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Review Article

Multiple Head and Neck Cancers: Molecular and Immunologic Underpinnings

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ABSTRACT

Although considerable advancements have been achieved in comprehending the molecular and clinical aspects of primary Head and Neck Squamous Cell Carcinoma (HNSCC), the emergence of multiple primary tumors, whether simultaneously or at different times, remains a major challenge in diagnosis, treatment strategy, and prognosis. The phenomenon of field cancerization is thought to be the cause of the frequent occurrence of multiple primaries. A complex interaction of genetic mutations, epigenetic changes, faulty signaling pathways, and immunological microenvironmental disturbances shapes the pathogenesis of different HNSCCs. These cancers frequently have both unique and shared mutational signatures, indicating a continuum ranging from divergent evolution from a common progenitor clone to full clonal independence. Their aggressive behavior and resistance to treatment are further exacerbated by the existence of cancer stem-like cells, resistance to apoptosis, Epithelial-Mesenchymal Transition (EMT), and immune evasion mechanisms. Patients with synchronous or metachronous HNSCCs frequently have poor clinical outcomes, with high rates of metastasis and recurrence, even with aggressive multimodal therapy methods. Therefore, in order to guide surveillance, therapeutic targeting, and biomarker-driven clinical decision-making, a better knowledge of the molecular etiology and tumor progression in these lesions is desperately needed.

Keywords: Head and Neck Squamous Cell Carcinoma, Multiple Primary Tumors, Field Cancerization, Carcinogens, HPV, Immunosuppressive Tumor Microenvironment.

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INTRODUCTION

Head and Neck Squamous Cell Carcinoma (HNSCC) constitute a biologically and clinically diverse set of epithelial cancers that develop from the mucosal surfaces of the oral cavity, pharynx, and larynx. Worldwide, HNSCC is responsible for over 890,000 new cases and over 450,000 deaths each year, making it the sixth most prevalent cancer globally.[1] The chronological appearance of independent tumors distinguishes synchronous and metachronous head and neck cancers as separate clinical entities. While metachronous lesions develop over a longer period of time, synchronous lesions appear concurrently or within six months after the initial tumor.[2] These lesions can appear across several mucosal sites, such as the esophagus, lungs, and aerodigestive tract, or they might form within the same anatomical subregion. The phenomenon of field cancerization, in which extensive regions of mucosal epithelium are preconditioned by long-term exposure to carcinogens, especially alcohol, tobacco, and oncogenic viruses like Human Papillomavirus (HPV), is thought to be the cause of the frequent occurrence of multiple primaries Schwartz.[3]

A complex interaction of genetic mutations (e.g., TP53, CDKN2A, PIK3CA), epigenetic changes, faulty signaling pathways (e.g., PI3K-AKT-mTOR, JAK/STAT3), and immunological microenvironmental disturbances shapes the pathogenesis of different HNSCCs.[4] Their aggressive behavior and resistance to treatment are further exacerbated by the existence of cancer stem-like cells, resistance to apoptosis, Epithelial-Mesenchymal Transition (EMT), and immune evasion mechanisms. Furthermore, it is still difficult to diagnose local relapses from real second primary, which has important therapeutic ramifications.

The tumor microenvironment represents a highly dynamic and interactive ecosystem, wherein cancer cells engage in continuous bidirectional communication with a variety of surrounding stromal components.[5] This intricate crosstalk enables malignant cells to reprogram nearby non-neoplastic elements, inducing

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the secretion of paracrine signals that support tumor cell proliferation, survival, and dissemination.[4]

In this review, we put together emerging insights into the molecular and immunologic underpinnings of synchronous and metachronous HNSCCs. In order to highlight how all of these factors come together to promote multifocal carcinogenesis, we concentrate on the functions of field cancerization, genetic and epigenetic changes, cancer stem cell dynamics, and the tumor microenvironment. The development of precision medicine strategies that can enhance outcomes for this high-risk patient population depends on the clarification of these pathways.

Etiological Risk Factors

The mucosal epithelium of the upper aerodigestive tract is the target of several, frequently concurrent carcinogenic events that contribute to the development of HNSCC. Exposure to tobacco-derived carcinogens, long-term alcohol use, and infection with oncogenic strains of the HPV, mainly HPV-16 and, less frequently, HPV-18, are the three main etiological pathways.[6] With a risk of tumor growth that is more than 35 times higher for tobacco users than non-users, tobacco use continues to be the most important modifiable risk factor for HPV-negative HNSCC. A complex combination of nitrosamines, including 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone, Polycyclic Aromatic Hydrocarbons (PAHs), and other mutagens mediate tobacco's carcinogenic effects.[7] When these substances are metabolically activated, DNA adducts are created. If these adducts are not adequately repaired, they can cause point mutations and chromosomal instability. In tobacco-associated cancers, mutations in tumor suppressor genes including TP53 and CDKN2A are very common.[8]

Alcohol causes cancer in both independent and synergistic ways. It functions as a solvent for carcinogens produced from tobacco and increases mucosal permeability.[9] Additionally, acetaldehyde, a highly reactive substance that generates DNA adducts and worsens genomic instability, is produced as a result of alcohol metabolism.[10] Chronic alcohol consumption is also linked to increased oxidative stress and immune surveillance suppression, both of which aid in the development and spread of tumors.[11]

Unlike alcohol- and tobacco-related HNSCC, which is caused by mutagens, HPV-positive tumors, primarily oropharyngeal carcinomas, are caused by the integration of viral DNA into the host genome.[12] HPV integration in HNC does not occur at a single site but at multiple splice breakpoints; studies on HNC cell lines suggest that these breakpoints are associated with genomic instability, an important feature of virus-induced carcinogenesis. Integration further promotes cell transformation by the subsequent production of viral oncoproteins E6 and E7.[13] Both p53 and RB1 are functionally inactivated by these viral proteins, which promote unchecked growth and genomic instability. While HPV-positive HNSCCs are linked to a better prognosis, their rising prevalence highlights how crucial it is to comprehend virus-mediated carcinogenesis.

Field cancerization, a word originally used to describe the common occurrence of synchronous and metachronous cancers, is a unifying principle that underlies multifocal carcinogenesis in the head and neck area. The existence of genetically modified but histologically normal-looking epithelium brought on by prolonged exposure to carcinogens is known as "field cancerization".[14] Even in clinically unaffected mucosa, molecular investigations have shown that these preconditioned regions frequently contain early mutations in TP53, CDKN2A, epigenetic silencing of tumor suppressor sites, and abnormal methylation patterns. Molecular evidence of clonal linkages between anatomically different cancers and between tumors and nearby dysplastic epithelium supports this idea. Multiple primary cancers with similar or different genetic profiles can form more easily when mutant epithelial progenitors clonally expand, frequently in the presence of compromised DNA repair. According to the hypothesis of clonal expansion, genetically altered epithelial cells of the basal layer begin to grow in a horizontal direction, along the basal and parabasal layers, forming at first a focus (patch) and later expanding into a field, which will eventually mutate focally into cancer.[13] This process is further accelerated by chronic inflammation, especially in tissues exposed to alcohol and tobacco, which produces growth hormones, cytokines, and reactive oxygen species that promote immune evasion, stemness, and mutagenesis. The high occurrence of many primary tumors in HNSCC can be explained mechanistically by these interconnected carcinogenic pathways and the biology of field cancerization. They also support the clinical necessity of genetic surveillance and individualized risk assessment.

Genetic and Epigenetic Alterations in Multiple Primary HNSCC

The molecular landscape HNSCC is characterized by a series of recurring genetic and epigenetic changes that support the formation of synchronous and metachronous primary tumors in addition to driving tumor initiation and progression. Multiple original HNSCC lesions frequently have overlapping mutations, according to genomic profiling. However, they can exhibit independent or divergent clonal evolution, which reflects a complex interaction between individual tumor biology and common etiological exposures.

Recurrent Somatic Mutations in Primary and Secondary Tumors

TP53, CDKN2A, PIK3CA, FAT1, and NOTCH1 are the genes most commonly changed in main and second primary HNSCCs.[15] These mutations impact key pathways related to differentiation, DNA damage response, and cell cycle regulation. A common field defect or clonal seeding from a pre-malignant precursor may be the cause of TP53 mutations, which are found

in up to 72% of HPV-negative tumors and are generally linked to exposure to tobacco-related carcinogens. These mutations are frequently seen in multiple synchronous or metachronous lesions within a single patient. Similarly, both early and advanced HNSCC lesions include CDKN2A deletions and promoter hypermethylation, which affect the activity of the $p16^{INK4A}$ tumor suppressor and may be a sign of field cancerization.[16] Since it helps to activate the PI3K-AKT-mTOR signaling axis, PIK3CA, which codes for the catalytic subunit of Phosphatidylinositol 3-Kinase (PI3K), is the most commonly mutated oncogene in HNSCC, especially in HPV-positive cancers.[17] FAT1 and NOTCH1, two additional commonly altered tumor suppressors, are implicated in both cell adhesion and epithelial differentiation.[18,19] Their change is frequently seen in separate initial tumors and has been connected to early carcinogenesis, indicating convergent evolution fueled by common environmental carcinogens.

HPV-Positive vs. HPV-Negative Multiple Tumors

Their HPV status represents a basic difference in the genetic architecture of several primary cancers. Instead of extensive mutagenesis markers, viral oncogene-driven processes are usually what define HPV-positive HNSCCs. E2F1 amplification, frequent epigenetic dysregulation, and PIK3CA mutations and deletions in TRAF3 are all more common in these tumors, which also show lower overall mutational loads.[20] Crucially, anatomically distinct but virally infected niches may give birth to HPV-positive synchronous or metachronous tumors, indicating multicentric carcinogenesis aided by immune evasion and sustained viral integration as opposed to traditional field cancerization. On the other hand, complicated genomic changes, such as mutations in TP53, CDKN2A, NSD1, and KEAP1, as well as localized amplifications in EGFR, FGFR1, and CCND1, are commonly seen in HPV-negative cancers linked to alcohol and tobacco consumption.[20,21] Synchronous and metachronous lesions in these situations could be the result of the growth of a genetically unstable precancerous field, which is frequently characterized by chromosomal instability and Loss of Heterozygosity (LOH). These molecular characteristics make it difficult to differentiate between recurrences and actual second primaries, especially in mucosal locations that are near to one another.

Epigenetic Alterations: Methylation, Histone Modifications, and ncRNAs

The pathophysiology of HNSCC and the formation of multiple primaries are significantly influenced by epigenetic dysregulation in addition to somatic mutations. Early transformation has been linked to aberrant DNA methylation, especially of tumor suppressor genes including CDKN2A, RASSF1, and MGMT.[22] This methylation can endure across separate tumor foci within the same anatomical field. The field cancerization concept is further supported by the frequent observation of LINE-1 hypomethylation and promoter hypermethylation of genes like CCNA1 and ITGA4 in HPV-negative tumors.[23] Histone alterations that alter transcriptional activity and chromatin accessibility include acetylation (H3K27ac) and methylation (H3K27me3).[24] In HNSCC, upregulation of EZH2, HDAC1, and DNMT1 is frequently seen, which helps to promote a stem-like phenotype and transcriptionally silence tumor suppressors.[25] These epigenetic regulators are also influenced by HPV oncoproteins; E7 increases histone methylation and EZH2 expression, while E6 upregulates DNMT1, which results in global methylation alterations. In HNSCC, non-coding RNAs (ncRNAs), namely long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), further alter gene expression. Treatment resistance, poor prognosis, and metastasis are all linked to dysregulation of miRNAs such miR-375, miR-21, and miR-99a.[26] It has been demonstrated that lncRNAs like HOTAIR, MALAT1, and HOXA11-AS control pathways like WNT/β-catenin and PI3K/AKT. These lncRNAs are becoming recognized as both functional and biomarkers of tumor progression in primary and secondary HNSCC lesions.[27] The need for thorough genomic and epigenetic profiling in clinical practice is highlighted by the molecular variability of both synchronous and metachronous HNSCCs. In addition to providing insight into the biological differences between recurrences and real second primary, this type of profiling creates opportunities for tailored therapy targeting according to the molecular characteristics of individual lesions.

Tumor Stemness and Clonal Evolution

In HNSCC, the development of multiple primary tumors is a result of both inherent cellular plasticity, especially in the form of tumor-propagating stem-like cells, and environmental carcinogenesis. Tumor-initiating cells, also known as Cancer Stem Cells (CSCs), are a tiny but biologically powerful subpopulation that can differentiate, self-renew, and start tumors. Through clonal proliferation, genetic divergence, and treatment resistance, these cells are believed to play a major role in both synchronous and metachronous carcinogenesis.

Cancer Stem Cell Markers and Functional Role

The expression of markers including CD44, CD133, ALDH1, and embryonic stem cell regulators like SOX2, OCT4, and NANOG characterizes CSC populations in HNSCC.[28] These indicators have a strong correlation with clinical outcomes in addition to conferring stem-like characteristics. For example, elevated CD44 expression is often enriched at the invasive front of tumors and is linked to tumor invasiveness, metastasis, and resistance to treatment.[29] The idea that CSC microenvironment interactions drive tumor propagation is also supported by the fact that $ALDH1^+$ cells are frequently found in perivascular niches and exhibit increased tumorigenicity in xenograft models.[30] Enriched populations of CSCs are frequently found in metastatic and second

main lesions, suggesting that stem-like cells may be the genesis of disseminated or clonally developed lesions in addition to supporting the primary tumor. Indeed, transcriptional variability within CSCs has been discovered by single-cell RNA sequencing investigations, indicating that epigenetic reprogramming and phenotypic plasticity play a role in tumor growth and treatment failure.[31]

Clonal Evolution and Intratumoral Heterogeneity

Although they may originate from a shared precancerous area, synchronous and metachronous cancers frequently have overlapping but distinct genomic profiles, suggesting that their evolutionary paths have diverged over time. According to the clonal evolution model, a genetically unstable progenitor clone is created by early driver mutations (TP53, CDKN2A, etc.) and then gives rise to several different tumor subclones as a result of selection pressures like immune surveillance, treatment, and exposure to carcinogens.[32,33] Clinical decision-making is made more difficult by the intratumoral and intertumoral heterogeneity that comes from this process. For instance, two tumors may have a similar morphology, but one may have a "stem-like" epigenetic profile due to PIK3CA mutations, while the other exhibits chromosomal instability and EGFR amplification, necessitating completely distinct treatment strategies. Clonal evolution in HPV-associated HNSCC is more frequently fueled by viral DNA integration and host cell cycle machinery dysregulation than by mutagenesis.[34] However, subclonal variety is evident in even HPV-positive malignancies, which may have consequences for recurrence and treatment resistance.

EMT and Stemness in Multifocal Tumorigenesis

In CSC-mediated tumor plasticity and metastasis, the Epithelial-Mesenchymal Transition (EMT) is crucial. Differentiated epithelial cells can more easily transform into mesenchymal, motile phenotypes with greater invasive potential thanks to EMT.[35] Transcription factors including TWIST, SNAIL, and ZEB1 control EMT in HNSCC.[36,37] Hypoxia (via HIF1α), cytokines (like IL-6), and signaling pathways like STAT3 and PI3K/AKT are often the main drivers of EMT.[38,39] Notably, EMT and the acquisition of stem cell characteristics go hand in hand.[40] Cells passing through EMT frequently show higher levels of CD44, CD133, and ALDH1, which lends credence to the idea that EMT serves as a useful pathway for the production of CSCs.[40,41] Their significance in multifocal tumor propagation is further supported by the fact that these EMT-CSCs are often enriched in second primary tumors and treatment-resistant recurrences.[42] Additionally, the EMT process is reversible. Metastasis is thought to require reversion to an epithelial phenotype (mesenchymal epithelial transition, MET) in order to colonize distant locales.[43] The intricate spatial and temporal behavior of HNSCC clones is thus explained by the dynamic flexibility between EMT and MET.[43,44] In conclusion, the multifocal nature of HNSCC is supported by tumor stemness and clonal evolution.[35] The common development of synchronous and metachronous cancers can be explained by their interaction with the immune milieu, carcinogen exposure, and therapeutic selection pressure.[44] Improving long-term results and lowering recurrence in HNSCC may be possible by focusing on CSCs and their regulatory mechanisms.[45]

Limitations

The genomic and epigenetic profiling in clinical practice of both synchronous and metachronous HNSCCs is not widely accepted, resulting in a lack of sufficient studies in this field.

CONCLUSION

HNSCCs are a prime example of the intricate linkages between tumor host relationships, genetic and epigenetic instability, and environmental carcinogenesis.[46] The process of field cancerization, which occurs when long-term exposure to the carcinogens alcohol, tobacco, and HPV causes extensive mucosal changes that predispose to separate tumorigenic events, is frequently the cause of these multifocal cancers.[47,48] Both primary and secondary tumor evolution are largely driven by epigenetic dysregulation, cancer stem cell proliferation, and important somatic mutations (TP53, CDKN2A, and PIK3CA) at the molecular level.[49,50] Recent developments in immunogenomics, single-cell technologies, and high-throughput sequencing have demonstrated that synchronous and metachronous tumors represent diverse clonal trajectories influenced by immune evasion, microenvironmental selection pressures, and therapeutic responses rather than being simple copies of a shared process.[51] Recurrence, metastasis, and treatment resistance are all made possible by the tumor microenvironment, especially its immunosuppressive and hypoxic elements.[52,53]

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CONFLICT OF INTEREST

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ABBREVIATIONS

HNSCC: Head and Neck Squamous Cell Carcinoma, **EMT:** epithelial-mesenchymal transition, **HPV:** human papilloma virus, **PAHs:** polycyclic aromatic hydrocarbons, **PI3K:**

phosphatidylinositol 3-kinase, **LOH:** loss of heterozygosity, **H3K27ac:** acetylation of lysine 27 on histone H3, **H3K27me3:** tri-methylation of lysine 27 on histone H3 protein, **ncRNAs:** non-coding RNAs, **lncRNAs:** long non-coding RNAs, **miRNAs:** microRNAs, **CSCs**: cancer stem cells, **MET:** mesenchymal epithelial transition,

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