Review

Higher incidence of nasopharyngeal carcinoma in some regions in the world confers for interplay between genetic factors and external stimuli

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Summary	**Nasopharyngeal carcinoma (NPC) is a rare variety of head and neck cancers. The risk factors include three major causes: genetic factors, viral infection, and environmental and dietary factors. The types of NPC show strong ethnic and geographic variations. The keratinizing and non-keratinizing types are prevalent in the lower incidence regions like North America and Europe; whereas the undifferentiated type is mostly found in the regions with higher incidences like China, North Africa, Arctic, and Nagaland of North-East India. These suggest a possible major role of the internal genetic factors for generation and promotion of this disease. Viral infections might accelerate the process of carcinogenesis by helping in cellular proliferation and loss of apoptosis. Diet and other environmental factors promote these neoplastic processes and further progression of the disease occurs.**

Keywords: Nasopharyngeal carcinoma, incidence, ethnic variation, undifferentiated NPC, North-East India

1. Introduction

Nasopharyngeal carcinoma (NPC) is a rare cancer which starts at the mucosal epithelium of the nasopharynx and in the minor salivary glands present there. It is a box-like chamber located near the base of the skull and lying above the soft palate of the mouth; and it covers the upper region of the throat behind the nose (Figure 1A). According to WHO (World Health Organization), there are 3 types of NPC based on histopathological classification: keratinizing squamous cell carcinoma, non-keratinizing differentiated carcinoma, and undifferentiated carcinoma. The incidence of each of these types shows regional preferences; each specific type is seen more often in some areas of the world than in others. It has been reported that the keratinizing and non-keratinizing types are prevalent in the lower incidence regions, whereas the undifferentiated type is mostly found in the regions with higher and/or moderately higher incidences (*1-8*). The diagnosis,

classification, and staging of NPC are done by microscopic examinations as well as by searching for the presence of distant metastases. In 2005, the updated edition of WHO classification of NPC describes three types of NPC: keratinizing squamous cell carcinoma, non-keratinizing carcinoma, and basaloid squamous cell carcinoma (*2*). The first variant is invasive type showing squamous differentiation with intercellular bridges and/or keratinisation over most of the tumor. Advanced tumors in local regions and low metastases to the lymph nodes are the key characteristics of this NPC. Lower association of EBV is also seen here. This type is further classified in well differentiated, moderately differentiated and poorly differentiated varieties. Non-keratinizing carcinoma of NPC is subdivided into the differentiated type of and undifferentiated type; of which the undifferentiated type is more prevalent in the high-incidence regions. Overall, this is the major type of NPC seen throughout the world. In the undifferentiated variety, the cells have vesicular nuclei, large nucleoli, and less distinct cell borders making the cell clusters look syncytial and overlapping. A huge amount of lymphocytes and plasma cells are found in these cell clusters; thereby disrupting the epithelial nature of the tumor. The differentiated variety shows cellular stratification and is uncommon in the high-incidence

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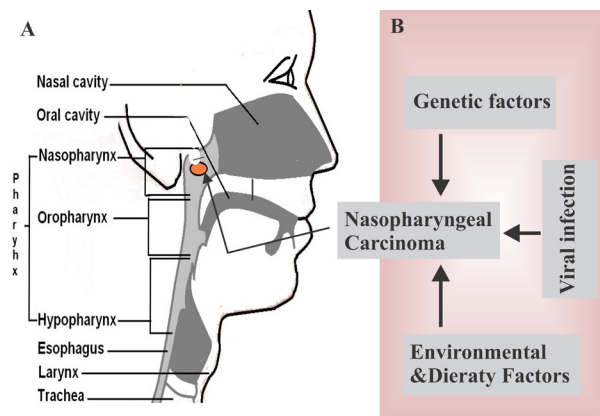E-mail: tathagata.choudhuri@visva-bharati.ac.in

Figure 1. Origin and causes of nasopharyngeal carcinoma. (A). Location of nasopharynx and site of origin of nasopharyngeal carcinoma (NPC). Nasopharynx is a box-like chamber located near the base of the skull and lying above the soft palate of the mouth; and it covers the upper region of the throat behind the nose. NPC generally starts at the mucosal epithelium of it. **(B).** The risk factors of NPC include genetic factors like HLA antigens, viral infection with Epstein-Barr virus (EBV) or Human papilloma virus (HPV), and environmental and dietary factors like ill-ventilation and consumption of preserved foods.

regions. The third type, basaloid squamous cell carcinoma, is very rare and harbor closely packed small cells with hyperchromatic nuclei without nucleoli, and a small amount of cytoplasm (2,6,9). Stages differ by the depth of invasion of the soft tissue and bony structures at and near the nasopharynx, degree of presence of affected cranial nerves, and the involvement of local and regional lymph nodes of the head and neck (1-3,9-11).

One of the major problems associated with NPC is the detection of the disease at an early stage, so that it can be cured. It is one of the most confusing and poorly understood diseases; and so, is commonly misdiagnosed. In most of the times the disease is detected at a very late stage; thereby recurrence cannot be avoided. Metastases form secondary tumors at distant places making the condition even worse. In females, formation of secondary tumors in cervix due to early metastasis sometimes helps in diagnosis and treatment plan. The signs and symptoms of NPC include lump(s) in the neck, hearing loss, recurrent ear infection, stuffiness, headache, blurred vision, nosebleeds, *etc*. Microscopic examination is the primary type of detection method. This is complemented by other tests like physical examinations, endoscopic nasopharyngeal examinations, and computed tomography (CT) imaging or magnetic resonance imaging (MRI). But confirmation by biopsy is the "gold standard" for the diagnosis of NPC (6,9,12-17). Once diagnosed, NPC is treated by radiation therapy (RT) as well as chemotherapy drugs like cisplatin, carboplatin, 5-fluorouracil, docetaxel, paclitaxel, *etc*. Sometimes a tumor is removed by surgery and then RT and/or chemotherapy are followed. Novel treatment techniques like precision radiotherapy, endoscopic surgery, transoral robotic resection, immunotherapy, *etc*. are being

practiced recently (18-24). Nowadays, the staging with any metastasis is diagnosed properly by the help of endoscopy, MRI, PET and CT scans; and then fusion images are formed from different modalities. These are then used for advanced targeted therapies like intensity-modulated RT (IMRT) and adjuvant chemotherapy. As NPC involves various signaling pathways inside the cells, a few inhibitors are being tested. For example, gefitinib and erlotinib for epidermal growth factor receptor (EGFR) and sorafenib for tyrosine-kinase are being tested. Recently, humanized monoclonal antibodies like nimotuzumab against EGFR and bevacizumab against vascular endothelial growth factor (VEGF) are also being tested in clinical trials (22-24).

The risk factors of this cancer includes *1*) family history and genetic factors (*25*); *2*) ethnicity, as the incidence is much higher in some populations compared to others (*4-7,25*); *3*) dietary factors, as consumption of saltine fish and meat is seen in affected populations (*6,7,12,25-27*); *4*) habits, like smoking and alcohol consumption (*25,28,29*); *5*) gender, as it is seen twice more often in males than females (*25,30-32*); *6*) and infection with EBV (Epstein-Barr virus) or HPV (human papilloma virus) (*25,29,33-34*) (Figure 1B). Globally, nasopharyngeal carcinoma (NPC) is an uncommon cancer accounting for about 0.7% of all cancers. In endemic areas like Southern China, Northern Africa, Alaska and Southeast Asia, the annual incidence is as high as 0.02-0.03% in males and about 0.01-0.015% in females, and the commonest form is undifferentiated carcinoma; whereas in North America and Europe, this incidence is much lower (0.001%) and the other forms of NPC are seen (*1-3,7*). In the present report, we will review the risk factors of NPC responsible for the higher incidence in some regions of the world and their consequences.

2. Dietary factors, lifestyle and environment contribute to the disease

NPC has a remarkable ethnic and geographical distribution; seen mainly in the Cantonese people in South East Asia, Eskimos in the Arctic, and Arabs in North Africa (*1-3,7,35,36*). The etiology of this disease involves a complex interaction of genetic factors, viral infection, and environmental factors including diet; of which, the environmental factors appear to have a relatively minor role contributing to the promotion of the disease (*35*). Diet, habits of smoking and/or alcohol consumption, and lifestyle contribute to the disease in the high-incidence races and each of these factors has gained experimental supports (*6,7,12,25-29*).

2.1. Dietary factors

Consumption of Cantonese salted fish has been suspected as a possible etiological factor in NPC. The

high incidence of this disease in Chinese population in China and Chinese migrated to other regions suggests that the dietary habit of these ethnic groups might play a contributory role in formation and/or promotion of this disease. Consumption of salt-preserved food, particularly at the time of weaning, is widely reported to be associated with NPC. Irrespective of the geographic location, two distinct peaks are seen: one in the early adolescence period (15-24 years), and the other in middle age (65-79 years). This suggests that internal genetic factors are the main players for the generation of the disease in these low-incidence populations. But in high-incidence regions, certain dietary materials and lifestyle add to the internal genetic factors, making the entire population much more susceptible to the disease (*4,7,8,10,25-35*). Children who were fed on salt-preserved fish and other foods starting at the time of weaning are more prone to get the disease at a younger age, generally within 14-19 years. On the other hand, children who were fed on fresh fruits and vegetables are less susceptible. In search for the reason behind such diet, presence of nitrosamines in Cantonese salted fish is suggested to be the main culprit as it can develop carcinoma of the nasal or paranasal cavities in rats fed on Cantonese salted fish (*37-40*). Continuous consumption of such nitrosamine containing foods helps accumulation of this chemical in the fat cells near nasopharynx. Later it promotes the detoxifying mechanisms of the cells of the body, particularly in the cells of the nasal and paranasal cavities as the smell of the food reaches these areas fast, and generation of procarcinogens are accelerated (*38-41*). Also, the inefficient process of salt preservation makes food items partially putrefied and such foods are consumed months later. *Staphylococci* and other bacteria including the nitrate-reducing bacteria grow during the process of salt preservation and turn the nitrates and nitrites of the salt to N-nitroso compounds like N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NYPR), and N-nitrosopiperidine. Chemical nitrosation after consumption of salt-preserved fish may also occur under the acidic pH of the stomach. As a result, nitrosamines are again accumulated in high amounts (*42,43*). Salt-dried fish also contains various mutagens, genotoxins, and EBV-reactivating substances like 2-chloro-4-methylthiobutanoic acid (CMBA) and tyramine (*42-46*). In high-incidence populations in Tunisia, South-China and Greenland, a relatively high level of volatile nitrosamine has been detected in common food items (*47*). Similarly, presence of NDMA, NDEA, NYPR, and benzo (a) pyrene (BaP) in smoke-dried fish and meat may serve as a cause of NPC in the Nagas of North-East India and other populations. People of Nagaland of North-East India consume smoke-dried meat which is used as a preserved food. Usually meat to be dried is placed on a bamboo shelf hanging over the fire place used for cooking. This process is again very inefficient and makes the meat partially putrefied. Extract of smoke-dried meat has been reported to be mutagenic in *Salmonella typhimurium* and clastogenic in mammalian test system. It can cause skin papilloma and systemic tumours in mice (*48,49*). Other preserved food items, including eggs, fruits, and vegetables may also increase the risk of NPC. Also, childhood exposure of preserved food items at weaning is strongly correlated with this disease. It has been suggested that consumption of fresh fruits, vegetables and meat might help in prevention of NPC, particularly in children (*50,51*). A report based on Turkish population shows that consumption of French fries, fried meat, and herbal tea are associated with the elevated risk of it. Moreover, in rural areas, people with low-income groups hardly follow a healthy and regular meal pattern; such an irregular meal pattern increases the risk of getting this disease (*52*). Volatile and non-volatile nitroso compounds found in preserved foods thus play a vital role in the formation of NPC.

2.2. *Habits*

Habits like smoking and alcohol consumption are also tested as the risk factors of NPC. For alcohol consumption, there is no conclusive data. Some literatures report no correlation (*26*) and some report a strong correlation with heavier intake of alcohol and association of NPC (*26,52,53*). Smoking, on the other hand, has been reported widely as a strong risk factor for developing NPC (*52,53-55*). Another habit of use of herbal nasal medicine by the people of Nagaland in Northeast (NE) India has been reported recently as one of the risk-factors for NPC (*49*). Use of herbal tea in China has also been included as a risk factor of NPC (*27*). These herbal products contain natural carcinogens like pyrrolizidine alkaloids (PAs), tannins, safrole, *etc*. Each of these has been found to be mutagenic on bacterial, shrimp, and mouse models. Furthermore, most of the rural Naga people and many Africans in Kenya live in ill-ventilated houses, and they burn oak, pine and other wood for heating, lighting and/or cooking purposes throughout the day; thereby inhale the thick smoke continuously. Such living and dietary habits contribute to the etiology of this cancer (*27,52,56,57*).

2.3. *Environmental inhalants*

A number of environmental inhalants may play a significant role in the promotion of nasopharyngeal carcinoma. Formaldehyde is well known to cause heritable mutagenic effects on DNA of mice and deletions in human DNA, while point mutations are more common in *E. coli* DNA. Base-substitution and frame-shift mutations are also reported for formaldehyde. Deposition of cotton dust or wood dust in the nasal epithelium may activate the detoxifying processes and

might generate procarcinogens. Various reports show that inhalation of formaldehyde in printing industries, exposure to cotton dust and combustion in textile industries, exposure to wood dust, and working in ill-ventilated rooms increase the risk of the disease (*57-59*).

3. Infectious factors control the interplay between external and internal factors

Epstein-Barr virus and human pappillomavirus, both have been reported to be associated with nasopharyngeal carcinoma (*25,29,33,34,60*). Epstein-Barr virus (EBV), a human gammaherpesvirus, has been classified as a group I carcinogen by the International Agency for Research on Cancer because of its association with NPC (*61*). This virus was first detected in the African patients with Burkitt's lymphoma and infects B-cells and epithelial cells (*62*). This virus is very well known for causing infectious mononucleosis (glandular fever) and various types of cancers including Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma, and gastric carcinoma (*62-64*). Both Type-1 and Type-2 EBVs are seen in Africa and the Type-1 variety is prevalent in rest of the world (*62,63*). EBVs can persist latently in the B-cells for the lifetime of a person after initial infection. All latency programs may exist in B-cells and can move into lytic phase; eventually giving birth of infectious virions which may infect epithelial cells of the same or other person(s) depending upon the route of infection (*65,66*). After initial infection, the EBV nuclear antigens (EBNAs) are expressed along with some cellular proteins. Different latency programs of EBV give rise to different sets of expressed viral antigens (*67,68*). *EBNA-2* is one of the first genes expressed upon infection of B-cells and is essential for establishment of latent infection and cell growth transformation (*69-71*). Soon after the EBNA-2 expression, the EBV latent membrane proteins 1 and 2 (LMP1 and LMP2) expressed (*71*).

3.1. *EBV infection in NPC*

A study reports that EBV can be activated by aqueous extracts of salt-dried fish of China. Another study reports that the combination of salted fish and EBV shows a strong association with NPC (*44,72*). Also, herbal medicines used by Chinese and Naga people can help the proliferation of EBV and thus may help develop NPC. A number of reports show higher levels of antibodies (EBV-IgG) against the virus capsid protein (VCP) in these populations (*73,74*). Various EBNAs have also been detected in tumor cells of NPC (*75,76*). One subtype of the viral immediate-early gene *BRLF1*, *BR1-C*, and its gene product Rta may be associated with the tumorigenesis of NPC. The DNA binding and transactivation domains of Rta has been detected to have multiple mutations. Three CTL (cytotoxic T lymphocyte) epitopes, NAA, QKE and ERP, have been reported to

have mutations (*77*). A 30-bp deletion in the *LMP1* gene and the loss of XhoI restriction site in *LMP1* gene have been detected in NPC tissues, whereas none of the non-malignant nasopharyngeal tissues harbour XhoI-loss and LMP1 30-bp deletion. This deletion is much frequent in Chinese population and is seen in undifferentiated NPC. Other studies have shown a higher proportion of LMP1 30-bp deletion is much more common in Inuit population (68%) rather than Chinese (30%) or Caucasians (20%). Distinct variations in C terminal, N terminal and transmembrane region in LMP-1 have been reported using molecular plylogenetic analysis of large panels of EBV isolated from southeast Asia, Papua New Guinea, Africa, and Australia where NPC is not endemic (*78,79*). The *BHRF1* gene, which is a homolog of the *bcl-2* oncogene, has been reported to express a particular variant of protein in NPC. This variant carries an L to V mutation in amino acid 88 and a no mutation in amino acid 79 (79V88V variant); thus contributing to the tumorigenesis (*80*). The *BZLF1* gene, which encodes the ZEBRA protein, has been reported to have a serine in place of alanine at the amino acid position 206 in NPC tissues (*78*). This may again explain the association of EBV with NPC. A high level of EBERs (96.67%) and LMP 1 (43.33%) expression and low amount of ZEBRA 1 (6.67%) expression have been detected in Spanish population. Variants of *EBER* genes have been identified in NPC in non-endemic regions, but its correlation with the formation of the disease has not been reported (81).

3.2. *HPV infection in NPC*

HPV is a DNA virus of the papillomavirus family and many of the types of this virus are transmitted sexually; thereby causing infections in the genital areas. Most such infections have no clinical symptoms and are resolved spontaneously. Some cause warts and precancerous lesions; which may eventually turn to cancers. Cervical cancer is the most common type of cancers caused by HPV; others are cancers of mouth and oropharynx, penis, anus, vulva, vagina, *etc*. Risk factors for HPV infection include early sexual experience, smoking, and poor immunity (*25,34*). Association of HPV with NPC usually results in overexpression of p16 and immunohistochemical methods are performed to detect this along with PCR of HPV DNA. In low-incidence areas, HPV-positive NPC cases are EBV-negative; but in endemic areas, co-infection by both of these viruses is common. Although the HPV-positive NPC cells generally show non-keratinizing morphotype, undifferentiated variety is less common and most cells are differentiated. The undifferentiated variety is seen in the endemic regions and in patients with EBV/HPV co-infection. The more infectious types of HPV, HPV16 and HPV18, are seen in NPC; though the HPV11 is also reported. Some of the HPV-positive NPCs actually originate from the oropharynx, but majority show the true nasopharyngeal

origin (*33,34,60*). The overall survival (OS) patterns are similar in HPV-positive, EBV-positive, and EBV-HPV co-infected NPCs. The involvement of viral gene(s) and interaction with the host genetic factors are to be examined in details and a long path is to be travelled to find out the mechanism of HPV infection in this rare disease.

4. Genetic factors render the body susceptible to the disease

As discussed earlier, a higher incidence of NPC is seen in some ethnic groups including Chinese people in China and Chinese migrated abroad. An intermediate incidence is seen in populations admixed with the Chinese. Therefore, this high incidence of NPC in specific ethnic groups indicates genetic factors controlling the disease. A number of reports have suggested the role of various genetic factors in association with NPC; particularly, polymorphisms in the histocompatibility locus antigens (HLA), cytochrome P450 2E1 (CYP2 E1), alteration of p53 codon 72 Arg>Pro, and some signaling pathways (*82-91*). It has been reported that human 8-oxoguanine DNA glycosylase 1 (hOGG1) gene and human MutY glycosylase homologue (hMUTYH) gene polymorphism is associated to the risk of NPC mainly among women in Chinese population (*92*).

4.1. *HLA antigens*

Studies on association of genetic factors with NPC have involved several NPC patients with different genetic systems. In all of those, only one group of antigens, the HLA, has been found to be associated with NPC (*85,86*). HLA genes express various proteins of the immune system and help it to process and present foreign antigens; thereby making those antigens vulnerable to immune lysis. In Chinese population, the HLA data reveal that the younger patients are genetically different from the older patients and may involve different mechanism for the onset of the disease. Susceptibility for NPC as well as survival after diagnosis, both are associated with the HLA antigens. Two different haplotypes, A2-C11-B46 and A33-C3-B58-DR3, have been diagnosed in NPC (*85,86,93*). Both these haplotypes are responsible for the susceptibility of the disease, and the A33-C3-B58-DR3 haplotype is associated with poor survival also. On the other hand, HLA-A2 without B46 or B58 and A2-B13 helps long-term survival. It has been inferred that HLA-A11, B13, and B22 are associated with lower risk for NPC development; whereas HLA-A2, C11, B14, B17, and B46 are associated with increased risk (*85-87,94,95*). In whites, HLA-A2 and A11 are found to be associated with lower risk of NPC (*95*). It has been suggested that in ethnic groups with higher incidence of NPC, HLA-A2, C11, B14, B17, and B46 show a reduced ability to process and present EBV

antigens; thereby confer an increased risk of developing NPC in individuals carrying these alleles. In contrast, HLA-A11, B13, and B22 are efficient in processing and presenting EBV antigens; thus confer a decreased risk of getting NPC (*96*). It has been reported that HLA-DRB1 allele polymorphism is associated with NPC in Asian, Tunisian, and Caucasian population. DBR1 locus, the most ubiquitous one, encodes a number of variable gene products. Genotyping of HLA 1 region and its association studies among 20 NPC cases in Northestern India have revealed the association of microsatellite markers HL003 (allele 121) and D6S2704 (allele 218) in the HLA class I region with high risk of NPC; while a protective effect of HL003 (allele 127) and D6S2678 (allele 255) have been conferred (*97*).

4.2. *Tumor suppressors and oncogenes*

More than fifty percent of cancers contain mutation in the tumor-suppressor gene *p53* which controls the cell cycle check point, apoptotic pathways, metabolic changes, DNA repair, senescence, and genomic stability. In NPC, mutations in *p53* are less common, although some single nucleotide mutations, frame shift, deletion and duplication may occur. Rather, overexpression/ accumulation of p53 in NPCs of endemic areas is well documented (*89*). But it has been reported particularly that a certain type of single-nucleotide polymorphism of *p53* at codon72 increases the risk of NPC. Substitution of proline (Pro) in place of arginine (Arg) at codon 72 of the p53 product plays a role in disease susceptibility. Individuals with Arg/Arg genotype have a lower risk of getting NPC compared to the individuals with Arg/ Pro genotype; and those with Pro/Pro genotype have a much higher risk of acquiring NPC (*90*). Recently, codon 72 Arg>Pro polymorphism and the risk of NPC has been reported in Northestern India. Moreover, p53 codon72 polymorphism is affected by the mutations seen in some other genes, like PIN-1, TNF-α (tumor necrosis factor alpha), and GST. Further, both EBV and HPV can modulate the effects of p53 in controlling apoptosis. It has been suggested that EBV-LMP1 can induce the expression of antiapoptotic genes BCL-2 (B-cell lymphoma 2) and A20; and thus help prevent apoptosis by p53. Though EBV-positive NPC cells have shown a higher amount of BCL-2, its direct relation to EBV infection and LMP1 expression needs to be studied in detail. It is suggested that higher amount of BCL-2 poses a synergistic effect on LMP1 function and helps the NPC cells to grow rapidly and the aggressive nature of the cancer depends on it. Metastases to different body parts including the lymph nodes and poor prognoses with recurrence in NPC are thus explained. Patients having low levels of BCL-2 in NPC stages III and IV experience higher rate of disease-free 5-year survival. Therefore the importance of BCL-2 overexpression is established in NPC, though the detailed molecular

mechanism is ill-understood (*91*). On the other hand, EBNA3C can bind directly to p53 and inhibit its DNA binding property; thereby prevents apoptosis by p53 pathway. But the interrelation between EBV infection and the susceptibility of the alteration of *p53* codon72 Arg form or codon72 Pro form needs to be investigated thoroughly (*90*). The involvement of TNF-α and HSP-70 (heat shock protain-70) in tumor immunity and cancer biology have been investigated. TNF as an interleukin have multifunctional role in cell survival, proliferation, differentiation and death. High expression of interleukins has been inferred with development of many cancers. HSP-70 is a chaperone and act as an anti tumor immune recognition by cytotoxic T lymphocyte. *TNF* genes are located towards the telomeric position of *HSP* gene members. Recently TNF-α (– 308 G>A), TNFβ (+ 252 A>G), HSP 70-1 (+ 190 G>C) and HSP 70-hom (+ 2437 T>C) genes polymorphism have shown a high association with NPC in Northeastern Indian population (*98*).

4.3. *Signaling pathways*

Various signaling pathways have been studied for their contributions in NPC (*85*). The Wnt signaling pathway, which regulates the developmental processes of embryo and tissue homeostasis of adults, has been suggested to be involved in the development and maintenance of NPC. Prolonged Wnt signaling activates dishevelled (DSH) family proteins, which in turn, inhibit axin, glycogen synthase kinase-3β (GSK3β), and the adenomatous polyposis coli (APC) proteins. Downregulation of GSK3β results in cytoplasmic β catenin accumulation in the cytoplasm and translocation into the nucleus. Cytoplasmic β catenin can bind to various transcription factors and E-cadherin, thereby help in cellular proliferation, differentiation, and adhesion. β-Catenin can activate interleukin-8 (IL-8), c-Myc and cyclin D1 expression, all of which contribute to carcinogenesis by causing cellular proliferation and/or angiogenesis. Higher levels of β-catenin have been reported in NPC and these levels are inversely related to the survival rates. β-Catenin levels are found to higher in NPC cells infected with EBV. Moreover, Wnt inhibitory factor (WIF), which can downregulate the Wnt signaling pathway, has been found to be decreased in NPC. The levels of inactivation of WIF by promoter hypermethylation are found to be related to the TNM stages in NPC; the higher the stage, the higher the inactivation of WIF.

Uncontrolled expression of phosphoinositide 3-kinases (PI3K) activates a serine/threonine protein kinase B (Akt), which in turn, upregulates cell proliferation and prevents apoptosis. Significantly higher expression levels of Akt are seen in undifferentiated NPC and these levels are inversely related to the OS of patients (*91*). This pathway is also activated by EBV-LMP1 and thus activates several downstream pathways leading

to various carcinogenic events like distant metastasis, lymph node involvement, advanced tumor stages, as well as worse prognosis. It has been found that NPC cells exhibit high level of Akt with low level of PTEN (phosphatase and tensin homolog protein). Inhibition of Akt with a PI3K inhibitor LY294002 results control of cell proliferation and inducing apoptosis. NPC metastasis by epithelial-mesenchymal transition occurs due to activated c-Src and Akt plays a vital role in this pathway. In NPC, Akt is also activated by the overexpression of UBE2T; thereby help proliferation, cell survival, invasion, and metastasis. The mitogen-activated protein kinase (MAPK) pathway, which regulates various cellular processes, has been shown to play an important role in cancer development. One of the MAPKs, the c-Jun N-terminal kinase (JNK), regulates cell survival. In NPC, constitutive activation of JNK results in p53 inactivation *via* phosphorylation and activation of DNA methyltransferase. This leads to inactivation of E-cadherin; thereby prevents apoptosis and alters cell adhesion. This finding also explains the loss of apoptosis *via* p53 even when the amount of p53 in NPC is mostly higher compared to normal cells. Another member of the MAPKs, the extracellular signal-related kinase (ERK), is found to be upregulated in NPC and it can induce transcription factors NF-κB, AP-1, and ETS. These, in turn, activate c-Fos, cyclin D1, and c-Myc resulting in cellular proliferation. The epidermal growth factor receptors (EGFRs) are also found to be upregulated in NPC and are associated with poor prognoses. Higher amounts of EGFRs then activate RAS/ERK signaling pathway leading to uncontrolled cell proliferation. Cytoplasmic interaction of cyclin D1 and cyclin E with EGFR helps in cellular proliferation by the induction of progression through G1/S phase. It has been shown that endocytosis and processing of EGFR is stimulated by EBV infection; thereby the translocated processed parts of EGFR act as transcription factors inside the nucleus and further activates signaling pathways that help cell proliferation (*22-24,91*). In latest studies, a few cellular micro RNAs like miR17 through miR92 and miR155, are reported to be upregulated in NPC. These miRs are known for their oncogenic properties and are involved in regulation of various genes in cellular signaling pathways like Wnt and apoptotic pathways. Downregulation of the tumor-suppressive miRs (like miR34 family, miR143, and miR145) may also regulate such pathways in NPC. Particularly, downregulation of miR29c, which targets genes for expression of extracellular matrix, helps in invasion and metastasis (*91*).

4.4. *Cytochrome P450 2E1*

Recently, the enzymes cytochrome P450 2E1 (CYP2E1) and glutathione S-transferase have been reported to be associated with NPC. Four varieties of cytochrome P450 (CYP1A1, CYP1A2, CYP2E1 and CYP3A4) can

activate procarcinogens which then produce reactive intermediates. These intermediates can damage DNA and play a vital role in chemical carcinogenesis. Case-control study and correlated study have shown that polymorphisms in CYP2E1 and CYP2A6 both contribute to the development of NPC in Thailand and South Chinese and Northeastern Indian population. Of these, CYP2E1 reacts with nitrosamine which is found in preserved foodstuffs. Inhaled xenobiotics and procarcinogens present in smoked foodstuffs may be activated by the oxidative and non-oxidative enzymes present in nasopharynx; thus contribute to the formation of NPC (*82,83,99,100*). The absence of glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) may also increase the risk. GSTs detoxify the body by helping glutathione (GSH) to bind to the xenobiotics and thus save the cellular proteins and nucleic acids. Therefore loss of GSTs may increase the risk of NPC as smoke and smoked foodstuffs contain various xenobiotics (*88*).

5. Conclusion

Studies on nasopharyngeal carcinoma (NPC) reveal three basic reasons of development of this rare disease: EBV infection (sometimes HPV), internal genetic factors, and environmental factors including diet. Incidence pattern and geographical distribution of NPC strongly suggest that the internal genetic factors are the main regulators for generation and maintenance of this cancer. Specific ethnic groups found in specific geographic regions support this phenomenon. Though environmental factors in those geographic regions are also suggested for their role in the disease, these might be minor players as it has been reported that Chinese people migrated to other regions show higher incidence; and the admixture of Chinese with others show moderately higher incidence levels. Thus, the genetic factors, that is, some specific allelic varieties found in susceptible ethnic groups might be the key regulators of NPC; EBV infections render these internal factors more active for causing the disease; finally, diet and environment stimulate the affected cells to promote the disease (Figure 2).

Different genes and gene clusters show distinct allelic variations among different ethnic groups. As for NPC, mainly Mongoloid people show higher incidence, as seen in Chinese of China and Nagas of North-East India; and higher or moderately higher incidences are seen in Eskimos and Arabians. Despite the geographic and ethnic variations, all studies show the involvement of the HLA genes and a few signaling pathways, as discussed
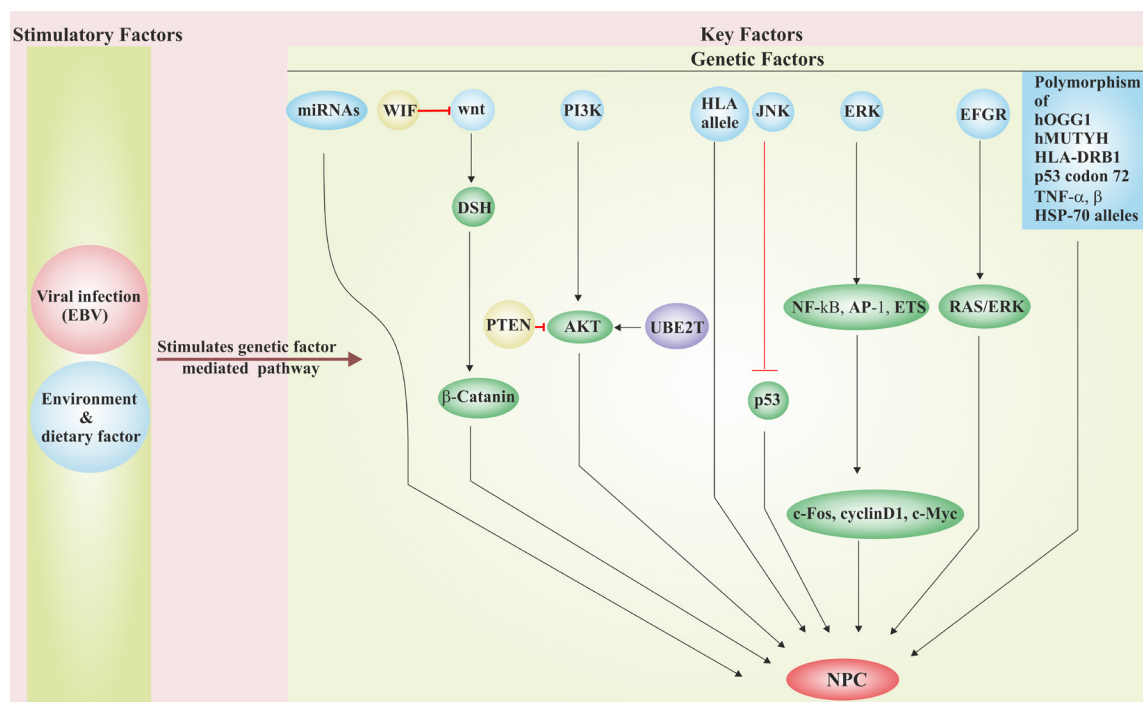

Figure 2. Mechanism of NPC formation. High incidence of nasopharyngeal carcinoma (NPC) in specific ethnic groups indicates internal genetic factors controlling the disease. Polymorphisms in the histocompatibility locus antigens (HLA), cytochrome P450 2E1 (CYP2E1), alteration of p53 codon 72 Arg>Pro, overexpression of BCL-2, and some signaling pathways like Akt, MAPK, and Wnt pathways are reported as the genetic players contributing to NPC. There are three basic reasons of development of NPC: EBV infection (or, sometimes infection with HPV), internal genetic factors, and environmental factors including diet. Specific alleles of HLA and alteration of the signaling pathway(s) render the body susceptible for getting the disease; some of the body cells in nasopharynx become neoplastic with uncontrolled proliferation. Carcinogenic and mutagenic agents, like nitroso compounds, present in the dietary and environmental factors add to the promotion of carcinogenesis as the nasal epithelium harbors a lot of detoxifying enzymes and can produce procarcinogens from those dietary agents. Infection with EBV and/or HPV makes the nasal epithelium more proliferative. Loss of activity of p53, overexpression of BCL-2, and activation of MAPK, Akt, and Wnt pathways lead to uncontrolled proliferation, angiogenesis, loss of apoptosis, and altered cell adhesion; and thus eventually help in invasion and metastasis.

Table 1. A global perspective of NPC distributions in respect to the incidence pattern (*1,5,10,12,32,56,72-75*)

Occurrence of NPC in respect to ethnic and geographical distribution		Factors responsible for NPC		
Geographical Distribution	Specific populations	Genetic Factors	Virus	Diet/Environment/lifestyle
Asian Continent	East and south Asian population/Arabian population	+	+	+
	Chinese population	+	+	+
	Naga Population in North East India	+	+	+
	Turkish Population	-	-	+
	Thailand population	+	-	-
Arctic continent	Arctic Eskimos/Inuit population	+	+	+
African Continent	North African population	+	+	+
	Population of Tunisia	+	-	+
	Population of Kenya	-	-	+
Australian Continent	Papua New Guinea	-	+	-
North-American Continent	Greenland	-	-	+
European Continent	Spanish population	-	+	-

+, incidence of a particular factor; –, no incidence of a particular factor.

earlier. The specific alleles of HLA and alteration of the signaling pathway render the body susceptible for getting the disease. The cells become neoplastic with uncontrolled proliferation and/or acquire EBV (or HPV) infection; which in turn, make the cells more proliferative. Carcinogenic and mutagenic agents present in the dietary and environmental factors add to the promotion of carcinogenesis, as the nasal cells can easily be infected with EBV and the nasal epithelium harbors a lot of detoxifying enzymes. People in poorly ventilated places are in continuous exposure to smoke which can deposit fine dust on nasal epithelium. This may then be detoxified by those enzymes and procarcinogens are generated in turn. Nitrosamines in smoke-dried food and salt-preserved food can contribute to the disease in the similar way. Global Distribution of NPC in respect to the incidence patterns is summarized in the Table 1.

The internal factors, that is, the genetic players, cannot be controlled. Infection with EBV is another factor which is almost uncontrollable. The only thing which can be controlled to prevent NPC is the third one. Environmental agents which may help carcinogenesis can be avoided, as well as the diet may be altered. Ventilation should be improved in houses, particularly in rural areas of Nagaland of North-East India and Africa. Proper ventilation in industries to reduce fumes, smokes and dusts may also decrease the risk of the disease. As the preserved food materials, mainly smoke-dried and salt-preserved foods, are well documented for causing NPC, these should be avoided and fresh fruits and vegetables should be consumed; particularly at the time of weaning. Delayed diagnosis and poor prognosis have made this disease a critical one; therefore intense research studies are needed to infer for the main causative factors and control measures to improve the

treatment procedures. Based on present knowledge, novel therapies are being tested in three routes: one is the targeted regulation of signaling pathways to control cell proliferation, angiogenesis, and metastasis; the other one is immunotherapy, again in a targeted manner; and the third one is a combination of targeted IMRT and adjuvant chemotherapy. But preclinical trials of such targeted therapies face a major problem of finding an authentic undifferentiated cell line and animal model in which EBV infection may also be established. The cell line C666-1 is generally used with all its limitations, and a few xenografts from NPC patients are also being maintained to test these therapeutic promises; but there is a long way to go for treating NPC to assure a disease-free survival.

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