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Review

The effect of human papillomavirus on DNA repair in head and neck squamous cell carcinoma

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ABSTRACT

Much of the current literature regarding the molecular pathophysiology of human papillomavirus (HPV) in head and neck squamous cell carcinoma (HNSCC) has focused on the virus's effect on cell cycle modulation and cell proliferation. A second mechanism of pathogenicity employed by HPV, dysregulation of cellular DNA repair processes, has been more sparsely studied. The purpose of this review is to describe current understanding about the effect of HPV on DNA repair in HNSCC, taking cues from cervical cancer literature. HPV affects DNA-damage response pathways by interacting with many proteins, including ATM, ATR, MRN, γ-H2AX, Chk1, Chk2, p53, BRCA1, BRCA2, RAD51, Rb-related proteins 107 and 130, Tip60, and p16INK4A. Further elucidation of these pathways could lead to development of targeted therapies and improvement of current treatment protocols.

Introduction

Emerging research continues to clarify the manner in which HPV-associated head and neck squamous cell carcinoma (HNSCC) contributes to a favorable prognosis. Evidence for improved outcomes in this cancer subset relative to HPV-negative HNSCC has generated interest in de-escalation treatment protocols in order to mitigate treatment toxicity while preserving survival rates [1–3]. However, scientists have only begun to elucidate the molecular mechanisms underpinning this relationship. A majority of current literature has focused on the contributions of oral HPV infection to cell cycle modulation and cell proliferation through the relationship of oncoproteins E6 and E7 to tumor suppressor proteins p53 and Rb [4]. However, downstream implications of these pathways with regards to DNA repair have largely been neglected. DNA repair alterations influence mutation acquisition and cellular resistance to DNA-damaging therapeutic modalities, and therefore, a greater understanding of the influence of HPV on DNA repair pathways could help inform clinical management of this cancer subset [5]. This review discusses DNA repair modulation in HPV-associated HNSCC and highlights gaps in the current literature.

Interaction of HPV with DNA-damage response (DDR) pathways

HPV, a simple circular double-strand DNA virus that encodes only 6–8 genes, heavily depends on host factors for viral replication, including host's DNA damage response and repair machinery. HPV infection has been associated with dysfunction of DDR pathways in HNSCC through several mechanisms. In fact, HPV can activate and dysregulate DDR pathways throughout various stages of their life cycles to replicate itself in host cells. Elucidation of mechanisms of hijacking and manipulation of host DDR machinery by HPV may help explain the tumorigenesis of HPV-positive HNSCC and lead to the development of more effective treatment for HPV-positive HNSCC.

DDR pathway activation by HPV

Several integral DDR pathways are modulated as a result of HPV infection, including ataxia telangiectasia-mutated kinase (ATM) and ataxia telangiectasia and Rad3-related protein (ATR). ATM, in particular, is activated primarily in response to double-stranded

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DNA breaks (DSBs) and has the ability to restart broken replication forks. ATR, conversely, functions as part of a response to replication stress and participates in nucleotide excision repair and homologous recombination (HR) [6]. Another part of this pathway, the Mre11-Rad50-Nbs1 (MRN) complex, contributes to non-homologous end joining (NHEJ) by activating ATM. HPV uses the Nbs1 protein to initiate the MRN complex in order to facilitate viral replication [7]. As tumorigenesis progresses, function of the Nbs1 gene is often abrogated. A decrease in function of the MRN complex consequently hinders the cell's ability to perform NHEJ, contributing to growth of the neoplasm [8]. These pathways are affected during two distinct stages of the viral life cycle, initial infection, and chronic infection prior to oncogenesis. Many studies point to the above pathways as potential targets for novel therapeutic strategies or possible use as biomarkers for early detection and improved treatment efficacy monitoring [9–11].

Several critical HPV proteins, notably E1 and E2, activate DDR pathways to promote viral replication. Numerous proteins associated with DDR (including ATR, ATM, γ-H2AX, Chk1, Chk2, p53, BRCA1, BRCA2 and RAD51) are found to co-localize with the HPV replication complex along with viral proteins, such as E1 and E2 [6]. γ-H2AX is phosphorylated by ATM in order to flag sites of DSBs. Chk1 is integral to ATR-mediated DNA repair, which primarily repairs ssDNA and resected DSBs. Chk2 works with MRN in the activation of the ATM pathway to perform DSB repair [5]. RAD51 participates in NHEJ and also functions independently in DSB repair through an inadequately elucidated mechanism [6,12]. p53 and BRCA1 have been shown to decrease and increase the activity of HR, respectively [13]. Although the direct interactions of E1 and E2 with these and other DDR proteins have yet to be fully described in the literature, they produce substantial dysfunction of DNA repair processes. Further exploration of these mechanisms could prove fruitful for the development of targeted therapies [10].

DDR deactivation by HPV

Activation of DDR response is important for initial HPV replication; however, ATM/ATR signaling activation ultimately would be detrimental for HPV replication as host cells would undergo growth arrest. Thus, in order to maintain long-term, stable viral replication, HPV viral proteins E6 and E7 hinder some of the DDR pathways, such as p53 and Rb, as part of the normal viral life cycle by arresting cell growth and preventing the induction of apoptosis. This subsequently allows time for viral DNA synthesis and replication. The mechanisms by which E6 and E7 manipulate the cell cycle are well discussed elsewhere in the literature [14,15], and therefore will only be addressed here in the context of interaction with DNA repair. E7 activity is associated with gradual accumulation of DSBs in rodent models, as evidenced by recruitment of DNA damage markers including γ-H2AX foci [16]. It has been postulated that suppression of DDR by E7 may prime the cell for additional DNA damage, which could partially explain why HPV-positive cells are more susceptible to radiotherapy [17]. Park et al. demonstrated in their rodent model that Rb and Rb-related proteins 107 and 130 are all targeted by E7. The disruption of each of these proteins led to decreased DDR, increasing cellular acquisition of mutations [16]. E6 and E7 have also been shown to induce hypermethylation of the promoter SMG-1, which may reduce DDR activity and impede DNA repair due to its role in mRNA surveillance [18]. In addition, Tip60, an acetyltransferase that acetylates and activates ATM, is degraded by E6 upon HPV genome integration [19].

The p16INK4A protein, nearly ubiquitous in HPV-positive HNSCC [20], is a well-known deregulator of the cell cycle. In addition, p16INK4a regulates the activity of HR. Overexpression of p16INK4a has been found to impair recruitment of RAD51 to the site of DNA damage, directly impacting HR-mediated DNA repair [21]. Moreover, a recent study has shown that HPV p16 overexpression repressed DDR activation mediated by TRIP12 and enhanced radiation-induced DSBs [22].

DDR in HPV-positive patients who are smokers

Although epidemiological data indicates that smoking is not associated with the risk of developing HPV-positive HNSCC, it has been well documented in the literature that patients who have exposure to cigarette smoke that go on to develop HPV-associated HNSCC have poorer outcomes [23–25]. Tobacco contributes to the oncogenesis of HNSCC through many molecular pathways, including DDR pathways which are often activated during both cancerous and non-cancerous cellular response to tobacco exposure [26,27]. Part of the additive risk from both exposures is the HPV-related downregulation of DDR pathways, which could otherwise mitigate damage associated with tobacco use. A recent study on molecular profiling implicated that HPV-positive cancer with smokers may be initiated by viral E6/E7 but the effects of tobacco-related mutations become more predominant as cancers progress. However, data regarding this hypothetical relationship is only just emerging [28].

Preferential disruption of DDR pathways by HPV

While the integration of HPV into the host genome is not targeted, selective pressures of the microenvironment cause some areas of the genome to be disrupted by HPV integration more than others [29]. Included in these affected areas are locations with known DNA fragility. Due to this non-random integration of the virus, integration occurs more often into specific sites in the host genome, including RAD51, causing dysregulation of the RAD51 protein and hindering DSB repair [30]. HPV can integrate directly into a gene, which disrupts function of the gene product, or adjacent to a coding region, causing duplication and/or deletion of nearby introns [31,32]. Through these mechanisms, integration of HPV can produce either loss of function or overexpression [33,34]. It has been suggested that the increased sensitivity of HPV-positive HNSCC to chemoradiotherapy may be in part due to disruption of many of the DDR pathways, such as Rad51, TP63, and NR4A and subsequent compromise of SSB and DSB repair after the treatment, which promote cell cytotoxicity [5,34,35]. The profound, preferred disruption of selective pathways, such as DDR pathways, in HPV-related HNSCC may be one of the reasons we see relatively low intra-tumor heterogeneity in these tumors, compared to HPV-negative tumors with smoking history [36]. Further research into specific pathway targeting is needed to investigate how HPV genomic integration could be manipulated for therapeutic benefit [10,37].

Lessons from cervical cancer literature

A substantial amount of research regarding the molecular pathophysiology of HPV in cervical SCC has accumulated since 1983 [38], providing a wellspring of literature that can be used as a springboard for research on HPV-related HNSCC. Numerous similarities between the cancer types indicate that findings in cervical carcinoma could inform research efforts in the realm of head and neck cancer [29,39]. Future research could explore other relationships shown in cervical cancer, including the impact of HPV on other critical DNA repair components such as the reappropriation of DNA-PKcs/Ku70/Ku80 during viral replication, the primary pathway involved in NHEJ [40,41]. However, differences in the roles of HPV infection do exist between the two cancer types, suggesting