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

Maria Li Lung, Arthur Kwok Leung Cheung, Josephine Mun Yee Ko, Hong Lok Lung, Yue Cheng and Wei Dai

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...
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[^46] |
| BCA1 (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 G1-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 (ectropic viral integration site 1) | Regulates chromatin remodeling[^77] |
| FGFR3/Int-2 (fibroblast growth factor 3) | Promotes cell growth and tumor growth and invasion[^78] |
| ERBB2 (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[^89-91] |
| PIK3CA (phosphoinositide 3-kinase, catalytic, alpha polypeptide) | Activates the activities of critical downstream cell signaling partners and enhances invasion[^72-79] |
| SATB1 (special AT-rich-binding protein 1) | Decreases cell proliferation and resistance to apoptosis[^94] |
| SP1 (SP1 transcription factor) | Regulates transcription of BMI1[^95] |
| TNFAIP3 (tumor necrosis factor, alpha-induced protein 3) | Inhibits apoptosis and negatively regulates inflammatory response[^96] |

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-kB) signaling is associated with NPC tumor formation[^95]. 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[^95].

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

[^46]: See reference [4-6] for details.
[^47]: See reference [71-75] for details.
[^48]: See reference [72,91-93] for details.
[^49]: See reference [74,79] for details.
### 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/2MYND10** (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]                                                    |
| **CD1** (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[104]                                                       |
| **CMTM3** (CKLF-like MARVEL transmembrane domain-containing 3) | Involves cellular chemokine signaling[105]                                                                 |
| **CRIp2** (cysteine-rich intestine protein 2)  | Inhibits angiogenesis by transcriptional repression[106]                                                       |
| **CRYAB** (alpha B-crystallin)                 | Suppresses tumorigenesis and epithelial-mesenchymal transition (EMT) by associating with adherens junction[107] |
| **DLC1/ARHGAP7** (deleted in liver cancer 1)   | Involves cell cytoskeleton organization, activates GTPase, signal transduction, and cell adhesion, inhibits cell invasion[108] |
| **DLEC1** (deleted in lung and esophageal cancer 1) | Inhibits cell growth and invasiveness[109]                                                                  |
| **DUSP6** (dual specificity phosphatase 6)     | Suppresses cell proliferation, induces apoptosis, inhibits EMT by negatively regulating the activity of ERK[110] |
| **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 metastatic 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)        | Arreets cell cycle transition[117]                                                                          |
| **MMP19** (matrix metalloproteinase 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) | Arreets 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
information regarding HLA associations with NPC are provided in different populations. On the other hand, HLA alleles associated with increased susceptibility for NPC in high-risk regions, such as HLA class I alleles HLA-A2 and HLA-B46, are most consistently confirmed by large-scale GWAS in different populations including Singaporean Chinese, Taiwanese, and southern Chinese. 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 associated with lower NPC risk in low-incidence areas, such as HLA-A2, are highly associated with lower NPC risk in low-incidence areas. 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.

| 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) [108]                |
| 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) [109]                             |
| Familial       | Guangzhou     | 15 families; 800 microsatellite markers covering 22 autosomes with average marker density of 5 cM   | Linkage to chr 5p13 (17 cM) [109]                                      |
| 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, 280 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) [112] |
| 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 [114] |
| Case-control   | Hong Kong     | 360 NPC cases, 360 controls; MassArray Sequenom SNP study with 233 SNPs confined to 6p            |                                      |

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 [15,23]. 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. 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...
Increasing evidence supports the involvement of LMP1 in altering host oncogenic signaling including the NF-κB, Akt, JNK1/2-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[33,36]. 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[38]. 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[39]. In addition to LMP1, the EBV latent protein EBNA1 can increase the activity of the AP1 transcription factor and

### 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.

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| Gene/function | Study |
|---------------|-------|
| Human leukocyte antigen (HLA) | Linkage analysis studies in Hong Kong and Singapore show HLA association with NPC[34]. HLA-A2-B46 haplotype is associated with NPC in Taiwan[35]. 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[35–36]. |
| 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[33]. PCR and direct sequencing of TLR polymorphism in Guangzhou study shows TLR3 polymorphism is related to NPC susceptibility but the effect is modest[34]. TLR4 SNP may modulate immune response to EBV and predispose to NPC[33,34]. |
| 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(CDKN1A) 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.
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[49].

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[40]. 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 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[42,43] |
|           |          | 2,499 subjects from 546 NPC families were genotyped | Association of SNP and increased NPC risk for individuals <46 years and with smoking history[144] |
|           |          | 547 NPC cases and 755 controls in Guangzhou |    |
| 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[45] |
| 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[46] |
| CYPE2B6, CYP2E1, 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[47] |
| CYP2E1, GSTP1, NQ01, 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[48] |
| 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[49] |
| GSTM1, GSTT1 (glutathione S-transferase theta-I) | 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[50] |
| 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[51] |

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].

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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.

### 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\(^{[152]}\). |
| 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 genotyping in 42 patients with NPC in Hong Kong treated with gemcitabine and oxaliplatin | ERCC1 polymorphism associated with NPC risk\(^{[154]}\); No associations between survival or response rate and ERCC1 genotype\(^{[156]}\). |
| 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.
in NPC cancer cell lines. Liu et al.[8] 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[56-58], the choice of analysis is not straightforward and depends on the specific aims and study design. Thus, data analysis and interpretation remain a statistical and computational challenge. Although 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.

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