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Current Understanding and Potential Immunotherapy for HIV-Associated Squamous Cell Carcinoma of the Anus (SCCA)

Christian Marin-Muller, BS[1,2], **Min Li, PhD**[1], **Changyi Chen, MD, PhD**[1], and **Qizhi Yao, MD, PhD**[1,2,*]

[1]Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas, USA

[2]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, USA

Abstract

Squamous cell carcinoma of the anus (SCCA) is a rare disease in the average population, but is of increasing concern among immuncompromised individuals such as the HIV seropositive. Coinfection with human papillomavirus (HPV) in this population is common. HPV infection is difficult to clear with a compromised immune system, which results in a greater risk of tumor development and a more aggressive progression of the disease. The recent approval of a prophylactic HPV vaccine for cervical cancer has sparked an interest in a search for improved immunotherapeutic multimodality therapies to combat anogenital tumors associated with the virus. In this review, we discuss the known mechanisms of action of HIV-associated SCCA, examine the current treatments for the disease, and focus on the potential of an immunotherapeutic vaccine approach for both prophylactic and therapeutic application.

Keywords

Squamous cell carcinoma of the anus; HIV; HPV; vaccine; immunotherapy

Introduction

Squamous cell carcinoma of the anus (SCCA) is an uncommon malignancy that represents 1.5% of all gastrointestinal cancers in the United States, accounting for only 3,400 cases per year [1-3]. Yet in the last few decades a marked increase in the incidence of anal cancer has been observed in both male (93%) and female (48%) populations [1]. This increase is even more marked within the immunosuppressed individuals [1,4]. While anal carcinoma is rare in the average population, it has an increased prevalence among homosexuals, immunosuppressed individuals, and those seropositive for human immunodeficiency virus (HIV) infection. The risk of SCCA in HIV-positive individuals is 120 times higher in these populations, making anal carcinoma the fourth most commonly reported malignancy among men with HIV infection [5,6]. Here, we will summarize the current understanding of SCCA; relationship of HIV to human papillomavirus (HPV)-associated SCCA; treatment of immunocompetent and immunocompromised patients; and vaccine candidates and immunotherapy for SCCA.

*Address correspondence to: Michael E. DeBakey Department of Surgery Baylor College of Medicine One Baylor Plaza, Mail stop: BCM360, Houston, TX 77030 Phone: (713) 798-1765 Fax: (713) 798-1705 qizhiyao@bcm.edu.

Current understanding of SCCA

SCCA tumors begin in the squamous cells that line the lower part of the anus and the majority of the anal canal. They are classified topographically according to the anatomy of the canal's three zones, the upper, middle, and lower parts, which are covered with rectal type mucosa, anal transitional zone mucosa, and partly keratinized squamous epithelial cells, respectively. Upper and middle region tumors are known as anal canal lesions, and lower region tumors are known as anal margin tumors and perianal lesions [7]. Anal cancers are further subdivided into various histological variants. Tumors have a predilection for the transformation zone, the transition site from the cylindrical to squamous epithelium of the anus, which makes squamous cell carcinoma the most common variant [8].

The pathogenesis of anal cancer is still poorly understood; yet many factors are known to be important in the development of the disease, including smoking, initiation of sexual life at an early age, having a large number of children, and the use of oral contraceptives for an extended time [9-11]. Viruses have also been implicated in the development of anal cancer. In addition, SCCA is known to share features with cervical carcinoma. Among subjects without a history of genital warts, for example, a history of seropositivity for herpes simplex virus type 2 (HSV-2) has been found to be associated with anal cancer [12]. While HSV and other viruses such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) have been investigated as potential agents [12], SCCA is most strongly associated with human papillomavirus (HPV) infection, a feature SCCA shares with cervical cancer [5]. The establishment of a persistent HPV infection is thought to be central to the development of the majority of cases of cervical cancer, since over 95% of cervical tumor specimens harbor HPV DNA [13]; as the result of a series of epidemiologic studies, it is now apparent that HPV infection is central to anal cancer development as well [2,14]. In fact, the presence of HPV DNA in SCCAs is so high (ranging in the literature between 35 to 60%) [15], suggesting that HPV presence may be a necessary cause for squamous cell carcinomas of the anal canal [16].

HPV are small, nonenveloped, double-stranded DNA viruses that infect skin or mucosal tissue, and are associated with a variety of clinical conditions ranging from innocuous lesions to cancer [17]. HPV infection is the most common sexually transmitted infection among sexually active couples, with an annual incidence of approximately 5.5-6.2 million. An estimated 75% of sexually active men and women have been exposed to HPV at some point in their lives [18]. HPV are subdivided into types based on their genome sequences, with each type identified by a number. Each HPV type occurs in the form of variants, differentiated by about 2% nucleotide differences in most genes and 5% in less conserved regions [19]. More than 100 HPV types have been identified to date.

The considerable variation present in the HPV genome results in variations in the strength of the association of different HPV types with different types of cancer [13]. Infection with many of these subtypes can lead to premalignant transformation of squamous epithelial cells [14], resulting in high-grade squamous intraepithelial lesions as well as invasive squamous cell carcinoma [20]. HPV-16 and -18, for example, account for about 70% of cancers of the cervix, vagina and anus, and for about 30%-40% of cancers of the vulva, penis and orophaynx [21]. HPV-16 has been shown to be present in the majority of anal cancers using PCR identification techniques [22,23]. In many cases, infection is not limited to only one subtype; in fact, infection with multiple HPV types is common among subjects with anal cancer. It is unclear whether multiple subtype infections are associated with a higher risk of malignancy or a more rapid rate of progression [9].

It is known that oncogenic strains of HPV (primarily HPV-16 and 18) integrate into the host cell's DNA and cause progression from the normal epithelium to carcinoma. They are thought to alter cell cycle control, causing chromosomal instability [24]. However, the exact mechanism of HPV-induced malignant transformation has not yet been determined, and may be different in HIV-negative and positive individuals [25]. High-risk HPV types encode for three oncoproteins with growth-stimulating and transforming properties, E5, E6, and E7 [26]. Evidence points to a critical role for the HPV E6 and E7 genes; high-grade squamous intraepithelial lesions have higher levels of expression of these particular proteins than do low-grade lesions. In addition, continued expression of both these proteins is required for maintenance of the malignant state [23,27], and keratinocyte transformation has been shown to require E6 or E7 protein expression [27]. It is also likely that variations in the E6 gene sequence may allow evasion from the host immune system, which would result in increased viral persistence [28]. A mechanistic possibility is that HPV induces high grade dysplasia by integrating into host DNA and/or inhibiting the p53 protein, possibly through the interaction of E6 proteins with host factor E6AP ubiquitin ligase, binding p53 and causing its degradation [15]. The *p53* gene is frequently wild type in HPV-related cervical cancers, and the same is likely in SCCA. It is therefore likely that E6-mediated abrogation of p53 protein-dependent apoptosis has the equivalent effect to inactivating mutations of the *p53* gene [26]. In addition, Hiller *et al*. [29] recently stated that several variants of HPV E6 protein may play a role in supporting increased viral persistence by avoiding immune responses.

E7 oncoprotein binds and degrades the retinoblastoma tumor suppressor protein (pRB), as well as additional targets, including cyclin-independent kinase inhibitors and several transcription regulators [30]. By inhibiting the functions of both E6 and E7 proteins with antisense mRNA, Hu *et al*. [31] were able to convert HPV-transformed cells back to the normal phenotype. E6 and E7 thus represent potential therapeutic targets. A more detailed understanding of the mechanisms of transformation and progression of SCCA is needed, in particular in light of evidence indicating possible alternate pathways in HIV-infected individuals [26].

Relationship of HIV to HPV-associated SCCA

HIV and HPV coinfected patients are at high risk of developing precancerous anal lesions and anogenital cancers [4,32]. The incidence of invasive anal cancer is 120 times higher in the HIV-infected patients than in the general population and is significantly increased in AIDS patients as well [21,33]. HIV increases the relative risk of anal carcinoma by 1.7 in women and 3.1 in men [4], particularly in the decade from 5 years before to 5 years after AIDS, compounded by more frequent relapses and a longer median time to a cure following treatment [34].

Anal cancer is preceded by high-grade squamous intra-epithelial neoplasias otherwise known as Bowen's disease [12,26,35]. HIV-positive patients with perianal Bowen's disease are more likely to progress from low-grade to high-grade SCCA within a given time period compared with HIV-negative individuals, and can subsequently progress to invasive SCCA within a short period despite multiple therapies [36,37].

It is thought that HIV increases the activity and duration of HPV infection, due to the ineffectiveness in clearing the infection in immunocompromised patients, thus leading to an increase in anal carcinoma and the aggressiveness of the tumors [1,38,39]. In immunocompetent individuals, HPV infection is a transient one, with an average duration of 8 months followed by spontaneous clearance by humoral and cellular immune responses against viral antigens [18,40]. HIV-positive patients, on the other hand, have persistent HPV infection in the anal canal which promotes a high rate of persistent shedding of HPV

particles. This persistent shedding is considered critical in the development of intraepithelial neoplasia, particularly in HIV positive women [40,41]. Prolonged HPV may also result in the reactivation of previously acquired HPV infection, and a subsequent loss of control of HPV viral replication [4].

In addition, several other factors come into play: HIV-1 tat seems to potentiate expression of E6 and E7 [42]; the increase in Langerhan's cells (LCs) in the anal mucosa which is normally seen during HPV infection is absent in HIV-positive patients, which may favor increased propagation of SCCA [43]; lastly, HIV alters dendritic cells, which are involved in early presentation of antigens for lymphocytes [44]. The prevalent HPV relapses in immunosuppressed populations are likely also a result of an HIV-mediated altered immune state, as evidenced by the increased prevalence of HPV and decreased CD4 count seen with prolonged HIV infection [39].

The reason for the relatively recent increase in SCCA among immunosuppressed individuals is most likely due to the advent of treatment with highly active anti-retroviral therapy (HAART). Although the medications included in this therapy do not cure HIV infection, they allow the immune system to reconstitute and have led to a dramatic reduction in mortality and to decreased morbidity [45]. In HIV-negative individuals, HPV-associated anal cancer has a latency period of 5 to 40 years. While it is thought that immunosuppression may accelerate the development of the malignancy, a similarly long latency period is still observed in HIV-positive patients [46]. HAART has reduced the prevalence of opportunistic infections, and as a result patients are living longer. The longer life expectancy of HAART patients correlates with an increased opportunity for invasive anal carcinoma to develop [4,46,47]. In addition, evidence shows that despite HAART-mediated immunoreconstitution, this therapy seems to have limited impact on the development of HPV-related lesions, which is consistent with an increase in lesion prevalence with extended time [48].

Treatment of SCCA in immunocompetent and immunocompromised patients

Three decades ago the primary treatment for SCCA was abdominoperineal resection (APR) with resultant colostomy [8]. APR involves removal of the anus, the rectum, and part of the sigmoid colon along with their draining lymph nodes, through incisions made in the abdomen and perineum [49]. APR still remains an often-used salvage procedure for treatment. However, the introduction of the Nigro protocol in 1974 began a transition towards non-surgical treatment of SCCA [50,51]. Nigro *et al*. [52] pioneered the use of neoadjuvant concomitant fluorouacil, mytomicin, and radiotherapy for anal cancer [52]. Since then, sphincter-preserving approaches such as chemotherapy and radiation treatments have gradually become the standard treatment [8,51], and the need for radical colostomy surgeries has dropped.

Chemoradiotherapy (CRT), as this combined treatment of radiotherapy and chemotherapy is known, has proven effective since the early 1990's, and is still advocated as a reasonable treatment, resulting in 5-year overall survival and colostomy-free survival rates of 50 to 78% and 61 to 76%, respectively [53]. CRT has replaced radical surgery and separate chemo and radiotherapy treatments as the initial treatment of choice for SCCA [47,53-55]. It improves local control, and has resulted in colostomy-free and even disease-free survival in large tumors [56]. However, this therapy has toxic side effects such as nausea, diarrhea, anal ulcers, and other chronic effects. Eighteen percent of patients in one study experienced acute grade hematologic toxicity [57], and even short-term toxicity is always a concern, since this requires patients to take breaks from radiation treatments [58]. Some improvements in

toxicity have resulted from the effective progression from the Nigro protocol, which used the addition of mytomycin C (MMC) to 5-fluorouracil (5-FU) and radiotherapy [50], to the less toxic combination of combined modality therapy with external beam radiation, cisplatin, and 5-FU [59]. Cisplatin is an effective substitute of MMC, resulting in favorable long-term survival without as many toxic effects [60]. Even with the toxicity issues, chemoradiotherapy as a treatment has been shown to be comparable to conventional APR [49,53] as far as overall patient survival rates are concerned, allowing surgical resection to be used solely as a salvage treatment. While a multimodality treatment (defined as local excision followed by chemoradiotherapy) [49] has improved the outlook for patients who might previously had only the radical surgery option, there is still much room for improvement in the treatment of SCCA [47]. Several approaches have been attempted at improving treatment results, such as dose intensification of different ratios of chemotherapy and radiotherapy. One approach has been to combine neoadjuvant chemotherapy followed by concomitant radiotherapy-chemotherapy, with the goal in mind that the initial induction chemotherapy may reduce tumor bulk prior to the concurrent chemoradiation treatment, which would allow for improvement of local oxygenation and thus result in a higher probability of local control post irradiation [61]. Salama *et al*. [52] recently studied an approach using concurrent chemotherapy and intensity-modulated radiation therapy, with results suggesting favorable tolerance of toxicity levels.

However, minor improvements have not yet yielded an ideal therapeutic scheme. Even after chemo and radiotherapy treatments, complications and local treatment failure often result in the need for APR in approximately one third of patients [62]. In these cases, the outlook is grim, as salvage surgery of this kind is associated with significant morbidity, with long-term survival achieved in just 39% of patients [63].

While chemoradiation rapidly became the standard in the treatment of SCCA for the immunocompetent, many clinicians were wary of adopting this approach in HIV seropositive individuals [55]. This resistance was based on reports of considerable levels of toxicity for these patients in the past, which found differences in diagnostic, therapeutic and prognostic results between HIV positive and negative patients [64]. More recent studies have demonstrated that with the advent of the HAART era, toxicity and treatment outcomes of chemo-irradiation are quite similar [14,47,55,65]. Thus chemoradiotehrapy has become the standard treatment in immunocompromised patients as well as in the immunocompetent [66].

The current view is that patients with CD4+ cell counts greater than 200/micromol, particularly those receiving HAART, should have their cancers managed in the same manner as their HIV-negative counterparts; treatment should not be withheld based on HIV status [65-67]. A recent study by Chiao *et al*. [65] revealed that in cases where there were no differences between the receipt of treatment for HIV-positive and HIV-negative individuals, the survival rate was the same for both groups. The 2-year observed survival rates were 77% and 75% among HIV-positive and HIV-negative individuals, respectively. In multivariate Cox analysis, significant predictors of survival were age, sex, metastasis at diagnosis, and comorbidity score, while HIV infection did not affect survival.

However, in patients with CD4 counts below 200/micromol, the claims that HIV positive patients experience greater toxicity to chemoradiation treatments may be warranted. Studies have found that these patients need longer treatment breaks and dose reductions as a result of the increased toxicity, making them more susceptible to recurrences and requiring modification of treatment regimes for such patients [3,31]. The value of combined modality in AIDS patients and those who are in advanced stages of the disease is questionable [56].

As with all cancers, prevention is the key, the higher rate of SCCA observed in HIV positive patients should be an indication that additional preventative screening is in order. Using cervical cancer prevention as a paradigm, anal Pap smear screening, high resolution anoscopy, colposcopy, and other screening methods have been proposed as part of routine HIV preventive care to detect precancerous anal lesions in the hope of decreasing anal cancer rates [10,14,48,67-70]. One study demonstrated that screening as part of routine HIV care revealed cytologic abnormalities in patients without obvious risk factors, suggesting that anal cancer screening should be performed on all HIV-infected patients regardless of gender or HIV risk factors, and not just in those with decreased T cell counts or chronic inflammation, as is common [71].

Vaccine candidates and immunotherapy

The most effective methods currently available for treating invasive anal carcinoma in HIV patients continue to have uncertain outcomes, making successful regimens for treatment of the disease difficult to implement [72]. Currently the campaign for more effective treatment of SCCA involves observing patients with premalignant lesions with the intent to detect and destroy early invasive lesions. The standard treatment after screening has been surgical resection or laser ablation, followed by CRT once the neoplasia becomes widespread [73]. However, dysplasia has been difficult to eradicate with these current treatments, and has a high level of recurrence, particularly among the HIV population [74]. New treatment options are clearly needed. The current consensus for cancer treatments is that an immunological strategy needs to be combined with chemotherapy and radiotherapy in an expanded multimodality combination treatment in order to the greatest effect on the reduction or elimination of tumor cells [75]. Thus there has been a recent drive for immunotherapeutic strategies that establish a balance between anti-tumor activity, reduction of side effects, and low levels of recurrence.

The search for an immunotherapeutic approach in recent years led to a milestone in the field of cervical cancer, with the development of the first approved HPV vaccine. The HPV vaccine is a quadrivalent vaccine that consists of recombinant viral-like particles (VLPs) of 4 HPV subtypes mixed with an aluminum-containing adjuvant [32]. HPV VLPs can be generated by synthesis and self-assembly *in vitro* of the major virus capsid protein L1 [76], a protein expressed during productive HPV infection. HPV L1 VLPs are practically identical to native virions from a morphological and antigenic standpoint, a feature that has allowed the technology to be exploited to produce subunit vaccines which elicit high titers of anti-L1 VLP antibodies [76]. The 4 subtypes of HPV found in the quadrivalent subunit vaccine include HPV 16 and 18, which cause 70% of cervical cancers, and HPV 6 and 11, which cause about 90% of genital warts, thus allowing the HPV vaccine to prevent most genital warts and most cases of cervical cancer [77]. The close association of SCCA with HPV indicates that SCCA may also be a good candidate for an anti-HPV prophylactic vaccination strategy.

The quadrivalent HPV vaccine has been a great step forward in the search for cancer immunotherapy in general, and may also have a direct impact on the treatment of SCCA. A phase III clinical trial was conducted to evaluate the efficacy of a prophylactic quadrivalent vaccine in preventing anogenital diseases associated with HPV-6, -11, -16, and -18 [78]. The quadrivalent vaccine significantly reduced the incidence of HPV-associated anogenital diseases in young women [79], and therefore has the potential to be used successfully against SCCA.

One drawback of this vaccine in its current form is that it is only useful from a preventative standpoint, and cannot be used to clear a preexisting infection, nor can it prevent an

infection with a different type of HPV [80,81]. Thus, the search continues for an antigen that would allow for an effective vaccine against SCCA that may act both as a preventative and therapeutic vaccine. The method of creating VLP-based vaccines should theoretically be expandable to additional antigenic molecules that may fit these criteria, and the approach can be applied to chimeric VLP vaccines against HIV-associated SCCA. The challenge is to find an antigenic molecule that would elicit an effective immune response. While VLP vaccines have shown prophylactic efficacy, their therapeutic potential is limited by the absence of capsid gene expression in tumors [82].

The viral oncogenes E6 and E7 of high-risk HPV types such as HPV-16 and HPV-18 are regularly expressed in anogenital precancerous lesions and cancers, and make for potential candidates for immunization [42]. Thus there is a clear interest in immunotherapy via therapeutic vaccination against these gene products, and multiple methods are being studied for effective treatments. Ohlschlager *et al*. [83] tested naked DNA containing a modified HPV-16 E7 for immunization against cervical cancer, and found that it induced an E7 wildtype-specific T cell response. Klencke *et al*. [73] examined the feasibility of using an agent containing a segment of HPV-16 E7 protein towards induction of an immune response against E7. However, this was a phase I trial designed to test safety, and thus the effectiveness of the treatment remains to be properly evaluated in future trial progressions. A successful phase II clinical trial was recently conducted using a vaccinia virus MVA E2 recombinant vaccine, which showed a successful regression of high-grade lesions in female patients [84]. More recently, Kaufmann *et al*. [85] combined the immunotherapeutic effects of L1 and E7 proteins in a chimeric VLP vaccine trial. The chimeric molecules were able to induce L1 and E7-specific T lymphocytes. The vaccination also resulted in the induction of new B cell clones, and a good safety profile was established. L2E7E6 chimeric vaccines are also being tested to neutralize HPV infection [82]. Future studies are needed to demonstrate the efficacy of chimeric molecules against SCCA, and the effect such a treatment may have on immunosuppressed patients.

Summary

Clearly, immunotherapeutic approaches utilizing E7 proteins or other as yet-to-be identified antigenic molecules are a promising alternative to the current limited treatments for SCCA. In particular, an antigenic marker might provide a control of intraepithelial lesions at different stages of cancer progression, and would make treatment of high-grade dysplasia a possibility. An antigenic molecule that raises an immune response to high-grade intraepithelial pre-cancerous lesions would give surgeons a welcome alternative to current therapeutic methodologies. The advent of microarray technology has allowed for the identification of many genes that are overexpressed in tumor cells of various cancers. Currently, gene expression patterns in pancreatic cancer have been studied using such platforms [86], and will undoubtedly be applied to SCCA tumors in future studies. However, it remains to be seen what impact these vaccines may have on immunosuppressed individuals, as little is known of the mechanisms of tumor progression in this population.

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