Chinese Journal of Cancer

Review

The interplay of host genetic factors and Epstein-Barr virus in the development of nasopharyngeal carcinoma

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Abstract

The interplay between host cell genetics and Epstein-Barr virus (EBV) infection contributes to the development of nasopharyngeal carcinoma (NPC). Understanding the host genetic and epigenetic alterations and the influence of EBV on cell signaling and host gene regulation will aid in understanding the molecular pathogenesis of NPC and provide useful biomarkers and targets for diagnosis and therapy. In this review, we provide an update of the oncogenes and tumor suppressor genes associated with NPC, as well as genes associated with NPC risk including those involved in carcinogen detoxification and DNA repair. We also describe the importance of host genetics that govern the human leukocyte antigen (HLA) complex and immune responses, and we describe the impact of EBV infection on host cell signaling changes and epigenetic regulation of gene expression. High-power genomic sequencing approaches are needed to elucidate the genetic basis for inherited susceptibility to NPC and to identify the genes and pathways driving its molecular pathogenesis.

Key words NPC genetics, Epstein-Barr virus, human leukocyte antigen, cell signaling, inflammation

Nasopharyngeal carcinoma (NPC) is an epithelial cancer whose etiology is associated with several factors, including infection with the ubiquitous human herpesvirus Epstein-Barr virus (EBV), host genetics, and environmental exposures. Although NPC is rare in most parts of the world, it is highly prevalent in southern Chinese populations. EBV plays an important role in the development of NPC, as most NPC tumors harbor this virus. This intimate relationship is attributed to the interaction of EBV with host cell genes for NPC development. It is now believed that EBV either selectively infects host cells with genetic alterations such as allelic loss[1] and aberrant cyclin D1 overexpression[2] or allows such cells to persist and eventually transform into cancerous cells.

Previous approaches for deciphering the molecular genetic basis for NPC included comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) studies to determine copy number gains and losses, epigenetic studies to identify critically silenced candidate genes, and functional studies to identify critical regions and candidate genes associated with NPC tumorigenesis. Expression profiling and transcriptome analysis have also identified several candidate genes and signaling pathways of interest. These studies have revealed both oncogenes (**Table 1**) and tumor suppressor genes (**Table 2**) that are important in NPC development, including genes that are involved in transcriptional regulation; cell adhesion, growth, proliferation, migration, and invasion; cell cycle and apoptosis; angiogenesis; and epithelial-mesenchymal transition and metastasis.

More recently, genome-wide association studies (GWAS) and single nucleotide polymorphism (SNP) analyses have identified candidate genes and aberrant pathways of importance in NPC. Multiple genetic regions were identified in familial and case-control studies (**Table 3**), indicating the possibility of multi-factorial risk factors and the combination of common low penetrance alleles having a role in NPC genetic risk.

Understanding the molecular pathogenesis of EBV infection and host gene aberrations will aid in our strategies for diagnostics and treatment of NPC. Human leukocyte antigen (HLA) affects host responses to EBV infection through viral antigen presentation. Inhibition of HLA expression may facilitate tumor cell evasion of the normal host immunosurveillance. Several studies support the link between the HLA complex, genetic susceptibility to NPC, and immune response to EBV (**Table 4**). Environmental exposure to carcinogens also plays a role in NPC development. Many studies have examined

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doi: 10.5732/cjc.014.10170

Table 1. Oncogenes involved in nasopharyngeal carcinoma (NPC) development

Gene name	Tumor-associated functions
AKT (v-akt murine thymoma viral oncogene homolog 1)	Induces metastasis[60]
BCAT1 (branched chain amino acid transaminase 1, cytosolic)	Induces cell proliferation, migration, and invasion[61]
BCL2 (B-cell CLL/lymphoma 2)	Inhibits apoptosis[62–64]
CCND1 (cyclin D1)	Promotes cell cycle G_1-S transition through regulation of pRb[65–67]
DeltaNp63/TP73L [tumor protein p73-like, p63 splicing variants lacking NH(2)-terminal transactivating domain]	Regulates Notch signaling, cell proliferation, and cell death[68–70]
EGFR (epidermal growth factor receptor)	Regulates cell signaling[71–75]
EIF4E (eukaryotic translation initiation factor 4E)	Promotes cell cycle progression by up-regulation of c-Myc and MMP9[76]
EVI1 (ecotropic viral integration site 1)	Regulates chromatin remodeling[77]
FGF3/Int-2 (fibroblast growth factor 3)	Promotes cell growth and tumor growth and invasion[78]
ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homologue 2)	Controls cell proliferation and angiogenesis[74,79]
HRAS (Harvey rat sarcoma viral oncogene homolog)	Induces cell cycle progression, regulates cell motility, and plays a role in cell signaling[72,80,81]
ID1 (inhibitor of DNA binding 1; dominant negative helix-loop-helix protein)	Regulates cell growth and senescence[82]
IL8 (interleukin-8)	Promotes metastasis through activation of epithelial-mesenchymal transition and Akt[83]
MACC1 (metastasis-associated in colon cancer 1)	Induces cell proliferation, migration, invasion, and colony formation[84]
MDM2 (MDM2 oncogene, E3 ubiquitin protein ligase)	Interacts with p53, to regulate its ability to control cell cycle and apoptosis[85,86]
MET (Met proto-oncogene)	Regulates cell proliferation and is involved in cancer signaling pathways[87,88]
MYC (v-myc avian myelocytomatosis viral oncogene homologue)	Regulates transcription of *BMI1*; induces cell proliferation, apoptosis, cell cycle progression; increases the radiotolerance of cancer cells[64,78,80,89,90]
PIK3CA (phosphoinositide 3-kinase, catalytic, alpha polypeptide)	Activates the activities of critical downstream cell signaling partners and enhances invasion[72,91–93]
SATB1 (special AT-rich-binding protein 1)	Decreases cell proliferation and resistance to apoptosis[94]
SP1 (SP1 transcription factor)	Regulates transcription of *BMI1*[89]
TNFAIP3 (tumor necrosis factor, alpha-induced protein 3)	Inhibits apoptosis and negatively regulates inflammatory response[95]

the link between NPC risk and carcinogen metabolism (**Table 5**) or DNA repair (**Table 6**).

NPC tumors characteristically contain large numbers of infiltrating lymphocytes, and EBV-induced inflammation is often associated with STAT3 activation. Latent membrane protein 1 (LMP1) is reported to sensitize NPC cells to genotoxic drugs. Nuclear factor kappaB (NF-κB) signaling is associated with NPC tumor formation[3]. In this review, we discuss the associations of NPC with HLA and inflammation and with aberrant cell signaling. We also describe in brief next-generation sequencing (NGS) approaches to better understand the associations between host genetics and EBV infection.

HLA and Inflammation

Host immune responses are important in determining the consequence of viral infection–related cancers. EBV plays an integral role in tissue inflammation and NPC development. The tumorigenic potential of viral infections is associated with their carriage of genes associated with cell transformation and their ability to induce chronic inflammation. The HLA system plays a central role in viral antigen presentation, which is key to determine the outcome of the host immune response to this lifelong viral infection. HLA genes are believed to play a role in NPC development because they have a functional impact on the innate and adaptive immune responses against the viral etiologic agent, EBV. NPC cells expressing specific EBV proteins, which are processed and the antigen presented in association with HLA class I alleles, may be recognized by EBV-specific $CD8^+$ cytotoxic T cells. Some evidence supports the hypothesis that EBV may down-regulate the expression of HLA alleles and result in immune escape of NPC cancer cells by decreasing the recognition of EBV-expressing cancer cells[4-6].

The genetic association of the major histocompatibility complex (MHC) region, in which HLA resides, is validated by a catalog of GWAS studies with numerous diseases and conditions including

Table 2. Tumor suppressor genes involved in NPC development

Gene name	Tumor-associated functions
ADAMTS9 (A disintegrin and metalloproteinase with thrombospondin motifs 9)	Inhibits angiogenesis by reduction of MMP9 and vascular endothelial growth factor A (VEGFA) expression[96,97]
ADAMTS18 (A disintegrin and metalloproteinase with thrombospondin motifs 18)	Activates diverse cell surface molecules, inhibits both anchorage-dependent and -independent growth[98]
BLU/ZMYND10 (zinc finger, MYND-type containing 10)	Involves inhibition of angiogenesis, transcription factor stress response, and tumor suppression[99–101]
BRD7 (bromodomain containing 7)	Regulates transcription and causes cell cycle arrest[102]
CDH1 (Cadherin 1, type 1, E-cadherin)	Inhibits proliferation, invasion, and metastasis[103]
CDKN2A/p16 (cyclin-dependent kinase inhibitor 2A)	Inhibits CDK4 kinase and causes cell cycle arrest[103]
CMTM3 (CKLF-like MARVEL transmembrane domain-containing 3)	Involves cellular chemokine signaling[104]
CRIP2 (cysteine-rich intestine protein 2)	Inhibits angiogenesis by transcriptional repression[105]
CRYAB (alpha B-crystallin)	Suppresses tumorigenesis and epithelial-mesenchymal transition (EMT) by associating with adherens junction[106]
DLC1/ARHGAP7 (deleted in liver cancer 1)	Involves cell cytoskeleton organization, activates GTPase, signal transduction, and cell adhesion, inhibits cell invasion[107]
DLEC1 (deleted in lung and esophageal cancer 1)	Inhibits cell growth and invasiveness[108]
DUSP6 (dual specificity phosphatase 6)	Suppresses cell proliferation, induces apoptosis, inhibits EMT by negatively regulating the activity of ERK[109]
FBLN2 (fibulin 2)	Interacts with extracellular matrix (ECM) proteins; inhibits cell proliferation, migration, and invasion; suppresses angiogenesis[110]
FBLN3 (EGF-containing fibulin-like extracellular matrix protein 1)	Suppresses migration and invasion of NPC cells and involves the regulation of Akt signaling pathways[111]
GADD45G (growth arrest and DNA damage-inducible, gamma)	Involves DNA damage response, inhibits cell growth and colony formation[112]
IGFBP-6 (insulin-like growth for binding protein 6)	Inhibits the proliferation, invasion, and metastasitic abilities; increases the apoptosis events of NPC cells; associates with the expression of EGR-1[113]
IRF8 (interferon regulatory factor 8)	Affects host defense, cell growth, differentiation, immune regulation and inhibits clonogenicity[114]
LARS (leucyl-tRNA synthetase)	Catalyzes ATP-dependent ligation of L-Leu to tRNA (leu) and is inactivated in NPC by both genetic and epigenetic mechanisms[115]
LTBP2 (latent transforming growth factor beta binding protein 2)	Reduces focal adhesion and cell migration, suppresses angiogenesis[116]
MIPOL1 (mirror-image polydactyly 1)	Arrests cell cycle transition[117]
MMP19 (matrix metallopeptidase 19)	Breaks down ECM to affect cell proliferation, migration, and adhesion[118]
PCDH10 (protocadherin 10)	Mediates cell-cell adhesion, induces apoptosis, and be involved in cell signaling[119]
PTPRG (protein tyrosine phosphatase receptor type G)	Arrests cell cycle, involves cell-ECM interactions, dephosphorylates kinases[120]
RASSF1A [ras association (RalGDS/ AF-6) domain family 1]	Involves cell cycle arrest, induces apoptosis, involves DNA repair, inhibits accumulation of cyclin D1[121]
THY1/CD90 (Thy-1 cell-surface antigen)	Involves suppression of tumor formation, cell proliferation, and invasion[122,123]
TSLC1/CADM1 (tumor suppressor in lung cancer 1/cell adhesion molecule 1)	Inhibits cell growth and induces apoptosis[124,125]
WIF1 (WNT inhibitory factor 1)	Inhibits WNT proteins, involves protein-tyrosine kinase activity[126]
ZNF382 (KRAB zinc finger protein)	Inhibits proliferation, induces apoptosis[127]

inflammatory, autoimmune, and infectious diseases; cancer; drug-induced hypersensitivity; and neuropsychiatric disease[7] . Historically, the first association of HLA alleles with NPC was reported in 1974[8]. For EBV-associated tumors such as NPC, previous candidate gene

Table 3. Familial and case-control genomic studies in NPC

Study type	Location	Details	Results
Familial	Guangzhou	20 families 382 microsatellite markers covering 22 autosomes with average marker density of 10 cM	Linkage to chromosome (chr) 4p15.1-q12 (14.21 cM)[128]
Familial	Hunan	18 families 20 microsatellite markers from chr 4p15-q12 (5), chr 3p (8), chr 9p (7)	Linkage to chr 3p21.3-21.2 (13.6 cM)[129]
Familial	Guangzhou	15 families 800 microsatellite markers covering 22 autosomes with average marker density of 5 cM	Linkage to chr 5p13 (17 cM)[39]
Case-control	Guangxi	350 NPC cases, 634 controls Study of chr 4p15.1-q12 region with 34 microsatellite markers	chr 4p not confirmed[130]
Case-control	Malaysia	111 NPC cases, 260 controls Genome-wide association study (GWAS) with 500,000 tag single nucleotide polymorphisms (SNPs)	Identified SNP in intron 3 of *ITGA9* (integrin, α9) on 3p21 (40 kb)[15]
Case-control	Taiwan	277 NPC cases, 285 controls GWAS with 480,000 SNPs; biological role for GABBR1 in NPC	Linkage to HLA region at 6p21.3 in *HLA-A & F/GABBR1* (GABA receptor 1) genes[14]
Case-control	Guangzhou, Guangxi	1,583 NPC cases, 1,894 controls GWAS with 464,000 autosomal SNPs	Linkage to 13q12 (*TNFRSF19*, TNF receptor superfamily 19), 3q26 (*MDS1-EVI1*), 9p21 (*CDKN2A-CDKN2B*) and reconfirm HLA on chr 6[13]
Case-control	Hong Kong	360 NPC cases, 360 controls MassArray Sequenom SNP study with 233 SNPs confined to 6p	*GABBR1, HLA-A*, and HCG9 were highly associated with NPC in single-marker association studies; microdeletions in *GABBR1* and *NEDD9* (neural precursor cell expressed developmentally down-regulated 9) were detected[131]

studies have shown a strong linkage between HLA associations and NPC risk[8-12]. These findings have been verified in more recent, large-scale GWAS in different populations[13-15]. Linkage analysis and several recent SNP studies indicate a strong association between HLA-associated genes and NPC risk[16-19]. The association of the HLA class I genes and risk of NPC development was reported in different ethnic Chinese populations including Singaporean Chinese[12,20], Taiwanese[11,16,21,22], and southern Chinese[9,23]. The HLA class I alleles HLA-A2 and HLA-B46 are most consistently associated with increased susceptibility for NPC in high-risk regions and among the Chinese population. On the other hand, HLA alleles HLA-A11, HLA-B13, HLA-B27, and HLA-A31 confer protective effects for NPC risk in high-risk areas. More details and comprehensive information regarding HLA associations with NPC are provided in several well-written reviews on NPC genetic predisposition[24-26]. However, the distribution of frequencies of HLA alleles or haplotypes varies among high-/intermediate-/low-risk regions and resulted in different, sometimes inconsistent, and even opposite associated alleles in different geographic areas. Such an example is HLA-A2, which is associated with lower NPC risk in low incidence areas[27,28]. This phenomenon may be due to an alternative hypothesis that HLA may only be a genetic marker, which is in close linkage with another NPC predisposition locus. The HLA region is highly polymorphic with enormous sequence diversity and gene density, and the genes are in extensive linkage disequilibrium. Thus, this complexity hinders the hunt for causal variants for NPC development. Standard molecular tools such as polymerase chain reaction–based methods for genotyping, including MassArray Sequenom, Taqman assay, and the GoldenGate Assay, have limited capacity, and probe designs are restricted by this difficulty for the HLA region. As most studies focus on 4-digit coding variation, the role of some functional non-coding variants within the HLA region may not have been addressed. There is a need for studying the role of non-coding variants of HLA and non-HLA genes and for identifying novel rare and common variants in NPC genetic susceptibility by applying deep resequencing approaches with the technological advances in NGS. To elucidate functional mechanisms and disease pathogenesis, it is crucial to identify the causal variants associated with NPC. HLA undoubtedly plays a substantial role in genetic predisposition for NPC development. Findings from previous studies that examined the specific EBV epitopes from LMP1 and LMP2 recognized by T cells in association with specific HLA alleles support the hypothesis that HLA alleles associated with NPC risk affect the processing and antigen presentation[6,29-31]. Specific HLA class I alleles and amino acid variants (HLA-A*11:01 allele, HLA-B*13:01, and B*55:02) in the HLA class I peptide-binding groove were observed to confer higher NPC

Table 4. Genetic risk for NPC: HLA and immune responses to Epstein-Barr virus (EBV)

Gene/function	Study
Human leukocyte antigen (HLA)	Linkage analysis studies in Hong Kong and Singapore show *HLA* association with NPC[18] *HLA-A2*-B46 haplotype is associated with NPC in Taiwan[16] RFLP study in Tunisia shows *HLA-G* (facilitates escape from cancer immunosurveillance); Ile 110 allele is less frequent among patients with lymph node involvement and more severe tumor stage, and deletion of C in codon 130 was associated with decreased NPC-free disease and survival[132]
T-cell receptor (*TCR*) and Toll-like receptor (*TLR*) in EBV infection and immune response	PCR-RFLP analysis of *TCR* gene in Singaporean study shows *TCR* polymorphism is associated with decreased NPC risk, particularly in patients with HLA B46[133] PCR and direct sequencing of *TLR* polymorphism in Guangzhou study shows *TLR3* polymorphism is related to NPC susceptibility but the effect is modest[134] TLR4 SNP may modulate immune response to EBV and predispose to NPC[135]
Host cell immune response to EBV	Microarray profiling of tumor and normal. EBV latent genes were confirmed to strongly associate with suppression of MHC class I HLA gene[4] *DC-SIGN* promoter SNP analysis in a Cantonese population shows two SNPs on *DC-SIGN* promoter are associated with high risk for NPC[136] *CTLA-4* polymorphism analysis in a Hangzhou study shows that *CTLA-4* SNP is highly associated with NPC susceptibility[137] *TNFα* and *HSP70-2* polymorphisms in a Tunisian study show the *HSP70-2* genotype is associated with increased NPC risk[138] PCR-RFLP analysis in a Taiwan study shows that $p21^{WAF/CIP1}$ and *TNFα* polymorphisms have no association with NPC; comparison between smokers and non-smokers shows the association of environmental factor with the p21 in NPC[139] *TNFα* polymorphism in Portuguese study shows NPC risk increased in undifferentiated NPC[140]
Miscellaneous	Genotyping in a Guangzhou study shows an association of EBV-positive serology and genetic factors represented by tag SNPs in 35 genes in homologous recombination repair involved in DNA repair among healthy individuals[141]

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

risk[10]. However, additional studies are needed to clarify how the HLA class I antigen recognition groove and the EBV epitopes interact. HLA class I alleles possess another function related to innate immunity which modulates the natural killer (NK) cells that kill cancer cells. HLA class I molecules induce the activation of killer-immunoglobulin-like receptors (KIRs), which in turn inhibit the function of NK cells. NPC risk is higher for EBV-seropositive individuals carrying at least five activating KIRs compared with those without activating KIRs[32]. By revealing the detailed insights into the HLA functional variants for NPC, potential novel therapeutic options will be developed.

EBV Latency Genes Induce Host Cell Signaling Changes

EBV usually undergoes different types of latency in various cell types. After EBV infection in the epithelial cell, EBV usually enters type II latency and expresses a specific panel of latent proteins including the LMP1, LMP2, and EBNA1. Although EBV infection is frequently observed in NPC, the contribution of these oncogenic latent proteins to the pathogenesis of NPC is not as clear as that in the B-cell transformation.

Increasing evidence supports the involvement of LMP1 in altering host oncogenic signaling including the NF-κB, Akt, JNK1/c-jun, and MEK/ERK-MAPK pathways in different cancers such as NPC. The C-terminal activating region 2 (CTAR2) of LMP1 is important for activation of the NF-κB signaling via the host cell proteins TRAF6 and TAK1[33,34]. LMP1 induces NF-κB–associated transcriptional activation to drive the expression of *TNFAIP2* and, thus, promotes migration[35]. On the other hand, LMP1 also showed inhibitory effects on tumor suppressive signaling pathways, including the LKB1-AMPK pathway. The LMP1 CTAR1 can activate the MEK/ERK-MAPK signaling pathway, resulting in phosphorylation of LKB1 and subsequent suppression of AMPK activity[36]. LMP1 was also found to induce the epithelial-mesenchymal transition (EMT) via the regulation of Twist, a master transcriptional regulator in embryogenesis and metastasis, through the NF-κB signaling pathway. The induction of EMT by Twist contributes to increased cell motility and invasiveness and, thus, resulted in more metastatic characteristics in NPC[37]. Angiogenesis is another important event for cancer development, and EBV also plays a role in regulating this process. LMP1 was reported to induce the expression of the principal pro-angiogenic factor vascular endothelial growth factor (VEGF) via the activation of JAK/STAT and MAPK/ERK signaling pathways[38]. In addition to LMP1, the EBV latent protein EBNA1 can increase the activity of the AP1 transcription factor and

Table 5. Genetic risk for NPC: carcinogen metabolism

Enzyme(s)	Function	Study	Results
CYP2E1	Carcinogen metabolism	50 NPC cases and 50 controls & 364 NPC cases and 320 controls in Taiwan were analyzed by PCR-RFLP	Increased NPC risk for homozygous variant genotype[142,143]
	Carcinogen metabolism	2,499 subjects from 546 NPC families were genotyped 547 NPC cases and 755 controls in Guangzhou	Association of SNP and increased NPC risk for individuals <46 years and with smoking history[144]
CYP2A6	Carcinogen metabolism	74 NPC cases and 137 controls in Thailand were analyzed by PCR-RFLP	5-fold increase in NPC risk with mutant allele[145]
CYP2A13	Carcinogen metabolism	The *CYP2A13* gen from 45 NPC patients in Guangzhou were PCR-amplified and sequenced	Identified novel SNPs, but no correlation between SNPs and NPC risk[146]
CYPE2B6, CYPE2E1, PRKDC, PCNA, CHEK2, NQ01	DNA repair, nitrosamine metabolism	31 NPC cases and 10 controls in Taiwan compared in a microarray targeting biological pathways for carcinogen metabolism, DNA repair, and chromosomal regions of interest	Differential expression in genes for DNA repair, nitrosamine metabolism, chromosomes 4p15-4q12 and 14q32[147]
CYP2E1, GSTP1, NQO1, MPO	Carcinogen metabolism	358 NPC cases and 629 controls in Guangzhou and Guangxi studied with Taqman genotyping and Tag SNPs	No significant difference between cases and controls[148]
GSTM1 (glutathione S-transferase M1)	Carcinogen metabolism	83 NPC cases and 114 controls in the US	No association with NPC risk, but absence of GSTM1 is associated with moderately increased NPC risk[149]
GSTM1, GSTT1 (glutathione S-transferase theta-1)	Carcinogen metabolism	350 NPC cases and 622 controls in Beijing studied with multiplex PCR	No significant association with NPC risk, but males with double null genotype had increased NPC risk[150]
GSTM1, GSTT1	Carcinogen metabolism	Meta-analysis of 85 published papers and selected 8 case-control studies of NPC	GSTM1 deletion is a risk factor for NPC; no association of GSTT1 with NPC risk[151]

Abbreviation as in **Table 4**.

induce AP1-mediated up-regulation of IL8, VEGF, and hypoxia-inducible factor-1α to enhance angiogenesis *in vitro*[39].

Although LMP2A plays a well-known, essential role in maintaining EBV latency in B cells, its role in epithelial cells is not well understood. Unlike *LMP1*, *LMP2A* is not normally regarded as a viral transforming gene and its expression is more consistently observed in NPC than LMP1. Indeed, the association of LMP2 with various oncogenic cell signaling pathways has been reported, suggesting that LMP2A may also participate in EBV-induced epithelial cell transformation[40]. For example, LMP2A can increase cell migration via the Akt signaling pathway by phosphorylating an inhibitory site on GSK3β, a Wnt signaling modulator downstream of Akt[41]. Indeed, LMP2A can activate the Akt signaling pathway in a PI3K-dependent manner and result in a PI3K-dependent nuclear translocation of β-catenin in the human foreskin keratinocytes[42].

EBV Impacts Stem Cell Signaling Pathways

Accumulating evidence indicates that understanding of cancer stem cell (CSC) behaviors holds great promise for the treatment of human cancers. However, CSC studies in NPC have been greatly hampered by the lack of suitable markers for investigation. Out of very few reports of the NPC CSC studies, the $CD44^{+}SOX2^{+}$ minor population was found to have stem-like properties in EBV-positive C666 NPC cells[43]. EBV seems to play an important role in regulating the stem-like characteristics, as the expression of the EBV latent proteins was associated with the activation of the Hedgehog (HH) signaling pathway and the expression of stemness-related markers and genes[44].

One important hallmark for both cancer and stem cells is their self-renewal capability. As yet, it is not fully understood how cells regulate their own proliferation and differentiation into various tissues and cells. Many signaling pathways have been linked to stem cell behaviors or self-renewal abilities. Among them, Wnt, HH, and Notch are prominent signaling pathways reported over the past decade. Evidence gathered to date indicates these pathways may have regulatory impacts on stem cell growth and differentiation. We recently found that Wnt signaling regulates self-renewal networks, cyclin D1 expression, p53 pathway, generation of stem-like cells, and growth abilities of NPC cells[45]. Because EBV has been regarded as a major cause of NPC, it is reasonable to speculate that EBV may directly affect both p53 and Wnt signaling during the development of NPC[46,47].

Table 6. Genetic risk for NPC: DNA repair

Enzyme(s)	Function	Study	Results
XRCC1 (X-ray repair complementing defective repair in Chinese hamster cells 1), hOGG1 (human 8-oxoG DNA glycosylase) (CYP2E1)	DNA repair	334 NPC cases and 283 controls in Taiwan were studied with PCR-RFLP	Increased odds ratio (OR) with multiple putative high-risk genotypes. Carriers with 1 putative high-risk genotype had OR=3; with 2, OR=4.3; and with 3, OR=25.[20]
XRCC1 XPD (xeroderma pigmentosum group D or ERCC2)	DNA repair	462 NPC cases and 511 controls in Guangzhou were studied with PCR-RFLP	*XRCC1* variant genotype associated with decreased NPC risk, especially among males and smokers[152]
		153 NPC cases and 168 controls in Sichuan were studied with PCR-RFLP	Increased NPC risk with *XRCC1* and borderline decrease in NPC risk with *XPD*; if both alleles are involved, then increase in NPC risk. No association with XRCC3[153]
ERCC1 (excision repair cross-complementing rodent repair deficiency, complementation group overlapping antisense sequence)	DNA repair	267 NPC cases and 304 controls in Sichuan were studied with PCR-RFLP	*ERCC1* polymorphism associated with NPC risk[154]
		ERCC1 genotyping in 42 patients with NPC in Hong Kong treated with gemcitabine and oxaliplatin	No associations between survival or response rate and *ERCC1* genotype[155]
RAD51L1 (RAD51 paralog B), BRCA2 (breast cancer 2, early onset), TP53BP1 (tumor protein p53-binding protein 1)	DNA repair	Discovery stage: 755 NPC cases and 755 controls in Guangzhou were studied by GoldenGate genotyping platform to investigate 676 tagging SNPs for 88 DNA repair genes. Validation stage: 1,568 NPC cases and 1,297 controls were analyzed by Sequenom DNA MassARRAY to validate 11 SNPs	Individuals with inherited defects in DNA repair genes have increased NPC risk; *RAD51L1* was the only gene validated[156]
N4BP2 (Nedd4-binding protein 2)	DNA repair	531 NPC cases and 480 controls in Guangzhou studied with PCR sequencing	Identified 3 novel SNPs associated with *N4BP2* in NPC susceptibility 4p15 locus; two haplotypes were associated with NPC[157]

Abbreviation as in **Table 4**.

EBV Induces Epigenetic Changes in NPC

Epigenetic modifications, including histone modification and promoter hypermethylation, are critical for NPC tumor development. The LMP1 protein was reported to induce up-regulation of DNA methyltransferase (*DNMT1*) expression[48]. *DNMT1* is mainly responsible for the maintenance of DNA methylation[49]. *DNMT1* expression was indeed induced by the LMP1 CTAR2 YYD domain via the JNK/AP1 signaling pathway. Thereafter, activated *DNMT1* expression resulted in promoter hypermethylation of the key epithelial marker E-cadherin[48]. On the other hand, EBV is also associated with histone modification in NPC. *LMP1* expression positively correlated with the degree of phosphorylation of the serine 10 residue in histone H3 (p-H3Ser10). As a result, this histone modification is associated with increased cell proliferation, foci formation, and AP1 activation in NPC[50]. In contrast, knockdown of histone H3 or overexpression of a dominant-negative mutant (*H3S10A*) reversed the above-mentioned phenotypes. This suggests an important role of EBV in regulating epigenetic changes in cancer-related genes of the host cells in NPC and highlights important interactions of host and viral genes.

NGS Approaches to Elucidating the Molecular Genetics of NPC

GWAS studies using SNP arrays show the association of *HLA* subtypes with NPC susceptibility and identified several additional susceptibility loci[10,13-15]. However, these studies are often limited by the loci on the array, with most being noncoding or far away from genes. Hence, these loci are not immediately informative and are difficult to be studied experimentally. Moreover, GWAS studies often target common variants and miss the rare ones that might underlie cancer genetics[51]. Therefore, additional GWAS are needed to understand the genetic basis of NPC. Recent advances in NGS approaches have allowed NPC researchers to systematically sequence expressed genes ("transcriptome"), known exons ("exomes"), and complete genomes in NPC, as well as the EBV genome, to decipher the regulatory network between EBV and host[52]. Recently, Szeto *et al*.[53] used RNASeq to characterize the sequence variants and the mRNA-microRNA regulatory network

in NPC cancer cell lines. Liu *et al*.[54] applied the NGS system to assemble the EBV genome using samples from patients with NPC in Guangdong province. An accumulation of such studies would help to elucidate NPC pathogenesis.

The tremendous data generated from NGS approaches provides a statistical and computational challenge. Although bioinformatics tools for computational analysis have developed rapidly[55-57], the choice of analysis is not straightforward and depends on the specific aims and study design. Thus, data analysis and interpretation remain the bottleneck of cancer genomic studies due to the complexities of NGS data and the cancer genome. Another challenge in NPC genomic study is significant heterogeneity and lymphocyte infiltration in the specimens, which might mask the true biological events in tumor cells[58]. To minimize false-negative errors, the International Cancer Genome Consortium (ICGC) guidelines suggest that the tumor cell content of a sample be at least 60%–80%. Meanwhile, increasing redundant coverage in sequencing can help compensate for low tumor purity[57]. It is also a great challenge for bioinformaticians to develop tools that allow sensitive detection of genomic changes in impure and heterogeneous cancer samples[59]. To minimize false-positives, verification resequencing is necessary to estimate the technology-relevant errors, and it is important for studies to report a verification rate. As proposed by ICGC, at least 95% of the mutations identified in each sample should be real.

To make biological sense of the findings from NGS data will require computational, biological, and clinical analyses to link the biological pathways and the functional relevance of the molecular alterations to NPC, and to evaluate the associations of genomic changes with NPC diagnosis, prognosis, and treatment strategies. To tackle the fundamental basis for NPC, basic scientists in the area of bioinformatics, EBV, and NPC must collaborate with oncologists who can provide clinical information and translate our findings to the clinic. Furthermore, establishment of a NPC tissue bank is necessary to provide key resources for NGS projects. We have established a Hong Kong–wide *Center for NPC Research* (www.cnpcr.hku.hk) that aims to use genome-wide NGS approaches to elucidate the genetic basis for NPC susceptibility and to identify the genes and the biological pathways that underlie aggressive, recurrent NPC.

Conclusions

Clarifying the roles of host and EBV genetics in NPC development is expected to enhance our understanding of NPC pathogenesis and to provide improved biomarkers for detection and novel targets for therapeutic intervention. Targeted therapeutics will enhance survival of NPC patients with metastatic disease. Therefore, elucidating the interactions between EBV and host genes is expected to improved strategies for the clinical management of this deadly cancer.

Acknowledgement

This work was supported by the Research Grants Council Area of Excellence grant (AoE/M-06/08) to MLL.

Received: 2014-09-03; accepted: 2014-10-11.

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