Nasopharyngyeal Carcinoma, Up-to-Date Review

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The Enigma of Nasopharyngeal Carcinoma

1. Viral Cancer
2. Geographic and ethnic distribution
3. Genetic susceptibility
4. Familial cases
5. Environmental factors
Nasopharyngeal Carcinoma, Outline

- Epidemiology
- Genetic susceptibility
- Environmental factors
- Targeted therapy
- New Prognostic indicators

Etiology
• 80,000 new cases per year, 0.75% of all cancers
• 18% of all malignancies in Hong Kong
Nasopharyngeal Carcinoma
Incidence in Saudi Arabia

- Similar to countries with high incidence
- Possible genetic etiology

A. Andejani, ET Al: Saudi Med J 2004; Vol. 25
Nasopharyngeal Carcinoma

Incidence in KSA

- Advanced stage.
- Lesser incidence in Saudi female
- Higher in Teen age group

A. A. Andejani, Saudi Med J 2004; Vol. 25
Figure 1 - Incidence of nasopharyngeal carcinoma among Singapore Chinese 1978 through to 1982.

Figure 2 - Incidence of nasopharyngeal carcinoma among Chinese, Shanghai 1979 through to 1982.

Figure 3 - Incidence of nasopharyngeal carcinoma among Saudis 1994 through to 1996.

Figure 5 - Incidence of nasopharyngeal carcinoma among Canadians 1978 through to 1982.
• 50-80% present with cervical node metastases
• Random biopsy positive in 70% of cases.
1. Squamous cell carcinoma (WHO-I)

2. Non-keratinizing carcinoma
   A. Differentiated non-keratinizing ca (WHO-II)
   B. Undifferentiated carcinoma (WHO-III)

3. Basaloid squamous carcinoma
Frequency of various histological subtypes of nasopharyngeal carcinoma

<table>
<thead>
<tr>
<th></th>
<th>High incidence population</th>
<th>Intermediate incidence population</th>
<th>Low incidence population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hong Kong (Queen Elizabeth Hospital, 2001-2003)</td>
<td>Singapore {Shanmugaram et al., 1979}</td>
<td>Tunisia {Cammoun et al., 1978}</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1%</td>
<td>17%</td>
<td>8%</td>
</tr>
<tr>
<td>Nonkeratinizing carcinoma</td>
<td>99%</td>
<td>83%</td>
<td>92%</td>
</tr>
<tr>
<td>- Undifferentiated</td>
<td>(92%)</td>
<td>(42%)</td>
<td>(76%)</td>
</tr>
<tr>
<td>- Differentiated</td>
<td>(7%)</td>
<td>(41%)</td>
<td>(16%)</td>
</tr>
<tr>
<td>Basaloid-squamous carcinoma</td>
<td>&lt;0.2%</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = Not available
NPC, WHO-III  EBER
Incidence of EBV and HPV in 38 NPC*

<table>
<thead>
<tr>
<th>Type</th>
<th>EBV(%)</th>
<th>HPV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO I (N=15)</td>
<td>13</td>
<td>27**</td>
</tr>
<tr>
<td>WHO II-III (N=23)</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

** = 1 HPV-11 and 3 HPV-16
NPC - Etiology

Interaction of all three etiological factors

1. Epstein Barr Virus (EBV) infection
2. Genetic susceptibility
3. Environmental factors

Liebowitz D ..ET AL: Semin Oncol 1994 Jun;21(3):376-81
EBV is an oncogenic human gamma-herpesvirus that persistently infects more than 90% of the human population.
Nasopharyngeal Carcinoma & 

EBV

- EBV is a group I carcinogen by IARC
- Serum IgA to (VCA) and (EA) with NPC
- Persistence of EBV DNA and EBNA in NPC

1. Henle and Henle, Int J Cancer 17: 1, 1976
Epstein Barr Virus & NPC,
Diagnosis

1. Swab + PCR based EBV *LMP-1* + *EBNA*
detection = Pathological Dx of NPC

2. EBV DNA load & *BARF1* glycoprotein
mRNA in NP *brushings* = Non-invasive Dx.

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Epstein Barr Virus, Major Antigens

1. Viral capsid Ag. (VCA)
2. EBV-induced membrane antigen, (MA)
3. Six EBV-associated nuclear antigens (EBNA, 1, 2, 3a, 3b, 3c, leader protein (LP)),
4. Early antigen (EA), diffuse (D) or restricted (R) forms
B-lymphocyte

CD 21

GP350/220
Epstein–Barr virus (EBV) infection in normal healthy virus carriers

Expert Reviews in Molecular Medicine ©2001 Cambridge University Press
Epstein Barr Virus - EBNA

1. EBNA-1
   - Episomal state.
   - Not recognized by host CD8 T cells

2. EBNA-2, EBNA-3c: Transformation of B cells
Epstein Barr Virus

- **LMP-1** is an oncogene essential in cell transformation and metastasis.
- Transformation of B lymphocytes
- Transform Rat-1 fibroblasts.
Screening Nasopharyngeal Carcinoma by Detection of LMP-1 in 308 Patients

- 55 pts. EBV LMP-1 positive (48 NPC, 2 lymphomas, 5 other pathology)

- 253 pts. negative (4 NPC) = specificity 98.4%

- Of 52 pts. with NPC, 48 positive for LMP-1 = sensitivity, 87.3%

LMP-1 in Irradiated NPC Patients

LMP-1 negative in 89% of pts before completion of therapy and 11% after completion

- Median disappearance time = 4.3 weeks (range 1.3-28 weeks)
- Median re-appearance time of LMP-1 and abnormal mucosal = 11.9 weeks (range 2.7 - 27.4 weeks)

Etiology, 
*Epstein Barr Virus & NPC*

1. Direct interaction between EBNA-5 and p63 → increase the stability of p63.
2. Over-expression of p53
3. Loss expressions of p16 and p27 proteins

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NPC - Etiology

Interaction of all three etiological factors

1. Epstein Barr virus (EBV) infection
2. Environmental factors
3. Genetic susceptibility

NPC – Etiology, Environment

A. Salted fish – nitrosamines
B. Cigarette smoking
C. Formaldehyde exposure
D. Occupational exposure to wood dust
E. Alcohol
F. Crowded conditions
• Biomass smoke has been implicated as a cause of nasopharyngeal carcinoma

• South American CC study, 784 cases:
  • Exposure to wood smoke as compared with cleaner fuels
  • Oral, pharyngeal and laryngeal cancer.
  • Adjusted odds ratio of 2.68 with 95% confidence

• Bulletin of the World Health Organization, 2000, 78 (9)
Fig. 2. A traditional home in KwaZula, Natal, South Africa with an open wood fire

Bulletin of the World Health Organization, 2000, 78 (9)
Fig. 1. A rural home in the highlands of Bolivia with walls blackened by smoke from an open wood fire
Formaldehyde is a product of many natural processes.*

Released during biomass combustion, such as forest and brush fires (Howard, 1989; Reinhardt, 1991).

*www.inchem.org/.../cicads/cicads/cicad40.htm
GENETIC ETIOLOGY
(Genetic susceptibility)
(Constant Factor)

HLA OR ANY OTHER?

NPC

Dietary factors
(Smoke-dried foodstuff
in Nagaland, India)
(in place of salted fish in
China)

Inhalatory factors
(Inhalation of smoke
continuously)
or smoking or others

VIRAL ETIOLOGY
(EBV infection)
(Constant Factor)

ENVIRONMENTAL FACTORS
Nasopharyngeal Carcinoma, Genetic Susceptibility

1. HLA
2. Polymorphism of Metabolic enzymes GSTM1, Cytochrome P540 2E1 (CYP2E1)
3. Polymeric Immunoglobulin Receptor (PIGR); T. Cell Receptors
4. Chromosome 4p, 4p15.1-q12
NPC – Etiology, HLA

**Increased Risk**
- HLA –A2 (Singapore)
- HLA A2-B38
- HLA A2-B16

**Decreased Risk**
- HLA –A11
- HLA –B13
- HLA –B22

**HLA B17 (South China, Singapore and Malaysia)**
**HLA&NPC**

- Predisposing gene in close linkage with the HLA locus (linkage studies)

- HLA ~ Susceptibility ~ Prognosis ~ Survival

**Lu QL et al: Genetic susceptibility to Nasopharyngeal carcinoma within the HLA –A locus in Taiwanese. Int J cancer 103:745-751**

Nasopharyngeal Carcinoma, Genetic Susceptibility

1. HLA
2. Polymorphism of metabolic enzymes
   Cytochrome P540 2E1 (CYP2E1)
3. Polymeric Immunoglobulin Receptor (PIGR); T. Cell Receptors
4. Chromosome 4p, 4p15.1-q12
Single Nucleotide Polymorphism
Cytochrome P540 2E1 (CYP2E1)

• Catalyses nitrosamines found in NPC-assoc. food.
• Leads to intermediates, damaging to DNA

• Taiwan case control study
• Homozygous SNP (C2/C2) genotype had a 2.6 fold risk for NPC relative to those with one or two copies of the wild type allele (*)

175 NPC cases and 317 controls
Divided into Thai, Chinese and Thai-Chinese
Evaluated two candidate genes by using 4 SNPs,

1. Complement receptor 2 (CR2) \( CR2\text{IVS2-848 C} \rightarrow \text{T} \)
2. Polymeric Immunoglobulin Receptor (PIGR)
   - \( PIGR1739 \text{ C} \rightarrow \text{T} \)
   - \( PIGR\text{IVS3-156 G} \rightarrow \text{T} \),
   - \( PIGR1093 \text{ G} \rightarrow \text{A} \)

R. Hirunsatit..et al: *BMC Genetics* 2003, 4:3
Polymeric Immunoglobulin Receptor (PIGR), Results:

- Role of the nucleotide PIGR1739
  - PIGR, 1739  C→T (EXONE 7)
  - PIGR IVS3-156 G→T,
  - PIGR 1093 G→A

- Significant increased risk in ethnic group
- Adjusted O.R. (95%CI) of 2.71 (1.72–4.23) and p < 0.00001.
Missense Mutation
C → T

Alanine → Valine

Affects Endo-proteolysis cleavage of PIGR extra-cellular domain

Altered efficiency in releasing IgA-EBV complex

Nature Reviews Molecular Cell Biology 3, 944-956 (December 2002) doi:10.1038/nrm972
Nasopharyngeal Carcinoma,
Histogenesis

- Somatic genetics
  1. Cytogenetics
  2. Comparative Genomic Hybridization
  3. Molecular genetic alterations
  4. Expression profile/Protiomics
Comparative Genomic Hybridization
High frequency major deletions on: 3p, 9p, 9q, 11q, 13q, 14q, 16q

Multiple mini-deletion are at 3p14-24.2, 11q21-23, 13q12-14, 13q31-32, 14q24-32, and 16q22-23.

Deletion of 3p and 9p have been shown in early events of NPC in almost all tumors. (1,2)

Molecular Genetic Alterations

LOH on 3p, 9p, and 14q in almost all tumors suggests a **tumor suppressor genes located in these regions**

- RASSF1A is a tumor suppressor gene on **3p21.3**
- RAS dependent growth control gene
- involved in multiple cellular regulatory processes:
  1. Transcription
  2. Signal transduction
  3. Cell adhesion
  4. RNA processing
  5. DNA repair system
High Frequency of Promoter Hypermethylation of RASSF1A in Nasopharyngeal Carcinoma

Kwok-Wai Lo, Joseph Kwong, Angela Bik-Yu Hui, Sylvia Yat-Yee Chan, Ka-Fai To, Andrew Siu-Chung Chan, Lillian Shuk-Nga Chow, Peter M. L. Teo, Philip J. Johnson, Dolly Poon Huang

Departments of Anatomical and Cellular Pathology [K-W. L., J. K., A. B-Y. H., S. Y-Y. C., K-F. T., L. S-N. C., D. P. H.] and Clinical Oncology [P. M. L. T., P. J. J.], Prince of Wales Hospital, and Institute of Molecular Oncology at the Sir Y.K. Pao Centre for Cancer [K-W. L., A. S-C. C., P. M. L. T., P. J. J., D. P. H.], The Chinese University of Hong Kong, Hong Kong SAR, China
Molecular Genetic Alterations

- High frequency of promoter hypermethylation of RASSF1A
- Tumor suppressor gene on 3p21.3, in 70-80% of all cases of primary tumors

SHORT COMMUNICATION

Identification of RASSF1A modulated genes in nasopharyngeal carcinoma

LS-N Chow\textsuperscript{1,5}, C-W Lam\textsuperscript{2,5}, SY-Y Chan\textsuperscript{1}, S-W Tsao\textsuperscript{3}, K-F To\textsuperscript{1}, S-F Tong\textsuperscript{2}, W-K Hung\textsuperscript{1}, R Dammann\textsuperscript{4}, DP Huang\textsuperscript{1,*} and K-W Lo\textsuperscript{1}

\textsuperscript{1}Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR; \textsuperscript{2}Department of Chemical Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR; \textsuperscript{3}Department of Anatomy, University of Hong Kong, Hong Kong SAR and \textsuperscript{4}Institut für Humangenetik und Medizinische Biologie, Martin-Luther-Universität Halle-Wittenberg, D-06097 Halle/Saale, Germany

- RASSF1A is frequently inactivated by promoter hypermethylation in NPC.

\textsuperscript{L S-N Chow ..et al: Oncogene (2006) 25, 310–316.}
Principle of cDNA microarray assay for gene expression (after Gibson & Muse 2002)

Red = "up-regulation"
Green = "down-regulation"
Black = constitutive expression
Molecular Genetic Alterations

- High frequencies of aberrant methylation detected:
  - EDNRB (90.5%)
  - RAPB2 (80%)
  - DAP-Kinase (76%)
  - RIZ1 (60%)
  - E-CADHERIN (52%)

Nasopharyngeal Epithelium

Germline mutation (major gene)
First “hit”
EBV Infection
Second “hit”
Inherited NPC

Gene polymorphism
Minor genes
First “hit”
EBV Infection
Second “hit”

EBV Infection
Environmental carcinogens

 Majority of NPC in high prevalence areas

Sporadic NPC
EBV latent infection (expression of viral proteins)

Normal Nasopharyngeal Epithelium → Low-grade Dysplasia → High-grade Dysplasia → Invasive Carcinoma → Metastasis

- Chromosomes 3p and 9p deletions
- Inactivation of p14, 15, p16 and RASSF1A
- Gain of chromosome 12 and loss of 11q, 13q
- p53 mutation, aberrant expression of cadherins

Environmental carcinogens
NPC, Therapy

• High-dose radiotherapy with adjunctive chemotherapy is the primary treatment

• Surgery,
  ➢ For nodes that fail to regress
  ➢ Nodes that recurrent after complete response.
• EBER-1 DNA in serum to monitor chemotherapeutic response. (1)

• The plasma EBV EBER-1 DNA load is proportionately related to the presence of NPC(2)

Molecular Targeted Therapy, siRNA technique

1. Transient transected bcl-xL
2. Epidermal growth factor receptor
3. Survivin, resisting apoptosis
4. EBV-encoded LMP-1

Targeted, Immunotherapy

- Boosting LMP2-specific CTL response

EBV, Vaccines

1. Major virus surface glycoprotein GP 220/350
   - MedImmune & GlaxoSmithKline (GSK)
   - Safe in humans but needs strong adjuvant
   - Clinical trials up to Phase 3
1. **Live recombinant vaccinia vectors** to express the gp220/350, protection in primates and in EBV-negative Chinese infants

2. **Clinical trials of an EBNA-3A peptide** conducted in Australia
NPC – Signs of Poor Prognosis

1. Male gender
2. Over 40 yrs of age at diagnosis
3. Advanced clinical stage (positive supraclavicular lymph nodes, 6 cm. or more, distant mets, etc.)
4. Cranial nerve involvement.
Actuarial Survival (%)

Stage I (n=63)
Stage II (n=237)
Stage III (n=461)
Stage IV (n=142)

$p = 0.0001$
Prognosis and Molecular Markers, Plasma EBV-DNA Levels & Risk of Death

- 139 patients NPC, uniform XRT
- Followed up 5.55 years
- Cox regression analysis
- Higher death relative risk of 1.6 for each 10-fold increase in serum EBV-DNA

Quantitative EBV DNA has adequate sensitivity and specificity to use as a screening test in areas where NPC is endemic

<table>
<thead>
<tr>
<th>EBV infection + genetic susceptibility = NPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental factors is more effective in genetically susceptible population</td>
</tr>
<tr>
<td>NPC incidence in KSA is similar to countries with moderately higher incidence</td>
</tr>
<tr>
<td><strong>Non-invasive NPC Dx:</strong> EBV DNA load + <em>BARF1</em> glycoprotein mRNA on NP brushing</td>
</tr>
<tr>
<td><em>LMP-1</em> is an oncogene playing an essential role in cell transformation and metastasis.</td>
</tr>
<tr>
<td>• HLA ~ Susceptibility ~ Prognosis ~ Survival</td>
</tr>
</tbody>
</table>
Single Nucleotide Polymorphism of Metabolic Enzymes: Cytochrome P540 2E1 & (PIGR)

LOH on 3p, 9p, and 14q in almost all tumors suggests a tumor suppressor gene in these regions.

Promoter hypermethylation of RASSF1A, a tumor suppressor gene on 3p21.3, in 70-80% of all cases of primary tumors.

Gene polymorphism
Major & Minor genes
First hit

EBV Infection
second hit

Majority of NPC in high Prevalence areas
Molecular, Targeted Therapy, siRNA technique
bcl-xL, EPGFR, Survivin, LMP-1

Targeted, Immunotherapy by boosting LMP2-specific CTL response

EBV, Vaccines against major virus surface glycoprotein gp220/350

Quantitative Plasma EBV-DNA Levels, to monitor response to XRT, Chemo, recurrence & death
Thank you
poor outcome in patients with nasopharyngeal carcinoma.
### Incidence of EBER-1 in 140 NPC*

<table>
<thead>
<tr>
<th>Type</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO I (N=5)</td>
<td>80</td>
</tr>
<tr>
<td>WHO II (N=73)</td>
<td>97.3</td>
</tr>
<tr>
<td>WHO III (N=62)</td>
<td>96.8</td>
</tr>
</tbody>
</table>

Tsai S-T, et al, Cancer 77:231, 1996
Expression of HER2 and C-KIT in nasopharyngeal carcinoma: implications for a new therapeutic approach.
Receptor on NP epithelium (PIGR) may have a single nucleotide polymorphism mutation in Chinese and Thai-Chinese.
Compliment receptor structure
Detection of KIAA1173 gene expression in nasopharyngeal carcinoma tissues and cell lines on tissue microarray

- NPC-associated tumor suppressor genes residing in chromosome 3p21-22
- KIAA1173 gene, locates at 3p22.1, a new carcinoma-related gene,
- 73 nasopharyngeal tissue samples (including 41 specimens of NPC, 18 atypical hyperplasia epithelia, and 14 normal nasopharyngeal mucosa epithelia) and 6 NPC cell lines using tissue microarray technique by in situ hybridization (ISH).
Detection of KIAA1173 gene expression in nasopharyngeal carcinoma tissues and cell lines on tissue microarray

- RESULTS: The positive rates of KIAA1173 mRNA were 21.9% (9/41) in NPC, 83.3% (15/18) in atypical hyperplasia epithelia, 92.8% (13/14) in normal nasopharyngeal mucosa epithelia, and 0 in all NPC cell lines.
- Its strongly positive rate was significantly lower in NPC than in atypical hyperplasia epithelia and normal mucosa epithelia (0 vs. 38.9% and 64.3%, P < 0.001).
- In 38 specimens of NPC with infiltrated lymphocytes, the positive rate of KIAA1173 mRNA was significantly lower in cancer cells than in tumor infiltrating lymphocytes (23.7% vs. 44.7%, P < 0.05);
- CONCLUSIONS: KIAA1173 gene is strongly expressed in normal nasopharyngeal mucosa epithelia, but down-regulated in NPC. It may be associated with the tumorigenesis of NPC.
Review of data on NPC suggested that EBV infection and genetic susceptibility are the constant etiological factors responsible for the higher incidence of NPC among various ethnic groups while other factors such as ingestants and inhalants may depend on the distinct dietary practices and living environment adopted by various ethnic groups in different geographical region of the world.
Nasopharyngeal carcinoma: molecular biomarker discovery and progress
William Chi-shing Cho*

*Molecular Cancer 2007, 6:1

Table 1: Biomarkers identified by proteomics technologies in nasopharyngeal carcinoma

<table>
<thead>
<tr>
<th>Technology</th>
<th>Primary use</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry</td>
<td>Diagnosis</td>
<td>Fibronectin, Mac-2 binding protein, Plasminogen activator inhibitor 1</td>
</tr>
<tr>
<td></td>
<td>Signaling target</td>
<td>Annexin A2, Heat shock protein 27, Stathmin, Annexin I, Basic transcription factor 3, Porin</td>
</tr>
<tr>
<td></td>
<td>Treatment response monitoring</td>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td></td>
<td>Diagnosis</td>
<td>Inter-α-trypsin inhibitor precursor</td>
</tr>
<tr>
<td>Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and tandem mass spectrometry</td>
<td>Treatment response monitoring</td>
<td>Platelet factor-4</td>
</tr>
<tr>
<td></td>
<td>Prognosis</td>
<td>Serum amyloid A</td>
</tr>
</tbody>
</table>
Epstein Barr Virus

- EBV genome is present in almost all NPC tissues
- Ideal tumor marker for NPC.

EBER
NPC & EBV

• Combination of salted fish and EBV was strongly associated with NPC, compared to EBV or salted fish alone.

• IgA-VCA was the most important predictor of NPC, followed by fish. (Zheng et al 60)
NPC – Treatment and Prognosis

• Radiation
• May take up to 10 weeks for tumor to disappear histologically
• If post-treatment biopsy is still positive after 10 weeks, additional treatment needed
• 20-60% mets below clavicles (lungs, liver and bones)
• Over 90% of local and distant failures appear within 3 years of treatment
Multiple dysregulated pathways in nasopharyngeal carcinoma revealed by gene expression profiling.

- Pathway analyses by microarrays revealed that upregulation of NF-κB2 and survivin played central roles in increasing resistance to apoptosis, as well as changes in integrin and Wnt/β-catenin signaling leading to uncontrolled proliferation.
- The role of survivin in resisting apoptosis in NPC was confirmed by RNAi, which suggested survivin as a novel therapeutic target for NPC.
Compared with the RASSF1A transfectants, an inverse expression pattern of activin bE, Id2 and ATF5 was shown in the immortalized nasopharyngeal epithelial cells treated with siRNA against RASSF1A.
EBV latent infection (expression of viral proteins)

Normal Nasopharyngeal Epithelium → Low-grade Dysplasia → High-grade Dysplasia → Invasive Carcinoma → Metastasis

- Chromosomes 3p and 9p deletions
- Inactivation of p14, 15, p16 and RASSF1A
- Gain of chromosome 12 and loss of 11q, 13q
- p53 mutation, aberrant expression of cadherins

Environmental carcinogens
Molecular, Targeted Therapy

- The transient transfected bcl-xL siRNA4 could effectively inhibit the growth of the cancer cells and induce their apoptosis suggesting that the siRNA technique could provide a new method for anti-NPC gene therapy.

Zhonghua Er Bi ; Yan Hou Tou Jing Wai Ke Za Zhi. 2005 May;40(5):347-51.
EBV-encoded LMP-1 & RNAi

- EBV-encoded \( LMP-1 \) was vulnerable to RNAi and selective inhibition of \( LMP-1 \) had anti-proliferation effect on NPC cell.
- RNAi could be a powerful tool in further investigations of \( LMP-1 \)
- A novel therapeutic strategy for associated NPC patients
Epidermal growth factor receptor silencing by RNAi could reduce the proliferation of NPC cells and induce cell cycle arrest at G1 phase, which shed light on the possible use of RNAi for further investigation of the pathogenesis and gene therapy of NPC.
Induction of c-Met proto-oncogene by Epstein-Barr virus latent membrane protein-1 and the correlation with cervical lymph node metastasis of nasopharyngeal carcinoma.

- close association of c-Met expression with cervical lymph node metastasis (P = 0.0272) in 39 NPC specimens studied immunohistochemically
- Epstein-Barr virus-encoding latent membrane protein-1 (LMP-1) is a primary oncogene and is suggested to enhance the metastatic property of NPC.
HLA & NPC

• HLA class 1 restricted cytotoxic T-Lymphocytes (CTL) play a major role in controlling EBV infection.
• LMP2-specific CTL can be detected in NPC.
• Treat by boosting LMP-2 specific CTL response.

A model for Epstein–Barr virus (EBV) infection and persistence

Expert Reviews in Molecular Medicine ©2004 Cambridge University Press
2- Polymorphism of Metabolic Enzymes GSTM1

- Glutathione S-Transferase M1
- Detoxifies benzopyrene and other carcinogens in tobacco smoke

A

PCR (specific primers) → restriction (specific enzyme) → DNA analysis

B

GeneChip® Mapping Assay Overview:

- Genomic DNA (250 ng)
- RE Digestion
- Adapter Ligation
- PCR: One Primer Amplification
- Complexity Reduction
- Fragmentation and End-Labeling
- Hybridization & Wash
- AA BB AB
Evidence linkage *Chromosome studies*: 
NPC predisposing gene to chromosome region 4p15.1-q12

- Whole genome scan for linking NPC on a 32 high risk NPC Cantonese pedigrees
- The marker D4S405 on chromosome 4p12-p15 yielded a maximum multipoint load score (MMLS) of 3.06,
- Disease susceptibility gene may be linked with D4S405 marker
- Fine mapping analysis has localized the NPC predisposing gene to chromosome region 4p15.1-q12

---

NPC, Cytogenetics

1. Rearrangements and deletions on chromosome 3
2. Common regions of loss include: 3p12-p21, 11q14-qter;
3. Common regions of gain: 7p15-p14, 7q11.2-q2, 8q21.1-q22, 12q22-q24.1 and 20q
Normal Nasopharyngeal Epithelium

Low Grade Dysplasia

- 3p & 9p deletions

Inactivation of p14, 15, p16 & RASSF1A

High Grade Dysplasia

- Gain on Chrom. 12, loss of 11q, 13q

Invasive Carcinoma

- P53 & Aberrant exp of Cadherin

Metastasis

NPC – Signs of Poor Prognosis

- Keratinizing histology
- Absence Of EBV
- Elevated pre-treatment CD-23
- IL-10, 8 positive tumors on IPEX
- Serum EBV DNA level
1. 96% of NPC patients and 7% of controls.
2. Advanced-stage pts → higher EBV-DNA
3. Monitoring response during radiotherapy and chemotherapy
4. Early detection of tumor recurrence

1. Lo YMD, Cancer Res 1999; 59: 1188–1191