The Molecular Mechanisms of Tobacco in Cancer Pathogenesis

The devastating link between tobacco smoke and human cancers results from nicotine and other carcinogens existing in cigarette smoke. Without either one of these chemicals, tobacco would be just merchandise, instead of being one of the greatest causes of cancer-related death in the developed countries. A striking number of evidence indicates that nicotine and other carcinogenic compounds in cigarette smoke not only activate or inactivate numerous signaling pathways in malignant cells, but also provide opportunities for cancer cells to proliferate and escape from apoptosis by inducing the epigenetic alteration in specific genes. Herein, the molecular mechanisms by which carcinogenic chemicals in tobacco promote cancer progression are summarized.

3.1.1. Tobacco and Genetic Changes

Alteration in the expression of oncogenes, tumor suppressors, DNA repair mediators, and last but not least, apoptosis-related genes are the most important phenomena involved in the development and pathogenesis of all kinds of cancers (16, 17). As a primary elucidation, tumorgenesis is usually associated with the activation of oncogenes and inactivation of tumor suppressor genes through several signaling pathways (18). Toxic and carcinogenic agents in cigarettes alter the expression of aforementioned genes through several mechanisms, such as point mutations, deletions, translocations and gene recombination (19). It is well-established that TP53, as the most important tumor suppressor gene, is commonly deregulated in many human cancers. Interestingly, TP53 mutations are detected in more than 50 % of lung cancer (20). Moreover, it has been indicated that both DNA repairment and induction of apoptosis are deregulated in cells harboring mutant p53. Extensive biochemical and genetic studies reported that there is a remarkable relation between cigarette smoke and mutation in *p53* gene. It is observed that PAH, a well-known chemical in cigarette, increases the frequency of thymine and guanine replacement in this gene. Likewise, a certain nitrosamine in cigarette smoke, Nicotin-derived nitrosamine ketone (NNK), has been shown to elevate substitution of guanine to adenine in exon 5 of p53 gene in lung cancer, highlighting the importance of this chemical in lung carcinogenesis (21). As another mechanism, it is known that cigarette smoke is a rich source of free radicals and reactive oxygen species (ROS). According to the reports, each pack of cigarettes produces approximately 5×10^4 free radicals, which eventually cause a wide range of cell damages, such as inactivation of enzymes, lipid peroxidation, and protein/lipoprotein oxidation. Evidence demonstrated that tobacco smoke could be involved in the pathogenesis of cancer through induction of oxidative stress and DNA damage, which ultimately leads to replication/transcription errors and genomic instability. Great damages and the base damage of single-stranded DNA induced by these chemicals are normally repaired by the NER (nucleotide excision repair) and BER (base excision repair) systems, respectively. Xrcc1 protein coded by XRCC1 gene is a necessary part of the BER system. This protein detects the single-stranded break of DNA, and

acts as a scaffold for binding other repair enzymes. Various studies demonstrated that there is a polymorphism in XRCC1 gene in the smokers, which reduces the activity of this protein and disturbs the DNA repair system (22). Another genetic polymorphism which is detected in smokers is placed in the promoter region of β-gelatinase (23, 24). β-gelatinase which is produced by alveolar macrophages, polymorphonuclear leukocytes, and osteoclasts plays an important role in the decomposition of collagen, elastin, fibronectin, and non-matrix molecules, such as pro-TNF-α, IL-8 and TGF-β. As a carcinogenic property of this enzyme, it is reported that β-gelatinase enhances the angiogenesis of tumor cells by releasing the pro-angiogenic factors (25). Moreover, β-gelatinase inhibits T cell proliferation and hinders immune system response through incising alpha interleukin-2 receptor, activating TGF-β and separating ICAM-1 (26).

3.1.2. Tobacco and Epigenetic Changes

Epigenetics modulation refers to heritable alterations in gene expression that are not due to changes in DNA sequence. Epigenetic changes include DNA methylation, histone modifications, histone and nucleosome changes, and gene regulation by microRNAs (27). All of these mechanisms regulate gene expression by altering the chromatin structure (28, 29). DNA methylation, as a normal reversible process, is observed mainly in regions with a high frequency of CG sites (CpG islands) and at 5′ end of the genes. It is of note to mention that the modification is generally repressive to transcription and is catalyzed by the activity a family of DNA methyl transferase enzymes (DNMTs) (30). Different patterns of methylation in infants whose mothers had smoked during pregnancy have been observed (31). It has been reported that nicotine could change the expression of DNA methyl transferases, such as DNMT1, DNMT3a, and DNMT3b, causing demethylation of SNCG (synuclein-gamma) oncogene. It is also observed that smoking is connected with creating a form of methylated tumor suppressor gene CDKN2A (p16) (32). MicroRNAs (miR) are noncoding small RNAs that regulate expression of several genes including oncogenes (Ras, Myc, and ALK), tumor suppressor genes (RB, TP53), genes related to cell cycle, anti-apoptotic genes (Bcl-2, XIAP), markers of angiogenesis (VEGF, VEGFR), and metastasis-related genes (E-cadherin, integrin 5α). In the human genome, about 1600 miRs have been identified. Alteration in the expression pattern of miRs have been reported in various kinds of diseases, such as cancer. MiRs play roles in two ways in the pathogenesis of cancer: 1) by regulating the expression of oncogenes and tumor suppressor genes, and 2) by acting as oncogenes, themselves. Of note, environmental mutagens such as UV, pollution, cigarette smoke and other factors affect the expression of these noncoding small RNAs. Among the mentioned factors, the influence of cigarette smoke on the expression of miR is very important. Indeed, cigarette smoke by increasing the expression of miR-504, an important miR for the expression of dopamine receptor gene (DRD1), induces addiction to tobacco. In addition, smoking increases hypermethylation and inactivation of tumor suppressor miRs, as well (33).

3.1.3. Tobacco and Growth Signaling Pathways

In normal cells, the mitogen growth signals, as essential factors for proper cell growth and proliferation, are transmitted via specific receptors. However, in cancer cells, due to certain gene abnormalities these receptors are badly adjusted. It has been reported that nicotine, as a major component of tobacco, binds to nicotinic acetylcholine receptors (nAchR) (34), epidermal growth factor receptor (EGFR) (35) and beta-adrenergic receptor (AR-β) (36), and plays an important role in cancer development. Nicotinic acetylcholine receptor has a pentameric structure that pass through the membrane. In non-neuronal cells, nAchR regulates growth, differentiation and cell migration (37, 38). Subsequent of nAchR activation by nicotine, wide range of signal transduction pathways, such as MAPK, AKT and PKC become activated, which in turn inhibit apoptosis, stimulate cell proliferation and induce angiogenesis (39). Nowadays, it is believed that activation and secretion of neurotransmitters through nAchR, which play an important role in cancer development, could be mediated by smoking. In addition to nAchR, nicotine could also bind to EGFR and AR-β and acts as a growth factor. Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor and plays an important role in growth, development, and tumorgenesis (40). NNK contributes to cancer cell proliferation not only by synthesis of thromboxane A2 (TXA2), but also by activating TXA2 receptor, as well. TXA2 activates the transcription factor CREB (cAMP response element-binding protein), enhances expression of Bcl-2, and also increases cell proliferation (41).

3.1.4. The Impact of Tobacco on Telomerase

For controlling the cell proliferative potential, telomerase, a well-known ribonucleoprotein, adds guanine riched sequences (TTAGGG) to the ends of chromosomes and, therefore, protects two ends of linear chromosomes against exonucleases. The compelling body of evidence indicates that abberent expression of telomerase results in immortalization of the cells, and in this respect, application of novel inhibitor of telomerase is shown to be very convenient approach to induce cell death in cancer cells (42, 43). Yim and colleagues (44) indicated that there is

a strong association between smoking and level of telomerase activity in bronchial epithelium. In fact, tobacco-induced telomerase activity in cells has been reported to elevate the risk of tumorigenesis through increasing cell survival.

3.1.5. The Impact of Tobacco on Cell Survival, Growth and Apoptosis

In healthy tissues, growth inhibitory signals is responsible for tissue homeostasis through arresting cells in G0 phase of cell cycle. Cell cycle progression from G1 to S is a key step in cell cycle regulation which is regulated by the CDK4/6-cyclin D and CDK2-cyclin E complexes. These complexes separate a tumor suppressor protein, retinoblastoma (Rb) from E2F by phosphorylation of Rb subunit of Rb-E2F complex. Disruption of the pRb pathway leads to the sensitivity of the cells to growth inhibitors. According to the reports, nicotine induces Raf-1 binding to pRb and inactivates the pRb. Furthermore, with activation of nAchR and β-AR, nicotine acts as a mitogen through cyclin D1 overexpression and cell cycle transition from G1 to S phase (45). Nicotine elevates the proliferative potential of the cells by the activation of PI3K/AKT signaling pathway, as a fundamental axis in tumorgenesis, tumor growth and drug resistance (46). Based on the previous studies, it is established that nicotine and NNK activate ERK and STAT pathways, and disrupt anti-growth signals in order to increase cell growth (45). Moreover, a study conducted by Charlesworth et al. (47) indicated that the cigarette chemicals suppress NK cells activity and proliferation. However, the results of this study also demonstrated this negative influence may be reversed as the exposure is removed, since within one month of smoking cessation, the number and the activity of NK cells elevated remarkably.

Apoptosis or programmed cell death takes part in controlling cell growth, homeostasis, and removal of abnormal cells. Defects in apoptosis pathway lead to cell survival and unlimited cell growth. In most cancers, resistance to apoptosis is observed mostly due to gene mutations. AKT and ERK1/2 pathways, as anti-apoptotic signals, phosphorylate Bad and eventually inhibit apoptosis. It is indicated that NNK and its metabolites inhibit apoptosis through up-regulating hemeoxygenase (HO-1), activating NF-κB and ERK pathway in lung tissue (41).

3.1.6. The Impact of Tobacco on Angiogenesis and Cancer Metastasis

Angiogenesis, the formation of new blood vessels from endothelial cells, is a critical event for nutrition and oxygen delivery to tumor cells. Tumor cells stimulate angiogenesis by up-regulating the expression of different growth factors, such as VEGF and bFGF. A compelling body of evidence indicated that one of the mechanisms by which nicotine takes part in angiogenesis is mediated through increasing the expression and secretion of nitric oxide (NO), a vasoconstrictor and angiogenesis mediator, from endothelial cells (48). Apart from NO, nicotine also increases the expression of endothelial growth factors, such as VEGF, bFGF, and PDGF in the endothelial cells (49).

The process of tumor metastasis, which is the principle cause of mortality among cancer patients, is the spread of tumor cells from one organ or part of the body to other parts. Clinical and epidemiological studies have shown that progression and metastasis of cancer in smokers is faster than others. Long-term use of nicotine decreases the expression of adhesion molecules such as E-cadherin and β-catenin in lung cancer cells. Another important molecule which is involved in metastasis is the extracellular protease. In fact, breaking the extracellular matrix using enzymes called matrix metalloprotease (MMP) is required for tumor cell metastasis and invasion (41). It seems that cyclooxygenase-2 (COX-2) elevates both angiogenesis and aggressive potential of tumor cells by increasing the production of prostaglandins and converting the pre-cancerous agents to carcinogens. It is demonstrated that nicotine increases metastasis of esophageal carcinoma by up-regulating and enhancing the activity of both MMP-2 and COX-2 (40). In another study, it is shown that nicotine plays an important role in migration of tumor cells mostly via increasing the expression of several chemokines/chemokine receptors, such as CXCR2, CXCR3, CXCR4 and CCL12 (50).

3.2. Tabacco and Different Human Cancers

Epidemiologic studies have convincingly linked cigarette smoking to the development and the pathogenesis of certain human cancers. In the following part of this article, we aimed to review the relation between tobacco smoke and the pathogenesis of different types of cancers.

3.2.1. Tobacco and Lung Cancer

Lung cancer is the second most common cancer in human beings (21). Smoking has a pivotal role in the pathogenesis of this malignancy and also is the reason for 90% of deaths in lung cancer patients (14). Based on the reports, mutation in p53 due to carcinogenic chemicals is the most common gene abnormality, which is detected in patients suffering from lung cancer (51). P16 is another molecule which is highly inactivated in lung cancer patients. It has been reported that tobacco smoke down-regulates p16 expression and provides an opportunity for lung cancer cells to proliferate and escape from apoptosis by methylating the promotor region of this gene. Moreover, it has been reported that both nicotine and NNK

not only increase the expression of neurotransmitters, adrenaline and noradrenaline, but also inhibit the expression of GABA (Gama amino butyric acid) in lung cancer cells (52). Also, carcinogens in tobacco increase the expression of β-gelatinase through NF-κB-dependent pathway and inactivation of histone deacetylase (23). Numerous studies have been conducted during the past decades to find the relation between genes involved in lung cancer pathogenesis and smoking. It has been demonstrated that tobacco impairs both the expression and the function of important enzymes, which are involved in the detioxification of carcinogens. Moreover, toxic chemicals in cigarette smoke develop the risk of lung cancer by affecting different signal transduction pathways (53). As mentioned earlier, tobacco contains different types of carcinogens such as PAH and N-nitrosamines. All these compounds are metabolized by microsomal enzymes of phase 1 and 2 of cytochrome P450 (CYP1A), NADPH quinine oxidoreductase-1 (NQO-1), glutathione S-transferase (GST), and sulfotransferases. It has been reported that different expression levels of CYP1A, due to several polymorphisms in this gene, are important criteria in determining a person's risk of lung cancer (54). In India, Shah and colleagues (55) reported that smokers with heterozygous genotypes TG and/or homozygous genotype CC are more prone to develop lung cancer than those with TT genotype.

3.2.2. Tobacco and Breast Cancer

Breast cancer is the most common cancer of women and is considered as the leading cause of cancer death in women all over the world. Studies have shown that indirect exposure to tobacco smoke increases the chance of developing breast cancer more than smoking tobacco or Hookah. It is identified that those who deal with indirect exposure to tobacco smoke from their childhood are 4.68-times more vulnerable to develop breast cancer than other individuals (56). Recent studies by Lee and colleagues (41) revealed that expression of nAchRα plays a key role in tumorgenesis in the advanced stages of breast cancer. Nicotine indirectly activates signaling pathway of nAchRα, which leads to aberrant expression of cyclin D3 in breast cancer cells. It has been also demonstrated that nicotine up-regulates the expression of nAchR through activation of PI3K/AKT signaling pathway, which in turn leads to phosphorylation of different adhesion molecules and induction of cancer metastasis.

3.2.3. Tobacco and Bladder Cancer

Smoking is the most important risk factor for bladder cancer (57). Smoking causes 50% of bladder cancer in men and 20% in women. Nicotine, as the main component of cigarette, is found in the urine of smokers (58). The most important and the most abundant gene abnormalities involved in the pathogenesis of bladder cancer are mutations in the FGFR3 and TP53 genes, both of them have been reported to be strongly associated with smoking (54). In addition, XRCC1 gene polymorphism is also regarded as one of the most common genetic changes in bladder cancer associated with smoking (59). 4-Ariel amine-biphenyle phenyl (4-ABP), a carcinogen in cigarette smoke, is able to induce TP53 gene mutation, chromosomal instability and cervical intraepithelial neoplasia (CIN) (60). Previous studies showed that nicotine stimulates cell growth in bladder cancer through induction and activation of nAchRα, AR-β, ERK1/2 and STAT3. Moreover, smoking during treatment impaired the healing process of patients with bladder cancer by inhibiting apoptosis of tumor cells (61).

3.2.4. Tobacco and Larynx Cancer

Cancer of the larynx accounts for approximately 1.5% of all cancers. It has been reported that smoking induces mutations in exons 5 to 8 of p53 gene in laryngeal cancer (21). Scientific evidence shows that cancer of the oral cavity, larynx and esophagus are directly associated with smoking. While the risk of laryngeal cancer is increased with smoking, cessation of smoke may decrease the risk of this type of cancer (62). Yilmaz et al. (63) reported that those who smoke heavily are likely to have larynx cancer around 1.23- to 5-times higher than individuals who do not smoke.

3.2.5. Tobacco and Colorectal Cancer

Among the carcinogens found in tobacco, nicotine is the most important risk factor for cancers of the digestive tract. It has been shown that nicotine increases the amount of both cyclooxygenase-2 (COX-2) and prostaglandin E1, which can act as the potent mediators for cell proliferation and metastasis of gastrointestinal cancer. Additionally, nicotine increases VEGF receptors, which stimulates angiogenesis of tumor cells (64). As mentioned earlier, polymorphisms in CYP1A, NQO1, and GST, as the most important detoxification enzymes, are also common feature for different types of malignancies, including colorectal cancer (54).

4. Conclusions

Despite a large number of carcinogenic chemicals that have been found in cigarette smoke, most research studies unequivocally introduced three basic components, including nicotine, NNK and PAH, as the most important cancer-causing factors in human. The correlation between these components and the incidence of a broad spectrum of human cancers, such as lung, breast, bladder, larynx, and

colorectal carcinomas is well-established in both in vitro and in vivo studies. Activation of non-neuronal nicotinic acetylcholine receptors (nAchR) signaling pathway, which has considerable implications for cancer and cardiovascular disease, is the main mechanism by which these components stimulate the growth and proliferative potential of malignant cells. Among the other underlying mechanisms that contribute to cigarette-mediated tumorgenesis, activation of some signaling pathways, including PI3K/AKT, STAT3, MAPK and NF-κB are studied the most. Recent studies also indicated that tobacco smoke alters the expression level of multiple tumor suppressors and oncogenes not only by inducing point mutations, deletions, translocations and gene recombination, but also by mediating epigenetic modifications. Although many facts about the carcinogenic character of tobacco are yet unknown, understanding the molecular mechanisms of cancer development associated with smoking could be promising for early detection, treatment, and reducing metastasis of tobacco-related cancers.

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Footnotes

Authors' Contribution: Davood Bashash designed the study; Mahnaz Abbasalizadeh, Parisa Sadreazami, and Ava Safaroghli-Azar did the literature review; Elaheh Nooshinfar wrote the manuscript, and Mohammad Esmaeil Akbari approved the final version.

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