CHAPTER II

REVIEW OF RELATED LITERATURES

Nasopharyngeal Carcinoma (NPC)

Histopathology

NPC is distinguished from other head and neck cancers by its histopathology, epidemiology, clinical characteristics, and therapy. The World Health Organization (WHO) classification subdivides NPC into three variants according to the predominant histological pattern. WHO type I is a keratinising squamous cell carcinoma similar to carcinomas that arise from other sites of the head and neck. This type is not common in endemic areas. WHO type II is a nonkeratinising keratinising squamous cell carcinoma while WHO type III is an undifferentiated carcinoma. Both types are referred to as lymphoepithelioma or Schminke tumors, squamous epithelial tumors with heavily lymphocyte infiltration and are common in endemic areas.[28]

Epidemiology and Evidence of Susceptibility

NPC is a rare disease in most parts of the world. It occurs with a high incidence in Southern China, Southern Asia, and other high-risk populations including North Africa and Eskimos. The aetiological factors of endemic NPC include EBV infection, genetic susceptibility, and environmental factors.[17,29] EBV association in NPC refers to those situations in which the viral genome or gene products are detected within tumor cells. This virus exists within tumor cells in a monoclonal form, with every viral episome carrying an identical number of terminal repeat regions. This suggests that such episome were derived from a single infected cell. EBV infection can cause a variety of diseases as a consequence of primary and latent infection. The virus primary infects the oropharyngeal epithelium. EBV then replicates, lyses, and infects in B-lymphocytes. After infection, the virus enters the lytic phase and causes

diseases ranging from mild self-limited illness in children to infectious mononucleosis (IM) in adolescences and adults. However, in some cells the virus will switch to the latent phase. These latently infected B-lymphocytes have the inclination to turn into lymphoma such as Burkitt's lymphoma (BL), Hodgkin's disease (HD) or can play an important role in the dissemination of infection of the nasopharynx. Consequently, this virus can induce NPC development (Figure1).[30-32] In addition to EBV, environmental factors is the ingestion of Chinese style salted fish, especially during childhood. Several carcinogenic volatile nitrosamines have been detected in Chinese salted fish. Besides salted fish, exposures to other preserved food products, such as salted shrimp paste, fermented soybean paste and various types of salted vegetable, have been shown to be related to NPC risk in Chinese.[15,17,33] Among non-dietary environmental exposes, tobacco smoking has been suggested to be a risk factor for NPC.[34-38]

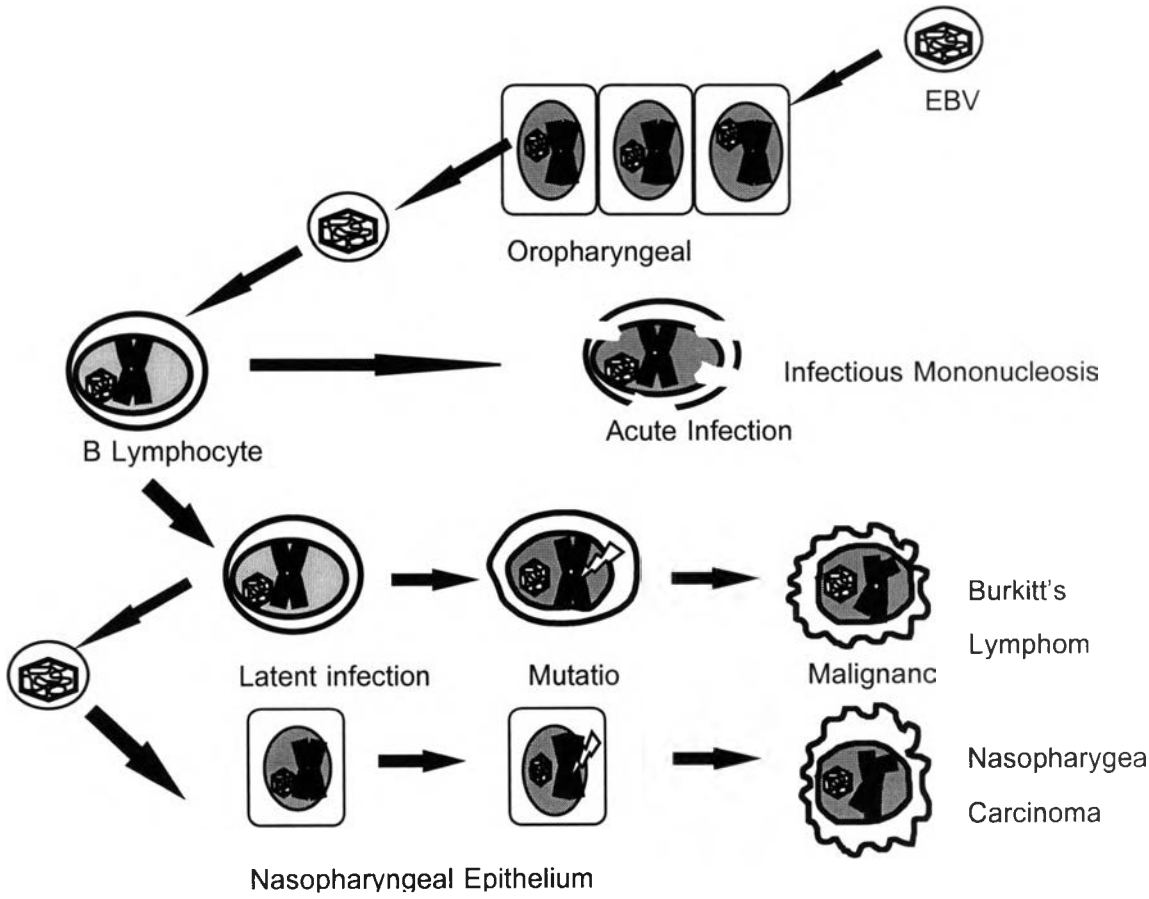

Figure1 A model of the Epstein-Barr Virus infection and disease development[32]

Susceptibility Genes and Nasopharyngeal Carcinoma

The distinct geographic variations in the incidence of NPC indicate a contribution of genetic or environmental factors, or both, to its development. The association between human leukocyte antigen (HLA) and NPC not only has first been discovered in Singapore but also confirmed in several countries in Asia.[39-40] Early evidence for a genetic determinant among Chinese was an HLA-associated higher risk for NPC, specifically the joint occurrence of HLA A2 and HLA BSin2 [relative risk (RR) = 2.35].[41] The risk for NPC would increase if HLA A2 and HLA Bsin2 antigen occurs frequently in Chinese people but not in white people. These data suggest that a gene closely linked to the HLA locus strongly influences disease susceptibility but not the HLA locus. The second NPC susceptibility gene is Cytochrome P450 2EI (CYP2EI) gene. The Taiwanese association study showed that individuals homozygous for an allele of the CYP2EI gene detectable by RsaI digestion were at an increased risk for NPC development [relative risk (RR) = 2.6].[42]

Mechanism of Nasopharyngeal Carcinogenesis

NPC involves multiple genetic alternations. The genetic alternations include the inactivation of tumor suppressor genes and activation of oncogenes. For example, previous study reported frequent allelic loss on chromosome 3, 9, 11,13 and 14, suggesting a potential location for essential tumor suppressor genes.[43] In addition to mutations, viral carcinogenesis involves interactions between viral oncoproteins and its host. Latent membrane protein 1 (LMP1) is a well-known viral oncoprotein from EBV. It can inhibit p53-mediated apoptosis through the induction of *A20* anti-apoptosis gene. LMP1 expression in epithelial cells activate transcription factor NF-κB.[44] Therefore, the upregulation of *A20* expression is a result of NF-κB activation. Interestingly, LMP1 has been shown to engage signaling proteins for the tumor necrosis receptor (TNFR). Activation of this signaling molecule by LMP1 could mimic ligand binding to this family of

receptors, and therefore subsequent induction of A20 may be important for the protection of apoptosis induced by activation of this pathway.[45] Additionally, LMP1 also induces expression of EGFR (epidermal growth factor receptor), leading to a deregulation of cellular growth control. The effect of LMP1 resembles those activated Ras molecules, suggesting a common biochemical pathway for these oncoproteins. Since overexpression of the EGFR by LMP1 could mimic an activated Ras pathway, LMP1 may exert its transforming effects in epithelial cells via deregulation of the EGFR.[46] In conclusion, that LMP1 is a cause of increased proliferate signals due to enhanced EGFR expression, as well as protection from cell death due to LMP1 induced A20 expression can contribute to development of epithelial malignancies.

Epstein-Barr Virus (EBV)

Classification

EBV was first isolated by Epstein and Barr in 1964 form a cultured Burkitt's lymphoma (BL) cell line using electron microscopy.[47] Initially, Dr.Denis Burkitt, a missionary doctor, described unique endemic jaw tumors in equatorial Africa in 1985.[48] Subsequently, Henle and Henle reported that EBV was the causative agent of infections mononucleosis (IM) because their laboratory technician eventually seroconverted against EBV during an episode of the disease.[49] In 1966, Old et al incidentally observed that patients with undifferentiated nasopharyngeal carcinoma (NPC) had remarkably elevated antibody titres to EBV, and they considered that this virus was etiologically linked to the development of this tumor.[50] EBV is a member of the herpesviridae family, subfamily gammaherpesvirinae, and genus lymphocryptovirus. Taxonomist has renamed EBV, human herpes virus 4. It has a narrow tissue tropism limited to B cell and epithelial cell of primate origin. It can immortalize B cells both in vitro and in vivo. Recent evidence has suggested that EBV might be subdivided into

two subtypes (A and B, or EBV-1 and EBV-2) based on differences at the protein sequence level and capacity to transform B cells.[51]

Virion structure and genome organization

All the herpesviruses have a similar structure consisting of linear double – stranded DNA, a nucleocapsid with 162 capsomeres, a protein tegument between the nucleocapsid and the envelope, and an outer envelope with external glycoprotein spikes (Figure 2).[52] The prototype laboratory strain named B95-8 EBV has been well characterized, and its complete nucleotide sequence was reported in 1984. The EBV genome has 172 kbp, and is divided mainly into terminal repeat (TR), internal repeat (IR1 - IR4), and largely unique sequence domains (U1 – U5) (Figure 3).[53-55] EBV isolates can be classified type 1 and 2 (originally called A and B) based on polymorphism of their EBNA 2, 3A, 3B and 3C genes. Furthermore, individual virus strains of the same type can be distinguished by the different sizes of their EBNA 1, 2, 3A, 3B, and 3C proteins visualized in immunoblots.[56-57] Especially, the EBNA 3B and 3C proteins are predominantly type – specific.[58]

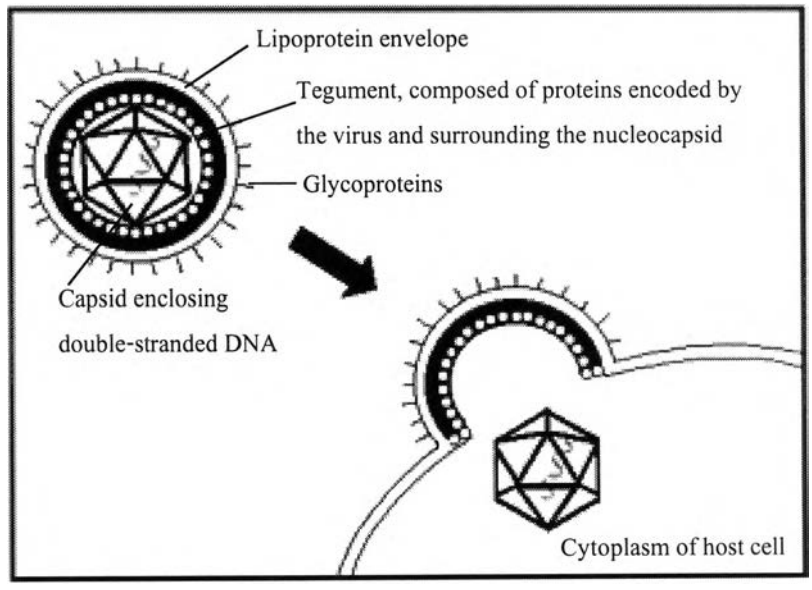

Figure 2 Adsorption and fusion of Epstein-Barr virus with cytoplasmic membrane for penetration.[52]

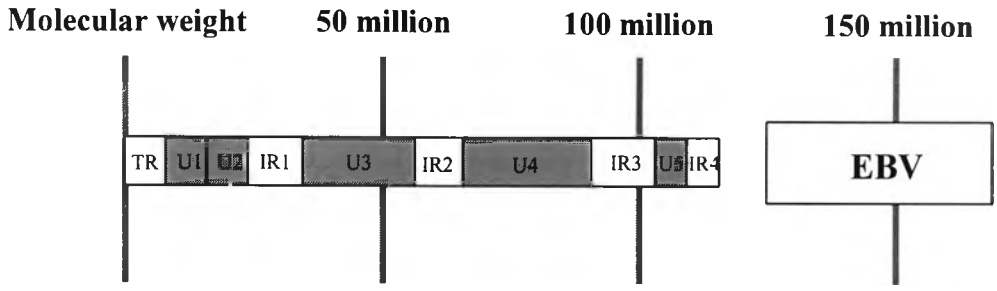

Figure 3 Genome organization of Epstein-Barr virus. TR ; Terminal repeat, IR1- IR4; Internal repeat1-4, U1- U5; Unique sequence domains1-5.

EBV infection and Pathogenesis

The Epstein – Barr virus has the ability to infect either B lymphocyte cells or salivary gland epithelial cells, the former being the primary target of infection for lytic infection. In latent infection, there are at least nine related proteins and two small non–polyadenylated RNAs encoded by EBV DNA. These nine proteins and two RNAs include Epstein–Barr virus nuclear antigens [EBNA-1, -2, -3, -3A, -3B, -3C, and leader protien (LP)], latent membrane proteins [LMP-1, -2A, -2B], and EBV – envelope RNAs [EBER-1 and –2], respectively. EBNA-1 is essential for the replication of the plasmid form of EBV, and EBNA-2 is highly association with cell proliferation, as is LMP-1.[59] Additionally, interesting differences in the expression of EBV – relate latent protein and RNAs are found at tissue level with three patterns of latency.[60] Latency I, in which only EBNA-1 and EBER, are found in EBV – infected cells, is seem in EBV genome positive BL and some gastric carcinomas. Therefore, these neoplastic cells easily evade immune surveillance because no target antigens of EBV–specific cytotoxic T–lymphocytes (EBV–CTL) are expresses. Latency II, in which EBNA-1, LPMs and EBERs are expressed, is observed in NPC, Hodgkin's disease (HD), and certain T – cell lymphoma. Latency III, in which all the EBNAs, LMPs and EBERs are expressed, is seen in Lymphoproliferative disorders (LPD) which are observed in immunocompromised individuals and in EBV – infected B – cell in the acute

phase of Infections mononucleosis (IM). These different types of latency are thought to be related to the development of the different phenotypes of EBV – associated diseases. In the lytic cycle, EBV may be transcribtionally activated spontaneously or by chemical induction to express genes for viral replication. The first such gene to be activated is BamHI -Z left – ward reading frame (BZLF) I, which express the transactivator function of BZLF I protein or BamHI – Z EB replication activator (ZEBRA). Then, EBV – related early protein, such as early antigen (EA) – diffuse (D) and EA – restricted (R), are expressed, Subsequently, viral DNA synthesis occur and viral capsid antigen (VCA) is seen, and finally virion are produced. The possible main pathogenetic mechanism in-patients with severe EBV infection are summarized in table1. These are: (1) neoplastic proliferation of latently EBV infected cell in cases of EBV genome–positive BL, HD and NPC; (2) neoplastic proliferation in cases with defective immune surveillance such ad in LPD affecting patients with primary immunodeficiency, recipients of transplants, and patients with AIDS; (3) EBV replication in infected cell in oral hairy leukoplakai; (4) the immune response against EBV infection itself in cases of severe or fatal IM and EBV–HLH; (5) both proliferation of latently EBV-infection cell and EBV replication such as in C(A)EBV and SCAEBV. More than one of the mechanism described above may operate together in each specific disease.[61]

Table1 Possible pathogenic mechanism of EBV-associated disease[61]

Main pathogenetic mechanisms	Disease
1. Proliferation of latently EBV-infected cells	Burkitt's lymphoma Hodgkin's disease Nasopharyngeal cacinoma
2. Proliferation of latently EBV-infected cells in immunodeficiency	Lymphoproliferative disorder in patients with primary immunodeficiency, in patients with transplantation, and in patients with acquired innunodeficiency syndrome
3. Replication of EBV in infected cells	Oral hairy leukoplakia in patients with acquired immunodeficiency syndrome
4. Immune response against EBV-infected cells	Infection mononucleosis , EBV-related haemophagocytic lymphohistiocytosis
5. Both proliferation of EBV-infected cell and replicaiton of EBV infected cells	Severe chronic active EBV infection syndrome

Identification of Susceptibility Genes

Functional Cloning

The functional cloning strategy is essential that there is some understanding of the biochemistry and pathogenesis of the genetic disorder under investigation. Thus, if the biochemical basis of an inherited disease is known, it may be possible to purify and partially characterize some of the gene

product. For example, the gene-specific oligonucleotide can be synthesized to correspond to peptide sequencing from the purified gene product, and can be used to identify the disease gene by screening cDNA libraries. Additionally, if there are antibodies specific for the desired protein, they can be used to screen cDNA expression libraries as called the specific antibodies.[62]

Positional Cloning

As the opposite pole to the functional cloning strategy, the positional cloning is a technique whereby genes are identified and isolated by chromosome location with no prior information in respect of biochemistry or function.[62] There are two essential requirements for finding disease genes. The first is genetic markers. If DNA marker link to the disease, it can tell the location of the disease gene. The genetic marker can be found easily now, because the Human Genome Project is completed. The second is sufficient numbers of families to establish linkage. Two general approaches have been used to studying with families. In one, linkage analysis is study association between disease locus and polymorphic marker in each family. When there is a small number of families and that individual family has a large number of patients. The advantage of this approach is that all affected members of the pedigree are known to have the same genetic disease, caused by a mutation at a single locus. On the other hand, if it has a large number of families, but individual family has few patients, it should use linkage disequilibrium analysis for localizing a gene. This approach is studying that association at the population level of a particular maker allele with a disease. That disorder has been occurred in donkey's year, and the location of which should be on the conserve region owing to a low mutation rate. Especially, linkage disequilibrium studies are well suited to both dominant and recessive disorders that do not appear to exhibit genetic heterogeneity.[63]

Candidate Gene Approach

In fact, the functional cloning strategy will rarely succeed because molecular pathology is too complicated, such that predictions of the biochemical function of an unknown disease gene are often imprecise.[62] In the part of positional cloning approach, it is inefficient because it is necessary to use all genetic makers on whole genome owing to unknown which one of DNA markers link a disease gene. Consequently, candidate gene approach is a new method for identified human disease gene by searching a relative gene with the disorder. This method is selection some genes for detected disorder locus, as called Candidate genes which may be suspected to have a role in the disease development.

Positional Candidate Gene Approach

Positional candidate gene approach combines the positional and candidate gene approaches. This strategy is more efficient since it is used to identification of over 50 disease genes, for example Huntington's disease.[62] In addition, Positional candidate gene approach is not essentially employ all of genetic markers on genome because it is useful for testing candidate susceptibility loci for linkage before starting to screen a candidate gene for mutation. Therefore, the identification of human disease gene by positional candidate gene approach is the most promising strategy at the present and in the near future.

Proposed Candidate Susceptibility genes

Genes interact with environmental agents

A large percentage of cancers are considered to be associated with environmental agents. All higher organisms have developed a complex variety of mechanisms by which they protect themselves from environmental insult.

Genetic reducing ability in these functions will result as susceptibility to cancer development. For example, almost all carcinogens in the environment are metabolically activated or detoxified by enzymes including cytochrome P4502E1 (CYP4502E1) and glutathione S-transferase (GSTs).[64] Therefore, any alteration in the activity of these enzymes would result in an altered chance for cancer development. As a result, there has been reported that association between polymorphism of these enzymes and cancer susceptibility. For example, the polymorphism of *CYP2E1* gene has correlated with NPC development in Taiwan.[42]

Genes function for epithelial EBV endocytosis

The interaction between EBV and epithelial cells has long been of special interest based on its close association with NPC. Genetic variation of this process could explain the high NPC prevalence in South Asia but not African, higher incidence of Endemic Burkitt's lymphoma. Entry of EBV into epithelial cells is necessary to use a receptor. Both CR2 and pIgR have been proposed to be the possible receptor proteins for EBV to enter nasopharynx. However, there is not sufficient knowledge to conclude which pathway EBV enters nasopharyngeal epithelium.

Genes function for immune response

The HLA (human leukocyte antigen) function is recognition of foreign antigens by T lymphocytes. As the physiological function of HLA presentation, one hypothesis, that these particular HLA Class I antigens may not present EBV antigens efficiently, thereby allowing the EBV to persist in nasopharyngeal epithelial cells, is the hypothesis of viral persistence. Indeed, EBV persistence is linked to several other cancers such as Burkitt's lymphoma and Hodgkin's disease.[65]

Genes, proto-oncogenes or tumor suppressor genes, interact with viral protein

The viral oncoprotein can interact with tumor suppressor gene products of human. In other word, polymorphic host interacting protein structure could result in distinct probability in cancer development. For example, LMP1 can interact with A20 and EGFR.[44,46] Thus, these protein structures of NPC patients may bind stronger to the viral protein than other EBV carriers.

Likage Disequilibrium and Hypothesis of Genetic Susceptibility

Although EBV infection exhibits worldwide distribution, NPC is found most often in Chinese population. In addition, emigrant Chinese populations in several countries continue to show a higher incidence of NPC than local populations, and the risk of NPC in patient's family is higher than other families. It is interesting to investigate why NPC is found most frequently in Chinese and the susceptibility gene of NPC. In this study, linkage disequilibrium analysis is used to identify disease gene by employing principle of candidate gene approach. Linkage disequilibrium is the tendency for certain alleles at two linked loci to occur together more often than expected by chance. For example, if ABO blood group is a marker locus linked to an assumed disease locus on the same chromosome. If majority of patients have A blood group but distribution of ABO blood group is equivalent proportion in normal population, this indicates that disease locus link to ABO blood group especially alleles A. By contrast, if dispersion of ABO blood group is equivalent proportion in both patients and normal population, it show that ABO blood group is not marker locus for this disease locus. The occurrence of linkage disequilibrium may be resulted from founder effect which is one of these early settlers carried the gene mutation. As a result, this genetic disease is of the highest frequency in population. Because we do not know which gene is susceptible to NPC development, candidate gene approach is used to find susceptibility genes. This approach studies the association between polymorphism of candidate genes and NPC phenotype. The polymorphism of candidate genes is detected by polymerase chain reaction - based restriction fragment length polymorphism (PCR-RFLP) method. In this study, three

candidate genes are chosen for the investigation. The first gene is *CYP2E1* that involves the metabolism of nitrosamine compounds. This gene has been proved to be associated with NPC in Taiwan. In addition, *CR2* and *pIgR* are selected because both may involve in the mechanism of EBV infection into epithelium cells. The linkage disequilibrium data between RFLP and NPC phenotype will help indicate whether *CYP2E1*, *CR2* and *pIgR* are responsible for NPC development.

Candidate Genes

The present study has chosen to investigate the role of three candidate genes in NPC development. They are

I. Cytochrome P450 2E1 (CYP2E1)

Function

Xenobiotics are foreign compounds to the body such as drugs, food additives, pollutants, etc. The metabolism of xenobiotics in humans occurs in two phases. In phase I, the major reaction involved is hydroxylation, catalyzed by the cytochrome P450 enzymes. Phase II reactions increase the water solubility of products in phase I by conjugation with glucuronic acid, sulfate, acetate, glutathione, or certain amino acids. Thus, most of these compounds facilitate their excretion from the body.[66] The cytochrome P450 enzymes are a multigene superfamily of monomeric mixed function mono-oxygenase, responsible for the phase I metabolism of a wide rage of structurally diverse substrates by ability to insert an atom of molecular oxygen into their substrates. The P450 superfamily is divided into ten subfamilies. Each subfamily member has greater than 40% sequence identity at the amino acid level. Subfamilies CYP1, CYP2, and CYP3 are primarily involved in xenobiotic metabolism, especially CYP2 family that is thought to have arisen as an adaptive response to environmental factors.[67-69] A single gene, *CYP2E1*, which is expressed in hepatic tissue and nasal epithelium, represents this

family in humans.[70] *CYP2E1* plays a major part in the metabolism of solvent carcinogens such as nitrosamines, benzene and ethanol.[71]

Correlation between CYP2E1 and NPC development

CYP2E1 enzyme, involving in the metabolic activation of procarcinogens into reactive intermediates capable of forming adducts and damaging DNA, is believed to play an essential role in chemical carcinogenesis.[72-73] Nitrosamine is a substrate of CYP2E1. It is believed that nitrosamine, once activated can lead to the development of numerous cancers.[74] Studies have also demonstrated that *CYP2E1* is expressed in the nasal epithelium of humans.[75] Evidence from previous epidemiological studies has suggested that salted fish is a food preferred by Chinese people and contains nitosamines and nitrosamine precursors. Therefore, *CYP2E1* is believed to render the nasal epithelium susceptible to NPC development. In the recent study, the association between *CYP2E1* and NPC was discovered first in Taiwan by using PCR-RFLP assay. The Taiwanese correlation study showed that individuals homozygous for the variant allele (-/-) of *CYP2E1* detectable by *RsaI* digestion were at an increased risk for NPC development (relative risk [RR] = 2.6; 95%confidence interval [CI] = 1.2-5.7).[42]

Genome structure and Polymorphic site

The human *CYP2E1* gene, spanning 11,413 base pairs and contained nine exons and a typical TATA box, was mapped to chromosome 10.[76] Analysis and characterization of polymorphism in this gene has involved mainly RFLP analysis by using the restriction endonucleases such as Dra*I*, Taq*I*, and *RsaI*. The polymorphic sites of the first both enzymes localized in intron 6 and intron 7 respectively, but *RsaI* polymorphic site localized in the 5' – flanking region of the gene. Polymorphisms detectable by Dra*I* and Taq*I* digestion are not affected transcription or function of the enzyme coded by

the gene. In contrast, the *RsaI* polymorphism is involved in the transcriptional regulation of *CYP2E1* expression. The previous study has suggested that homozygous for the variant allele (-/-) of *CYP2E1* detectable by *RsaI* digestion is expressed at higher level than the wild-type (+/+) form of the gene.[78]

II. Complement receptor type 2 (CR2)

Function

Complement receptor type2 (CR2, CD21) is an integral membrane glycoprotein of size approximately 145 kDa.[79] It is found primarily on mature human B cells and also on follicular dendritic cells, epithelial cells, and some T cell.[80-84] The nature ligand for CR2 is complement component C3d or C3dg, although iC3b will also interact with CR2.[85] The EBV coat glycoprotein gp 350/220 is also a ligand for CR2, in which its binding site overlaps with that for C3d.[86] Thus, EBV can bind and infect B lymphpcytes and epithelail cells through CR2 receptor. The previous study suggested that cell-to-cell contact provide a general mechanism for EBV to infect epithelail cells in vitro, and perhaps also in vivo. Since CR2 has been detected in some epithelial tissues, including the pharyngeal mucosa these epithelial cells may be effective targets for EBV infection in vivo if they express CR2 and contact virus-producing lymphocytes. The importance of cell-to-cell contact for EBV infection may be explained by several mechanisms. Firstly, interaction of surface molecules between virus donors and recipients may tigger some signaling events essential for infection. Secondary, close contact may transiently generate a local bridge for the intercellular transport to EBV. Finally, cell-to-cell contact may facilitate the accessibility of virus particles to a novel low-affinity receptor on the target cells. On the other hand, cell-free EBV particles could entry epithelial cells exhibiting endogenous CR2 receptor expression or following transfection of CR2 cDNA, representing a cell contact-independent route of infection.[87-88]

Correlation between CR2 and NPC development

The EBV is one of aetiological factors for NPC. Owing to, it has been found in NPC tumor cells and preinvasive lesions but is absent from normal naspharyngeal epithelial cells, which suggests that EBV can infect epithelial cells. In addition, it can replicate and transformation of these cells.[89] Generally, the viral infection is essentially using a receptor and some studies reported low level of CR2 expression at nasopharyngeal epithelium.[90] As mentioned above, that mechanisms of epithelial EBV endocytosis both cell contact-independent infection and cell-to-cell contact infection may use CR2 receptor. Therefore, CR2 may involve in NPC development.

Genome structure and polymorphic site

CR2 is a member of the regulators of complement activation (RCA) gene family found on band q32 of chromosome 1.[91] The entire extracellular portion of the CR2 protein is composed of short consensus/complement repeat (SCR) domains, also known as complement control protein domains. CR2 contains either 15 or 16 SCR domains. Exon 11 is the alternately utilized exon found in some but not all CR2 cDNAs due to a deletion of exon 11 some alleles. Each SCR domain contains 60-70 amino acid recidues. The following of final SCR is a 28 amino acid transmembrane domain and a 34 amino acid cytoplasmic tail. In CR2, the 16 repeats occur in four homology groups, each containing four SCR. Group one [SCR1-4] is encoded by a fused SCR exon (exon1, 2), a single SCR exon (exon3), and splite exons (exon 4a and 4b, which are split in an identical point). Group two [SCR5-8] and group three [SCR9-12] are analogous to group one except group four [SCR13-16] owing to the absence of a split SCR exon in exon 16.[92] The polymorphisms of the CR2 gene were seen when genomic DNA was cut with the restriction enzyme *TaqI* and *HaeIII*. In this gene, the *TaqI* polymorphic site is near the fused exon for SCR1-2, and *HaeIII* polymorphic site is near

the single exon for SCR15. Interestingly, the N-terminal pair of SCR domains of CR2 [SCR1-2] is a binding site for C3d and gp 350/220 EBV ligands.[93]

III. Polymeric immunoglobulin receptor (pIgR)

Function

The epithelial polymeric immunoglobulin receptor (pIgR), also known as the transmembrane secretory component (SC), plays a crucial role in mucosal immunity by translocating polymeric IgA and pentameric IgM through secretory epithelial cells into external body fluids.[94] The pIgR is synthesized by mucosal epithelial and glandular cells and expressed on the basolateral plasma membrane.[95] The cytoplasmic segment of pIgR apparently contains all the information needed for appropriate vectorial sorting within these polarized epithelial cells. The sorting signals act in a temporal and hierarchical way to direct the pIgR through the various intracellular compartments, and its migration has regulated protein trafficking in epithelial cells. The epithelial transport of pIgR can be divided into three discrete step: 1) binding and endocytosis at the basolateral surface, 2) transcytosis from basolateral to apical endosomes, and 3) delivery to the apical cell surface followed by proteolytic cleavage of the pIgR with release of its extracellular part into secretions either as bound SC in SIgA and SIgM, or as a variable excess of free SC (Figure 4).[96-97] The cytoplasmic domain mutation of this gene in Madin-Darby canine kidney (MDCK) cells at Ser664Ala exhibits slowly transcytosis and increase recycling at the basolateral plasma membrane. In contrast, if Ser664 is mutated to an aspartate the receptor is transcytosed at a rate that is greater than the normal pIgR.[98] Since this receptor mediates the endocytosis and transcytosis of polymeric IgA, which allows IgA to function in host defense at three anatomic levels in relation to mucosal epithelium: 1) in the luminal secretions, 2) within the epithelial cells during trancytosis, and 3) in the

lamina propria beneath the epithelium (Figure 5).[94] Additionally, Free SC is important not only to stabilize IgM in the secretions, but has recently also been shown to inhibit epithelial binding of *E. coli* and certain bacterial toxins. Both S_IgA and free SC have been reported to interact specifically with a surface protein (SpsA) of *Streptococcus pneumoniae*.[99]

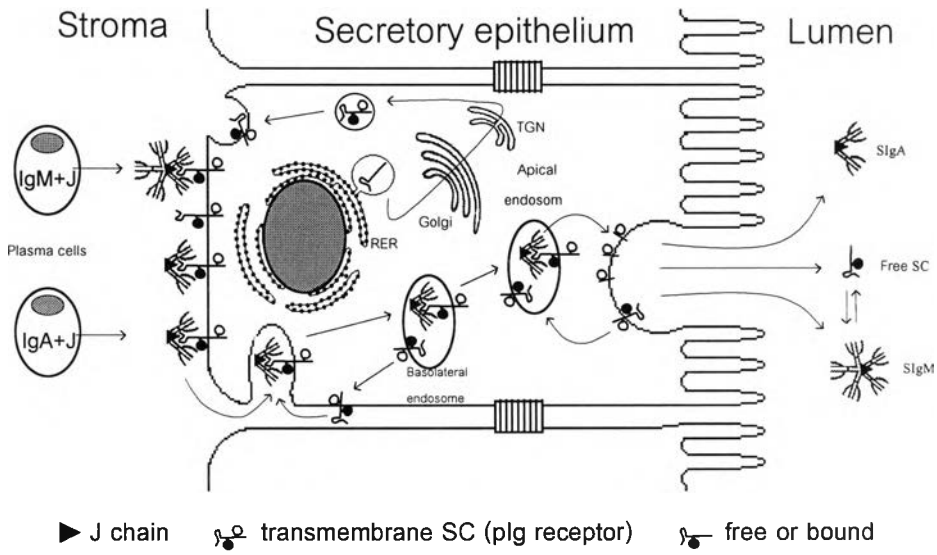

Figure 4 Schematic representation of transported polymeric IgA and pentameric IgM across epithelial cells by using pIgR receptor. J-chain containing polymeric IgA (IgA+J) and pentameric IgM (IgM+J) are produced by local plasma cells (left). The pIgR is synthesized by secretory epithelial cells in the rough endoplasmic reticulum (RER) and matures in the Golgi complex by terminal glycosylation. In the trans-Golgi network (TGN), pIgR is sorted for delivery to the basolateral plasma membrane. After endocytosis, ligand-complexed and unoccupied pIgR is delivered to basolateral endosomes and sorted for transcytosis to apical endosomes. However, some recycling from basolateral endosomes to the basolateral surface may take place for unoccupied pIgR as indicated, and recepter recycling may also occur at the apical surface (not show). Although, most pIgR is cleaved and SIgA, SIgM, and free SC are released to the lumen. During epithelial translocation, covalent stabilization of SIgA regularly occurs, whereas free SC in secretions apparently stabilizes the noncovalently bound SC in SIgM.[97]

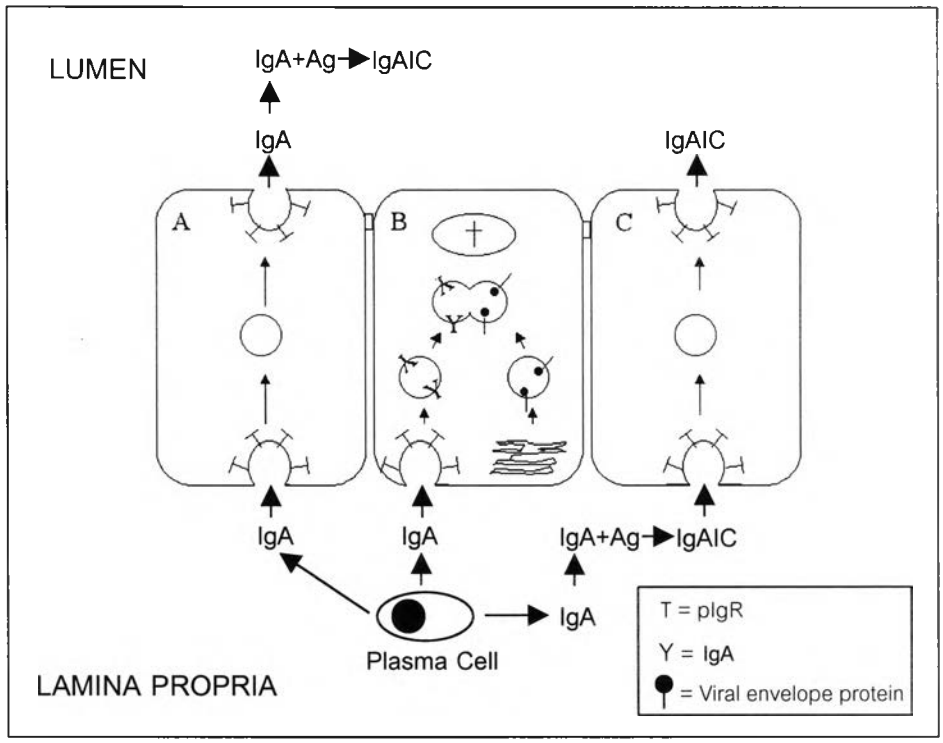

Figure 5 Schematic representation of three levels at which pIgA or SIgA may provide immune protection. The plasma cells in lamina propria secrete pIgA. *Cell A* shows that pIgA can be endocytosed at basolateral surface by pIgR, transcytosed, and secreted into the lumen, where it can combine with antigen (Ag) to form immune complexes (IgAIC). In *cell B*, which has been infected by a virus, it is suggested that a transcytotic vesicle containing IgA antibody to viral envelope protein can fuse with a post-Golgi vesicle containing newly synthesized envelope protein, which provides an opportunity for the antibody to disrupt production of new virus. In the lamina propria below *cell C*, IgA antibody combines with antigen. The immune complex is endocytosed by pIgR and transported intact across the cell and into the lumen.[94]

Correlation between pIgR and NPC development

EBV is a persistent human gammaherpesvirus causally associated with nasopharyngeal carcinoma. Virus entry into human epithelium is less well defined. However, demonstration that pIgR can mediate internalization of infectious IgA-EBV complexes suggests that antibody-enhanced infection plays a role in EBV pathogenesis in Chronic virus carriers. In additional, it is proved that EBV can infect into both nonpolarized epithelial cells[100] and NPC[101] by using EBV-IgA-pIgR complexes.

Genome structure and polymorphic site

Characterization of the *pIgR* gene will supplement present knowledge of the structural organization of related members in the Ig supergene family. Moreover, the human *pIgR* gene is assigned to chromosome1, region 1q31-q41.[102] This gene has altogether 11 exons covering the entire coding region, but the first exon is not translated while the open reading frame is encoded by exons2-11.[103] The exon size ranged from 59 to 657 nucleotides and exon-intron junctions followed known consensus sequences. As showed in the figure 6, three of the five extracellular Ig-related domains (D1, D4, and D5) were confined to one exon each (E3, E5 and E6), whereas D2 and D3 were encoded by the same exon (E4). The membrane-spanning segment was confined to part of one exon (E8). The cytoplasmic tail was encoded by four exons (E8 – E11).[97] The previous study reported that the polymorphic restriction site of this gene located in the third intron, detected by *PvuII* nuclease.[94]

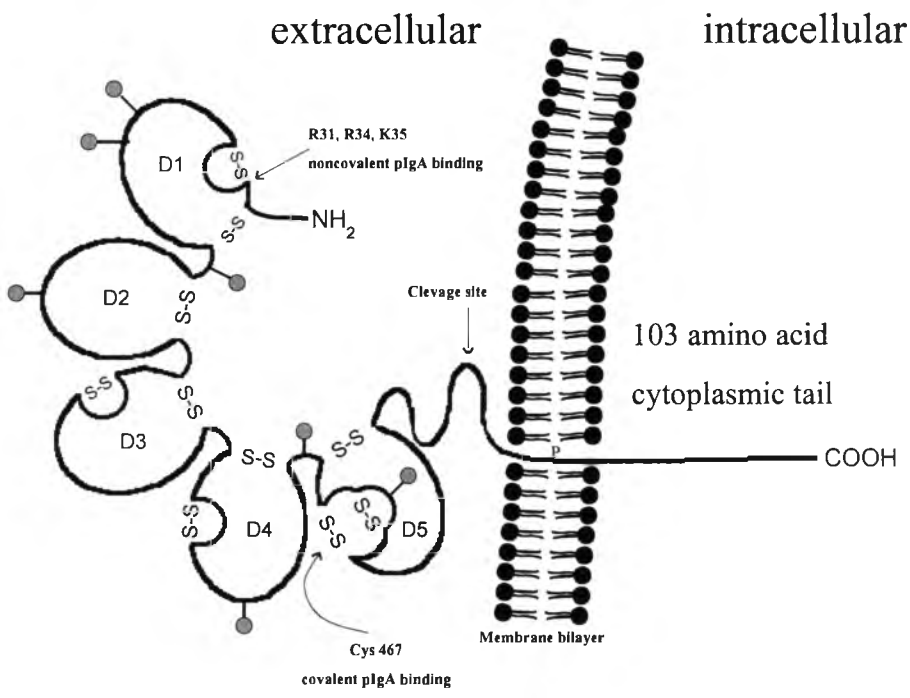

Figure 6 Schematic structure of human pIgR. The pIgR has the five extracellular domains, a single membrane-spanning segment and cytoplasmic tail. Arrows and text indicate functionally important extracellular region. The pIgR contains 20 cysteins confined to the five extracellular domains, each being stabilized with one (D2) or two (D1, D3, D4, and D5) disulfide bridges (S-S). Glycosylation sites (●—) are also depicted. The kink-inducing proline (P) in the membrane-spanning segment suggests signal-transducing capacity of the receptor.[97]