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REVIEW ARTICLE

TGF-β SIGNALING IN HPV-POSITIVE HEAD AND NECK CANCER: FROM EARLY TUMOR SUPPRESSION TO METASTASIS AND RESISTANCE

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ABSTRACT

The TGF-β signalling pathway is an enigmatic player with a tumor suppressor effect in HPV-positive head and neck cancer during the initial stages of cancer, but has a pro-cancer effect during later stages. Specifically, HPV-positive oropharyngeal carcinomas are characterized by the suppression of TGF-β signalling due to the virus's changes in the cells. In the initial stage of cancer, TGF-β works pro-apoptotic and anti-proliferative, while in the later stages, it is involved in immune checkpoint, metastasis, and chemoresistance. This duality presents major problems for therapeutic intervention as modulation of the tumor-promoting effects of TGF-β requires sparing its tumor-suppressing properties. However, recent studies have reported novel therapeutic approaches such as selective inhibitors, immune checkpoint blockade, and combined targeted therapy for HPV oncoproteins and the TGF-β pathway. These approaches present some promise for enhancing clinical results; however, more research is required to realize these benefits for the benefit of the patients. This review examines the complex role of TGF-β signalling in HPV-positive head and neck cancers and discusses its implications for future therapeutic development.

Keywords: TGF-β signalling, head and neck cancers, Tumor progression, Immune evasion

1. INTRODUCTION

Head and neck cancers (HNCs) are a term used to describe a group of cancers originating from the head and neck region, including the larynx, pharynx, and oral cavity. These cancers are of great concern to the global health care system, claiming about 650000 new cases and 330000 deaths per year [1]. HPV-associated OPSCC and, specifically, OPSCC are a newly recognized subcategory that exhibits distinct molecular, clinical, and pathological characteristics [2,3]. HPV-positive head and neck cancers have been on the rise in the last few decades, attributed to the oncogenic HPV types, out of which the most common type is HPV-16, which accounts for more than 90% of HPV-positive OPSCC. In this manner, HPV-16 has emerged as a focus in the therapeutic studies [4].

While traditional contributing causes for HNCs include poor oral hygiene, alcohol consumption, and tobacco use, a growing number of HPV-related oropharyngeal cancers have been observed, particularly among young, non-smoking individuals [5]. Epidemiological data show a notable rise in HPV-positive OPSCCs, especially in North America and Europe, contrasted with a decline in HPV-negative HNCs due to reduced tobacco use [6]. HPV-positive HNC patients tend to be younger, have fewer comorbidities, and exhibit better overall survival rates, even at advanced stages [7,8]. This improved prognosis is attributed to the strong immune response typically induced by HPV-positive tumors [9]. Molecularly, these cancers are marked by p16 protein overexpression due to HPV E7 oncoprotein, causing retinoblastoma (Rb) protein degradation and leading to uncontrolled cell cycle and tumour growth [10].

The TGF-β signalling pathway is essential in developing and progressing HPV-positive cancers. It is a highly conserved pathway responsible for cell growth, differentiation, apoptosis, and immune response [11]. TGF-β acts as an anti-oncogene or a tumor suppressor in normal healthy cells since it controls cell proliferation and induces cell death [12,13]. However, in cancer, the pathway has a dual nature, where the same path, which is helpful for the normal functioning of cells, also leads to uncontrolled growth of the cells [14]. Initially, it inhibits tumor growth. However, when the disease advances, TGF-β signalling turns to oncogenic actions, including invasion, metastasis, and immune escape [15].

This paradox is true, especially in HPV-positive neck and head cancer patients. The involvement of HPV oncoproteins E6 and E7 in regulating the TGF-β signalling pathway adds complexity to the pathways' crosstalk [16]. For instance, the E7 oncoprotein can inhibit the Smad-dependent pathway, thus intervening in the canonical TGF-β signalling cascade and promoting tumorigenesis [17]. This disruption boosts cancer cell growth and the invasion of the adjoining tissues. Therefore, this review aims to provide a detailed discussion of the multiple and antagonistic functions of TGF-β signalling in HPV-positive HNSCC [18,19]. While the role of the TGF-β pathway is to act as an anticancer pathway in the early stages of cancer development, it plays a pro-tumoral role in the later stages of cancer, thus posing a therapeutic challenge [20]. So, it is critical to comprehend this paradox to design therapies that would selectively abolish the oncogenic properties of TGF-β while maintaining its tumor-suppressive properties [21].

Hpv Infection In Head And Neck Cancers HPV Epidemiology and Types

HPV has been a known carcinogenic factor in several other cancers, such as cervical cancer, anal cancer, and, more recently, in the HNSCC [22]. There has been an increase in HPV-associated HNS cancers, mainly OPSCC, in the world, especially in developed countries [23,24]. Tobacco and alcohol are generally known risk factors for most HNSCC, but HPV-positive tumors are becoming more and more frequent, an epidemiological change [25]. Of the more than 200 identified types of HPV, HPV-16 and, to a lesser extent, HPV-18 are the most oncogenic strains associated with HNSCC [26,27]. Notably, HPV-16 contributes to over 90% of HPV-positive cases of oropharyngeal cancers, typically affecting the oropharynx, base of the tongue, and tonsils [28]. This pattern of HPV-driven malignancy contrasts with other HPV-related cancers, which are less influenced by lifestyle factors such as smoking and alcohol consumption, setting them apart from HPV-negative neck and head cancers [29].

Distinctive Molecular, Clinical, and Pathologic Features

Patients with HPV-positive HNSCC generally present at younger ages and have more favourable survival outcomes compared to those with HPV-negative cancers [30]. The enhanced outlook in HPV-positive cases is primarily due to the immune system's improved ability to respond effectively to virally infected tumor cells. In addition, HPV-positive tumors tend to harbour fewer genetic mutations than their HPV-negative counterparts, which are often driven by prolonged contact with cancer- causing agents like alcohol and tobacco [31,32].

On a molecular level, the HPV-positive tumor exhibits viral oncoproteins E6 and E7, which inhibit some essential tumor suppressors, namely p53 and Rb[33]. These oncoproteins lead to the growth of the infected cells by inhibiting the suppression of the cancerous tumors. HPV-negative cancers, on the other hand, are precipitated by environment-related carcinogens and, therefore, exhibit higher mutation rates and aggressive tumor behaviour [34].

Mechanisms of HPV in Carcinogenesis

The carcinogenic nature of HPV, especially in HNSCC, is mediated through the integration of viral DNA in the human genome. This integration disrupts key regulatory signals and twists cellular signals that allow uncontrolled growth and the development of tumorigenesis [35]. Two viral oncoproteins, E6 and E7, are the most important in the carcinogenic process of HPV-positive cancers [36]. Persistent infection is achieved when the HPV genome integrates into the host cell; this makes the oncoproteins continually expressed [37,38]. This constant interaction impairs the cellular processes necessary for evaluating the cell and the apoptotic mechanisms that keep it in check, directly inhibiting the apoptotic equipment or modifying the working of main signalling pathways known to be suppressive to tumor growth [39]. In addition, through the integration process, which is required for viral DNA replication, aneuploidy is encouraged and supplies the source for other mutations that fuel the carcinogenic process [40]. The immune system does not efficiently eliminate integrated HPV, whereas transient HPV is successfully eliminated, and therefore, oncogenic activity persists [41]. The E6 and E7 oncoproteins have been identified as key causes of tumor development in HPV-infected SCCs [42]. E6 degrades the tumor suppressor protein p53 by binding to the ubiquitin ligase E6AP. Consequently, it inhibits its tumour-suppressive actions of p53 protein, referred to as tumor suppressor protein, which is usually involved in cell cycle arrest or apoptosis if DNA is damaged [43]. The degradation of p53 by E6 function increases the growth of cells with damaged DNA since a backup system is removed by E6 [44,45]. Likewise, E7 inactivates another important tumor-suppressor pathway by binding to the Rb, which is central to cell cycle regulation [46]. Since the E2F protein is the protein product of a gene that is positively regulated by cells that have passed the G1 checkpoint, this interaction leads to the release of E2F and hence the cyclin E and subsequent passage to the S phase of the cell cycle, thus enabling cell proliferation [47].

In the process of malignant transformation, E6 and E7 of HPV continuously collaborate to knock out the normal growth control regulation system [48].The TGF-β signalling pathway, which is involved in cancer as a tumour suppressor and a tumour promoter, also works with these viral oncoproteins to encourage the development of cancers from the HPV+ population [49,50]. E7 has been reported to inhibit the Smad-dependent TGF-β signalling pathway from functioning, thus dampening the suppressive effects of TGF-β on the growth of tumor cells [51]. Through altering this pathway, HPV-positive cells can escape growth inhibition and enhance immune escape, cell motility, and metastasis [52](Fig. 1).

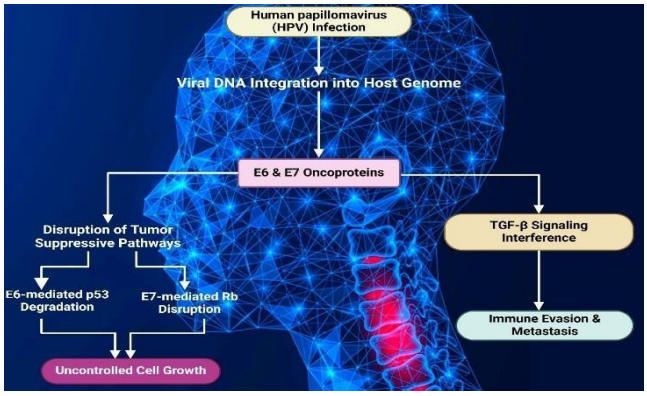

Figure 1. The image depicts the process by which Human papillomavirus (HPV) infection leads to uncontrolled cell growth. It shows how the viral DNA integrates into the host genome, producing E6 and E7 oncoproteins, which disrupt tumor suppressive pathways, ultimately causing immune evasion and metastasis.

TGF-β Signalling Pathway

A TGF-β signalling pathway regulates many cellular processes such as cell growth, differentiation, cell death, and immune response; that is considered one of the principal cellular signalling pathways [53]. This pathway operates through two primary mechanisms: the conventional Smad-dependent signalling and Smad-independent pathways, which incorporate PI3K, MAPK, and Rho-like GTPases [54]. TGF-β in the canonical pathway combines its receptor and phosphorylates Smad2 and Smad3 proteins. These phosphorylated Smad proteins with Smad4 form a complex that is then transported to the nucleus to modulate gene expression, which may involve processes like apoptosis, cell cycle arrest, and cell differentiation [55]. On the other hand, the Smad-independent pathway recruits the succinate/succinyl-CoA pathway, which plays a role in regulating cell survival and motility and engaging the extracellular matrix [56].

TGF-β is an important signalling molecule that has a biphasic effect in cancer. During the initial steps of tumorigenesis, TGF-β protects against cancer through cell growth arrest and death [57]. It also assists in regulating genomic rates of cell division so as not to go overboard and cause the cell to become malignant. Nonetheless, at the advanced stage of cancer, the roles of TGF-β as a tumor suppressor are abolished and instead contribute to cancer development [58]. These changes in cell behaviour from extracellular matrix remodelling, epithelial-mesenchymal transition, and tumor suppression to tumor promotion indicate characteristics of cancer, such as HPV-positive head and neck cancers [59]. The change in the TGF-β signalling from a tumor suppressor to a promoter of cancer progression is due to changes in the tumor microenvironment and genetic mutation within the cancer cells as cancer progresses [60]. In its tumour-promoting phase, TGF-β assumes a commanding role in invasion, metastasis, and immune evasion processes, especially in cancers with an HPV background [61]. This cytokine can also interfere with other signalling pathways, including PI3K and MAPK, to enhance the tumorigenic impacts of TGF-β [62]. An essential part of TGF-β's tumorigenic effect is its participation in EMT induction, which increases cancer cells' migratory ability [63].Also, in the immune system, TGF-β is known to promote the effect of Tregs that suppress tumoricidal immune responses and dampen T cells' cytotoxic action [64]. Such an immune modulatory effect and its ability to induce EMT help increase tumor growth, metastasis, and resistance to chemotherapeutic agents in HPV-positive carcinomas [65]. Thus, TGF-β is regarded as multi-factorial in developing terminal-stage neoplasms [66] (Figure2).

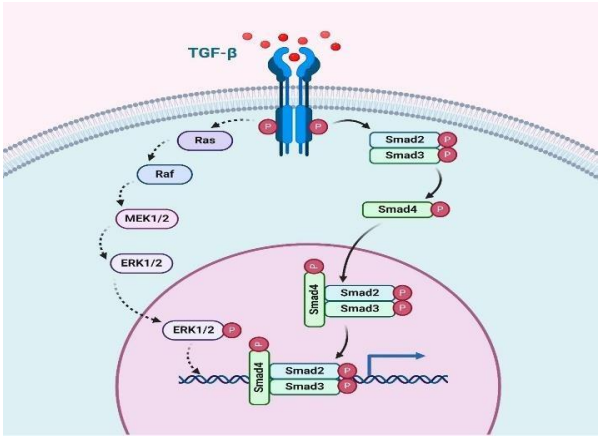

Figure 2. The image illustrates the TGF-β signaling pathway. It shows TGF-β binding to its receptor, activating Smad2/3 proteins, which then partner with Smad4 to regulate gene expression alongside a parallel Ras-Raf-MEK1/2-ERK1/2 signaling cascade.

Therapeutic Implications Of Tgf-B In Hpv-Positive Head And Neck Cancers

Therapies targeting TGF-β

Viruses like HPV and Epstein-Barr virus (EBV) have been linked to the growth of tumors. Cytokines are very important in causing inflammation and cancer [67]. Elevated levels of cytokines, including TGF-β, can be detected in both serum and saliva using ELISA assays, providing potential biomarkers for the early identification of cancer [68]. Polz-Dacewicz et al. demonstrated that patients with oropharyngeal cancer exhibited significantly elevated levels of TNF-α, VEGF, TGF-β, and IL-10 compared to healthy controls, particularly in HPV-positive cases, suggesting that salivary cytokines may serve as early indicators for OPSCC [69]. Similarly, a multivariable Cox regression analysis study by Zhang et al. also revealed that p16 positivity, one of the characteristics of HPV-positive OPSCC, was an independent predictor of overall survival in HIV-positive patients with head and neck cancers. Furthermore, TGF-β expression was associated with clinical outcomes, thus validating its serve as a prognostic marker in identification of neck and head cancer risks [70]. These findings highlight the potential of biomarker panels in predicting disease progression and guiding personalized treatment approaches by analysing biological factors such as gene expression and mutations [70].

In addition to cytokines, FcGBP, a type of Fc receptor involved in immune responses, has been identified as a potential prognostic marker in HPV-positive HNSCC. Initially discovered in the small intestine, FcGBP plays a role in cell adhesion and immune regulation [71]. Wang et al. demonstrated that high FcGBP expression combined with low TGF-β levels in HPV-positive HNSCC correlated with increased macrophage infiltration and improved overall survival. This underscores the inverse relationship between TGF-β and FcGBP, where TGF-β downregulates FcGBP expression, thereby inhibiting cell migration, and emphasizes FcGBP's functional significance in cancer progression and prognosis [72].

MicroRNAs (miRNAs), another class of small ncRNA molecules that control gene expression, also have potential in cancer treatment [73]. Even though miRNAs were initially recognized as growth inhibitory molecules, recent studies indicate that they can behave as bona fide tumor suppressors or oncogenes depending on their targets [74]. Thus, anti-HPV positive HNSCC has developed into a significant factor of miRNAs as both therapeutic and diagnostic tools [75].

Wang et al. demonstrated that HPV-positive HNSCC patients had numerically fewer counts of CAFs, while this phenomenon was considered to be a predictor of a higher survival rate. In particular, exosomes containing miR-9-5p derived from HPV-positive HNSCC cells reduced fibroblast transformation and TGF-β1-induced increases in NOX4 and ROS levels [76]. These results indicate that miR-9-5p is crucial in inhibiting TGF-β signalling and enhancing patient survival in HPV-positive cases [76] (Figure 3).

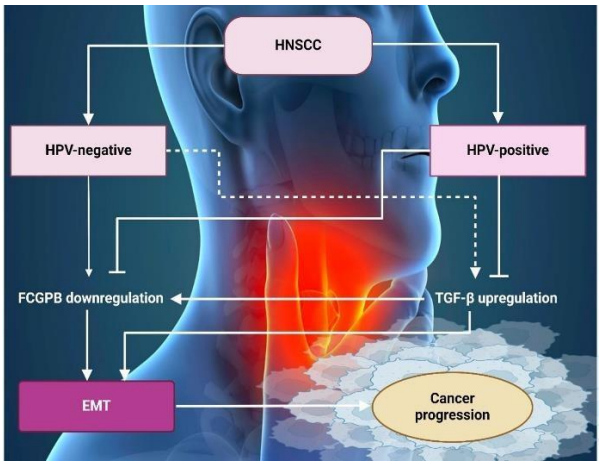

Figure 3. The image portrays the differences in pathways between HPV-negative and HPV-positive head and neck squamous cell carcinoma (HNSCC). It highlights the key mechanisms like FCGPB downregulation in HPV-negative cases and TGF-β upregulation in HPV-positive instances, leading to cancer progression.

Immune checkpoint inhibitors and their interaction with TGF-β

ICIs, encompassing CTLA-4, PD-L1, or PD-1, are molecules that obstruct proteins used by cancer cells in their effort to escape the immune system [77]. As such, ICIs prevent the binding of these proteins, thus allowing the immune system to reactivate T cells and help identify cancerous cells for destruction [78]. Clinical trials have indicated that HPV-positive patients have an improved response to PD-1/PD-L1 inhibitors as compared to HPV-negative patients, making HPV status a potential biomarker for categorizing head and HNSCC and identifying candidates for immunotherapy [79].

Wang et al. conducted a meta-analysis showing that HPV-positive HNSCC patients experienced greater benefits from PD-1/PD-L1 inhibitors, reflected in improved overall survival (HR = 0.71) and response rates (ORR = 21.9% vs. 14.1%) compared to HPV-negative patients. This enhanced response was attributed to increased T-cell infiltration, immune activation, and cytolytic activity in HPV-positive tumors. This indicates that the HPV-positive status correlates with a favourable prognosis for ICI treatment, regardless of PD-L1 expression or tumor mutation burden [80]. Thus, the immune landscape of HPV-positive tumors, driven by HPV-mediated immune modulation, could be pivotal in the success of ICI therapies [80].

In another study, Mi et al. explored the resistance mechanisms to TGF-β in human keratinocytes immortalized with HPV type 16 DNA (HKc/HPV16). These cells, representing advanced stages of malignancy, displayed growth factor independence and resistance to differentiation. Mi et al. reported that as these keratinocytes progressed toward malignancy, they exhibited a marked decrease in TGF-β receptor type I, reducing sensitivity to TGF-β-mediated proliferation suppression. However, re-expression of the TGF-β receptor type I in these resistant cells restored their responsiveness to the growth-suppressing properties of TGF-β. This study highlights the critical role of TGF-β receptor expression in mediating resistance to growth inhibition during HPV-driven cancer progression, suggesting that modifications in the TGF-β pathway play a role in immune evasion and resistance to therapies [81].

Combination Therapies

Combination therapies in cancer treatment utilize a cocktail approach, where two or more treatments are used simultaneously to maximize therapeutic benefit and address mechanisms of resistance that often arise with single-agent therapies [82]. This approach aims to target multiple cancer growth and survival pathways, thereby enhancing the overall effectiveness of treatment [83]. One innovative example is bintrafusp alfa (M7824), a bifunctional fusion protein engineered by fusing the external domain of the human TGF-β receptor II to a PD-L1-blocking IgG1 antibody. From the in vitro and in vivo experiments in murine models, bintrafusp alfa was shown to have greater antitumor activity as evidenced by diminished tumor volume and metastasis compared to TGF-β trap or PD-L1 inhibition. Several clinical trials demonstrate that bintrafusp alfa at doses of up to 20-mg / kg is characterized by acceptable tolerance in patients with heavily pre-treated advanced solid tumors. Importantly, this bifunctional protein exhibited potency in HPV-related neoplasms, including cervical carcinoma, anal carcinoma and SCCHN [84]. Cho et al. obtained an ORR of 33% with bintrafusp alfa for HPV-positive SCCHN patients,

contrasting this with an ORR of 13% using bintrafusp alfa in all SCCHN patients. The toxicity was tolerable; 34% of patients experienced any grade 3 treatment-related AE, and no grade 4 AEs or death were observed [85]. These findings suggest that bintrafusp alfa, by simultaneously targeting PD-L1 and TGF-β, may offer a potential treatment avenue for HPV-positive cancers [85].

Another approach in combination therapy involves sorafenib, a multikinase inhibitor targeting several key kinases, including VEGFR, PDGFR, and RAF, which are crucial for tumor cell proliferation and angiogenesis [86,87]. Sorafenib has shown efficacy in treating liver, renal, and thyroid cancers. Combined with cetuximab, an EGFR inhibitor, the aim was to block both angiogenesis and cancer cell growth [88]. However, Gilbert et al. found that this combination therapy had limited clinical benefit for patients with recurrent/metastatic HNSCC, as both treatment arms achieved an ORR of only 8%. Further analysis revealed that p16-negative tumors, or those homozygous for the–509C mutation, and patients with low plasma TGF-β1 levels, had better progression-free survival. Elevated TGF-β1 levels were associated with worse outcomes, suggesting that targeting TGF-β could be critical in optimizing combination therapies for specific patient subsets [89,90].

Integrin alpha 6 (α6), a transmembrane protein that forms heterodimers with integrin beta 1 or beta 4 subunits, has been identified as a prospective treatment target in HPV-positive HNSCC. These integrins play vital roles in cell adhesion, migration, and intracellular signalling [91]. An et al. found that ITGα6 overexpression in HPV-positive HNSCC cells enhanced susceptibility to HPV infection and promoted cancer stem cell-like properties, at least partially via the AKT pathway. High levels of ITGα6 were linked to a bad outcome, especially in HPV-positive patients. This suggests it could be used as a treatment approach for tumor stem cells in this group [92,93].

Targeted Therapy In A Preclinical Model In Vivo

SMAD proteins serve a vital function in mediating the actions of TGF-β in signal transduction [94]. These proteins enable the formation of a Smad complex, composed of phosphorylated Smad2/3 and unphosphorylated Smad4, which translocates to the nucleus to regulate target gene expression. In HNSCC, alterations in TGF-β signalling have been implicated in disease progression [95]. Loss of function in DPC4/Smad4 has been observed in various tumors, including HPV-positive HNSCC [96,97].

Báez et al. demonstrated that Smad4 expression was much lower in tumors that were positive for HPV (56%) compared to HPV-negative tumors (39%), suggesting that the loss of Smad4 contributes to HPV-driven carcinogenesis [98]. These findings highlight the potential of targeting TGF-β signalling by restoring or inhibiting Smad-dependent pathways as a therapeutic strategy in HPV-positive HNSCC [98,99].

TGF-β1 plasma levels have been demonstrated to be influenced by polymorphisms in the TGF-β1 gene, which in turn affects the progression and susceptibility of cancer [100]. The C509T polymorphism in the promoter region of the TGF-β1 gene is linked to almost double the plasma levels of TGF-β1 in persons homozygous for the T allele relative to those with the wild-type C allele [99,101]. Additional polymorphisms, including G915C and T869C, induce nonsynonymous amino acid alterations in the regulatory region of the TGF-β1 gene, causing fluctuations in circulating TGF-β1 levels. Research has shown that the T869C variation correlates with less TGF-β1 production, while the G915C allele is related to increased production [102]. Guan et al. discovered that specific SNPs, especially T869C, were strongly correlated with HPV16-positive malignancies in patients with SCCOP. Individuals who are non-smokers, non-drinkers, and younger, possessing variant TGF-β1 genotypes, exhibited a higher likelihood of developing HPV16-positive tumors compared to non-carriers, indicating that these genetic variations contribute to tumor susceptibility [103,104].

Changes in DNA methylation at gene promoter regions, particularly hypermethylation or hypomethylation, are recognized as key mechanisms in tumorigenesis and cancer progression [105]. Worsham et al. validated the impact of differential promoter methylation in HPV-positive HNSCC, confirming earlier findings in an independent series. Their study revealed that seven out of eleven genes were differentially methylated, with MSX2 identified as an anchor gene within the framework of the study. The study further reinforced the importance of pathways related to serotonin signalling, semaphorin signalling, and TGF-β signalling in the distinct biological mechanisms of HPV-positive HNSCC [106,107].

People with the CT/CC genotype are more likely to develop cancer due to changes in TGF-β1 production and function caused by polymorphisms at the miRNA-187 binding region [108].

Tao et al. showed that young people who don't smoke or drink much are more at risk of developing HPV16-associated OSCC when the TGF-β1 rs1982073 polymorphism is paired with HPV16 L1 seropositivity. To reduce the likelihood of malignancies caused by HPV, the results highlight the need for early detection methods and genetic screening in high-risk groups [109,110].

Mechanisms Of Therapy Resistance

Immune evasion and resistance to immunotherapy

Although HPV infection is widespread, only a tiny fraction of the afflicted people will develop cancer. Studies have shown that cervical cancer risk increases with genetic susceptibility, indicating that genetic factors influence the likelihood of developing HPV-induced cancer [111]. Host immunity also performs an essential function in susceptibility, mainly in HPV-associated tumors. Higher rates of cervical and oropharyngeal cancers among individuals with HIV/AIDS suggest that immune suppression increases the risk of HPV-related malignancies [112]. Levovitz et al. proposed that variations in immune-related genes may serve as predictive biomarkers for HPV-associated cancers, including oropharyngeal, cervical, and anal cancers. Specifically, TGFβR1 signalling, which operates through the p38-MAPK axis, was found to be increased in HPV-positive cancers, contributing to differential susceptibility to HPV-induced malignancies by affecting immune dysregulation and host-virus interactions [113,114].

HPV-positive OPSCC is linked to a more favourable outcome than its HPV-negative equivalent, partly due to increased immune infiltration and higher ERα expression [115]. Bolyos et al. showed that HPV-positive OPSCC subjects exhibited significantly better survival than HPV-positive non-OPSCC patients. In HPV-positive OPSCC, downregulation of TGF-β signalling and ECM genes led to increased immune infiltration, further contributing to a favourable prognosis. The unique immune response signatures observed in HPV-positive OPSCC, including elevated ERα mRNA expression, correlate with enhanced survival [116,117]. Immunotherapy has significantly increased overall survival for HNSCC patients, mainly when targeting the tumor microenvironment [118].

Wang et al. phase II clinical trial of cetuximab and nivolumab in individuals with reoccurring/metastasized HNSCC revealed that p16 expression, as determined by immunostaining, was related to an elevated response rate.

However, it did not impact overall outcomes. In p16-positive patients, lower tumor-tissue HPV DNA counts correlated with higher RR and longer overall survival. The analysis of immune infiltration in HPV-positive HNSCC exhibited a higher presence of macrophages, CD4+ effector T cells, plasma cells, and B cells compared to HPV-negative cancers. Notably, high FCER2 expression and the plasma cell presence in tumours positive for HPV were linked to better patient outcomes. FCER2 inhibits the proliferation and migration of HPV-positive tumors, demonstrating its potential role in combating tumor growth [118]. Macrophages and neutrophils, innate immune cells, are typically linked to poor outcomes, whereas adaptive immune cells, like lymphocytes, correlate with improved survival [119]. Sahaf et al. found that OPSCC patients had significantly higher tumor-associated neutrophil levels than HPV-positive OPSCC, a difference driven by elevated CXCL8 levels and IL-1 upregulation. Inhibiting the IL-1/IL-1R axis using anakinra decreased chemokine secretion and neutrophil recruitment, highlighting a potential mechanism behind the poor prognosis of HPV-negative OPSCC and identifying potential therapeutic vulnerabilities in these patients [120].

Role in chemoresistance and radiotherapy

TGF-β is essential for detecting DNA damage and managing key cell fate decisions such as apoptosis, cell cycle arrest, and repair [121]. Deletion of TGFβ1 or suppression of TGF-β signalling impairs these processes, as demonstrated by in vivo and cellular assays [122]. This impairment occurs because TGF-β signalling is required for the activation of ATM kinase and the expression of ligase IV, both of which are essential elements of the NHEJ mechanism for DNA repair [123]. Additionally, TGF-β regulates the expression of BRCA1, a key player in homologous recombination repair. The broad TGF-β effects on DNA repair constituents enhance the translational potential of TGF-β inhibitors in cancer therapy. Studies have shown that inhibiting TGF-β sensitizes preclinical breast, brain, and lung cancer models to radiotherapy and chemotherapy [124]. Liu et al. further demonstrated that HPV-positive HNSCC cells exhibit impaired TGF-β signalling, which disables HRR and increases dependence on alternative, error-prone end-joining repair pathways. Consequently, HPV-positive cells become more sensitive to radiotherapy, cisplatin, and PARP inhibitors. This suggests that TGF-β inhibitors could be an effective therapeutic strategy in

HPV-positive head and neck cancers by exploiting DNA repair vulnerabilities [125]

TGF-β1 polymorphisms, such as the TGFβ1 rs1800469 C509T variant, have been associated with increased TGF-β1 plasma levels. Individuals containing the T allele variant have nearly double the TGF-β1 plasma levels compared to those with the wild-type C allele [126]. Other polymorphisms, such as T869C and G915C, also affect TGF-β1 levels through nonsynonymous amino acid changes. Earlier research indicates that increased levels of TGF-β1 are linked to the T869C variant and the G915C wild-type allele [127]. These polymorphisms have been explored in the context of HPV-related cancers, including SCCO. The TGFβ1 rs1982073 CT/CC variant alleles were shown to be strongly linked with better survival outcomes, including disease-specific and disease-free survival, in HPV16-positive SCCOP patients who had chemoradiation treatment, according to Tao et al. The rs1982073 polymorphism reduced the risk of death and recurrence, further highlighting its relevance in the clinical course of HPV16-positive SCCOP [128]. HPV is known to regulate the host DNA damage repair (DDR) pathway, promoting viral replication by modulating DNA repair mechanisms [129]. SMAD4, a key mediator of TGF-β signalling, is essential for preserving cell survival after genotoxic stress [121].

Citro et al. demonstrated that HPV-positive neck and head malignancies upregulate SMAD4, leading to increased expression of DDR proteins such as CHK1 and Rad51, which promote DNA repair and viral replication. Silencing SMAD4 in HPV-positive cells has been shown to increase sensitivity to cisplatin, suggesting that targeting SMAD4 may improve treatment efficacy in HPV-positive cancers [130].

Several GWAS have recognized risk loci for HNSCC, with the HLA locus being particularly notable. These studies suggest that genetic polymorphisms related to TGF-β1 and TGFβR1 contribute to variability in immune and inflammatory responses, which may influence susceptibility to HPV-positive OPSCC [131]. Niu et al. analyzed the correlation between TGFβ1 and TGFβR1 polymorphisms and treatment outcomes in HPV-positive OPSCC patients receiving chemoradiotherapy. Their findings underscore the importance of genetic polymorphisms in predicting treatment response and failure, with direct clinical implications for optimizing therapy [132]. TGFβ1 genotype has emerged as a predictive prognostic marker in HNSCC patients undergoing chemoradiotherapy, independent of p16 status [133]. Lundberg et al. confirmed that the TGFβ1 polymorphism is linked to improved disease-free survival rate in patients with HNSCC, independent of EGFR expression. These results prove that TGFβ1 might be a useful diagnostic indicator for HNSCC treatment [134] (Table 1)

Table 1. This table outlines therapeutic approaches targeting TGF-β in HPV-positive head and neck cancers, detailing therapies, mechanisms, biomarkers, and prognostic impacts in HNSCC.

Therapy Type	Target/Mechanism	Cancer Type	Biomarkers/Genes Involved	Prognosis	Reference
Immune Checkpoint Inhibitors	PD-1/PD-L1 blockade	HPV-positive HNSCC	PD-L1, TGF-β, CD4	Higher response rates in HPV-positive patients	[80]
Combination Therapy (Bintrafusp Alfa)	TGF-β trap and PD-L1 blockade	SCCHN, HPV-positive	TGF-β, PD-L1	33% response rate in HPV-positive tumors	[135]
Sorafenib + Cetuximab	EGFR and multi-kinase inhibition	Recurrent/Metastatic HNSCC	TGF-β, p16	Nosignificant effect; elevatedTGF-β associated with worse PFS	[136]

FcGBP Protein	Interaction with immune cells	HPV-positive HNSCC	FcGBP, TGF-β	Increased survival with high FcGBP and low TGF-β levels	[137]
miRNA Therapeutics	miR-9-5p targeting NOX4 and ROS	HPV-positive HNSCC	miR-9-5p, NOX4, TGF-β	Inhibition of TGF-β signalling, improved survival	[76]
TGF-β Inhibition + DNA Damage Repair	TGF-β inhibition	HPV-positive HNSCC	SMAD4, TGF-β, DNA repair	Enhanced radiosensitivity and chemotherapy response	[125]
Promoter Methylation Studies	Differential methylation	HPV-positive HNSCC	MSX2, TGF-β	Highlighted unique TGF-β signalling in HPV-positive cancers	[106]
TGF-β1 Polymorphisms	Polymorphisms affecting TGF-β1	HPV-positive SCCOP	TGF-β1, p16	Better survival with TGF-β1 rs1982073 CT/CC variant	[138]
Integrin alpha 6	Overexpression in CSCs	HPV-positive HNSCC	ITGα6, AKT	Poor prognosis with high ITGα6 expression	[139]
SMAD4 and DNA Repair	SMAD4 in DDR pathway	HPV-positive HNSCC	SMAD4, CHK1, Rad51	Enhanced DNA repair and resistance; low SMAD4 improves treatment	[140]
TGF-β1 and p16 Genotype	TGF-β1 polymorphisms	HPV-positive HNSCC	TGF-β1, p16	TGF-β1 polymorphisms linked to better outcomes	[134]

Future Directions And Perspectives

Developing more tailored therapeutics requires careful consideration of TGF-β's dual function in cancer, as a cancer suppressor in the initial stages and as an inducer of tumours in mature stages, according to the current consensus [141]. The focus is designing genetic and molecular approaches that selectively inhibit the tumor-promoting pathways of TGF-β while preserving its tumor-suppressive functions [142]. RNA interference (RNAi) technologies and CRISPR-Cas9 gene-editing methods are being developed to block key elements of TGF-β signalling contributing to cancer development [143]. Additionally, efforts are underway to create more potent and dual-specific TGF-β inhibitors with reduced toxicity [144]. Most noteworthy, there are bifunctional fusion proteins that simultaneously antagonize both TGF-β and PD-L1, and these seem to be planning for future preclinical and early clinical studies [142]. These strategies for targeting might mean inhibiting the tumour-promoting signals of TGF-β while restoring

antitumor immunity that was previously discussed, including immune checkpoint inhibitors [145]

The concepts of the new personalized medicine approaches promote incorporating TGF-β-targeting drugs into the anticancer therapy [146]. Tumors HPV-positive, for instance, should have different sensitivity to the treatment based on the targeting of TGF-β, since these types of cancer proved to have different immune contexts [9]. More targeted biomarkers, such as the TGF-β receptor expression levels or specific genetic mutations, may define the populations who can benefit from these drugs [147]. It also enables one to provide better therapy outcomes for the patient while at the same time avoiding more treatments, thus minimizing side effects. The improvements in biomarker-directed therapies are promising for the cancers because TGF-β is a crucial factor for tumour promotion [148].

Conclusion

TGF-β is a cytokine that has essential functions in contributing to damage and cellular migration regulation. Because of this, it is easier to design trials targeting the diverse role of FAK in tissue remodelling and tumorigenesis. Nevertheless, TGF-β inhibitors' anti-tumor effects are still unproven clinically owing to the lack of non-orientation and minimal use of these inhibitors in the clinical treatment of HNSCC. Promising outcomes in the HPV-positive population have been noted. Combinational strategies targeting TGF-β and common agents such as PD-L1 or EGFR inhibitors have provided uneven results. Several biomarkers have been identified to reflect the immune context as a key factor in determining the response to treatment indicated for advanced cancer, for instance, & no effort to implement them in clinical practice has been easy.

Due to the dual role of TGF-β, future therapeutic models must target the tumour-promoting action of TGF-β but spare its tumor suppressor actions. Further developing biomarkers and the new field of "-medicine'' will be paramount in creating an individualized therapy plan covering scientific aspects such as the patient's HPV status and TGF-B activity. The success of implementing TGF-β inhibition in clinical management will therefore depend on furthering these strategies and the characterization of the tumor microenvironment. Countering the complexities of the TGF-β signalling is critical in developing a better approach to cancer treatment especially for HPV-positive cancers, which are regulated by this cytokine.

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