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Epstein-Barr Virus; A Nascent Viral Link with Oral Squamous Cell Carcinoma

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MWS and QJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FAB managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is one of the most common malignancy among both sexes worldwide. Incidence rates have been found to be increasing throughout the world from past few decades. Other than known etiologies like alcohol, smoking and tobacco, viral association has been found to be significant in some cases. Recently, a few studies have stressed on the significance on the role of Epstein - Barr virus (EBV) in OSCC as a risk factor. In this review article, we try to merge facts and link of EBV with OSCC on the basis of multiple significant past studies.

Keywords: Epstein-bar virus (EBV); Oral squamous cell carcinoma (OSCC); carcinogenesis; virology.

1. INTRODUCTION

Amongst the most common malignancies in the world, oral squamous cell carcinoma (OSCC) stands at number six. It accounts for approximately 5% of the malignant tumors in developed countries and its incidence has been shown to be on a rise throughout the world [1]. It contributes to nearly 90% of total head and neck squamous cell carcinomas (HNSCC) [2]. OSCCs

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usually originate from the non-keratinizing stratified mucosal epithelium and show morphological similarity to squamous cell carcinomas of other body regions, like those of cervix, anus, or bronchi.

Lifestyle and environment, both play a significant role in the development of OSCC directly and indirectly. Habits such as tobacco products use, betel nut chewing and alcoholism play an important role in its prevalence. Although these are considered to be major risk factors, it has been seen that these do not always show a positive correlation in the development of OSCC. Some people in fact develop the condition without any obvious risk factors [3]. This suggests that there may be factors other than the aforementioned, which could play a role in the process of carcinogenesis in the oral mucosa.

The role of diet and genetic predisposition also cannot be ruled out. Some researchers have noted that apart from habits, chronic irritation, chemicals, certain types of radiations, as well as some viruses do play a role in the process of carcinogenesis [4–7]. Some of these viruses are human papilloma viruses (HPV), herpes simplex virus (HSV), hepatitis C virus and Epstein–Barr viruses (EBVs) [8–10].

2. VIRUSES

In 1966, Rous was awarded the Nobel Prize for his discovery of tumor-inducing viruses. Concomitantly, the US congress launched the Virus Control Program (VCP) in 1964. Although, the program could not find a human carcinogenic virus, it still succeeded to discover novel information about the mechanism through which viruses converted normal living cells into altered forms [11-12].

Multiple cohorts from the past century indicate an increase in the incidence of head and neck cancers. This could largely be attributed to exposure to individual and environmental carcinogens, but the role of oncogenic viruses cannot be ruled out. Studies have indicated a significant synergistic association of the aforementioned risk factors with other factors like diet, body mass index, oral hygiene and viral infections in the development of oral cancers [13]. The viruses most commonly associated with this transformation are human papilloma virus (HPV) [14,15], herpes group viruses [16], adenoviruses [17], and hepatitis C viruses [18-19].

In the early decades of the last century, the role of viruses in carcinogenesis started getting highlighted. Their oncogenicity was first demonstrated in chicken, rabbits and rodents [20]. In the latter portions of the same century, the role of viruses in the carcinogenic process became a well-established fact. Since then, different viruses have been attributed to development of carcinomas and sarcomas in various parts of the body.

Among the identified viruses, HPV and herpes have been the most thoroughly studied. These are now considered to be the most likely “synergistic viruses” involved in human oral cancer development. The herpes viruses most often linked to oral cancer are the Epstein-Barr virus (EBV), human herpes virus- (HHV-) 8, and cytomegalovirus (CMV) [21].

In this review, we will discuss the approaches taken by various researchers worldwide to discover human cancer viruses and the methods for detecting new viruses. We will also discuss the cause-effect relationship between the co-factors and the virus-associated cancers.

2.1 Guidelines for Viral Carcinogenesis

The various carcinogenic viruses share certain common feature. Topmost of these features include non-lethal infection of the host cell and the ability of long term domestication therein [22]. They then evade the host immune response to prevent it from attacking them. Chronic inflammation and host cell mutations play a critical role in this transformation process [23].

Different guidelines have been proposed to aid in establishing a causal relationship between viruses and human cancers [24–27].

2.1.1 Evans and Mueller Guidelines [25]

2.1.1.1 Epidemiologic guidelines

(1) Geographic distribution of viral infection corresponds with that of tumor, adjusting for the presence of known cofactors.
(2) Viral markers are higher in case subjects than in matched control subjects.
(3) Viral markers precede tumor development, with a higher incidence of tumors in persons with markers than those without.
(4) Tumor incidence is decreased by viral infection prevention.

2.1.1.2 Virologic guidelines

(1) Virus can transform cells *in vitro*.
(2) Viral genome is present in tumor cells but not in normal cells.
(3) Virus induces the tumor in an experimental animal.

2.1.1.3 Hill Criteria for Causality [26,27]

(1) Strength of association (how often is the virus associated with the tumor?).
(2) Consistency (has the association been observed repeatedly?).
(3) Specificity of association (is the virus uniquely associated with the tumor?).
(4) Temporal relationship (does virus infection precede tumor genesis?).
(5) Biologic gradient (is there a dose response with viral load?).
(6) Biologic plausibility (is it biologically plausible that the virus could cause the tumor?).
(7) Coherence (does the association make sense with what is known about the tumor?).
(8) Experimental evidence (is there supporting laboratory data?).

3. EPSTEIN BARR VIRUS

In 1964, Tony Epstein and Yvonne Barr were able to isolate a virus from Burkett's lymphoma samples. It was hence called Epstein Bar Virus (EBV). The EBV was found to a double stranded DNA virus with 172,000 base pair DNA molecule. This molecule is divided into internal repeat and terminal repeat domains [28]. The genome encodes for 80 discovered proteins. Most of the genes have a similar mechanism of action as that of herpes simplex virus, the difference being in the genes expressed in the latent phase of B-cells which do not have recognized counterparts in other human herpes viruses. All phases of the EBV life cycle are associated with human disease. The DNA is surrounded by a protein nucleocapsid. This nucleocapsid is enclosed by a protein, which in turn is covered by an envelope containing lipids and glycoproteins, essential for infecting host cell [29].

4. INFECTION & PATHOGENESIS

Infected cells will increase in number in the immunocompromised individual. Eventually B-cell growth control pathways will be activated leading to malignancies such as nasopharyngeal carcinoma (NPC), Burkett's lymphoma (BL), post-transplant lymphomas, and gastric carcinomas [30].

There are several viral proteins encoded in EBV that have the potential to transform. Some of these include EBV latent membrane proteins 1 and 2 (LMP1 and LMP2) and EBV nuclear antigens 2 and 3 (EBNA2 and EBNA3). LMP1 has the ability to transform a wide range of cell types; including rodent fibroblasts [31]. It plays a pivotal role in making the EBV able to make B-cells immortal [32]. The multiple transmembrane-spanning domains along with the carboxyl terminus of LMP1 interacts with several tumor necrosis factor receptor associated factors (TRAFs) [33,34] resulting in high levels of activity of NF-jB, Jun, and p38 in LMP1-expressing epithelial and B-cells [35–36]. The expression of numerous adhesion and anti-apoptotic genes is also up regulated by LMP1.It also activates the expression of fibroblast growth factor-2 (FGF-2), IRF-7 [37] and matrix metalloproteinase-9 (MMP-9) [38].

LMP2 is another viral membrane protein which also is dispensable for the transformation of native B-cells. It is required for transformation of post-germinal center B-cells. It interacts with Lyn and Syk to mimic B-cell receptor (BCR) signaling, including activation of the PI3 K/AKT survival pathway [39]. These pathways are active and facilitate proliferation through a normal cell cycle cascade [40].

5. PREVIOUS STUDIES

The presence of EBV was discovered in various squamous cell proliferative lesions of the oral cavity by Horiuchi et al. [41]. Using Polymerase Chain Reaction (PCR) and *in situ* hybridization they were able to detect the presence of EBV DNA and EBV encoded small messenger RNA in OSCC. EBV genome was found to be present almost 60% of OSCC, but none of papillomas demonstrated EBV genome. Similarly, oral hairy leukoplakia lesion seen in patients with AIDS has been proven to be associated with EBV. On this basis, Horichi gave two possible conclusions; 1. EBV may be carcinogenic and 2. The presence of the virus is coincidental to epithelial cells of squamous cell carcinoma, carcinoma *in situ,* and leukoplakia.

In another study, a positive correlation was found between different grades of OSCC and EBV DNA positivity. It also showed that the percentage positivity of EBV DNA increases from well-differentiated OSCC to poorly differentiated OSCC [42].

Table 1. Studies showing association of EBV with OSCC

No.	Finding	Author	Year
1.	EBV genome was found to be present almost 60% of OSCC, but none of papillomas demonstrated EBV genome	Horiuchi et al.	1995
2.	A positive correlation was found between different grades of OSCC and EBV DNA positivity	González-Moles et al	1998
3.	Out of 54 participants who were diagnosed with OSCC, 39 were EBV positive.	Higa et al	2002
4.	It revealed that 82.5% EBV positive cases expressed the genes for EBNAs, LMP2A, and 2B and certain structural proteins.	Li et al	2009
5.	EBV infection is not influenced by age, smoking or alcohol use.	Sand et al	2002
6.	This expression also showed a positive correlation with an increase in infection by EBV	Jiang et al	2012
7.	The virus leads to extensive methylation of both the host and viral genome, and these changes inhibit apoptosis, promote viral persistence and propagation, thus inducing malignant transformation in the infected B-cell	Tempera et al	2014

In a study carried out in Okinawa, Japan, out of 54 participants who were diagnosed with OSCC, 39 were EBV positive. In the same study however, 41 participants from mainland Japan did not show any significant correlation between OSCC and EBV [43].

A study was carried out in Taiwan by microarray analysis to detect EBV infection and gene expression in OSCC. It revealed that 82.5% EBV positive cases expressed the genes for EBNAs, LMP2A, and 2B and certain structural proteins. These genes fabricated at the spots 61 (BBRF1, BBRF2, and BBRF3) and 68 (BDLF4and BDRF1) on EBV-chip were actively expressed in a significantly greater number of OSCC exhibiting exophytic morphology or ulceration than those tissues with deep invasive lesions [44].

Sand et al examined the oral mucosa of a total of 119 subjects; 23 with Oral Lichen Planus (OLP), 29 with OSCC and 67 healthy subjects. They used PCR for EBV DNA analysis and found 37.9% prevalence of EBV in OSCC and 26.1% prevalence of EBV in patients with OLP. Combining the two, the overall prevalence of EBV in both types of oral lesions was 32.1%. In the control group this was 7.3%. The difference between the two groups was found to be statistically significant. There was no significant difference of prevalence of EBV found between the smokers and non-smokers or the alcoholics and non-alcoholics. No significant difference was also noted between the different age groups. This brought them to the conclusion that EBV infection is not influenced by age, smoking or alcohol use [45].

Another group of researchers isolated epithelial cells by laser capture micro dissection in patients with oral dysplasia and squamous cell carcinoma. The levels of CD21, CK19, and EBV RNA were measured by quantitative reverse transcriptase PCR. Results showed that the frequency and intensity of expression of CD21 increased as the oral epithelial cells became more dysplastic. This expression also showed a positive correlation with an increase in infection by EBV. It was also noted that lesions that carry EBV also generally express higher levels of CK19 than those that do not [46].

Another study shows EBV binds to CD21 receptor on inactive B-lymphocytes through envelope glycoprotein and enters the cell. The viral capsid dissolves and the viral genome is transported to the cell nucleus .The virus leads to extensive methylation of both the host and viral genome, and these changes inhibit apoptosis, promote viral persistence and propagation, thus inducing malignant transformation in the infected B-cell [47].

We, therefore conclude that dysplasia makes cells more susceptible to infection by EBV and that the infection can alter the phenotype of the infected cell in a manner which could affect the overall prognosis.

6. CONCLUSION

Viruses are significant risk factor for oral cancers and precancerous lesions. One should always keep in consideration the significant role of virus in etiopathogenesis during the diagnosis of such kind of lesions. Many of the studies showed the significant association of EBV with OSCC.

Analyzing the published researches has led to substantial increase in our understanding of the oncogenic involvement of Epstein Barr virus in OSCC. The numbers of studies are still not significant enough to prove the direct mechanism. Additional work still needs to be done in this domain so as to fill all the gaps which are still not proven.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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