Molecular Mechanisms of Tobacco-derived Nitrosamines in Oral Squamous Cell Carcinoma (OSCC)

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a prevalent malignancy of the head and neck, predominantly driven by tobacco consumption. The risk is exacerbated when combined with alcohol. Among the various carcinogens in tobacco, tobacco-specific nitrosamines (TSNAs), such as N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), stand out as the most potent in relation to OSCC.

Methods: This study explores the molecular pathways through which TSNAs contribute to OSCC.

Findings: Metabolically activated TSNAs can form DNA adducts that may lead to mutations, especially when affecting genes that control cell growth or apoptosis. These mutations can activate oncogenes or inactivate tumor suppressor genes, facilitating cellular transformation and the progression of tumors. Concurrently, tobacco products initiate chronic inflammation, releasing reactive species that damage DNA. This inflammation also promotes cell proliferation and angiogenesis, bolstering tumor growth. Furthermore, TSNAs can induce epigenetic changes, such as DNA methylation and histone modifications, which can alter gene activity and contribute to carcinogenesis. Additionally, TSNAs have been found to activate the PI3K/Akt/mTOR pathway, pivotal for regulating cell growth and preventing apoptosis, thereby promoting an environment conducive for tumor survival and growth.

Implications: Understanding these molecular pathways may lead to the development of early detection biomarkers. This knowledge also presents opportunities for creating targeted therapies specific to the pathways involved in OSCC progression. However, the primary preventive strategy remains abstention from tobacco products. Increased awareness regarding the molecular repercussions of tobacco consumption can serve as a potent deterrent.

Conclusion: The presence of TSNAs in tobacco plays a significant role in the onset and progression of OSCC through various molecular mechanisms. This in-depth molecular understanding is invaluable for devising both preventive strategies against tobacco use and the development of therapeutic treatments for OSCC.

Keywords: *Tobacco-specific nitrosamines (TSNAs), Oral squamous cell carcinoma (OSCC), DNA adducts*

Introduction

Oral Squamous Cell Carcinoma (OSCC) refers to a form of malignant tumor that arises from the squamous epithelial cells lining the oral cavity [1], [2]. These cells are flat and scale-like in appearance, and when they undergo malignant transformation, they can lead to the development of a carcinoma. OSCC is the most predominant type of oral cancer, accounting for a significant majority of all malignancies in the oral region. The relevance and urgency of discussing and understanding OSCC cannot be understated. Oral cancer not only poses a significant threat to health, impairing essential functions such as speaking, eating, and breathing, but it also has profound psychosocial implications. The face, which is a focal point for human interaction, can undergo disfiguring changes due to the disease and its treatments. Additionally, early detection and awareness about OSCC are crucial for timely intervention, better treatment outcomes, and improving the quality of life of affected individuals [3], [4].

OSCC commonly originates from various sites within the oral cavity. Some of the most frequent sites include the lateral and ventral surfaces of the tongue, the floor of the mouth, the lower and upper gingiva (gums), the retromolar trigone (an area behind the wisdom teeth), and the buccal mucosa (the inner lining of the cheeks). Each of these sites represents a unique microenvironment within the oral cavity, and the specific location can influence both the clinical presentation and prognosis of the tumor [5], [6].

The different oral tissues and their susceptibilities play a significant role in the manifestation of OSCC [7]. The oral cavity is lined with mucosa that consists of a stratified squamous epithelium. This epithelium undergoes constant exposure to various environmental factors such as tobacco, alcohol, and pathogens, which can instigate carcinogenesis. Some areas of the oral cavity might be more prone to irritation or injury, making them more susceptible to malignant changes. For instance, the lateral borders of the tongue are more frequently involved than the dorsum due to the increased friction and exposure to irritants. The floor of the mouth, another common site, is thin and has a rich vascular supply, factors that might contribute to its vulnerability. Recognizing the specific susceptibilities of these tissues can guide preventive strategies and targeted screenings, potentially mitigating the risk of OSCC development.

The etiology of Oral Squamous Cell Carcinoma (OSCC) is multifactorial, with numerous risk factors contributing to its onset. Foremost among these are tobacco and alcohol consumption. Tobacco, in its various forms, including smoking and chewing, contains a plethora of carcinogens that can instigate changes at a cellular level, leading to cancer. Alcohol acts synergistically with tobacco, enhancing its carcinogenic effects. Regular and heavy consumption of both significantly heightens the risk. Another emerging and noteworthy risk factor is the infection with the Human papillomavirus (HPV), especially the high-risk HPV-16 subtype. HPV-related OSCC typically presents in younger patients and often originates in the oropharynx, the part of the throat just behind the mouth. Chronic inflammation and irritation, whether from ill-fitting dental

appliances, sharp teeth, or habitual cheek biting, can facilitate the malignant transformation of oral tissues. Additionally, genetic predispositions, where individuals inherit mutations that decrease their resistance to the development of OSCC, also play a pivotal role [8].

OSCC can present with a myriad of signs and symptoms, depending largely on the site and stage of the tumor. Commonly, the initial presentation might include non-healing ulcers, white (leukoplakia) or red (erythroplakia) patches, or lumps in the oral cavity. These lesions might appear innocuous initially but can progressively become indurated or hard. As the disease advances, patients might experience symptoms like pain or a burning sensation, especially during chewing or swallowing. Difficulty in swallowing, voice changes, unexplained weight loss, and even mobility issues with the tongue or jaw can emerge [9], [10]. Lymph node enlargement in the neck may also be palpable, indicating potential metastasis. It's imperative to note that many of these signs and symptoms can also be attributed to benign conditions, but their persistence or progression warrants a thorough evaluation.

The importance of regular dental and medical check-ups in the context of OSCC is paramount. A routine oral examination can identify early changes suggestive of malignancy, enabling early intervention and better prognostic outcomes. If any suspicious lesions are noted, a biopsy, where a small sample of the tissue is taken and examined microscopically, is the gold standard for diagnosis. Further, imaging studies like X-rays, CT scans, MRI, or PET scans can be employed to assess the extent of the tumor and any potential metastatic sites. A crucial component of OSCC management is the staging of the disease, which informs treatment strategies and provides prognostic insights. The TNM classification, which stands for Tumor size (T), Lymph Node involvement (N), and Metastasis (M), is widely used to stage OSCC. Each parameter is graded based on the extent and severity, with higher numbers usually indicating more advanced disease [11], [12]. The collective information from this staging helps guide the therapeutic approach and aids in patient counseling.

Tobacco-derived Nitrosamines and OSCC

Tobacco products have been widely used for centuries, but their associated health risks have been a focal point of research only in the past few decades. At the heart of these concerns are the various carcinogenic compounds present in tobacco. Carcinogens are substances capable of causing cancer in living tissue. Tobacco smoke contains more than 7,000 chemicals, of which hundreds are harmful and at least 70 are known carcinogens. These numbers are staggering and have instigated extensive research into the specific agents within tobacco that contribute most significantly to its carcinogenicity [13], [14].

Among the vast array of carcinogens found in tobacco products, tobacco-specific nitrosamines (TSNAs) stand out due to their high potency in inducing tumors [15]. These compounds are unique to tobacco and are not found in significant amounts in other food items or consumables. TSNAs are formed during the curing, fermentation, and aging of tobacco. Specifically, they are a result of the chemical reaction between

nicotine and other alkaloids in the tobacco leaf with nitrite and other nitrogen-containing compounds present. Their concentrations can vary depending on the type of tobacco product, its manufacturing process, and storage conditions.

Two of the most studied TSNAs in the realm of tobacco-induced carcinogenesis are N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). These compounds have drawn particular attention because of their strong carcinogenic properties. NNN has been shown to induce esophageal and nasal tumors in rodents, while NNK is a potent inducer of lung tumors. NNK is particularly concerning because, apart from its direct carcinogenic effect, it can also be metabolized in the body to form other carcinogenic compounds, increasing its overall potential to cause harm. The International Agency for Research on Cancer (IARC), which is a part of the World Health Organization (WHO), classifies both NNN and NNK as Group 1 carcinogens, meaning there's sufficient evidence to deem them as carcinogenic to humans [16], [17].

The knowledge of the potent carcinogenic properties of TSNAs, especially NNN and NNK, has posed challenges and opportunities for regulatory authorities worldwide. It is a daunting task to regulate and reduce the levels of these carcinogens in tobacco products because their formation is intricately linked to the natural processes of tobacco cultivation and production. However, many countries have taken steps to implement measures that limit TSNA content in smokeless tobacco products, given the direct correlation between TSNA levels and the risk of cancer. These measures often include best practices in tobacco cultivation, handling, and processing to minimize TSNA formation [4], [18], [19].

Molecular Insights:

DNA Adduct Formation is a profound process instigated by Tobacco-specific nitrosamines (TSNAs) found in tobacco and its smoke. When individuals consume or inhale these TSNAs, and they are metabolically activated within the human body, they can potentially form DNA adducts. These adducts are problematic aberrations in the DNA structure, resulting from external or internal chemicals binding covalently to the DNA molecule. If left unattended or if the cellular repair mechanisms fail to address these adducts, mutations in the DNA sequence can ensue. The real danger manifests when these mutations occur in genes responsible for vital cellular operations, such as cell growth or apoptosis, paving the way for carcinogenesis, the onset of cancer [20].

Conversely, the DNA in our cells boasts a wide array of genes, ensuring the cell's health and systematic functioning. Among this vast genetic ensemble are oncogenes and tumor suppressor genes. Initially, oncogenes are proto-oncogenes, a dormant version of the gene. But DNA adducts and other damaging agents can activate these proto-oncogenes, transmuting them into oncogenes capable of converting a normal cell into a tumor cell. Tumor suppressor genes, acting as the cell's defensive mechanism, usually protect cells from veering into a cancerous state by inhibiting cell division or prompting apoptosis in compromised cells. But the inactivation of these genes through mutations removes

this protective layer, allowing for unchecked cell proliferation and potential tumorigenesis [21], [22].

Chronic inflammation, often associated with tobacco product consumption, isn't a mere passive state. This enduring inflammatory condition generates reactive oxygen and nitrogen species, volatile molecules that can inflict harm upon cellular structures, including DNA [23], [24]. The DNA damage from these reactive species can expedite the carcinogenesis process. Beyond the direct impact on DNA, chronic inflammation accelerates cell proliferation. With cells dividing at a more frenetic pace, the likelihood of errors during DNA replication heightens. Unaddressed errors can culminate in mutations that, especially in conjunction with DNA adduct-induced mutations, can thrust a normal cell into a malignant transformation [25].

Furthermore, unchecked cell division means that cells have more opportunities to amass mutations propelling them towards a cancerous state. Chronic inflammation plays a crucial role in this rapid cell division. Each division increases the risk of errors in DNA replication. If these errors aren't corrected, they can contribute to mutations. Such mutations, particularly when paired with the DNA damage caused by adducts and reactive species, can collectively push a cell from a state of normalcy to malignancy.

Lastly, another concerning facet of chronic inflammation is its facilitation of angiogenesis, the birth of new blood vessels. While angiogenesis is naturally essential for growth and healing, it's a double-edged sword in the tumor context. New blood vessels nourish tumors with oxygen and vital nutrients, enabling them to proliferate and expand. A tumor with a robust vascular network grows more swiftly and holds a higher propensity to metastasize or invade distant body parts. This means that the inflammation induced by tobacco not only directly harms DNA and fosters cellular growth but also equips tumors with the infrastructure they require to thrive [26], [27].

Epigenetic Changes are one of the remarkable yet subtle ways that our genes can be regulated without altering the actual DNA sequence. When we talk about TSNAs, or Tobacco-specific nitrosamines, we aren't merely discussing their ability to damage DNA directly, but also their potential to instigate these epigenetic changes. Epigenetics is akin to a set of switches on our genes, determining which ones are active and which ones remain silent. Modifications such as DNA methylation, where a methyl group is added to the DNA molecule, and histone modifications, which alter the proteins around which DNA is wrapped, are primary mechanisms of these epigenetic alterations. For instance, increased methylation in certain regions of a gene can inhibit its expression, essentially "turning it off." On the other hand, various histone modifications can either tighten or loosen the DNA's wrapping around histones, making genes less or more accessible for transcription, respectively. These processes, influenced by TSNAs, have profound implications for carcinogenesis. If genes that regulate cell growth or apoptosis are turned off or on inappropriately due to these modifications, it could set the stage for uncontrolled cell proliferation, a hallmark of cancer.

The PI3K/Akt/mTOR pathway is one of the most critical signaling conduits in our cells, intricately choreographing various cellular processes. Central among these is its role in directing the cell cycle, fostering cell growth, and deterring apoptosis, the programmed death of cells. Given the pathway's fundamental roles, any perturbations to its regular functioning can have significant repercussions. Enter TSNAs. Evidence has shown that TSNAs have a proclivity for activating this signaling pathway. Once activated, the pathway can bolster the cellular milieu in ways that are conducive to tumor growth and survival. PI3K, or phosphoinositide 3-kinase, initiates the signaling cascade, leading to the activation of Akt, a kinase that, when active, promotes cell survival and growth. This, in turn, stimulates mTOR, another kinase that supports cellular protein synthesis, nutrient uptake, and overall cell growth. When TSNAs instigate the unwarranted activation of this pathway, cells might evade apoptosis when they shouldn't, or they might proliferate at rates that are abnormal. Over time, this can give rise to a population of cells that have a survival and growth advantage, attributes that are precursors to tumor development [28], [29].

Epigenetic changes underscore the fact that DNA damage is not the only route to malignancy; the dysregulation of gene expression through alterations in the epigenetic landscape can also be a driving force. Concurrently, the activation of vital cellular pathways, such as PI3K/Akt/mTOR, by TSNAs spotlights the interconnectedness of cell signaling and how disturbances in one pathway can cascade into overarching cellular changes. Both these phenomena underscore the significance of understanding TSNAs' impacts on the cellular environment, particularly when charting strategies for cancer prevention and therapy.

Conclusion

Oral squamous cell carcinoma (OSCC) is among the leading malignancies of the oral cavity, and its prognosis is significantly influenced by the stage at which it is detected. Late-stage diagnosis often results in poorer outcomes, making early detection paramount for effective treatment. Understanding the molecular processes that give rise to OSCC is a pivotal step towards this goal. As our knowledge of the genetic, epigenetic, and proteomic alterations that underpin the development and progression of OSCC deepens, it offers the potential to identify unique biomarkers that can serve as indicators of early disease onset. Biomarkers can be detected through non-invasive or minimally invasive methods, like saliva tests or tissue biopsies, providing clinicians with valuable tools to monitor individuals at high risk and ensuring timely intervention [30].

As with many forms of cancer, OSCC's pathogenesis is rooted in aberrant molecular pathways that promote unchecked cellular growth, invasion, and metastasis. With the advent of advanced genomic sequencing techniques, it is now possible to identify specific genetic and molecular anomalies associated with OSCC. These insights pave the way for the development of targeted therapies—drugs or treatment strategies specifically designed to interfere with particular molecular pathways or processes that are deregulated in OSCC. Unlike traditional chemotherapy, which often has a broad and sometimes indiscriminate action, targeted therapies offer the promise of greater efficacy

by homing in on the cancer's specific vulnerabilities, while simultaneously minimizing side effects on healthy tissues. The end result is a more tailored and potentially more effective therapeutic approach [31].

While molecular advancements promise a future with improved detection and treatment methods for OSCC, the adage "prevention is better than cure" remains true. Primary prevention, or stopping the onset of the disease before it begins, is a crucial strategy in combating OSCC. Among the most prominent risk factors for OSCC is the use of tobacco products. Chewing tobacco, smoking cigarettes, cigars, or pipes introduce a cocktail of carcinogenic compounds directly to the oral cavity, causing DNA damage, promoting cellular mutations, and initiating the cascade of molecular events that can culminate in OSCC [32], [33].

Oral squamous cell carcinoma (OSCC) is a malignancy that, although influenced by multiple factors, has a strong association with tobacco use. Central to this association are tobacco-derived nitrosamines. These are a group of carcinogens found abundantly in tobacco products, and their involvement in the etiology of OSCC is profound. Their mode of action is multifaceted. A primary mechanism by which tobacco-derived nitrosamines exert their carcinogenic effect is through the formation of DNA adducts. These are molecular structures that form when these nitrosamines bind covalently to the DNA molecule, leading to structural modifications. Such DNA adducts can disrupt the normal functioning of genes, potentially leading to uncontrolled cellular growth, a hallmark of cancer [18], [19].

Beyond DNA adduct formation, tobacco-derived nitrosamines are also implicated in directly inducing mutations. Mutations are permanent alterations in the DNA sequence, and when they occur in genes that regulate cell growth, differentiation, and death, they can contribute to cancer development [34]. Additionally, the carcinogenic impact of these nitrosamines extends to the epigenetic realm. Epigenetics refers to modifications that alter gene expression without changing the underlying DNA sequence. By influencing epigenetic processes, tobacco-derived nitrosamines can silence tumor suppressor genes or activate oncogenes, further propelling the pathogenesis of OSCC.

At a cellular level, the journey from a normal cell to a cancerous one often involves the aberrant activation of signaling pathways that regulate vital cellular processes. Tobacco-derived nitrosamines have been found to activate key cell signaling pathways that promote cell proliferation, inhibit apoptosis (programmed cell death), and foster invasion and metastasis. This pathway modulation establishes an environment conducive to cancer initiation and progression.

The comprehensive molecular understanding of how tobacco-derived nitrosamines contribute to OSCC offers valuable insights for therapeutic development. Recognizing the specific DNA lesions, mutations, epigenetic changes, and activated pathways allows researchers to devise targeted therapies. These therapies can be designed to rectify or inhibit the molecular aberrations induced by these nitrosamines, thus potentially halting or even reversing the cancerous transformation. Additionally, this understanding

bolsters the rationale for preventive strategies. If the molecular footprint of tobacco-derived nitrosamines can be detected early, it could serve as a predictive marker, guiding interventions even before overt cancer develops.

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