



Thoughts on Beta-integrins and a postulated pathogenesis for nasopharyngeal carcinoma

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Abstract: The Epstein-Barr virus (EBV) has been definitively implicated in the development of nasopharyngeal cancer (NPC). However, little is known about how specifically EBV gains entry into the cells to effect carcinogenic change, and why this affects certain ethnic populations more than others. The current dogma regarding EBV infection in NPC has been that of late infection, followed by multiple postulated mechanisms for immune evasion of EBV against the host defences, leading to eventual trigger of carcinogenesis. This paper postulates that beta-integrin 6, which is primarily expressed during embryonic development and possibly in neonatal life, may play a role in the development of NPC by facilitating entry of EBV into cells within the nasopharynx during the perinatal period, and this, coupled with ethnic differences in cell-mediated and innate immunity, leads to latent infection within the nasopharynx. Subsequent accumulation of further environmental and/or oncogenic changes leads to the eventual development of NPC in the patient in later life.

Keywords: Nasopharyngeal carcinoma (NPC); beta-integrin 6; neonate

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Introduction

Nasopharyngeal carcinoma (NPC) is a disease endemic in certain specific regions of the world, such as in southern China, Southeast Asia, the Arctic region, and in certain populations within the Middle East and North Africa. Despite its intriguing disease distribution, much is still unknown about the pathogenesis and mechanism of development of NPC. Current theories include different dietary preferences—with those high in nitrosamines, such as preserved foods and salted fish observed to have an increased frequency of consumption in populations more susceptible to NPC (1,2), as well as more recently, a suggestion of genetic susceptibility in patients with certain human leukocyte antigen (HLA)—A and B haplotypes, cytogenetic abnormalities (3,4), as well as genetic

polymorphisms in toll-like receptor 8 (TLR-8) (5).

It is widely known that the Epstein-Barr virus (EBV) has been definitively implicated in the development of NPC (6,7). In view of the fact that EBV is a ubiquitous human herpesvirus carried by more than 90% of the global population, this raises questions as to how specifically does EBV gain entry into the cells to effect carcinogenic change, and why this affects certain ethnic populations more than others.

Antibodies against the EBV antigen were first identified in NPC patients in the 1960s, and subsequently it was proven that higher levels of anti-EBV antibodies were found in NPC patients than in the general population without the disease (8,9). Since then, the current dogma regarding EBV infection in NPC has been that of late infection, followed by multiple postulated mechanisms for immune evasion

of EBV against the host defences (10), leading to eventual trigger of carcinogenesis. However, while this mechanism has been proven in B lymphocytes with the establishment of latent infection and subsequent reactivation into lytic replication (11,12), current research has yet to definitely discover the mechanism by which EBV is able to enter the epithelial cell and maintain its latency.

In this paper, we postulate that beta-integrins may play a role in the development of NPC, specifically through facilitating entry of EBV into cells within the nasopharynx during the perinatal period, and this, coupled with other carcinogenic factors such as a nitrosamine—rich diet, together with ethnically—determined genetic susceptibilities, leads to the eventual development of NPC in the patient.

What are beta-integrins?

Integrins are a group of cell surface receptors that mediate cell adhesion to each other or to extracellular matrix substrata; and also have been shown to have effects on cell differentiation, gene expression, and survival and proliferation of cells (13,14). Each integrin is composed of 2 transmembrane glycoproteins; one alpha-subunit and one beta-subunit. In total, 15 alpha-subunits and 8 beta-subunits have been defined.

Of the beta-integrin subunits, beta-integrin 6 was found to be of special interest due to the observation that colon carcinoma cells transfected with the beta-integrin 6 cDNA attained increased potential to proliferate and survive *in vitro* (15). In a study by Breuss *et al.*, it was found that the alpha-v-beta-6 receptor is the only known integrin that is restricted exclusively to epithelial cells. More importantly, this integrin is expressed primarily during embryonic development and is downregulated and not expressed constitutively in normal differentiated healthy adult epithelia (16). However, the alpha-v-beta-6 integrin is up-regulated during tissue remodeling and re-expressed in injured and inflamed epithelia, and in at least some types of epithelial derived tumours. Furthermore, beta-integrin 6 was found to be up-regulated in parallel with morphogenetic events, tumorigenesis, and epithelial repair (17).

Many viruses, including EBV, use integrins to facilitate entry into mammalian cells by facilitating virus attachment, stimulation of endocytosis and induction of signaling pathways (18-20). Chesnokova *et al.* showed that the direct interaction between an EBV surface glycoprotein, gHgL,

and epithelial cell surface integrins containing the beta-6 subunit can provide the trigger for fusion of EBV with an epithelial cell by direct virus fusion with the cell plasma membrane (21).

Beta-integrins allow EBV infections in early neonatal life

While beta-integrins have been shown to be primarily expressed during embryonic development, study of Rhesus monkey models suggest that there is some persistent residual expression of beta-integrin 6 in early neonatal life (16,17). Naturally, the same has not been verified in human neonates—however, the Rhesus monkey has long been used in biomedical research due to their anatomical and physiological similarities to humans, thus making a similar presence of residual beta-integrin 6 expression in human neonates a logical inference.

The presence of beta-integrin 6 in neonates thus allows a pathway for EBV to enter epithelial cells and persist chronically. As seen in a mouse model with murine gammaherpesvirus, long term persistence of the virus is age-dependent, demonstrating that infection during the neonatal period leads to chronic persistence of infection through entry into the epithelial cells of the mice (22). Compared with EBV infection that occurs later in life which is lymphotropic for human B cells, and only seems to affect epithelial cells in acute diseases with productive lytic replication such as infectious mononucleosis, but does not lead to persistent or latent disease within the epithelial cells (23); the presence of beta-integrin 6 in only neonatal life provides a plausible explanation for the entry of EBV into the epithelial cells, and its subsequent persistence, as explained below.

Significance of neonatal EBV infection and subsequent NPC development

The presence of the EBV virus was confirmed within epithelial cells of NPC (8,24), which we postulate to have been able to enter the epithelial cell during the neonatal period, via beta-integrin 6, and lie latent within the cell. The virus is not cleared in affected individuals due to the paucity of the immune response during neonatal life (25). This reduction in immunity is true for both cell-related and innate immunity, and this may further be extrapolated to partially explain the ethnic distribution of NPC via genetic differences in HLA II phenotypes (26,27) and

TLR 8 polymorphisms (25,28) found in East Asians, which has been hypothesised to increase their susceptibility to NPC (29). To further underpin this genetic association, this similar TLR—8 polymorphism was also found in the Mozabite Berber population (in North Africa), a separate ethnic group with Intermediate NPC prevalence (30).

The entry of EBV into the epithelial cell is a necessary, but insufficient step in the eventual carcinogenesis of NPC. The early neonatal infection of the nasopharyngeal epithelium results in latency in the epithelial layers (23) during early life. It has been demonstrated that in the nasopharynx, there is a transitional zone between the ciliated columnar respiratory and the stratified squamous epithelium (31). However, the presence of this transformational zone is only active during the period of fetal development to the first 10 years of life (32); thus, any changes to the epithelium induced by viral insults must occur early in life, which would coincide with the period when TLR8 is the only active regulator of the host immune response. Subsequently, these cells undergo malignant progression over extended periods of time, as a result of exposure to carcinogens or when additional oncogenes are expressed, similar to the “HPV-cervical cancer” model, occurring with a long latency and slow progression rate (29), resulting in the presentation of NPC later in life, with a peak age of presentation in their 40s to 60s (33).

Thus, our proposed pathway for NPC carcinogenesis involves firstly early neonatal latent EBV infection through the entry of EBV into epithelial cells via beta-integrin 6, followed by a gradual accumulation of further carcinogenic changes through environmental factors, as well as possible activation of other oncogenes, leading to development of NPC cancer stem cells and leading to clinical cancer (29).

Conclusions and future directions

To lend credence to our proposed NPC carcinogenic pathway, we need to first establish the presence of beta-integrin 6 persisting into early neonatal life and not just at the embryonal/fetal stage; and then subsequently, that EBV entry into the epithelial cell mediated by beta-integrin 6 can lead to persistence of the virus into adult life.

We propose a number of mouse studies into this area which can help to support our theory, utilizing wild-type mice contrasted with beta-integrin 6—deleted mice.

- (I) Patterns of beta-integrin 6 expression in neonatal mice

The distribution of b6 mRNA in adult primate

tissues has been studied by in-situ hybridization and was found to only be detected in epithelial cells within the nasopharynx, salivary glands, lungs, mammary glands, kidneys, at very low or undetectable levels (16). We propose that the distribution pattern of beta-integrin 6 can be studied in neonatal mice, to establish especially if beta-integrin rich epithelial cells can be found in the nasopharynx; utilizing fluorescence hybridization with anti-beta-integrin 6 in wild type mice; and contrasting that with beta-integrin 6 deleted mice.

- (II) Effect of beta-integrin 6 on ability of neonatal mice to acquire herpes virus infections

This can be carried out using either gamma herpes virus or polyoma virus on mice, which serves as a model for studying human gammaherpesvirus pathogenesis, as EBV is a strictly human pathogen; using wild type neonatal mice as compared with beta-integrin 6-deleted neonatal mice. The ability of beta-integrin 6 to result in latent infection can be studied using real-time quantitative polymerase chain reaction (Q-PCR) to measure viral loads at different time points, as previously conducted by Ptaschinski and Rochford (22). This can further be contrasted with that of similar infections in adult mice, where we aim to show that latent infections do not occur.

Establishing the role of beta-integrin 6 in EBV entry into neonatal epithelial cells will allow us to gain further insights into the virus as well as the carcinogenic pathways used—not just in NPC but also in other EBV related cancers and conditions. Hopefully this will provide us with insights into novel therapeutic mechanisms for intervention against this oncovirus.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/anpc.2018.04.04>). JTSW serves as an unpaid editorial board member of *Annals of Nasopharynx Cancer*. SSP has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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