MEDICAL RADIOLOGY

Radiation Oncology

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Nasopharyngeal Cancer

Multidisciplinary Management

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Medical Radiology · Diagnostic Imaging and Radiation Oncology
Series Editors:
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Continuation of Handbuch der medizinischen Radiologie
Encyclopedia of Medical Radiology

ISBN: 978-3-540-92809-6 e-ISBN: 978-3-540-92810-2

DOI: 10.1007/978-3-540-92810-2

Springer Heidelberg Dordrecht London New York

Medical Radiology · Diagnostic Imaging and Radiation Oncology ISSN 0942-5373

Library of Congress Control Number: 2009931698

Cover design: Publishing Services Teichmann, 69256 Mauer, Germany

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

Springer is part of Springer Science+Business Media (www.springer.com)

Foreword

Carcinomas of the nasopharynx represent a relatively uncommon disease process in Western countries, but are more frequently diagnosed as a head and neck malignancy in Southeast Asia. Most of these tumors are epithelial in origin. The nonkeratinizing, poorly or undifferentiated squamous cell carcinomas are the more commonly diagnosed pathologies in Asia, accounting for almost 95% of all cases. However, 75% of cases are World Health Organization Type 1 in North America, whereas those in Southeast Asia are Types 2 and 3. Radiation therapy is the primary treatment for nasopharyngeal carcinomas, and since these tumors tend to present with regional metastasis, combined chemotherapy and radiation therapy is commonly pursued. This is particularly appropriate in patients who have locally advanced disease.

This book, edited by Lu, Cooper, and Lee, discusses the recommendations for diagnosis and staging procedures for nasopharyngeal cancer, staging systems and prognostic factors, and management using radiation therapy for early-stage disease and combined treatment modalities with radiation and cytotoxic chemotherapy for more advanced disease. The supporting scientific evidence clearly indicates that these are the appropriate approaches for the treatment of carcinomas of the nasopharynx.

The volume also deals in detail with techniques of radiation therapy, including intensity-modulated radiation therapy, and outlines appropriate follow-up care and surveillance for those individuals who survive.

Even though nasopharyngeal cancer represents a relatively uncommon tumor in the Western world, it is a common tumor in Southeast Asia and the book by Lu, Cooper, and Lee constitutes a landmark volume identifying the appropriate approaches for the management of this disease process.

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Preface

Nasopharyngeal cancer is a unique type of head and neck malignancy. Essentially unresectable because of proximity to the skull base, nasopharyngeal cancer historically had been treated by radiation therapy alone. Although cure rates for early-stage disease have been relatively good, the substantially worse outcome for locoregionally advanced disease and the not insubstantial risk of disseminated disease clearly indicated that a more effective therapeutic strategy was needed for more advanced tumors.

The use of concurrent chemotherapy with radiation therapy, popularized by the landmark Intergroup trial (INT0099), has significantly improved the outcome of advanced nasopharyngeal cancers. This trial can be therefore viewed not only as a proof of principal, but also as a starting point for the refinement of chemotherapy-enhanced radiation therapy in the management of this disease, a quest that continues to the present time.

Similarly, technical advances in radiation therapy, particularly the development of intensity modulated radiation therapy (IMRT) and image guided radiation therapy (IGRT), have also improved our abilities to place the radiation dose precisely in three-dimensional space, ensuring adequate coverage of the gross tumor and clinical target volumes while simultaneously sparing normal tissues. As the anatomic location of the nasopharynx is in close proximity to critical organs at risk, appropriate beam shaping and placement previously had been (at times insurmountable) challenges for the radiation oncologist.

However, as occurs in any rapidly evolving field, numerous unanswered questions and controversies remain. The optimal schedule, timing, and specific chemotherapy regimen (both concurrent and adjuvant) are still unknown. The delineation of ideal target volumes for IMRT is both an opportunity and a challenge for radiation oncologists who are specialized in the management of this malignancy. Similarly, recent developments in molecular biotechnology herald the prospect of better diagnosis and/or individualized treatment of the disease. Yet, the practicing physician cannot wait for these answers and must make crucial decisions on his/her patients' behalf today, based on the information available. Clearly, with all these opportunities and challenges, sound understanding of the updated current knowledge of nasopharyngeal cancer is essential.

Hence, we initiated this international collaborative effort to provide a comprehensive review of all key knowledge practicing physicians currently need to know about the management of nasopharyngeal cancer, arranged in four sections. The first part of the

book (Chaps. 1–9) discusses the biologic concepts: epidemiology/etiology, pathogenesis, clinically pertinent molecular biology, clinical presentation, diagnosis, and staging. The second part (Chaps. 10–17) details the current concepts of definitive (often multidisciplinary) therapy for nondisseminated nasopharyngeal carcinoma. Critical analyses of the clinical trials that form the basis of currently available evidence-based medicine, current state-of-the art treatment strategies, and novel approaches that promise further improvements in outcome are explained in the chapters of this section. In the third section (Chaps. 18–21), management of more desperate situations, failure after initial treatment, and palliation of distant metastasis are discussed. Patients' long-term quality of life after treatment (Chap. 22), the fortunately rare occurrence of nasopharyngeal cancer in early life, and the staging of the disease (Chap. 24) are reviewed as well. We consider that such an arrangement not only provides appropriate coverage of the core of knowledge and discussions that are crucial to clinical management of nasopharyngeal carcinoma, but also facilitates a structural and systemic way of studying and understanding this knowledge.

We greatly appreciate the expertise and authoritative contributions of all of the included authors, each reflecting their dedication to improve the outcome of care of future patients. Consequently, we have intentionally allowed the authors to address some of the same key issues in different chapters to provide different perspectives of unresolved issues. In the end, the success of this publication must be measured primarily by how well we elicit ideas and provoke thoughts for future research in the clinical management of nasopharyngeal carcinoma.

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Contents

The Epidemiology of Nasopharyngeal Carcinoma

1

Jun Ma and Sumei Cao

CONTENTS

1.1 Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor of nasopharyngeal epithelium. It is the main type in nasopharyngeal malignant tumors in both endemic areas and regions with low incidence. Epidemiological studies in NPC with the focus on etiology and biological behavior of the disease were strongly encouraged as a result of the International Union against Cancer (UICC) Symposium on Cancer of Nasopharynx held in Singapore in 1964 (Muir et al. 1967), and investigations in the past four decades have produced many important findings in those aspects. NPC has unique epidemiological features, including obvious regional, racial, and familial aggregation. The aim of this chapter is to detail the incidence and distribution of NPC, as well as risk factors of the development of the disease.

1.2 Regional and Spatial Distribution

Nasopharyngeal cancer is a type of tumor with extremely unbalanced endemic distribution. It can be seen in many countries and areas of the five continents. However, the incidence of NPC is lower than $1/10^5$ in most areas. High-incidence areas are centralized in the southern part of China (including Hongkong). The highest incidence is found in Guangdong province, and the incidence in male can reach 20–50/100000. According to the data of International Agency for Research on Cancer (IARC), approximately 80,000 cases of NPC were newly diagnosed worldwide in 2002, and about 50,000 cases deceased, with Chinese accounting for 40%. Intermediate rates were seen in local inhabitants of

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Table 1.1. Incidence of nasopharyngeal carcinoma (NPC) in some cancer registries of five continents in 1998–2002 (N, $1/10^5$)

Region and population	Age-standard-incidence rate	
	Male	Female
China		
China, Zhongshan	26.9	10.1
China, Guangzhou	22.2	9.8
China, Hong Kong	17.8	6.7
China, Shanghai	4.1	1.5
China, Nangang District, Harbin City	1.1	0.5
Southeast Asia		
Malaysia, Sarawak	15.0	6.5
Malaysia, Penang	9.3	3.3
Singapore	11.0	3.6
Singapore: Chinese	12.8	4.1
Singapore: Indian	1.8	0.1
Singapore: Malay	5.5	2.0
Philippines, Manila	5.8	2.4
Thailand, Chiang Mai	3.9	1.5
Thailand, Songkhla	2.7	0.9
Thailand, Lampang	2.5	1.5
USA		
USA, Hawaii: Chinese	9.9	1.1
USA, Hawaii: Filipino	3.3	1.3
USA, Hawaii: Hawaiian	2.0	0.2
USA, San Francisco: Chinese	8.1	4.0
USA, San Francisco: Filipino	3.1	1.0
USA, Los Angeles: Chinese	6.0	1.9
USA, Los Angeles: Filipino	3.8	0.8
Middle East/North Africa		
Algeria, Setif	5.4	1.7
Tunisia, Sousse	4.6	1.9
Uganda, Kyadondo	2.3	1.3
Kuwait: Kuwaitis	1.7	0.8
Europe		
Austria	0.4	0.2
Finland	0.3	0.1
Arctic		
Canada, Northwest Territories	4.3	1.3
USA, Alaska	1.5	0.9

Southeast Asia, Eskimos from the Arctic area, and inhabitants from North Africa and the Middle East (Table 1.1) (Parkin et al. 1992, 2002; Waterhouse et al. 1982; Muir et al. 1987).

There is prominent difference in the incidence of NPC between the Northern and Southern parts of China. High-incidence areas are centralized in five Southern provinces (Guangdong, Guangxi, Hunan, Fujian, Jiangxi). The incidence is highest in Guangdong province, so NPC is also called "Canton tumor." Within Guangdong province, the Pearl River delta and Xijiang River basin, especially Zhaoqing, Foshan, and Guangzhou, form a high-incidence core region.

1.3 Gender and Age Distribution

The incidence of NPC is higher in males than that in females, and the ratio is 2–3:1 (Parkin et al. 1992, 2002; Waterhouse et al. 1982; Muir et al. 1987). The predominance in male gender is observed in both endemic and low-incidence areas. However, the distribution of the age of patients diagnosed with NPC differs substantially in areas with various incidences. In low-incidence areas, the incidence of NPC is increased with age, while in the endemic areas, the incidence is increased obviously after 30-years, peaked at 40–59-years, and decreased thereafter (Zong et al. 1983). It has also been reported that in low- to medium-incidence area, the incidence of NPC has a relatively small peak among adolescents and young adults (Burt et al. 1992).

1.4 Racial Distribution

The incidence of NPC is highest in Xanthoderm, then in Melanoderm, and lowest in Caucasian. High-incidence areas are mostly habitations of Xanthoderm, such as Southern China, Hong Kong, and Southeast Asia. Eskimos in the Arctic area also belong to Xanthoderm.

In the same area, the incidence of NPC is different between different races. For example, the incidence is twofold higher in people speaking Cantonese than in people speaking other dialects, such as Hakka, Hokkien, and Chiu Chau (Li et al. 1985). Even after immigrating to other countries in Southeast Asia,

the incidence in Cantonese speaking people is still twofolds higher than in other people from Southern China (Lee et al. 1988). In the United States, the incidence is highest in Chinese people living abroad, then in Filipinos, Japanese, black people, Spaniards, and lowest in white people (Burt et al. 1992).

Migrant epidemiological data showed that people from high-incidence areas of Southern China still kept high incidence even after they immigrate to America, Australia, Malaysia, or Japan (Parkin et al. 2002; Armstrong et al. 1979; Mccredie et al. 1999). Similarly, the incidence of NPC in immigrants and their offsprings from North Africa, where the incidence was relatively high, was still higher than local inhabitants after they immigrated to Israel, a low-incidence area (Parkin and Iscovich 1997). However, the incidence in second and third generation of immigrants was decreased to only half of that before immigration (Warnakulasuriya et al. 1999). On the contrary, the incidence of NPC in Caucasian who were born in China or Philippines is obviously increased, when compared with those born in North America, (Buell 1973) and the incidence in French who were born in North Africa was also obviously higher than that in inhabitants from Southern France (Jeannel et al. 1993).

The results of migrant epidemiology suggest that both genetic factors and environmental factors may play an important role in the pathogenesis of NPC. In addition, the distribution of pathological type is also different in different races. Ninety percent of the NPC in Southern China, Hong Kong, Taiwan, and Singapore are undifferentiated or differentiated nonkeratinizing Carcinoma (Zong et al. 1983). While in nonendemic areas, keratinizing squamous cell carcinoma is predominant, it is concluded that the etiological factors may be different in high- and low-incidence areas (Vaughan et al. 1996).

1.5 Familial Aggregation

NPC is a disease with obvious familial aggregation. There have been reports of high-incidence families in high-, medium-, and low-incidence areas. Furthermore, the ratio of cancer family history in high-incidence areas is higher than that in low-incidence areas. For example, the ratio of NPC family history reported in Hong Kong (Yu et al. 1986) and Guangzhou of China (Yu et al. 1990) is 7.2% and 5.9%, respectively. In Greenland, 27% of the patients with NPC have cancer family history, and most are NPC (Albeck et al. 1993). In the city of Shanghai in eastern China, a medium incidence area, the ratio of NPC family history is 1.85% (Yuan et al. 2000). In cancer family, most patients with NPC are first-degree relatives of the probands. The incidence in first-degree relatives of the patients with NPC is 4–10-folds of that in control population. The reason for familial aggregation of NPC may be similar hereditary susceptibility or living environment of the family members. Complex segregation analysis on NPC family in Southern China shows that NPC belongs to multigenic hereditary tumor (Jia et al. 2005).

1.6 Time Tendency

Recently published data demonstrated that the incidence of newly diagnosed NPC has been decreased in certain high-incidence areas. For example, the incidence and mortality of NPC has clearly decreased in Hong Kong from 1970s, in Taiwan from 1980s, and in Singapore from 1990s. The decreased incidence in Chinese people living in North America was also obvious. However, obvious ascending tendency has been observed in a few areas or populations such as Malay people living in Singapore (Wang et al. 2004). The incidence of NPC was stable or slightly increased in

Table 1.2. Comparison between the average annual age-standardized (world population) incidence rates of nasopharyngeal cancer (per 100,000 person-years) in Hong Kong and Sihui City, Guangdong, China

Period	Average annual incidence			
	Hong Kong Chinese		Sihui City of Guangdong, China	
	Male	Female	Male	Female
1973–1977	32.9	14.4		
1978–1982	30.0	12.9	28.1	12.3
1983–1987	28.5	11.2	28.7	14.8
1988–1992	24.3	9.5	28.7	13.4
1993–1997	21.5	8.3	28.0	11.8
1998–2002	17.8	6.7	30.9	13.0

endemic areas of Southern China including Guangdong and Guangxi provinces (Jia et al. 2006) (Table 1.2).

The change in epidemiologic tendency of NPC in these areas may be related with the change in exposure of corresponding population to risk factors. It is generally considered that changes in smoking, consumption of pickled food, and immigration may have great influences on the incidence in Hong Kong, Singapore, and Taiwan. Epidemiologic studies found that smoking was the main reason for keratinizing squamous cell carcinoma, while it had little relationship with nonkeratinizing squamous cell carcinoma. Further investigations found that the decrease of incidence in Hong Kong and North America was due to the decrease of keratinizing squamous cell carcinoma, while the incidence of nonkeratinizing squamous cell carcinoma kept stable (Jia et al. 2006; Sun et al. 2005; Tse et al. 2006). The decrease in smoking frequency in these areas may account for the decrease in incidence of NPC.

In the past 30 years, rapid economy development has been observed in high-incidence areas of NPC in Southern China, such as Guangdong and Guangxi. The eating and living habit has greatly changed. However, the stable incidence rate of NPC in these endemic areas indicate that the risk factors seem unchanged, as more than 90% of NPC cases belong to nonkeratinizing carcinoma.

1.7 Risk Factors

1.7.1 Epstein–Barr Virus

Antibodies to Epstein–Barr virus (EBV) were detected in the serum of patients with NPC by Old et al. in 1966 (Old et al. 1966). Subsequent studies showed that the level of anti-EBV antibodies was significantly increased in NPC from different races and areas, compared with the control. Viral DNA can be detected in the nucleus of epitheliums using in situ hybridization technique, while it was not obvious in infiltrating lymphocytes.

In prospective population studies, it was found that increased IgA antibody to Epstein–Barr (EB) viral capsid antigen (VCA) and neutral antibody to EBV DNAse were specific markers of NPC in high-incidence area, since they can effectively predict the development of NPC. For example, Chien et al. (2001) found that risk factor of people in Taiwan who were positive for both of the above. For example, in population studies, Chien et al. found that elevated IgA antibody against VCA and EBV DNAse are highly specific markers for NPC cases in Taiwan area, predicting a 32.8-fold (95% CI: 7.3-147.2) increase for those with both markers positive. Recently, Ji et al. conformed that there was a window phase of about 3 years from seropositivity of EBV to the development of NPC (Ji et al. 2007).

Although globally most people were infected by EBV, only a small portion of them developed NPC, which meant that the genesis of NPC was multifactorial. Currently, it was confirmed that many factors can result in the activation of EBV, such as environmental carcinogens and/or immune deficiency (Friborg et al. 2007; Stowe et al. 2001). It is still unclear about the mechanism for EBV entrance into epithelial tissue. The pathogenesis in NPC is detailed in Chap. 2.

1.7.2 Salty Fish and Pickled Food

One of the most potent and confirmed risk factors for NPC is salty fish consumption. Fish and other food that conserved in salt are heavily consumed in Southern China and areas with moderate risk factors for NPC such as Southeast Asia and North Africa. These foods contain a known carcinogen N-nitrosamine and its precursor. In different populations, the relative risk for developing NPC in people who eat salty fish on daily basis after adulthood was estimated to be 1.8–7.5, compared with those who did not or only eat a little salty fish (Yu et al. 1989; Lee et al. 1994). In addition, the relative risk for developing NPC in people who eat salty fish on daily or weekly basis during weaning period or infancy was estimated to be 1.1–37.7, compared with those who never eat or only eat a little salty fish (Yu 1991). On the contrary, eating more fresh fruits and vegetables can reduce the risk of NPC by 30%–50%, which may be due to the effect of antioxidant and antinitrosamine components in Vitamin C and E. However, evidences on the relationship of salty fish and pickled food with NPC from perspective studies are still lacking.

1.7.3 Smoking and Drinking

Results from a number of prospective epidemiological studies revealed that chronic smoking was a risk factor for NPC. In addition, the development of NPC

depended on the severity of smoking measured by pack-year. In general, risk of NPC in smokers was 2–6-fold of that in nonsmokers (Friborg et al. 2007; Hsu et al. 2009). The risk of NPC potentially induced by cigarette smoking was lower than the risk for lung cancer or squamous cell carcinoma of the larynx. However, smoking was the main risk factor for squamous cell carcinoma NPC, while its association with undifferentiated or nonkeratinizing NPC has not been demonstrated (Vaughan et al. 1996). This finding indicated that the decrease in morbidity of NPC in North America and Hongkong might result from reduced smoking frequency. Smoking-induced NPC was less significant than lung cancer or laryngeal cancer, which may be due to the lower sensitivity of nasopharyngeal epitheliums to carcinogens in tobaccos than epitheliums in other regions, or the lower content of tobaccos in pharynx nasalis than the respiratory tract.

Most studies performed in China and the United States showed that alcohol drinking was unrelated to the development of NPC. Results from a perspective study performed in Singapore also confirmed this viewpoint (Friborg et al. 2007). However, the results were not completely consistent, since positive results were found at least in two case control studies (Vaughan et al. 1996; Nam et al. 1992). The inconsistency may be caused by experimental design, susceptibility of different people, and other confounding factors.

1.7.4 Effect of Hereditary Susceptibility

The epidemic features of NPC suggested that genetic factors contribute a lot to the genesis and development of NPC. Many investigations have focused on the possible pathogenic effect of human leucocyte antigen (HLA), which is involved in the presentation of foreign antigens, including viral polypeptide, so as to facilitate their directive lysis by immune system. Since EBV can be found in almost all patients with the disease, the risk factor for NPC may be increased in individuals who inherited HLA allele with weaker presenting ability of EBV antigen. On the contrary, the risk factor for NPC was relatively low in individuals who inherited HLA allele with effective presenting ability of EBV antigen (Hildesheim et al. 2002). It was reported currently that HLA-A2-Bw46 and B17 can increase the risk factor for NPC by 2–3-fold. HLA-A11, B13, and A2 can reduce the risk factor for NPC by 1/3–1/2.

Epidemiologic studies also determined the correlation between polymorphism of some genes with the risk of NPC, including homozygous variant derived from cytochrome P4502E1 (CYP2E1), null allele of glutathion S-transferase M1 (GSTM1), (Kongruttanachok et al. 2001; Hildesheim et al. 1995; Nazar-Stewart et al. 1999) T cell receptor polymorphism (TCR), poly immunoglobulin receptor (PIGR), candidate tumor suppressing gene GX6, DNA repair gene hOGG1, and XRCC1. The relative risk was estimated to be 2.0–5.0. CYP2E1 and GSTM1 are involved in the metabolism of nitrosamine and cigarette smoke, respectively. Their etiological effects may be different because of the difference in environment; in other words, they may have different biological interaction with environmental factors, such as salty fish and smoking.

Studies on chromosomal abnormality, heterozygote deficiency, and gene expression, as well as main NPC susceptible sites on No.4 chromosome found by whole genome scanning in familial study performed in Southern China also provided potential opportunities to determine NPC susceptible genes (Feng et al. 2002).

1.7.5 Traditional Chinese Medicine

In certain epidemiological studies performed in Southeast Asia and Southern China, use of traditional Chinese medicine has been associated with an increase in NPC by 2–4-fold (Zheng et al. 1994; Hildesheim et al. 1992). Certain plants and medicinal materials in Chinese traditional medicine can induce the activation of latent EBV. Such features can be attributed to tetradecanoylphorbol acetate (TPA)–like substances in plants and earth. TPA-like substances, in combination with N-butyrate, a product of anoxybiontic bacteria found in pharynx nasalis, can induce the synthesis of EBV antigen in mice, increase EBV-mediated B cell transformation, and promote the genesis of NPC (Tang et al. 1988).

1.7.6 Professional Exposure

Formaldehyde is a well-known carcinogen that can induce carcinoma of nasal cavity in rodents. Meta analysis on more than 30 epidemiological studies showed that exposure to formaldehyde was significantly

associated with the genesis of NPC, and a dose–response relationship was demonstrated (Partanen 1993). In 1995, formaldehyde was suggested to be an etiological factor for NPC by IARC.

Middle-sized (5–10 μm) dust particles are easily absorbed to pharynx nasalis. Several epidemiological studies have found that the risk factor for NPC was increased in people exposed in wood dust, and it was dependent on exposure time and dose (Luce et al. 2002). In addition, the risk factor for NPC was reported to be increased in people exposed to extreme temperature and work environment with combustibles. However, the exposure ratio for these professional exposures was relatively low in most endemic areas, so it cannot be confirmed whether these factors are independently important in the high incidence in these areas.

1.7.7 Chronic Upper Respiratory Disease

Most epidemiological studies showed that the risk for NPC was increased by about twofold in people with chronic ear, nose, throat, and upper respiratory disease (Zheng et al. 1994). It may be due to the conversion of nitrate to nitrite by bacteria present in these regions, since nitrite is the component of carcinogen *N*-nitroso.

1.7.8 Trace Element

Nickel is one of the carcinogens to human. Surveys performed in high-incidence areas found that the content of nickel in rice, drinking water, and hair of local inhabitants was significantly higher than that in low-incidence areas. In high-incidence areas, nickel content in NPC patients was also higher than in healthy population. Epidemiological surveys also found that trace elements zinc and cadmium were positively related with the genesis of NPC, while magnesium, calcium, and strontium were negatively related (Bolviken et al. 1997).

1.8 Summary

NPC is a disease with unique epidemiological features. The distribution of the disease demonstrates a clear regional, racial, and gender prevalence. The incidence of the disease is relatively high among local inhabitants of Southern China, Southeast Asia, Eskimos from the Arctic area, and inhabitants from North Africa and the Middle East, with the highest incidence found in Guangdong province of China, and the incidence in male reaching 20–50/10^5. NPC is associated with a number of risk factors. It appears that individuals with hereditary susceptibility were infected by EBV in the early period of life, then EBV was activated under the synthetic action of multiple environmental factors, and eventually NPC was developed. In addition to EBV, other environmental factors such as trace elements and dietary habits may be associated with the initiation and development of the disease. However, changes in lifestyle in the past several decades in Southern China had little association with the etiology of NPC. Further epidemiological investigations will be needed to detect the changes in the trend of NPC and the underlying risk factors, so that prevention and/or early detection of the disease can be realized.

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Pathogenesis and Etiology of Nasopharyngeal Carcinoma

2

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CONTENTS

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2.1 Introduction

Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma (SCC) that usually develops around the ostium of the Eustachian tube in the lateral wall of the nasopharynx (Sham et al. 1990). This disease was initially reported in 1901 and characterized clinically in 1922 (Wei et al. 2005). NPC is a disease with a remarkable geographic and racial distribution worldwide. This is a rare human malignancy with an incidence below 1/100,000 populations per year in Caucasians from North America and other Western countries. In contrast, the highest incidence is noted in the Southern Chinese population of Guangdong, Inuits of Alaska, and native Greenlanders (Chou et al. 2008; Parkin et al. 1992); particularly, among the Cantonese who inhabit the central region of Guangdong Province in Southern China, the incidence is 15–25 cases per 100,000. NPC is also called the "Canton tumor" in Guangdong Province (Hepeng 2008; Yu et al. 2002). Southern Chinese migrants, irrespective of their country of migration, also exhibit high rates of NPC (Yu et al. 2002), but the rate of NPC among ethnic Chinese born in North America is considerably lower than those born in China (Buell 1974). An intermediate incidence has been reported in Alaskan Eskimos and in the Mediterranean basin (North Africa, Southern Italy, Greece, and Turkey), ranging from 15 to 20 cases per 100,000 persons (Chan et al. 2002). Independence of race–ethnicity, the rates of NPC in men are two to three folds higher than those in women for most populations (Yu et al. 2002). Overall, NPC can occur in all age groups, but has a bimodal age distribution. The incidence peaks at 50–60 years of age, and a small peak is observed during late childhood (Jeyakumar et al. 2006).

The distinct difference in the incidence among geographic and population area implies that both

environmental factors and genetic susceptibility play roles in the development of NPC (Lo et al. 2004). The early age incidence peak noted among Southern Chinese may suggest that exposure to the putative carcinogens occur very early in life (Yu et al. 2002). Epidemiological studies have linked childhood intake of locally consumed preserved foods to NPC development in all four groups of populations exhibiting increased risk of NPC – Chinese, natives of Southeast Asia, natives of Arctic region, and Arabs of North Africa (Yu et al. 2002). Moreover, environmental factors may also accelerate to the development of NPC. For example, exposure to smoke or chemical pollutants, including trace elements (e.g., nickle), have been reported to be associated with the development of NPC (Wu et al. 1986; Yu et al. 1981). Therefore, the development and progression of NPC disease is multifactorial with geographic areas, genetics, diet, and environmental exposure.

2.2 Histological Subtypes of NPC

The World Health Organization (WHO) classifies NPC into three histopathological types based on the degree of differentiation. Type 1, SCC, is seen in 5%–10% of cases of NPC and is characterized by well-differentiated cells that produce keratin and demonstrated the presence of intracellular bridges when observed under the electron microscope. Type 2, nonkeratinizing squamous carcinoma, varies in cell differentiation (from mature to anaplastic cells) but does not produce keratin. Type 3 or undifferentiated NPC constitutes the bulk of the tumors seen in patients with NPC, is also nonkeratinizing, but is less differentiated, with highly variable cell types (clear cell, spindle cell, anaplastic) (Shanmugaratnam 1978).

Types 2 and 3 NPC are Epstein–Barr virus (EBV) associated and have better prognoses than type 1; EBV infection is generally absent in type 1, especially in nonendemic areas (Marks et al. 1998). However, more recent data suggest that almost all NPC tumors in the endemic areas, regardless of histologic subtype, have comorbid EBV infections, which is a strong evidence for EBV as the etiology of NPC (Vasef et al. 1997). Undifferentiated NPC or type 3 was frequently characterized as lymphoepithelioma owing to the heavy infiltration of the primary tumor with lymphocytes. In endemic areas such as Southern China, WHO Type 3 accounts for more than 97%, while keratinizing SCC is more common in the Western countries (up to 75%) (Marks et al. 1998). There is no uniform morphological characteristic of NPC-affected tissues; thus, diagnosis of undifferentiated NPC is usually based on the location of the tumor in the nasopharynx and the presence of EBV transcripts in the tumor cells (Gullo et al. 2008). Clonal EBV genome is present in the early preinvasive dysplastic lesion or carcinoma in situ, illuminating that the development of malignant invasive tumor drop behind the infection of EBV (Pathmanathan et al. 1995). This close association with EBV is what makes NPC unique from other head and neck cancers.

2.3 Etiologies and Pathogenesis

In endemic regions, NPC presents as a complex disease caused by an interaction of the oncogenic gammaherpesvirus EBV chronic infection, environmental, and genetic factors, in a multistep carcinogenic process. The highest incidence of NPC in Southern Chinese strongly indicates that both genetic susceptibility and environmental factors contribute to the tumorigenesis of NPC in its development and progression. In addition, a small population of cells sharing properties of normal stem cells (NSC) within tumor has been suggested to be involved in the etiology of NPC. This chapter will focus mainly on three major etiological factors including genetic, environmental, and viral factors.

2.3.1 Genetic Factors

While nasopharyngeal carcinoma is a rare malignancy in most parts of the world, it is one of the most common cancers in Southeast Asia including areas such as Southern China, Hong Kong, Singapore, Malaysia, and Taiwan. The reported incidence in these countries ranges from 10 to 53 cases per 100,000 persons. The incidence is also high among Eskimos in Alaska and Greenland and in Tunisians, ranging from 15 to 20 cases per 100,000 persons (Chan et al. 2002). Familial clustering of NPC has been widely observed in both the Chinese population (Jia et al. 2004; Zeng et al. 2002), and non-Chinese patient cohort (Levine et al. 1992). The familial risk of NPC

is among the highest of any malignancy (Suarez et al. 2006). The described relative risk of NPC in first-degree relatives is about 8.0 (Friborg et al. 2005). The high risk of NPC to Cantonese population and the people with familial NPC history suggest that genetically determined susceptibility may play an important role in the etiology of NPC.

An important characteristic of familial cancers is the early age onset of NPC (Zeng et al. 2002). Several linkage analyses studies suggested the association of susceptibility human leukocyte antigen (HLA) haplotypes with NPC development. Most studies conducted among the Chinese population demonstrated an increased risk of NPC for individuals with HLA-A2. A recent study detected a consistent association between NPC and the prevalent Chinese HLA-A2 subtype (HLA-A*0207), but not the prevalent Caucasian subtype (HLA-A*0201) (Hildesheim et al. 2002). The HLA types of AW19, BW46, and B17 have also been reported to be associated with an increased risk, whereas HLA-A11 is associated with a decreased risk (Liebowitz 1994). Significant complex multiple chromosome aberrations are often demonstrated in NPC, as well as in other solid tumors. The finding of translocation, amplification, and deletion of 3p, 5p, and 3q indicates that a minimal region of breakpoints is possible for contributing to NPC (Shih-Hsin Wu 2006; Tjia et al. 2005). Breakpoints have been frequently observed in 1p11–31, 3p12–21, 3q25, 5q31, 11q13, 12q13, and Xq25 (Lo et al. 1997). Inactivation of tumor suppressor genes on 3p, 9p, 11q, 13q, 14q, and 16q and alteration of oncogenes on chromosomes 8 and 12 are important in the development of NPC (Hui et al. 1999; Lo et al. 2000). A recent study provides evidence for the linkage of NPC to chromosome 3p and a fine map of NPC susceptibility locus to a 13.6 cM region on 3p21.31–21.2 (Xiong et al. 2004). These results are in agreement with several previous studies that suggest that the deletion of chromosomes 3p is a common genetic event in NPC (Deng et al. 1998; Lo et al. 2000). Many tumor suppressor candidate genes such as *CACNA2D2*, *DLC1*, *FUS1*, *H37*, *HYAL1*, *RASSF1A*, *SEMA3B*, and *SEMA3F* and tumor susceptibility genes such as *hMLH1* have been isolated from the region (Xiong et al. 2004). These studies indicate that genes in the 3p21 may play a critical role in tumorigenesis of familial NPC.

Some studies suggested that genetic polymorphisms in genes that metabolize carcinogens are associated with NPC susceptibility. Cytochrome P450 2E1 (CYP2E1) is one of the cytochrome P450s and is responsible for the metabolic activation of nitrosamines and the related carcinogens. Case-control studies have shown a strong association of the variant form of CYP2E1 (c2 allele) with increased risk of this disease in Chinese populations (Hildesheim et al. 1997; Hildesheim et al. 1995). Other nitrosamine metabolizing genes, such as Cytochrome *P450 2A6* (*CYP2A6*), have also been suggested to play a role in NPC susceptibility (Tiwawech et al. 2006). Phase II detoxification enzyme, glutathione *S*-transferase M1 (GSTM1), was found to be a synergistic risk factor for NPC (Friborg et al. 2007; Nazar-Stewart et al. 1999). The association of other DNA repair genes with NPC susceptibility has also been implied. While a reduced risk for NPC was observed with polymorphism of the *XRCC1* gene (Arg280His), polymorphism of the *hOGG1* gene (Ser326Cys) was shown to be associated with an increased risk for NPC in the Taiwan population (Cho et al. 2003).

2.3.2 Environmental Factors

A large number of case-control studies conducted in diverse populations (Cantonese, other Southern Chinese, Northern Chinese, and Thais) residing in different parts of Asia and North America have confirmed that Cantonese-style salted fish and other preserved foods containing large amounts of nitrosodimethyamine (NDMA), *N*-nitrospyrrolidene (NPYR), and *N*-nitrospiperidine (NPIP) may be carcinogenic factors for NPC (Armstrong et al. 1998; Ning et al. 1990; Sriamporn et al. 1992; Yu et al. 1988; Yuan et al. 2000). Earlier age at exposure in salted fish has been shown to a high risk of NPC in Southern Chinese (Yu et al. 2002). In animal studies, nasal and nasopharyngeal tumors could be induced in rats by feeding them Chinese salted fish (Ning et al. 1990; Yu et al. 1988). Moreover, cigarette smoking and occupational exposure to formaldehyde and wood dust are recognized risk factors as well (Yu et al. 2002). Several studies conducted in high- and low-risk populations during the past decade have obviously implicated the nasopharynx as a tobacco-susceptible cancer site (Yu et al. 2002). Ever smokers exhibit a roughly 30%–100% excess risk relative to life-long nonsmokers (Yuan et al. 2000). Formaldehyde is a recognized nasal cavity carcinogen in rodents. Smoke particles from incomplete combustion of coal, wood, and other materials are also of the size and weight to be deposited mostly in the nasopharynx (Armstrong et al. 2000). The use of certain Chinese

medicinal herbs has been suggested to increase the risk for NPC by reactivating EBV infection in the host (Zeng et al. 1994).

2.3.3 Epstein–Barr Virus

It was in 1966 when Old et al. first discovered the relationship between EBV and NPC, using in situ hybridization and the anticomplement immunofluorescent (ACIF) assay (Old et al. 1966). Subsequent studies by others demonstrated the expression of EBV latent genes – Epstein–Barr virus nuclear antigen (EBNA), latent membrane protein-1 (LMP-1), LMP-2, and EBV-encoded small RNAs (EBER) – in NPC cells (Baumforth et al. 1999) confirming the infection of tumor cells by EBV. Intriguingly, expression of EBV early antigen (EA) is positively correlated with the consumption of salted and preserved food, suggesting that development of EBV-positive NPC could be related to dietary habits (Shao et al. 1988), and provides another link to the epidemiological studies with NPC. Approximately 90% of the adult population undifferentiated nasopharyngeal carcinomas (UNPC) all over the world are EBV-positive by serology. In most NPC patients, higher EBV antibody titers, especially of IgA EBV-associated cancer, are observed. So, measuring patients' EBV-specific IgA antibodies is a useful method in screening for early detection of NPC (Cohen 2000). EBV infection is an early, possibly initiating event in the development of nasopharyngeal carcinoma (Pathmanathan et al. 1995). A current hypothesis proposes that EBV plays a critical role in transforming nasopharyngeal epithelial cells into invasive cancer (Lo et al. 2004). Wu et al. (2003) found that EBV-positive tumors grew faster than EBV-negative tumors, and also had clonal EBV terminal repeat sequences. Preinvasive lesions of the nasopharynx are infected with EBV. Since EBV infection indeed precedes clonal expansion of malignant cells (Raab-Traub et al. 1986), EBV is thought to contribute, at least in part, to the overall pathogenesis of NPC. Many studies have demonstrated that UNPC are invariably EBV-positive, regardless of geographical origin (Niedobitek et al. 1991; Weiss et al. 1989).

2.3.3.1 EBV Structure

In 1964, EBV was identified in tumor tissue from a patient who had African Burkitt lymphoma, a fatal malignancy of the B lymphocyte (Epstein et al. 1964). EBV is a γ-herpes virus (Wan et al. 2004) present in over 90% of adults worldwide. It is a member of the *Lymphocryptovirus* genus – viruses that are closely related members of herpesvirus family. The EBV genome is large exceeding 172 kb pairs of linear double-stranded DNA, as in other herpesviruses, the molecule is divided into unique, internal repeat, and terminal repeat domains. EBV was the first herpesvirus to have its genome completely cloned and sequenced (Baer et al. 1984). During growth transformation, the virus does not replicate and produce progeny virions, but rather is replicated by the host DNA polymerase as an extra chromosomal episome (Raab-Traub 2002).

2.3.3.2 EBV Infection in NPC

EBV was the first human virus identified to be associated with human cancers, including lymphomas as well as epithelial tumors (Epstein et al. 1966). The association between EBV infection and NPC is well documented and particularly close with EBV genome present in virtually all NPC cells. Primary EBV infection normally occurs in early childhood, is usually asymptomatic, and results in life-long virus persistence, but when exposure is delayed until adolescence, infection mononucleosis often ensues provoking an infection during early adulthood. EBV has a strong tropism for human lymphocytes and for the epithelium of the upper respiratory tract, where it can remain latent (Borza et al. 2002). This virus has been associated with different neoplastic diseases, like polyclonal B lymphoproliferation in immunosuppressed patients, Burkitt lymphoma, or Hodgkin's disease (Niedobitek et al. 1994). However, the tumor showing the strongest worldwide association with EBV is nasopharyngeal carcinoma (Liebowitz 1994; Pathmanathan et al. 1995). Elevated titers of IgA antibody to EBV viral capsid antigen (VCA) are usually found in patients with NPC. The rise in IgA titers to these antigens can be noticed before the development of UNPC and correlates with tumor burden, remission, and recurrence (Mazeron 1996; Zheng et al. 1994). Therefore, this method of measuring patients' EBV-specific IgA antibodies is useful in screening for early detection of NPC (Cohen 2000).

In almost all cases of EBV infection, the oropharynx is the primary site of infection, as well as the site of viral replication. EBV infects primary resting B

lymphocytes to establish a latent infection and yield proliferating, growth-transformed B cells in vitro. In vitro studies demonstrate that EBV infects and potentially activates B cells by binding to the type 2 complement receptor (CR2, or CD21), the putative EBV receptor (Baumforth et al. 1999). Hence, EBV appears to home to the oropharynx, and more specifically, the B cells within the oropharynx. The strain B95-8 can be found in EBV-positive cell lines such as Raji, Namalwa, and CA 46 (Chang et al. 1990). These cell lines are all of B-lymphocyte lineage. This strain has been used as a benchmark to check for EBV positively in NPC.

In vitro, EBV infects resting human B lymphocytes and transforms them into lymphoblastoid cell lines (LCLs), a process that is termed growth transformation and a hallmark of this virus (Altmann et al. 2005). *vBcl-2* genes are essential for the initial evasion of apoptosis in cells in vivo in which the virus establishes a latent infection or causes cellular transformation or both (Altmann et al. 2005). In vivo and in vitro EBV's latent state is characterized by the absence of virus synthesis and maintenance of the viral genome as plasmids in the infected cell. EBV genome delivery to the nucleus as a key rate-limiting step in B-cell transformation, and highlights the remarkable efficiency with which a single virus genome, having reached the nucleus, then drives the transformation program (Shannon-Lowe et al. 2005). NK cell activation by DCs can limit primary EBV infection in tonsils until adaptive immunity establishes immune control of this persistent and oncogenic human pathogen (Strowig et al. 2008).

Four different models have been proposed to explain the transition of EBV from the latent reservoir of infection in blood-borne B lymphocytes to sites of productive replication in oral epithelium. Model 1 proposes that B lymphocytes carrying latent EBV infection migrate from the blood to the epithelium, where the EBV reactivates and infects adjacent epithelial cells (Imai et al. 1998); Model 2 proposes that EBV virions produced by B lymphocytes in the oral submucosa bind submucosal EBV-specific dimeric immunoglobulin A (IgA) and enter basal oral epithelial cells by endocytosis via the polymeric Ig receptor (Sixbey et al. 1992); Model 3 proposes that EBV virions produced by B lymphocytes in oral lymphoid tissues gain access to and infect middle- and upper-layer oral epithelial cells as a result of microscopic traumatic epithelial injury (Niedobitek 2000); Model 4 proposes that blood-borne pre-LC are latently infected with EBV and that oral epithelium cells are likely to be LC harbor EBV infection that can reactivate into productive EBV replication (Walling et al. 2007). However, the process of EBV entry into keratinocytes and NPC cells is more complex, as both keratinocytes and NPC cells express only low levels of CR2 receptor (Billaud et al. 1989). In addition, the relevance of serological tests for EBV infection in predicting the occurrence of NPC is presently still unclear.

2.3.3.3 EBV Subtype in NPC

EBV ubiquitously infects more than 95% of adult population worldwide, but NPC is an endemic disease. One possibility is that there are malignancy-associated EBV subtypes, which are prevalent in NPC endemic area. The first full-length sequence analysis of an NPC-derived EBV strain from a patient with NPC in Guangdong, China, has been analyzed. This EBV strain was termed GD1 (Guangdong strain 1). Compared with prototypical strain B95.8, there are many sequence variations in GD1 when compared with 43 deletion sites, 44 insertion sites, and 1,413 point mutations. The selected GD1 mutations detected with high frequency in Cantonese NPC patients suggest that GD1 is highly representative of the EBV strains isolated from NPC patients in Guangdong, China, an area with the highest incidence of NPC in the world (Zeng et al. 2005).

EBV can be classified as type 1 (A) and type 2 (B) based on sequence divergence in EBNA2, 3A, 3B, and 3C genes (Adldinger et al. 1985; Sample et al. 1990). Type 1 EBV contains an extra *Bam*HI site in the *Bam*HI F region ("f" variant) and loss of a *Bam*HI site at the *Bam*HI W//I boundary ("c" variant) (Bouzid et al. 1998; Lo et al. 2007; Lung et al. 1991). Type 1 EBV is more prevalent in most Southern Chinese patients with NPC or other head and neck tumor patients, while Type 2 EBV or the coexistence of type 1 and 2 EBV are seen only occasionally (Abdel-Hamid et al. 1992; Chen et al. 1992; Choi et al. 1993; Sung et al. 1998; Zimber et al. 1986). But an early report stated that type 2 virus occurs mainly in Africa, and that type 1 is distributed widely in the world (Young et al. 1987). It was found that "f" variant might have an association with the development and/or maintenance of NPC among Southern Chinese (Lung et al. 1991). More evidence showed that these types were just related with EBV geographical distribution (Sandvej et al. 1997). An *Xho*I restriction site

loss and a 30 bp deletion within the *LMP1* gene define an EBV strain that is associated with increased tumorigenicity or with disease among particular geographical populations (Li et al. 1996; Sandvej et al. 1997; Trivedi et al. 1994). However, these notions have recently been challenged by the fact that virus with a deleted version of LMP1 is present in the general population in endemic regions, which can also be found in nonendemic areas within Asia (Itakura et al. 1996) and in Western countries (Sandvej et al. 1997). Moreover, it has been shown that the *LMP1* gene from nonendemic Russian NPC harbors a nondeleted version of *LMP1*, whereas the gene with a deletion can be found in healthy subjects from the same area (Hahn et al. 2001). According to *LMP1* C-terminal variants, Edwards et al. (1999) have defined EBV into at least seven substrains: Ch1, Ch2, Med, Ch3, Alaskan, NC, and B95.8. They found among Asian isolates, the Ch1 and B95.8 strains were in normal specimens and the Ch1 and Ch2 strains in NPC. They also found Ch1 strain prevalent in the NPC patients from areas of endemicity and nonendemicity (Edwards et al. 1999). It was also proved the Cantonese population is susceptible to the predominant Ch1 strain in the nasopharyngeal carcinoma endemic region of China, but its relationship with the host remains to be characterized further (Li et al. unpublished data). EBNA-1 can also be classified into five subtypes: P-ala, P-thr, V-val, V-leu, and V-pro based on the polymorphism of amino acids at position 487 (Bhatia et al. 1996; Gutierrez et al. 1997; Snudden et al. 1995), which has been associated with geographical location or disease status (Imai et al. 1998; Zhang et al. 2004). *LMP1* is an EBV oncogene expressed frequently in EBV-associated malignancies (Eliopoulos et al. 1997; Kilger et al. 1998; Wang et al. 1985). A new typing standard based on 155849nt in RPMS1 of EBV may represent a specific EBV subtype (A type) in the NPC endemic region, and could serve as a valuable indicator for a high risk of NPC in Southern China (Li et al., unpublished data).

2.3.3.4 EBV Expression in NPC

In nonkeratinizing NPCs, the virus is detectable in almost all cancer cells, where it is present as a monoclonal episome (Niedobitek et al. 1996; Raab-Traub et al. 1986). Moreover, monoclonal viral genomes have also been detected in *in situ* NPCs (Pathmanathan et al. 1995). Expression of the viral genome in nonkeratinizing NPC has been studied extensively. During latency, up to 11 viral genes are expressed that encode up to nine proteins and EBV infection in NPC is classified as latency type II, which is characterized by expression of the EBERs, EBNA1, LMP1, LMP2A, and the Bam H1 A transcripts, despite LMP1 is only expressed in up to approximately 65% of nasopharyngeal carcinoma tumors (Niedobitek et al. 2000; Young et al. 2000). While remaining latent, EBV can induce RNA and protein production. Recent evidence demonstrated the association of distinct lytic promoter sequence variation with NPC in Southern Chinese and suggested the participation of a lytic-latent switch of EBV in NPC carcinogenesis (Tong et al. 2003).

In NPC cells, the virus is in the form of episome and not integrated into the host genome. *LMP1* and *BARF1* have profound effects on cellular gene expression and may contribute to EBV-mediated tumorigenesis. In vitro, *LMP1* expression in epithelial cells can induce or upregulate the expression of intercellular adhesion molecule 1 (ICAM-1), CD40, and cytokines such as interleukin 6 (IL-6) and IL-8 (Dawson et al. 1990; Eliopoulos et al. 1997). Expression of *LMP1* in immortalized nasopharyngeal epithelial cells induces an array of genes involved in growth stimulation, enhanced survival, and increased invasive potentials (Lo et al. 2004). Blocking the important signaling activities of *LMP1* abrogates transformation and testifies to the importance of such events in the transformation process (Murray et al. 2000). *BARF1* is able to immortalize primate epithelial cells and enhance growth rate of the transfected cells (Wei et al. 1997). A family of rightward transcripts from the *Bam*HI A region was initially identified in cDNA libraries from NPC where they are abundantly and consistently expressed (Gilligan et al. 1991).

2.3.3.5 The Role of EBV in the Pathogenesis of NPC

Biology of EBV Infection

Approximately 90% of the adult population throughout the world is EBV-positive by serology (Lo et al. 2004). After primary infection at early age, persistent EBV latent infection is found in some resting B cells, but has not been detected in the nasopharyngeal epithelia of healthy individuals (Babcock et al. 1998; Tao et al. 1995). However, EBV infection has been demonstrated *in situ* carcinomas of the nasophar-

ynx, which are presumed precursor lesions of NPC (Niedobitek et al. 1996). These findings seem to suggest that EBV infection takes place before invasive growth begins, but probably does not represent the first step in the pathogenesis of NPC. The EBV latent proteins include the six nuclear antigens (EBNAs 1, 2, 3A, 3B and 3C, and EBNA-LP) and the three latent membrane proteins (LMPs 1, 2A, 2B). EBNA-LP is transcribed from variable numbers of repetitive exons. LMP2A and LMP2B are composed of multiple exons located on either side of the terminal repeats (TR) region, which is formed during the circularization of the linear DNA to produce the viral episome. EBER1 and EBER2 are highly transcribed nonpolyadenylated RNAs and their transcription is a consistent feature of latent EBV infection. A pivotal biologic property of the virus is the ability to alter B-lymphocyte growth in vitro, leading to permanent growth transformation.

EBV entering in B lymphocytes is mainly mediated by binding of the major viral envelope glycoprotein, gp350/220 (gp350), to CD21 receptor on the surface of B cells (Nemerow et al. 1987), and through the binding of a second glycoprotein, gp42, to HLA class II molecules as a coreceptor (Borza et al. 2002). EBV encodes more than 100 genes, at least ten genes are for EBV replication, and viral genes include three integral membrane proteins. Apart from latent membrane proteins 1, 2A, and 2B (LMP) and six EBNAs (EBNA1, 2, 3A,3B, 3C, and EBNA-LP), two small untranslated EBV RNAs (EBERs) (Rickinson 2002; Rowe 2001; Roy et al. 2004; Smith 2001) are also encoded by EBV. These viral proteins profit to cell immortalization and malignant transformation by various signaling mechanisms. For example, *LMP1* encoded by EBV is a membrane protein that activates multiple signaling pathways and transcription factors, including nuclear factor-kappaB (NF-κB). NF-κB activation is necessary for Hodgkin/Reed-Sternberg (HRS) cells to proliferate and inhibit apoptosis (Bargou et al. 1997). Furthermore, activation of NF-κB is essential for B-cell immortalization by EBV and *LMP1*-mediated transformation of fibroblasts (He et al. 2000). To understand the pathogenic properties of EBV in NPC, the state of EBV infection and viral gene expression have been determined in biopsy samples of NPC (Raab-Traub 2002).

The EBV-Encoded Nuclear Antigens

Cells infected EBV express a group of nuclear proteins, which influence both viral and cellular transcription. EBNA1 is expressed in all transformed lymphoid cell lines and EBV-associated tumors. This protein is required for the replication and maintenance of the episomal EBV genome through binding to the plasmid origin of viral replication, OriP. EBNA1 can also interact with certain viral promoters as a transcriptional transactivator and has been shown to upregulate the LMP1 promoter (Lin et al. 2002). Humme et al. has reported that EBNA1 does not have a crucial function in in vitro B-cell transformation beyond the maintenance of the viral genome by Gene-knockout method (Humme et al. 2003). EBNA2 is an acidic phosphoprotein, which transcriptionally activates both cellular and viral genes, thus upregulating the expression of certain B-cell antigens, CD21 and CD23, as well as LMP1 and LMP2 (Lin et al. 2002). Two types of EBNA2 have been identified, encoded by divergent DNA sequences, EBNA2A or 2B, that distinguish two types of EBV, EBV1 or 2 (Dambaugh et al. 1984). The three members of the EBNA3 family, EBNA3A, 3B, and 3C, all appear to have a common origin and encode hydrophilic nuclear proteins, which contain heptads repeats of leucine, isoleucine, or valine that can act as dimerization domains (Lin et al. 2002). The EBNA2 and 3 proteins are the major targets of cytotoxic T-lymphocytes that eliminate latently infected, growth-transformed B-cells (Murray et al. 1992). EBNA-LP is encoded by the leader of each of the EBNA mRNAs and encodes a protein of variable size depending on the number of BamHI W repeats contained by a particular EBV isolate (Lin et al. 2002). A role for EBNA-LP in enhancing EBNA2-mediated transcriptional activation has been proposed (Lin et al. 2002).

The EBV-Encoded Latent Membrane Proteins

LMP1 is an integral membrane protein with oncogenic potential encoded by the *BNLF-1* gene (also called *LMP1* gene) of EBV (Hudson et al. 1985), and is the first EBV latent gene found to transform established rodent cell lines and alter the phenotype of both lymphoid and epithelial cells (Martin et al. 1993; Moorthy et al. 1993; Wang et al. 1985). LMP1 is often present in nasopharyngeal carcinomas and was detected in preinvasive lesions of the nasopharynx (Pathmanathan et al. 1995).

EBV-encoded LMP1, expressed in most of NPC, has been suggested to have an important role in the pathogenesis and development of NPC and its expression correlates with poor prognosis (Gullo et al. 2008). The high percentage of detection of LMP1 in

NPC samples, independent of histological type or tumor location (Burgos 2005), supports a role for EBV in the pathogenesis of different types of NPC, where LMP1 overexpression could be an important factor in the development of the disease. LMP1 is an integral membrane protein containing a cytoplasmic amino terminus, six transmembrane domains, and a long cytoplasmic carboxy terminal portion (Raab-Traub 2002). LMP1 functions as a constitutively active tumor necrosis factor receptor (TNFR), activating a number of signaling pathways in a ligand-independent manner (Eliopoulos et al. 1997; Kilger et al. 1998). Functionally, LMP1 resembles CD40 – another member of the TNFR superfamily – and can partially substitute for CD40 in vivo, providing both growth and differentiation signals to B cells (Uchida et al. 1999). The two distinct functional domains, C-terminal activation regions 1 and 2 (CTAR1 and CTAR2), have been identified within the cytoplasmic carboxy terminus of LMP1, which both can activate the NF-κB transcription factor (Liebowitz et al. 1992). The consequences of NF-κB activation are numerous and include the upregulation of antiapoptotic gene products. These roles suggest that LMP1 is, directly or indirectly, a key modulator of the apoptosis process. Expression of LMP1 plays an essential role in immortalization of human B cells through the activation of a number of cellular signaling pathways, including NFκB, JNK, JAK/STAT, p38/MAP, and Ras/MAPK (Young et al. 2004). In human epithelial cells, LMP1 alters many functional properties that may involve in tumor progression and invasions.

Unlike LMP1, the LMP2 protein is not essential for B-cell transformation in vitro (Longnecker et al. 1992). However, the constant expression of this viral gene in EBV-carrying memory B cells from healthy individuals indicates that LMP2 may play an important role in mediating virus persistence (Babcock et al. 1998). The LMP2 proteins are encoded by highly spliced mRNAs that contain exons located at both ends of the linear EBV genome (Laux et al. 1988). LMP2A and 2B are the two forms of LMP2. The LMP2A protein contains a unique 119-amino acid N-terminal cytoplasmic tail that is absent from LMP2B. LMP2A is shown to inhibit the switch from latency to lytic EBV replication induced by BCR triggering (Longnecker 2000). This effect has been related to the ability of LMP2A to interfere with BCR signaling and possibly plays a major role in mediating EBV persistence in the infected host (Dolcetti et al. 2003). It has been approved that the interaction of epithelial cells with extracellular matrix proteins triggers LMP2A phosphorylation, indicating that this EBV protein is involved in signaling pathways activated by cell adhesion (Scholle et al. 1999). Ectopic expression of LMP2A in a human keratinocyte cell line resulted in enhanced proliferation, clonogenicity in soft agar, and inhibition of differentiation (Dolcetti et al. 2003). LMP2B might function by increasing the spacing between LMP2A N-terminals, causing the release of the Src and Syk protein tyrosine kinases and restoring BCR signal transduction (Baumforth et al. 1999).

The EBV-Encoded Noncoding RNAs

Viral microRNAs (miRNAs) were first shown to exist following the cloning of small RNAs from a B cell line latently infected with EBV (Pfeffer et al. 2004). A recently appreciated property of EBV is that it encodes about 30 mature miRNAs from 20 pre-miRNAs, which, given the size of its genome (approximately 165 kb), represents a 1,000-fold enrichment of this class of genes relative to those in its human host (Cai et al. 2006; Grundhoff et al. 2006; Landgraf et al. 2007; Pfeffer et al. 2004). EBV's miRNAs have been detected by Northern blotting or cloning (Cai et al. 2006; Grundhoff et al. 2006; Landgraf et al. 2007; Pfeffer et al. 2004). Among these, BART7, 10, and 12 have been most frequently detected and BART15 and 20–5p rarely or not at all (Cai et al. 2006; Edwards et al. 2008; Grundhoff et al. 2006; Kim Do et al. 2007; Lo et al. 2007). A recent report has shown that by using quantitative, stem-loop, real-time PCR to measure the expression of EBV's miRNAs and found them to differ nearly 50- and 25-fold among all tested cell lines and among EBV-positive Burkitt's lymphomas, respectively (Pratt et al. 2009). There is little or no increased expression of its miRNAs when EBV's lytic cycle is induced (Pratt et al. 2009). EBV does not regulate its productive cycle by altering the levels of its miRNAs exception to BART2 (Barth et al. 2008).

EBV encodes multiple miRNAs from two primary transcripts, the BHRF1 and the BARTs. The expression of BHRF1 miRNAs is dependent on the type of viral latency, whereas the BART miRNAs are expressed in cells during all forms of latency (Pratt et al. 2009). EBV was found to encode five miRNAs clustered within two genomic regions. miR-BHRF1–1, 2, and 3 are located within the untranslated region (UTR) of BHRF1, an antiapoptosis Bcl-2 homolog. miR-BHRF1–1 is located within the 5′UTR with miR-BHRF1–2 and 3 encoded within the 3′UTR.

miR-BART-1 and 2 are located within intronic regions of the BART family of transcripts, which are extensively spliced. The BART family of transcripts has been suggested to encode a number of proteins, although it remains to be convincingly demonstrated that these proteins are expressed during viral infection (Smith et al. 2000). Subsequent investigation identified a further 22 EBV miRNAs not identified in the original study, due to a large deletion in the B95–8 strain used for the initial cloning strategy (Cai et al. 2006; Grundhoff et al. 2006). Three miRNAs encoded within the BART region, miRBART16, miR-BART17–5p, and miR-BART1–5p, have since been shown to target sequences within the 3′UTR of the viral latent membrane protein 1 (LMP1) following the transient transfection of an LMP1 expression plasmid and various BART-derived miRNAs (Lo et al. 2007). In latent infection, EBV also expresses at least 14 BART miRNAs, which are encoded by a genomic region, which also encodes the noncoding BamHI A rightward transcripts (BARTs) (Cai et al. 2005; Grundhoff et al. 2006; Pfeffer et al. 2004). Seven of EBV's microRNAs are closely related to microRNAs of an EBV-related monkey virus (*Rhesus lymphocryptovirus*) and provide a first example of miRNA conservation within the herpesvirus family (Cai et al. 2006).

EBV may also use cellular miRNAs to regulate gene expression. There are at least two cellular miRNAs with pleiotropic cellular effects that may be induced by EBV proteins. LMP1 activates the promoter for mir-146a, a cellular miRNA, which downregulates a large number of interferon-responsive genes (Lo et al. 2007). Thus, EBV may cooperate a cellular miRNA pathway involved in modulating the interferon response to enhance EBV replication in vivo. miR-155 is a cellular miRNA derived from BIC, a noncoding RNA whose expression is upregulated in a variety of B cell malignancies including diffuse large B cell lymphomas, CLL, and Hodgkin's lymphoma (Eis et al. 2005; Fulci et al. 2007; Kluiver et al. 2005). Consistent with an important role for miR-155 in B cell malignancy, transgenic mice carrying an miR-155 transgene develop B cell lymphomas (Costinean et al. 2006). This indicates that EBV induces cell miRNAs with effects on immune responses and oncogenesis.

The most abundant RNAs in EBV-infected cells are small nuclear EBER RNAs that are present at approximately 10^5 copies per cells but are not necessary for lymphocyte transformation (Arrand et al. 1982; Swaminathan et al. 1991). EBER1 and EBER2 have 167 and 172 nucleotides, respectively. The EBERs are expressed in many of the malignancies linked to EBV and presumably contribute in some way to the maintenance of latency in vivo (Raab-Traub 2002). The evolutionary conservation of EBERs among primate homologs of EBV and their ubiquitous expression suggests that they play a critical role in EBV biology, such as apoptosis, lymphomagenesis, cell transformation, and some genes expression (Swaminathan 2008).

In nasopharyngeal carcinoma cells, the EBER2 promoter was stronger than the H1 and U6 promoters in shRNA synthesis, leading to more effective knockdown of the target genes. The EBER promoters fundamentally different from those of H1 and U6 can be used to drive the intracellular expression of shRNAs for effective silencing of target genes in mammalian cells and particularly in EBV-infected cells (Choy et al. 2008).

2.4 NPC Stem Cells

In normal organs, stem cells are defined as a subset of cells with the capacity of self-renewal to maintain the stem cell reservoir and of differentiation to generate various types of cells in the tissue. By self-renewal, stem cells divide symmetrically and perpetuate themselves by generating daughter cells with identical stem cell abilities of parent. By differentiation, stem cells give rise to a hierarchy of limitedly proliferative but functional mature cells. This model was first established in hematopoietic system, in which a small number of donor cells identified with stem cell characteristics can reconstitute the bone marrow by transplantation. After that, tissue-specific stem cells were isolated from multiple organs including lung, skin, liver, and brain. The stem population principally stays quiescent in specialized niches under physiological conditions while actively entering a proliferation state and differentiating in response to specific stimuli. The balance of self-renewal and differentiation is accurately orchestrated to maintain the tissue homeostasis. Zhang and coworkers first described the identification of stem-like cells in normal mouse nasopharyngeal epithelium with the well-established label-retaining cell (LRC) approach, which is based on the evidence that stem cells are able to retain nucleoside analog including bromodeoxyuridine (BrdU) (Tumbar et al. 2004; Zeng et al. 2007). In mouse nasopharyngeal stratified squamous epithelia, less than 3% of cells were long-term BrdU LRCs, of which 64.12% localized in

the basal layer and 35.88% were in the superbasal layer. Besides, approximately 12% of LRCs were recruited into cell cycle progression, demonstrated by double-label with BrdU and 3H-TdR. These findings suggested the existence of stem cells in normal nasopharynx.

Borrowed from the view of NSCs, cancer stem cells (CSC) are proposed to be a small population of cells within the tumors, which are capable of self-renewal and generating heterogeneous progeny to constitute the tumor bulk. The idea of CSC is not new but has received increasing attention and enthusiasm in the fields of oncology. This enthusiasm is justified by the identification and characterization of rare tumor-initiating cells analogous to NSCs within hematopoietic malignancy and solid tumors including those of breast, lung, colon, and brain. Several lines of evidence support the existence of CSCs in NPC. First, LRCs were also found in the mouse NPC xenografts in addition to normal mouse pharynx, with a similar percentage of less than 0.5% in mouse xenografts generated by three human NPC cell lines. Second, Wang and coworkers isolated side population (SP) cells fitting the criteria of CSCs from human NPC cell lines by using a cell-permeable DNA-specific bisbenzimidazole dye Hoechst 33342 and further investigated biological characteristics of SP cells including proliferation, self-renewal, and tumor-initiation. In one of the poorly differentiated NPC cell line CNE-2, SP cells accounted for less than 3% of the whole population. After being sorted in vitro, SP cells showed more extensive proliferative potential and generated significantly more clones than non-SP cells. In addition, SP cells can give rise to non-SP cells, whereas non-SP cells were unlikely able to generate SP cells. Furthermore, increased resistance to conventional therapies including chemotherapy and radiotherapy was observed in SP cells. More importantly, in vivo assays revealed that SP and non-SP cells were different in the ability to initiated tumors. Ten thousand SP cells were sufficient to form tumors while 200,000 non-SP cells were required (Friborg et al. 2007).

If the hypothesis is proved to be true about the existence of stem cells in normal nasopharynx and NPC, it will open a new frontier for exploring the oncogensis, progression, and treatment-resistance of NPC. This concept challenges the more traditional view about tumor development, by which tumor cells are assumed equal as for tumorigenesis, and the heterogeneity of tumor evolves from random mutations or an environmental selection progress. Whereas CSC hypothesis proposes that tumor is initiated and maintained by a minority of tumor cells and the heterogeneity of tumor comes from the aberrant "differentiation" of these cells.

Cancer stem cell hypothesis sheds much light on the origin of NPC. Self-renewal is crucial in stemness of CSCs. If the CSC proliferates but fails to self-renew, the CSC pool will be inevitably exhausted. In consideration of the observed similarities between CSCs and its normal counterpart in terms of self-renewal and cell surface markers, it is reasonable to assume that CSCs origin from a mutated NMS, which escapes from proliferation control but spare the self-renewal. Actually, several lines of evidences indicate that this may be the case. For example, Kim et al. 2005 isolated putative bronchioalveolar stem cells (BASC) from mouse lung. Oncogenic *K-ras* was found to activate the expansion of BASCs and transform these cells into adenocarcinoma precursors. Moreover, PTEN deletion in mouse hematopoietic stem cells has been suggested to result in a myeloproliferative disorder and followed by acute T-lymphoblastic leukemia (Guo et al. 2008). Besides, there is another possibility that a restricted progenitor or terminally differentiated cell, which experiences a serial of mutations and acquires the ability of self-renewal, gives rise to the subset of tumor cells with some features of NMSs. Actually, many pathways important for the maintenance of NMSs are found disregulated in a variety of cancers. For example, *Bmi*-1, a member of Polycomb group (PcG) genes, is required for the maintenance and self-renewal of embryonic and somatic stem cells. In the context of NPC, *Bmi*-1 is found highly expressed in both nasopharyngeal carcinoma cell lines as well as NPC samples, which is negatively related to the prognosis of NPC patients. Experimentally, overexpression of *Bmi*-1 sufficiently immortalizes normal nasopharyngeal epithelial cells by induction of telomerase reverse transcriptase activity and inhibition of p16 (Ink 4a) expression (Song et al. 2006).

2.5 Molecular Alterations

The genetic, environmental, and viral causative factors, either acting alone or in combination, would lead to multiple genetic and epigenetic alterations (Lo et al. 2004). The development of NPC involves accumulation of multiple genetic and epigenetic changes leading to the evolution of clonal cell population that possesses growth advantages over

other cells (Lo and Huang 2002). By comparative genomic hybridization (CGH) analyses, a large number of primary NPC have recently been examined for the gain and loss of genetic material in the genome, including gain at chromosome 1q, 3q, 8q, 12 and loss at 3p, 9p, 11q, and 14q (Chen et al. 1999; Chien et al. 2001; Fang et al. 2001). The highest frequencies of allelic loss were found on 3p (75%) and 9p (87.0%). Using array-based CGH, frequent amplifications were detected for several oncogene loci, including MYCL1 at 1p34.3 (66.7%), TERC at 3q26.3 (46.7%), ESR at 6q25.1 (46.7%), and PIK3CA at 3q26.3 (40%) (Hui et al. 2002). Loss of heterozygosity analysis (LOH) on 9q, 11q, 13q, and 14q was found in over 50%% of the NPC samples (Lo and Huang 2002). The most frequent LOH were observed at chromosome 3p, 9p, and 14q, which is in agreement with the CGH-based findings. In addition, some karyotyping-based studies have been performed on NPC, where many structural and numerical alterations found on 1p, 3p, 3q, 5q, 9p, 12, 11q, 13q, 14q, 16q, and X (Bernheim et al. 1993; Chang et al. 1989; Hui et al. 1998; Lin et al. 1993; Zhang et al. 1982). Among these alterations, deletion of 3p and gain of 3q are the most frequent events (Lo et al. 1997; Lo and Huang 2002). A recent spectral karyotyping (SKY) analysis on NPC cell lines confirmed most of the abnormalities identified previously by CGH and LOH and illustrated additional breakpoints on a number of apparently balanced chromosomes, including 3p21, 3q26, 5q31, 6p21-p25, 7p14-p22, and 8q22 (Wong et al. 2003).

Genes located on chromosomes 9p21 (p14, p16) and 3p21.3 (RASSF1A) were found to be defective owing to deletion or promoter hypermethylation (Kwong et al. 2002; Lo et al. 1996). Frequent promoter hypermethylation of cancer genes is an important feature of NPC (Lo et al. 2004). The tumor suppressor properties of p16 and RASSF1A have also been demonstrated in NPC cells (Chow et al. 2004; Wang et al. 1999). Induction of epigenetic alterations of cellular genes was proposed as one of the mechanisms for enhancing the transformation of nasopharyngeal epithelial cells by EBV infection (Lo and Huang 2002).

In NPC cells, the apoptosis process may be interfered by multiple genetic changes. Overexpression of Bcl-2 and inactivation of p53 pathway is believed to be the major mechanisms for the reduction in apoptosis in this cancer (Lo and Huang 2002). Bcl-2 product has a high degree of homology with BHRF-1, an open reading frame product in the EBV genome (Cleary et al. 1986) that disturbs epithelial cell differentiation (Dawson et al. 1995). The high prevalence of Bcl-2 detected in NPC is consistent with the frequent immunoreactivity of this oncoprotein exhibited by the basal layer cells of nasopharyngeal normal mucosa, at the same time that Bcl-2 protein produces a significant extension of cell survival may be considered a key event either in cell transformation or in tumor growth (Korsmeyer 1992). EBV can use viral proteins influence the expression of Bcl-2, as LMP1 (Burgos 2005). However, studies on the value of p53 in NPC are controversial; some studies showed that p53 protein accumulation may be a common event in carcinogenesis (Niedobitek et al. 1993; Niedobitek et al. 1994; Sakai et al. 1992), but in the last few years strong evidence indicates the low incidence in p53 modifications in this cancer (Kouvidou et al. 1997; Nasrin et al. 1994; Niemhom et al. 2000; Spruck et al. 1992; Sun et al. 1992). To reveal the tumorigenesis pathway, several studies have examined the early events in NPC development. Some reports have demonstrated clonal proliferation, overexpression of Bcl-2, telomerase activation, and EBV infection in the precancerous lesions (Chang et al. 2000; Jiang et al. 1996; Pathmanathan et al. 1995; Sheu et al. 1997).

From the view of tumor development, emerging data suggest that several pathways essential in development, such as Sonic hedgehog and Wnt/β-catenin, disregulated in CSCs. In NPC, Smo is activated in SP cells and the resistance of SP cells to radiotherapy is partially reversed by cyclopamine, which blocks SHH signaling pathway through binding to Smo (Friborg et al. 2007). Gene expression profiling indicated that multiple components of Wnt/β-catenin pathway were upregulated (Zeng et al. 2007). EBV latent membrane protein 2A (LMP2A) and epigenetic inactivation of Wnt inhibitory factor 1 were suggested to contribute to the aberrant activation of Wnt/β-catenin pathway (Chan et al. 2007; Morrison et al. 2005). These data indicate that Wnt/β-catenin pathway may play an important role in the tumorigenesis and progression of NPC.

2.6 Summary

The geographically constrained distribution of EBV-associated NPC in Southeast Asian populations suggests that both viral and host genetics may influence disease risk (Gullo et al. 2008). NPC undergoes a

multistep carcinogenesis. The consistent expression of specific viral genes and the detection of latent membrane protein in every cell in NPC samples and in premalignant lesions suggest that these viral gene products contribute to the abnormal proliferation, as they may serve as specific tumor markers and targets for novel therapy strategies (Lo et al. 2004). Moreover, the induction of EBV replication in latently infected cells is being evaluated as a therapeutic approach to stop malignant cell proliferation (Ambinder et al. 1999). The high frequencies of epigenetic alterations in this cancer suggest the potential application of novel inhibitors targeting DNA methylation and histone acetylation. Additionally, the identification of cancer stem cells in NPC provides important insight into understanding NPC biology and may also help develop new strategies to fight NPC more efficiently, which will ultimately improve the prognosis of NPC patients.

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