19th Scientific Meeting of the International Academy of Pathology, Arab Division, Dec. 7-9, 2007, Aleppo- Syria

# Nasopharyngeal Carcinoma, Up-to-Date Review

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# *The Enigma of Nasopharyngeal Carcinoma*

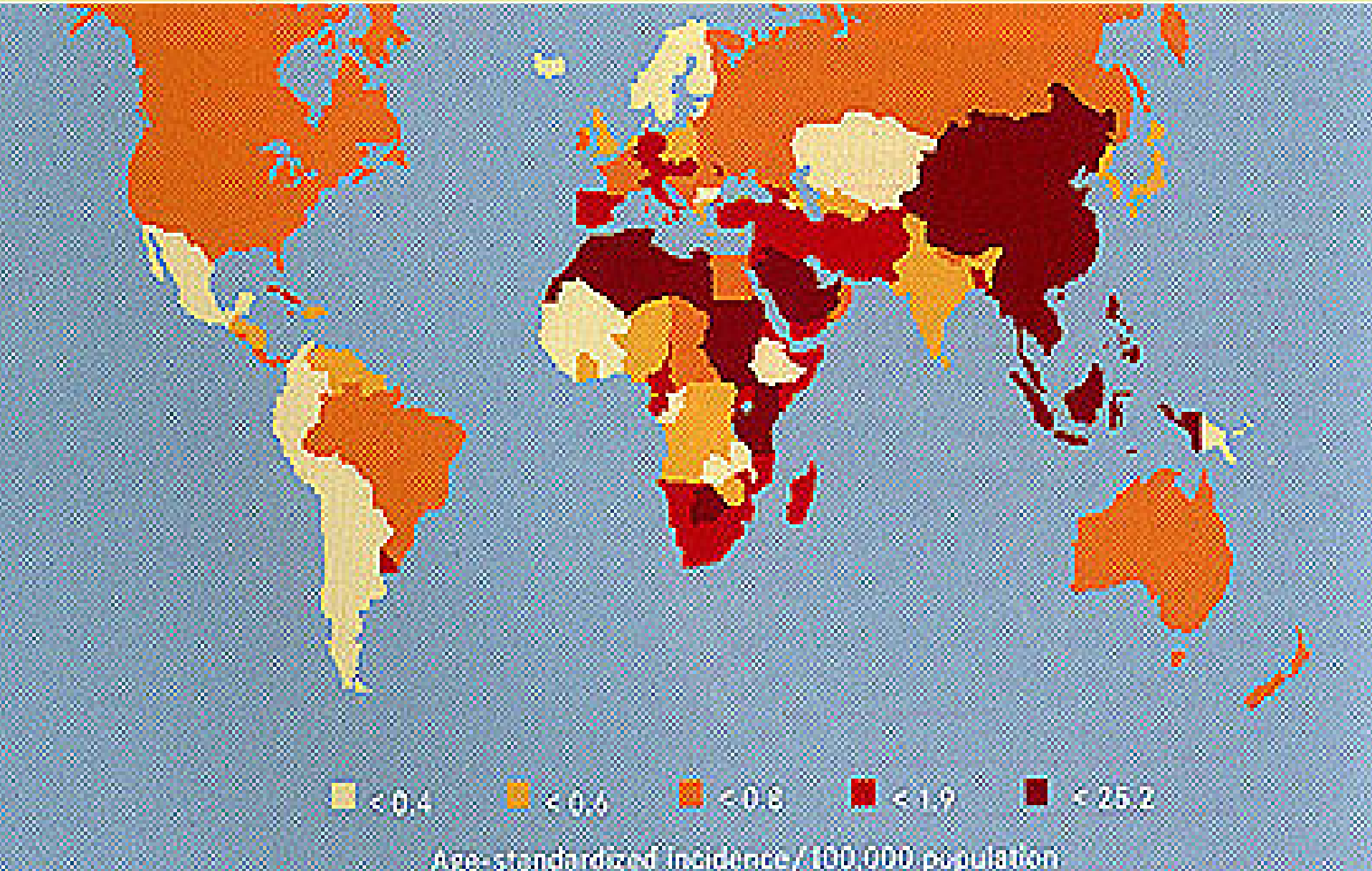
1. Viral Cancer
2. Geographic and ethnic distribution
3. Genetic susceptibility
4. Familial cases
5. Environmental factors

# Nasopharyngeal Carcinoma, Outline

Etiology

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

- 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

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

Amer MH : ***Ann Saudi Med*** 1982; 2: 203-215.

# *Nasopharyngeal Carcinoma*

## ***Incidence in KSA***

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

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***A. A. Andejani,: Saudi Med J 2004; Vol. 25***

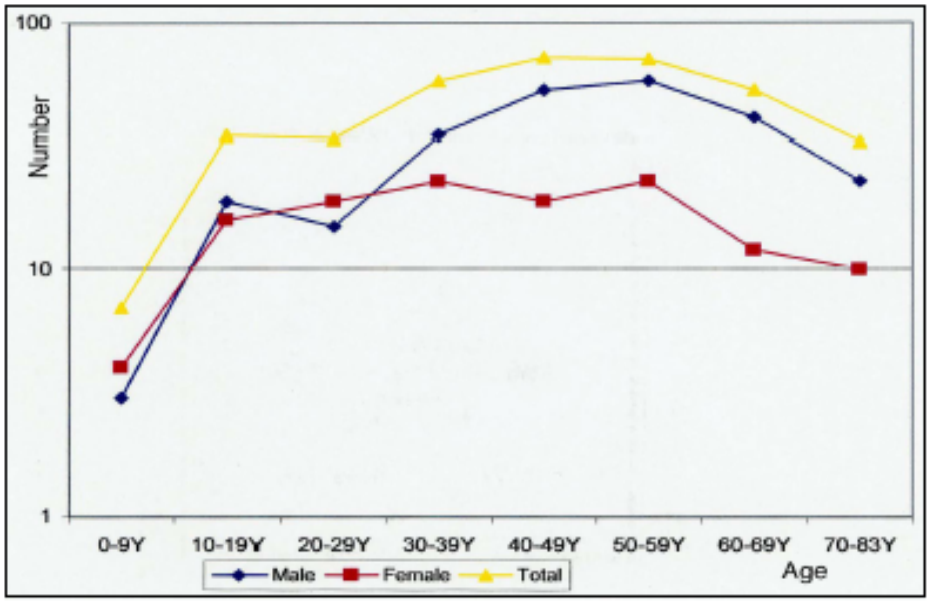



**Figure 3 -** Incidence of nasopharyngeal carcinoma among Saudis 1994 through to 1996.

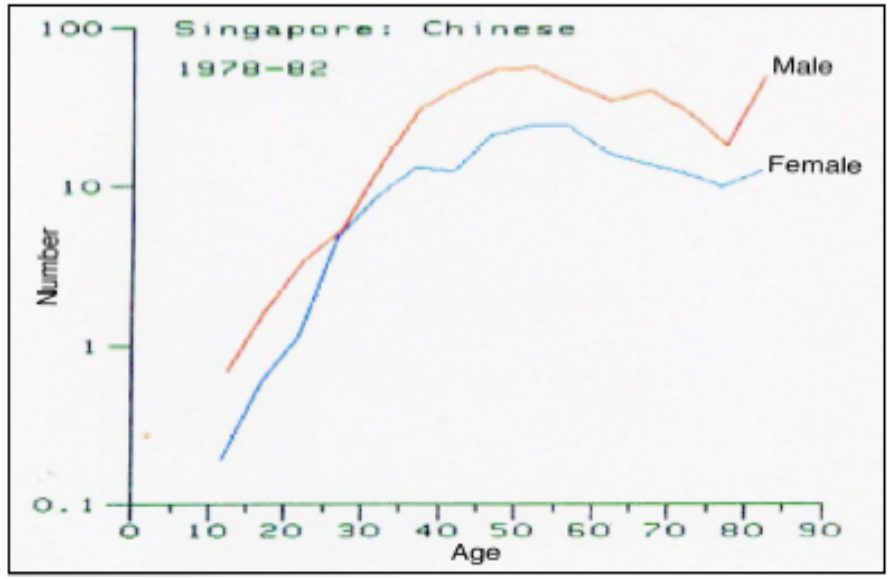



**Figure 1 -** Incidence of nasopharyngeal carcinoma among Singapore Chinese 1978 through to 1982.

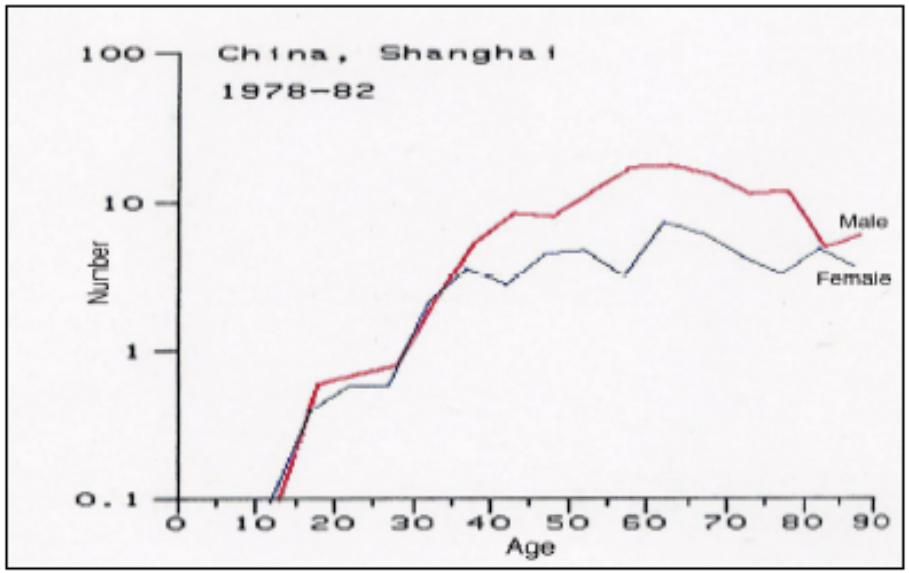



**Figure 2 -** Incidence of nasopharyngeal carcinoma among Chinese, Shanghai 1979 through to 1982.

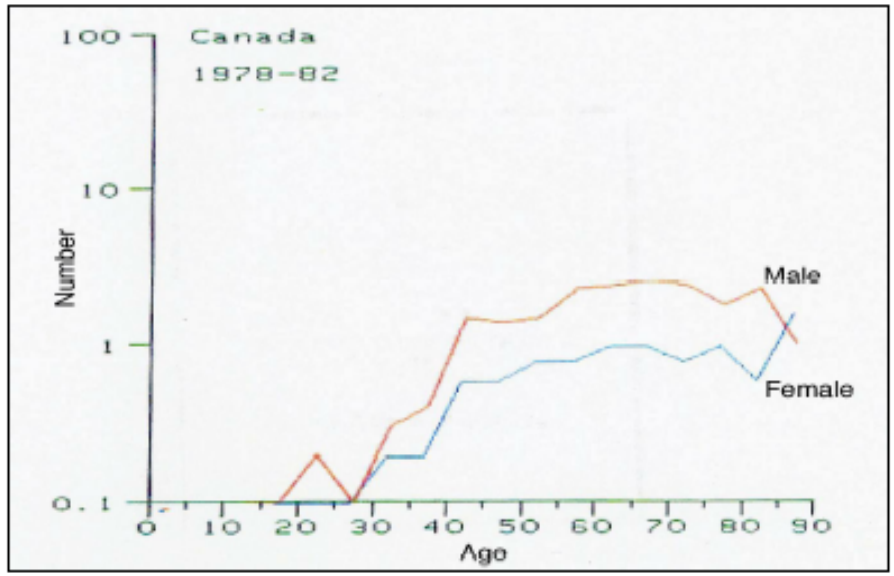
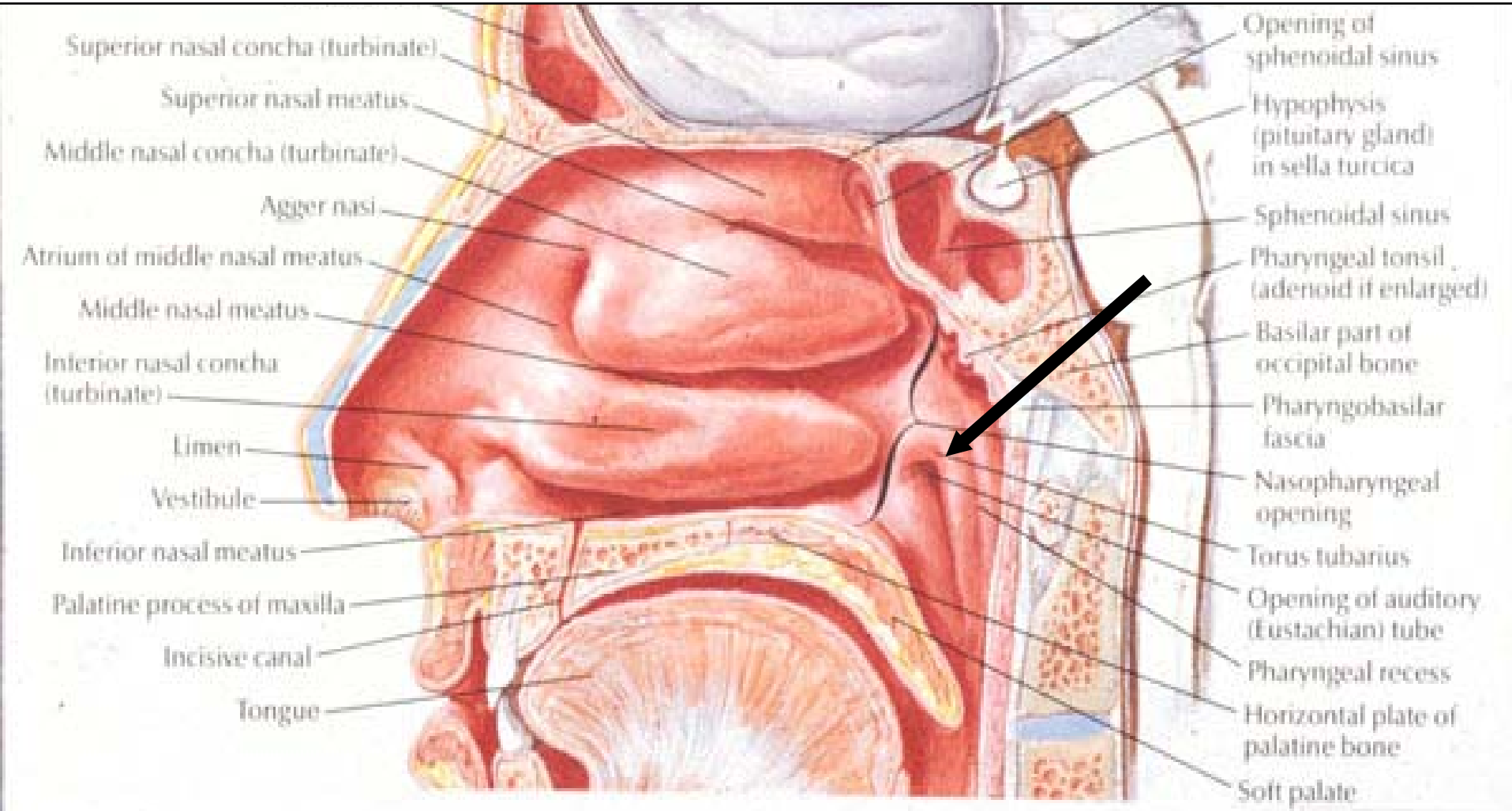



**Figure 5 -** Incidence of nasopharyngeal carcinoma among Canadians 1978 through to 1982.

- **50-80% present with cervical node metastases**
- **Random biopsy positive in 70% of cases.**



# WHO (2004)

1. Squamous cell carcinoma (WHO-I)

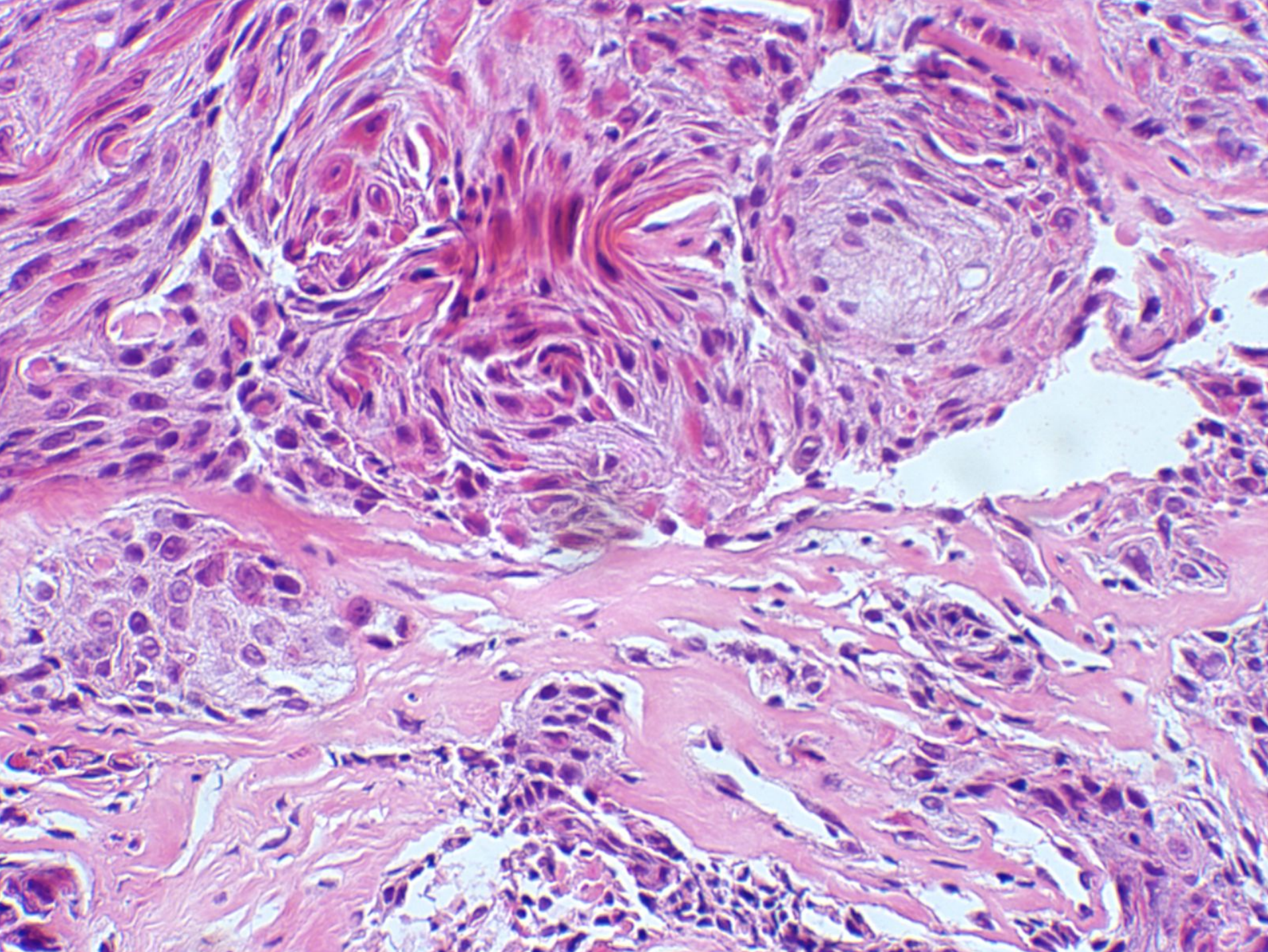
2. Non-keratinizing carcinoma
   - A. Differentiated non-keratinizing ca (WHO-II)
   - B. Undifferentiated carcinoma (WHO-III)

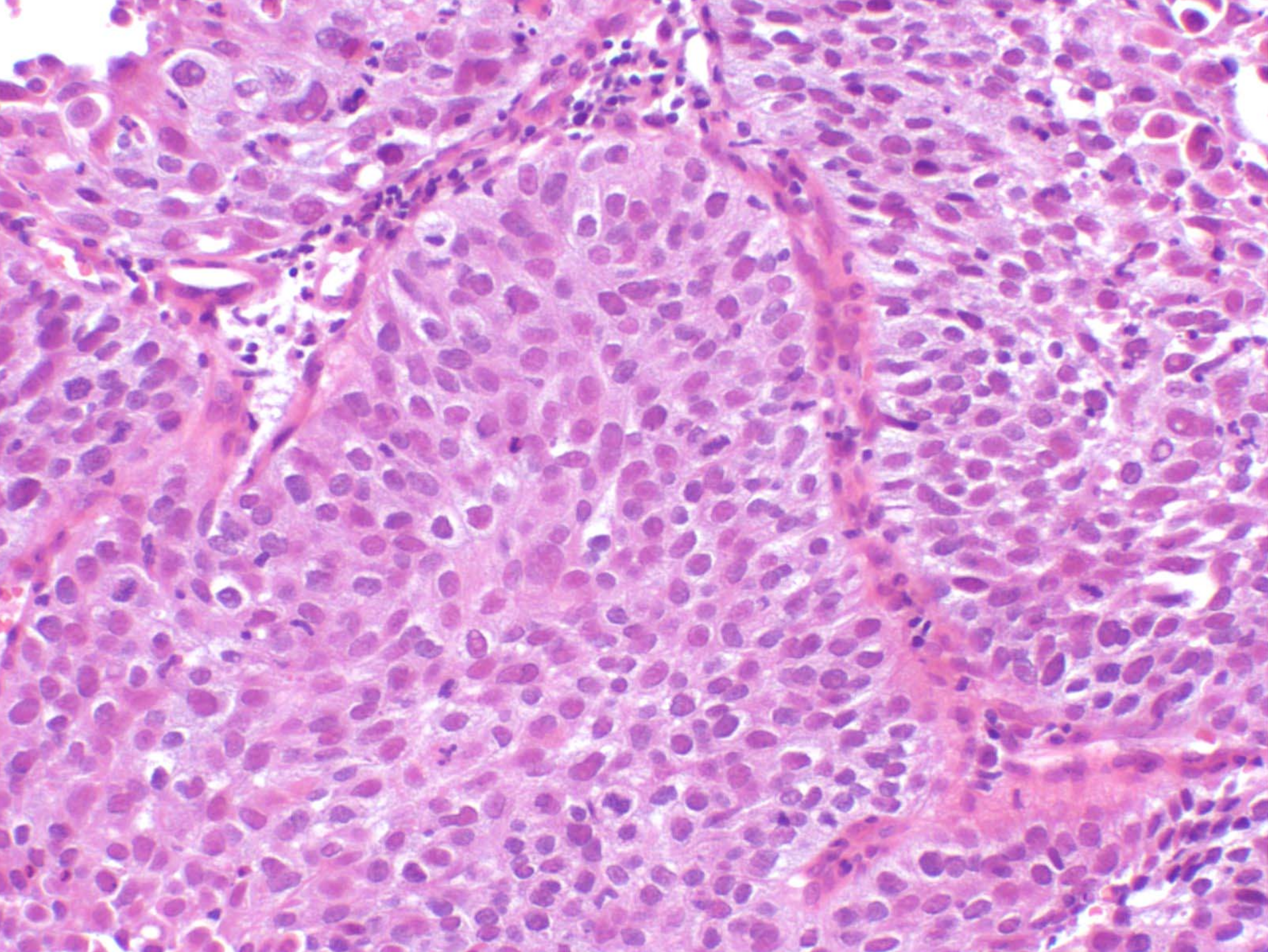
3. Basaloid squamous carcinoma

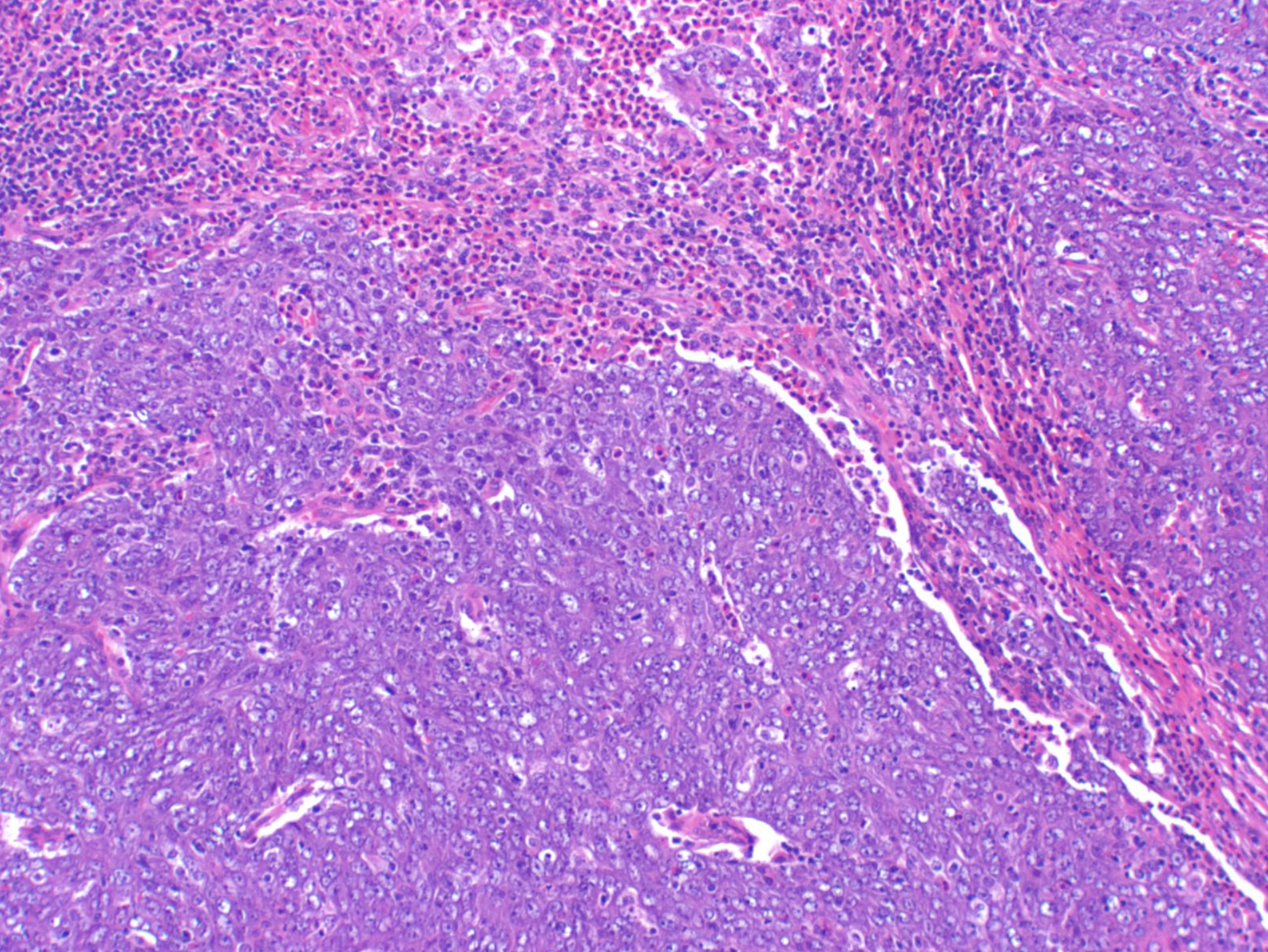
## Frequency of various histological subtypes of nasopharyngeal carcinoma

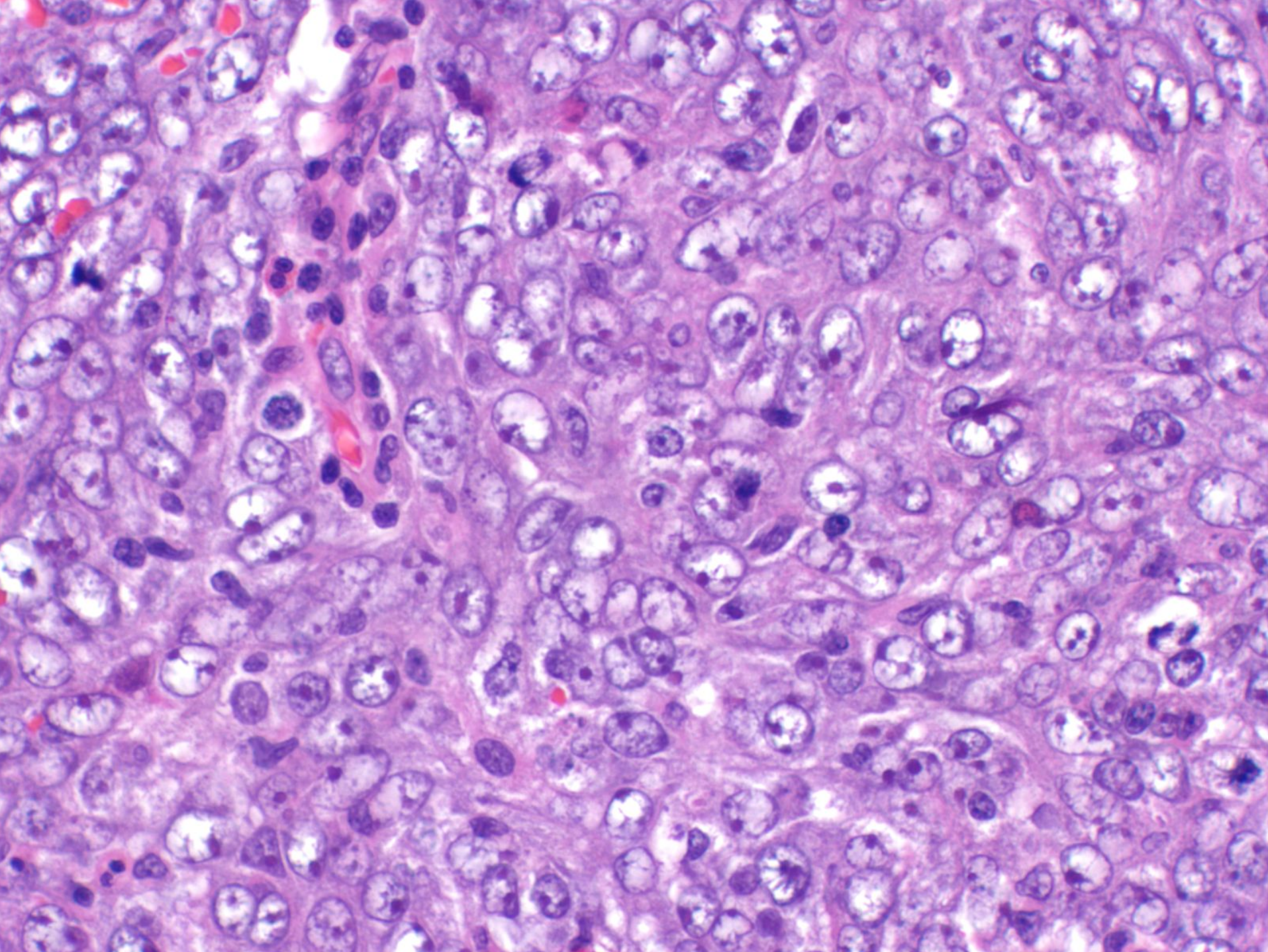
| | High incidence population | | Intermediate incidence population | Low incidence population | |
|---|---|---|---|---|---|
| | Hong Kong (Queen Elizabeth Hospital, 2001-2003) | Singapore {Shanmugaratnam et al.,1979} | Tunisia {Cammoun et al.,1978} | Japan {Sugano et al.,1978} | U.S.A. {Weiland,1978} |
| Squamous cell carcinoma | 1% | 17% | 8% | 13% | 25% |
| Nonkeratinizing carcinoma | 99% | 83% | 92% | 87% | 75% |
| - Undifferentiated | (92%) | (42%) | (76%) | | |
| - Differentiated | (7%) | (41%) | (16%) | | |
| Basaloid-squamous carcinoma | <0.2% | NA | NA | NA | NA |

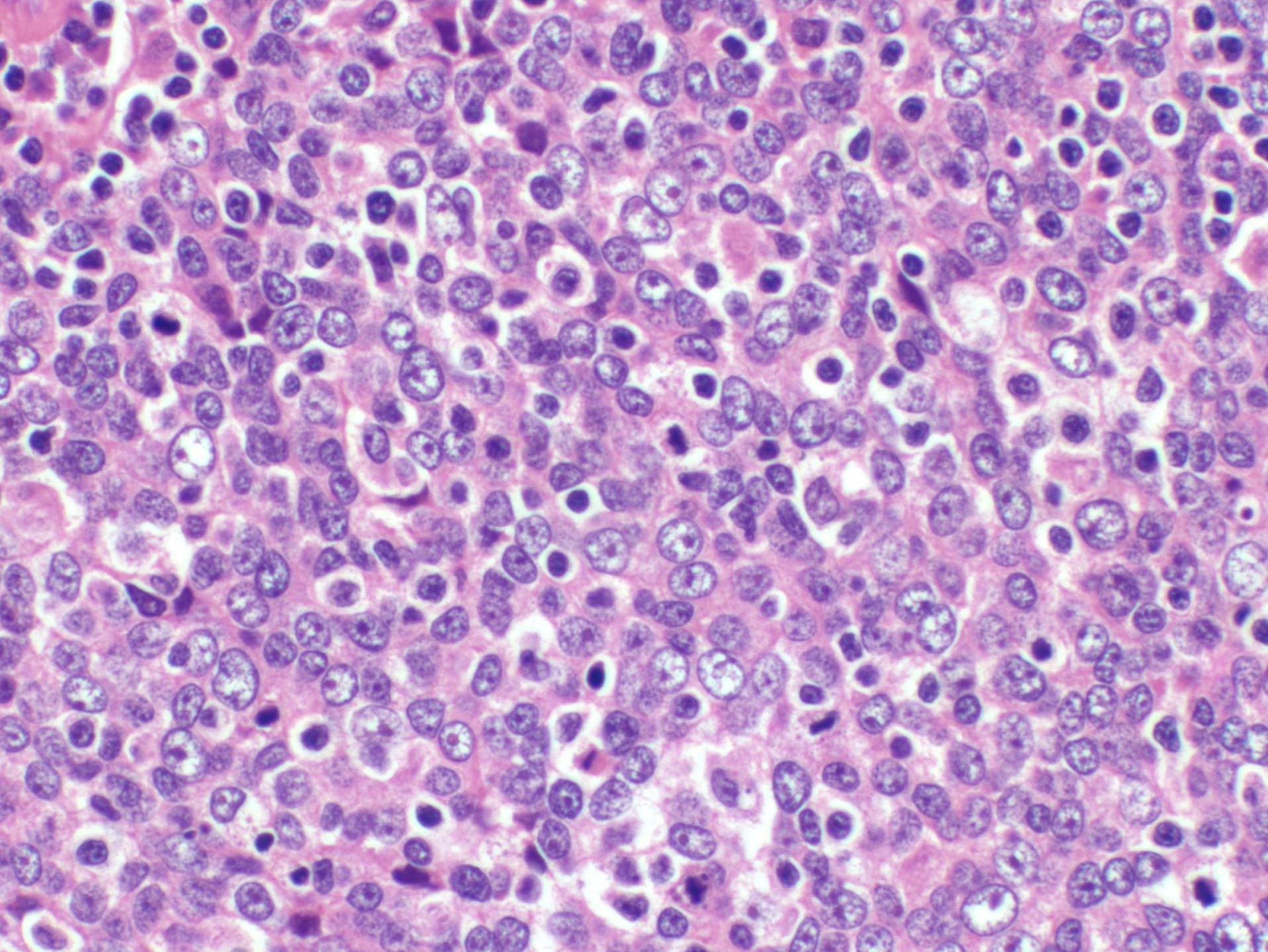
NA = Not available

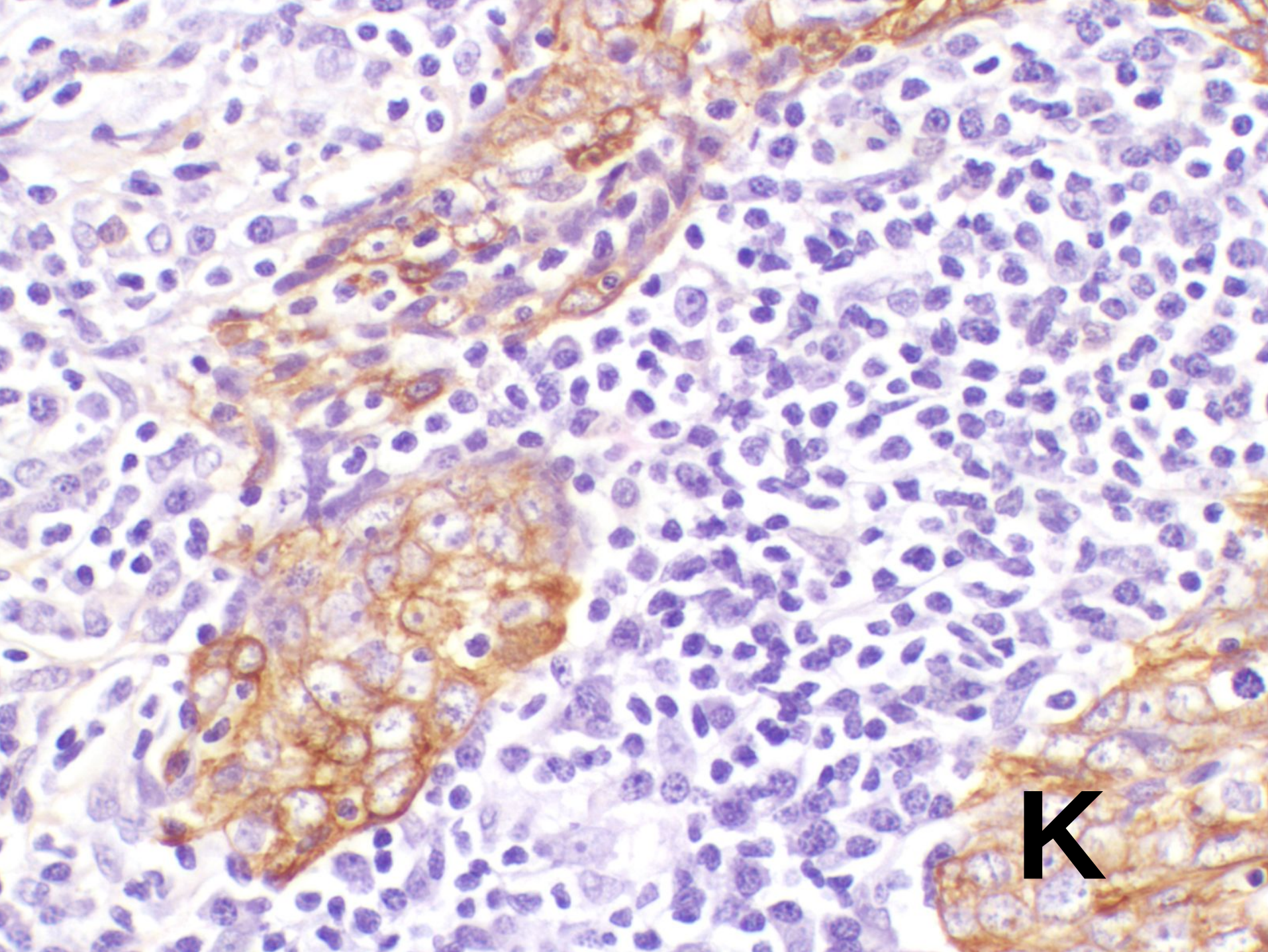




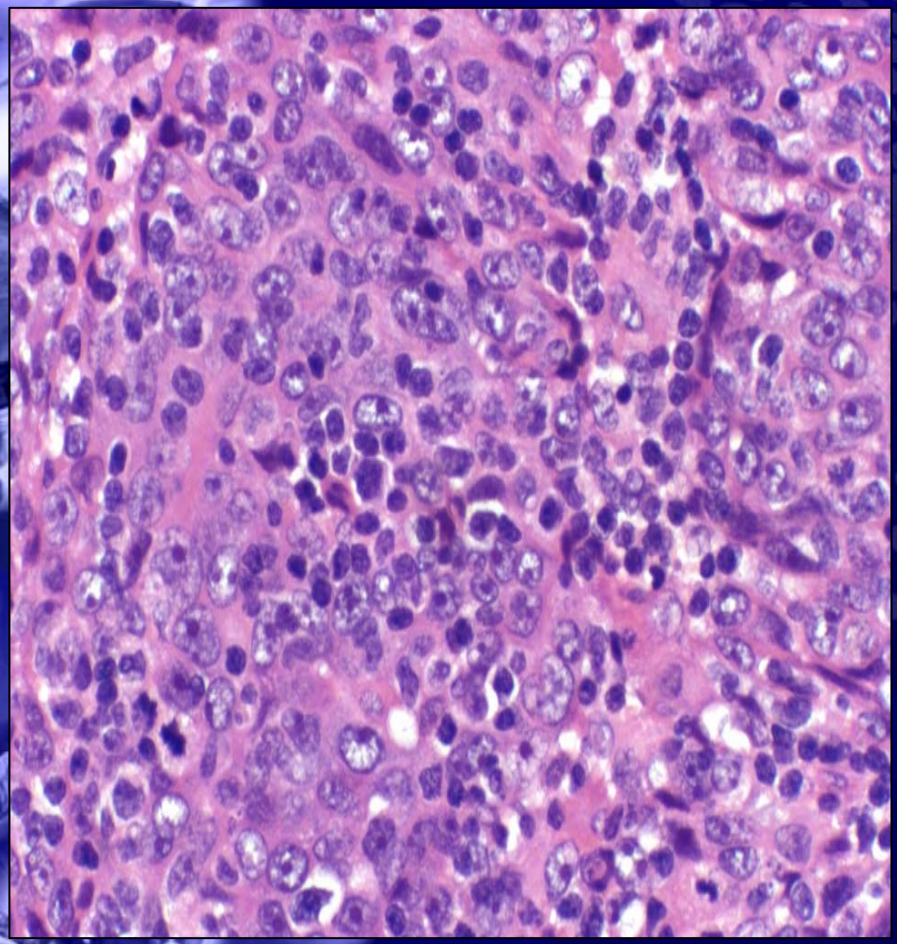




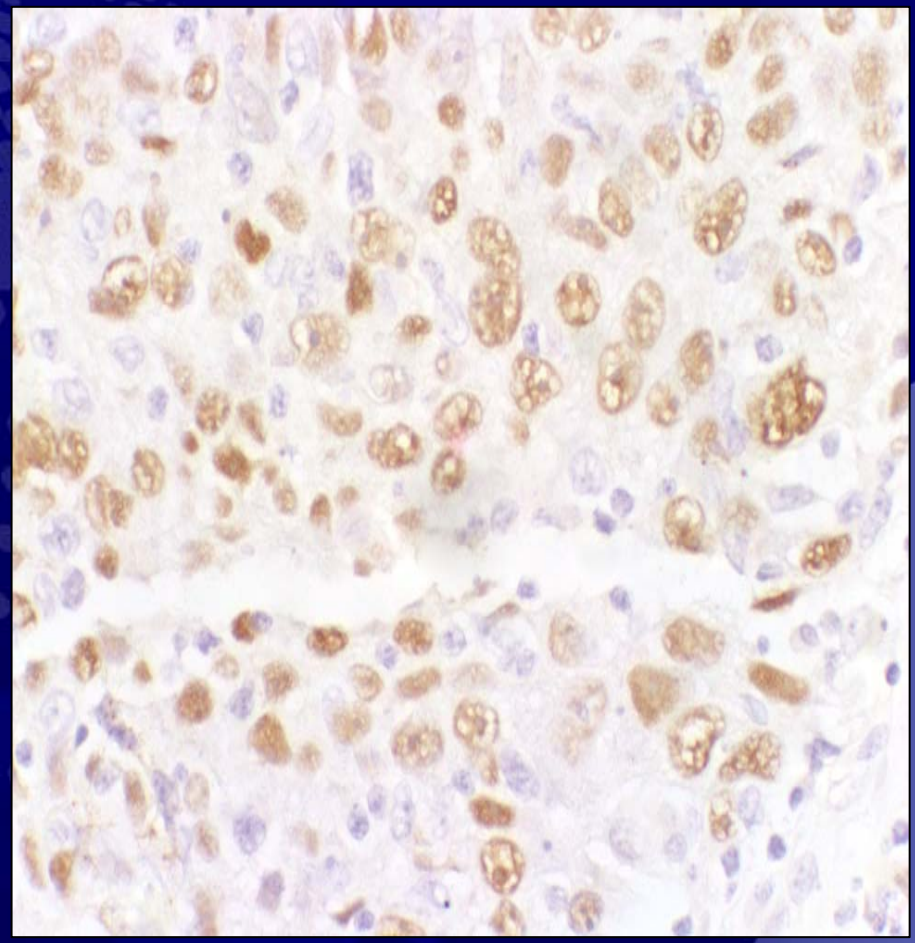



K

# *NPC, WHO-III*



# *EBER*



# *Incidence of EBV and HPV in 38 NPC**

| Type | EBV(%) | HPV(%) |
|---|---|---|
| WHO I (N=15) | 13 | 27** |
| WHO II-III (N=23) | 100 | 0 |

*= Hording U, et al, Laryngoscope 104:99, 1994

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

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# *NPC - Etiology*

**Interaction of all three etiological factors**

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

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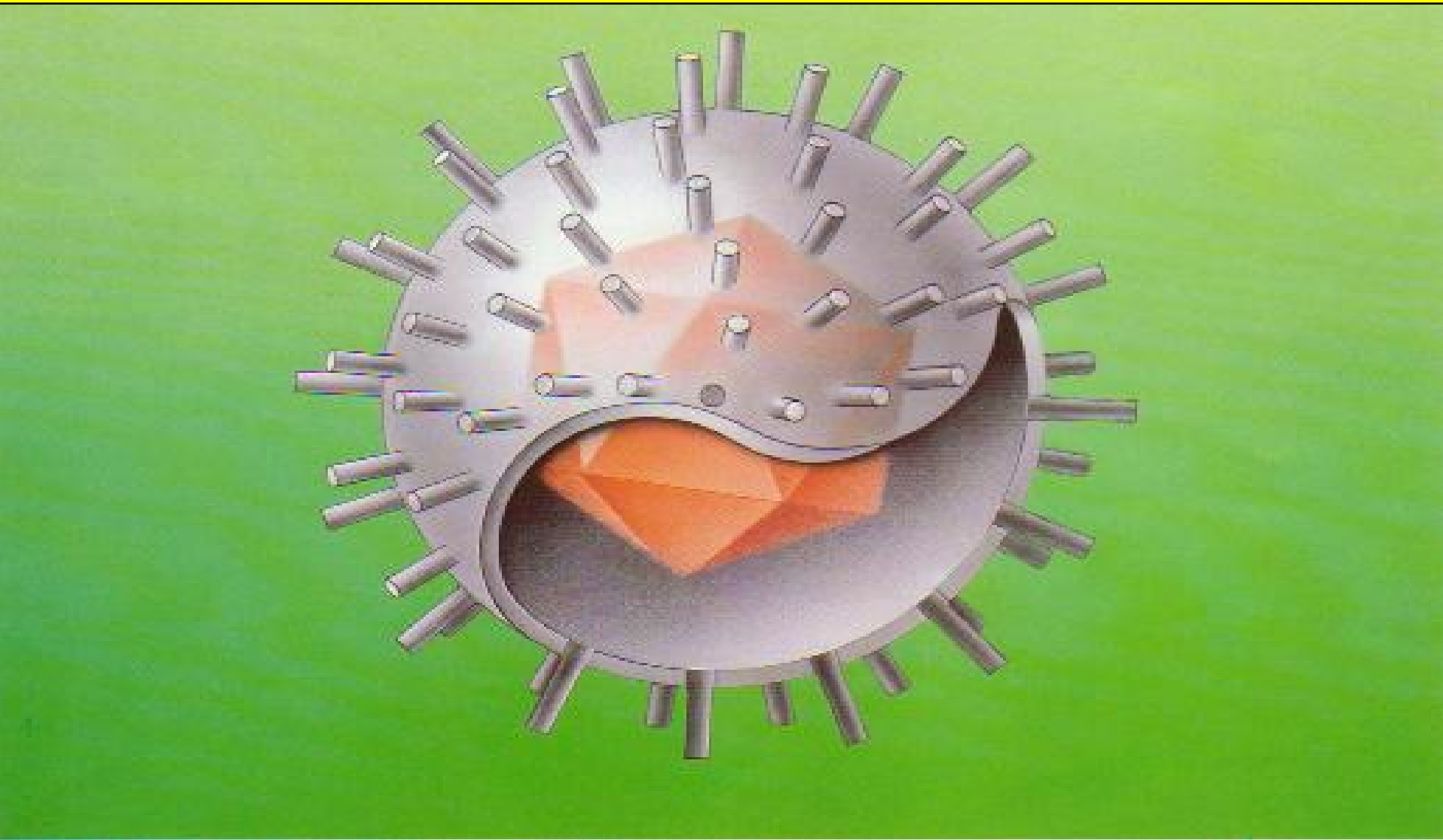
Liebowitz D ..ET AL: Semin Oncol 1994 Jun;21(3):376-81

Yuan JM .et al.Int J Cancer 2000 Feb 1;85(3):358-63

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

1. Serum IgA to (VCA) and (EA) with NPC
2. Persistence of EBV DNA and EBNA in NPC


1. Henle and Henle, Int J Cancer 17: 1,1976
2. Huang D.P; Int J Cancer 14: 580, 1974

# *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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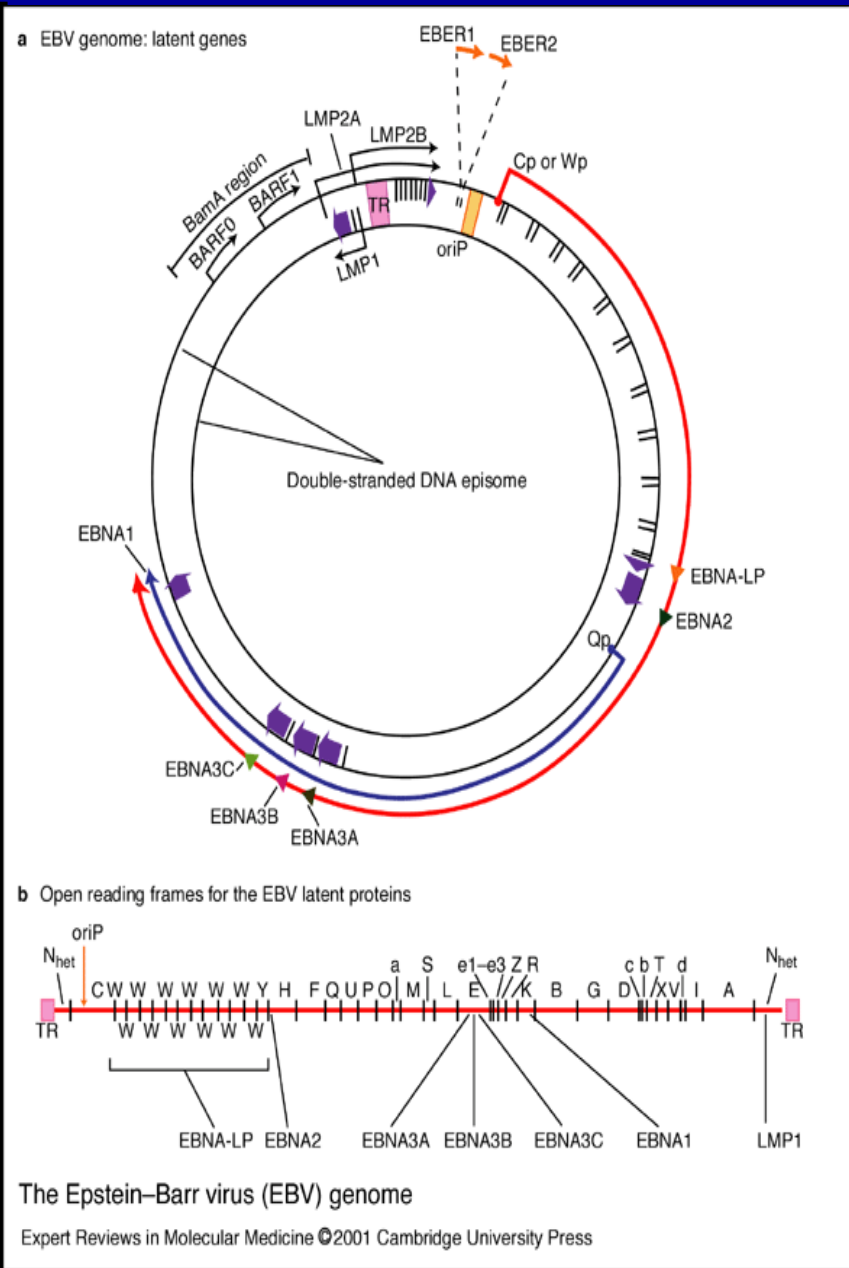
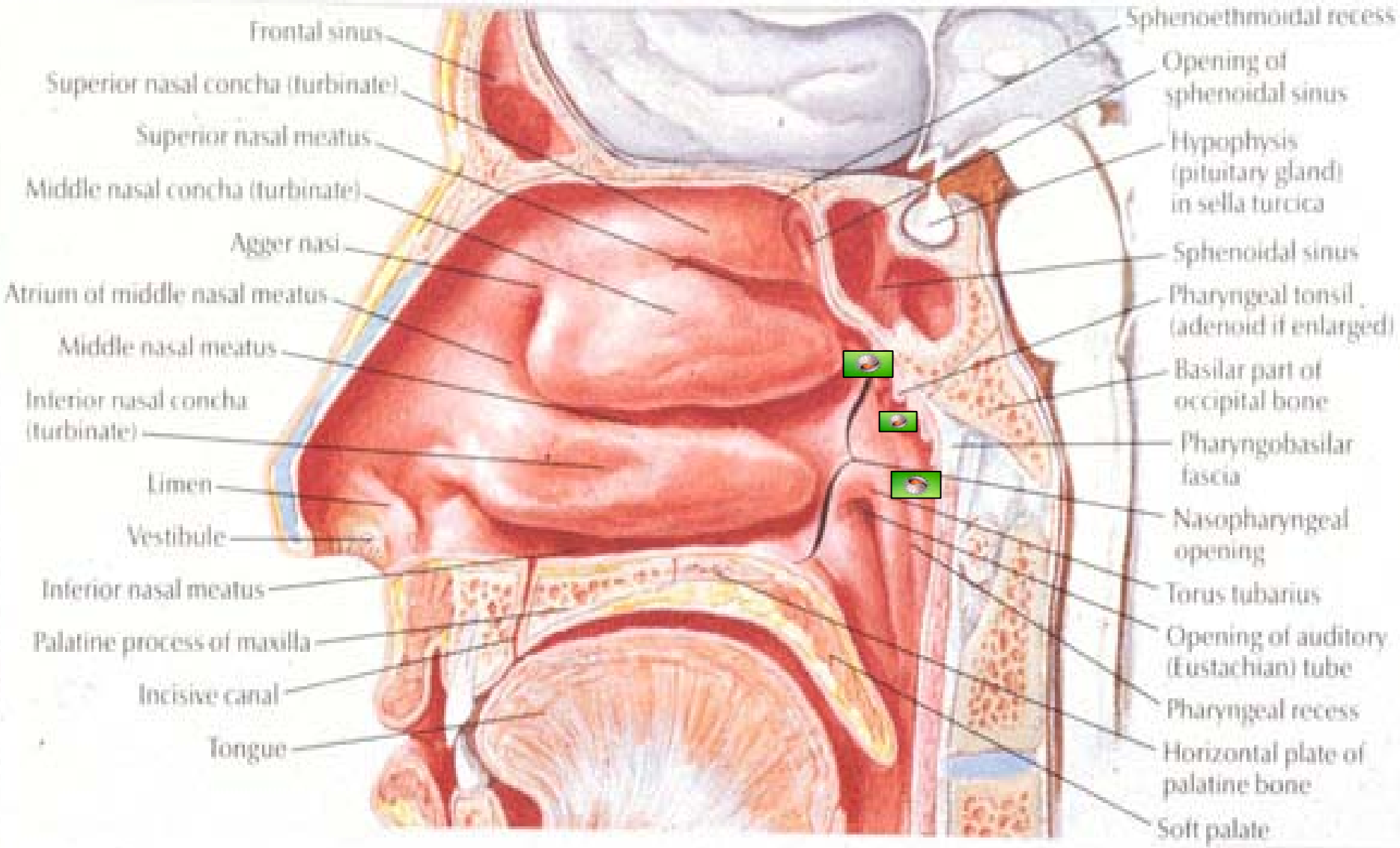
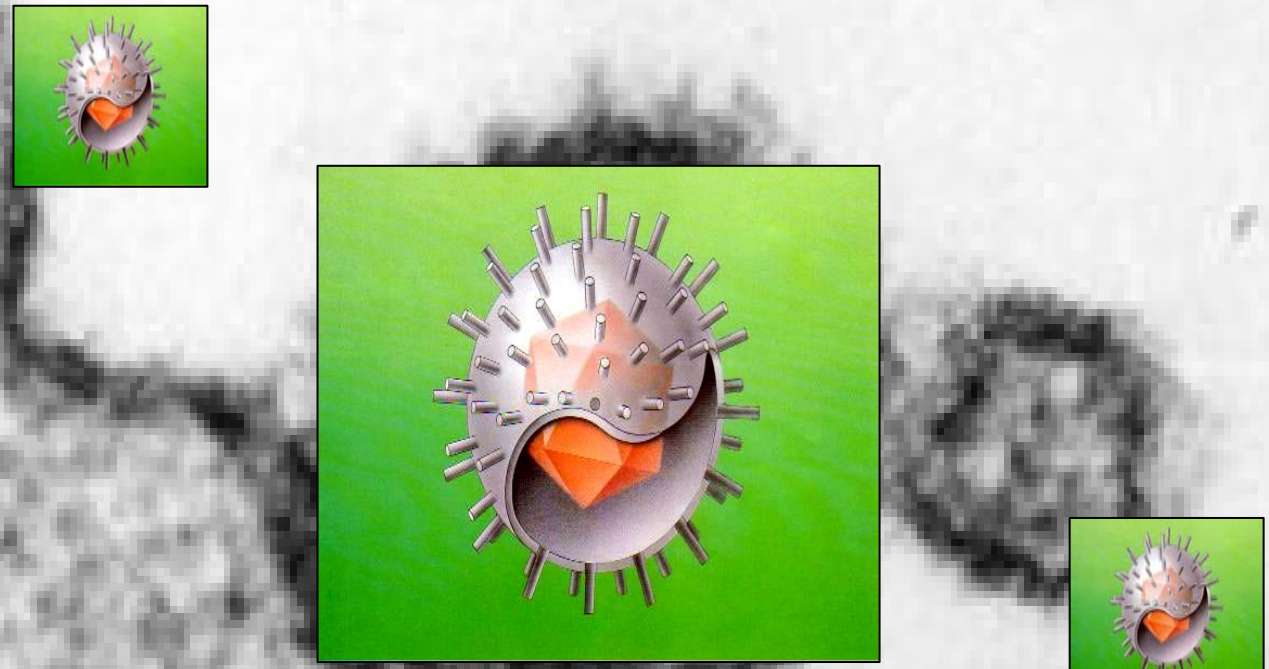
1. *Hao SP, Otolaryngol Head Neck Surg 2004, **131:**651-654.*
2. *Stevens SJ, Int J Cancer 2006, 119:608-614.*

# *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**



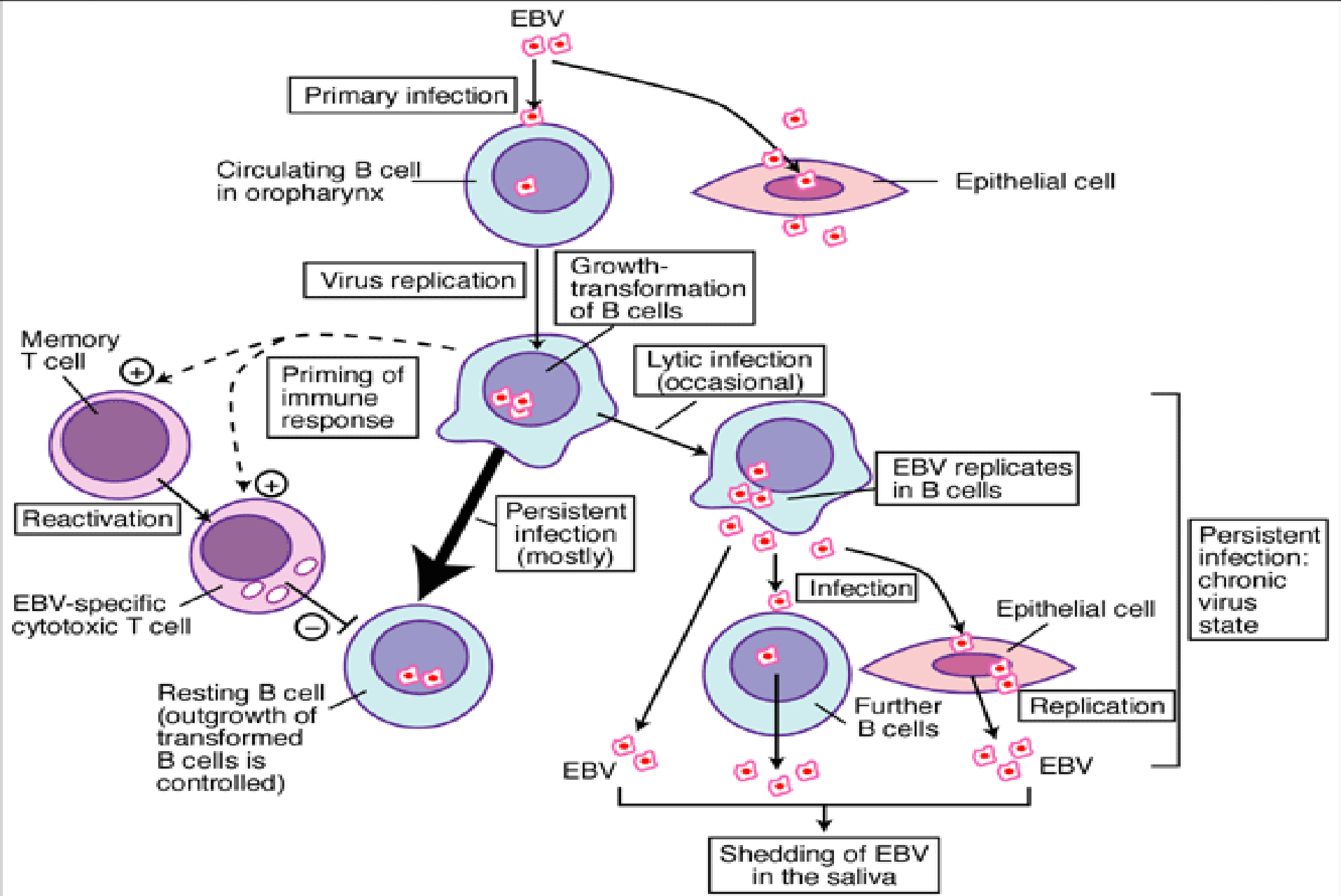

Frontal sinus
Superior nasal concha (turbinate)
Superior nasal meatus
Middle nasal concha (turbinate)
Agger nasi
Atrium of middle nasal meatus
Middle nasal meatus
Inferior nasal concha (turbinate)
Limen
Vestibule
Inferior nasal meatus
Palatine process of maxilla
Incisive canal
Tongue
Sphenoethmoidal recess
Opening of sphenoidal sinus
Hypophysis (pituitary gland) in sella turcica
Sphenoidal sinus
Pharyngeal tonsil (adenoid if enlarged)
Basilar part of occipital bone
Pharyngobasilar fascia
Nasopharyngeal opening
Torus tubarius
Opening of auditory (Eustachian) tube
Pharyngeal recess
Horizontal plate of palatine bone
Soft palate



CD 21

GP350/220

# B-lymphocyte

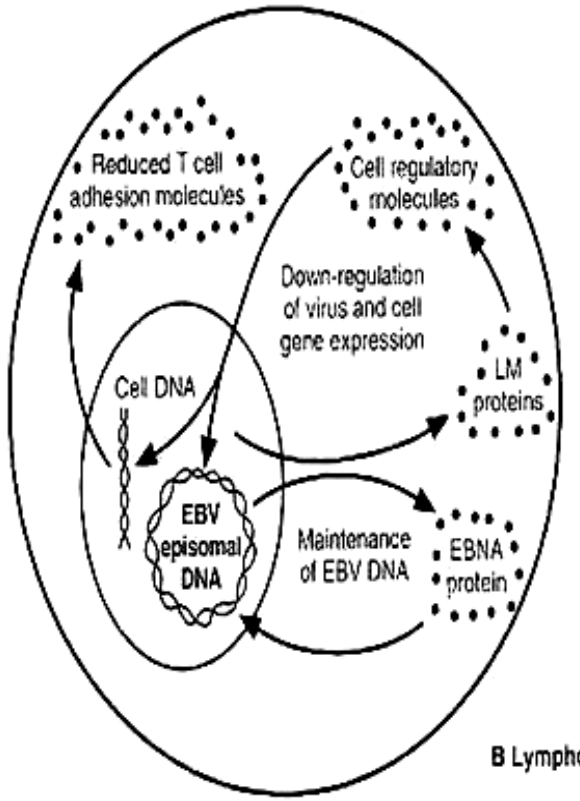



Epstein–Barr virus (EBV) infection in normal healthy virus carriers

# *Epstein Barr Virus- EBNA*

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

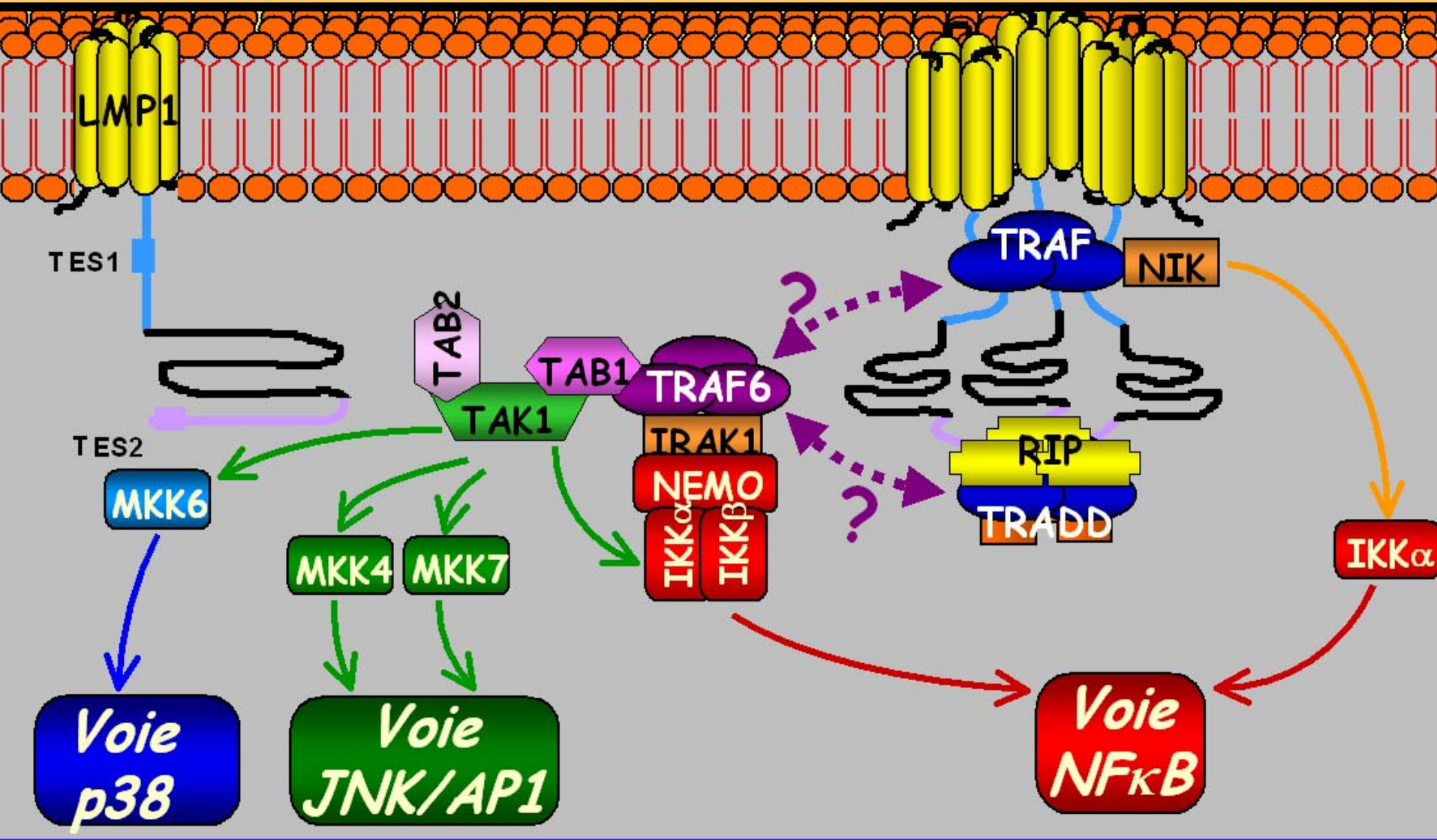
2. **EBNA-2, EBNA-3c:** Transformation of B cells



# *Epstein Barr Virus*

- *LMP-1 is an* oncogene essential in cell transformation and metastasis.
- Transformation of B lymphocytes
- Transform Rat-1 fibroblasts.

Yan G, *Proteomics* 2006, 6:1810-1821.




LMP1-triggered signaling pathways

# *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%

- ***Hao S-P, et al. Cancer 97:1909, 2003***

# *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)

Tsang N-M, et al. Cancer 98:2385, 2003.

# *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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Guo C, *J; Trans / Med* 2006, **4:**23.

Fan SQ, *Hum Pathol* 2006, **37:**593-605.

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# *NPC - Etiology*

**Interaction of all three etiological factors**

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Liebowitz D ..ET AL: Semin Oncol 1994 Jun;21(3):376-81

Yuan JM .et al.Int J Cancer 2000 Feb 1;85(3):358-63

# *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

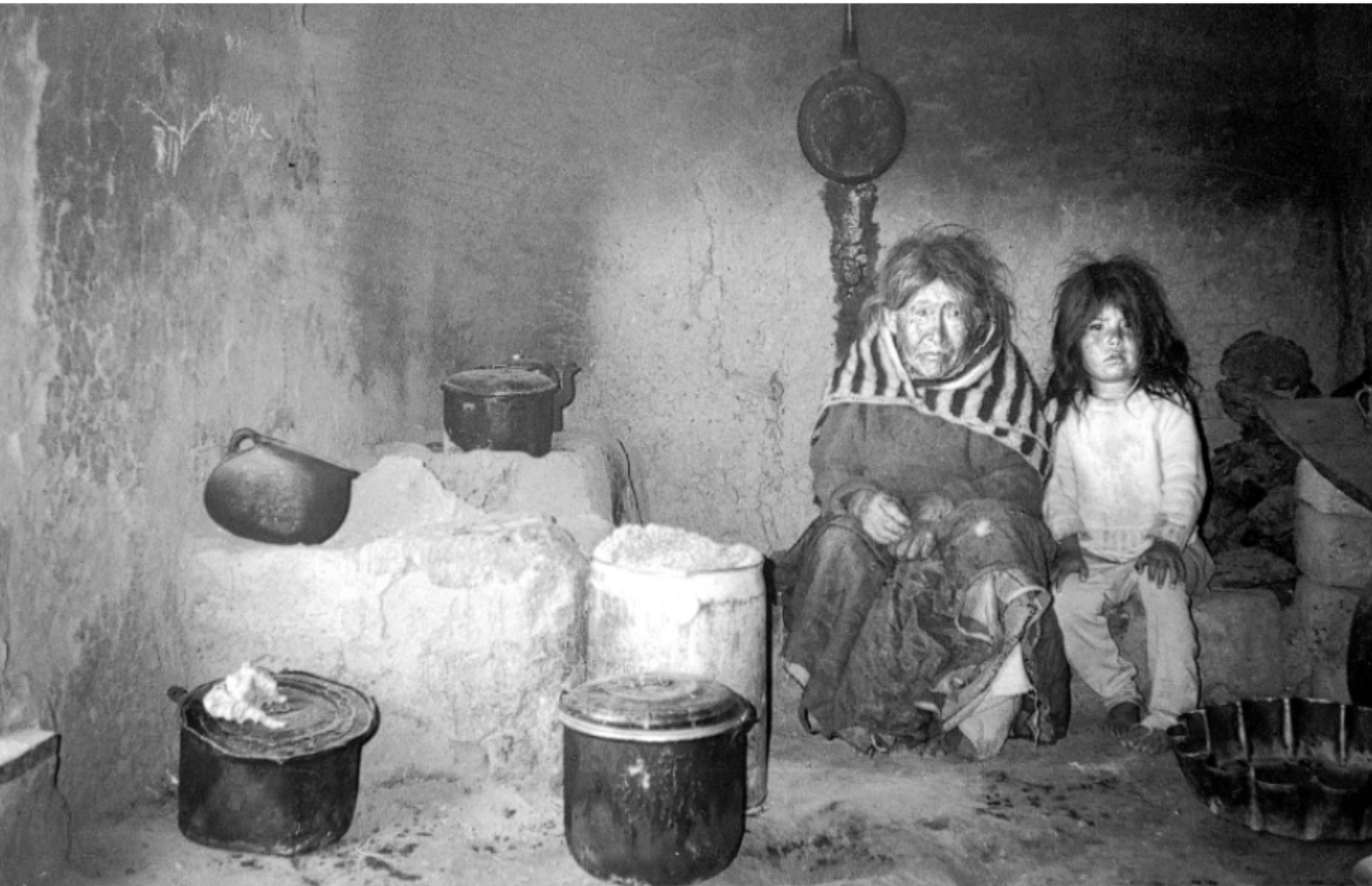
***Pintos J et al. Journal of Epidemiology, 1998, 27 (6): 936–940**

•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**

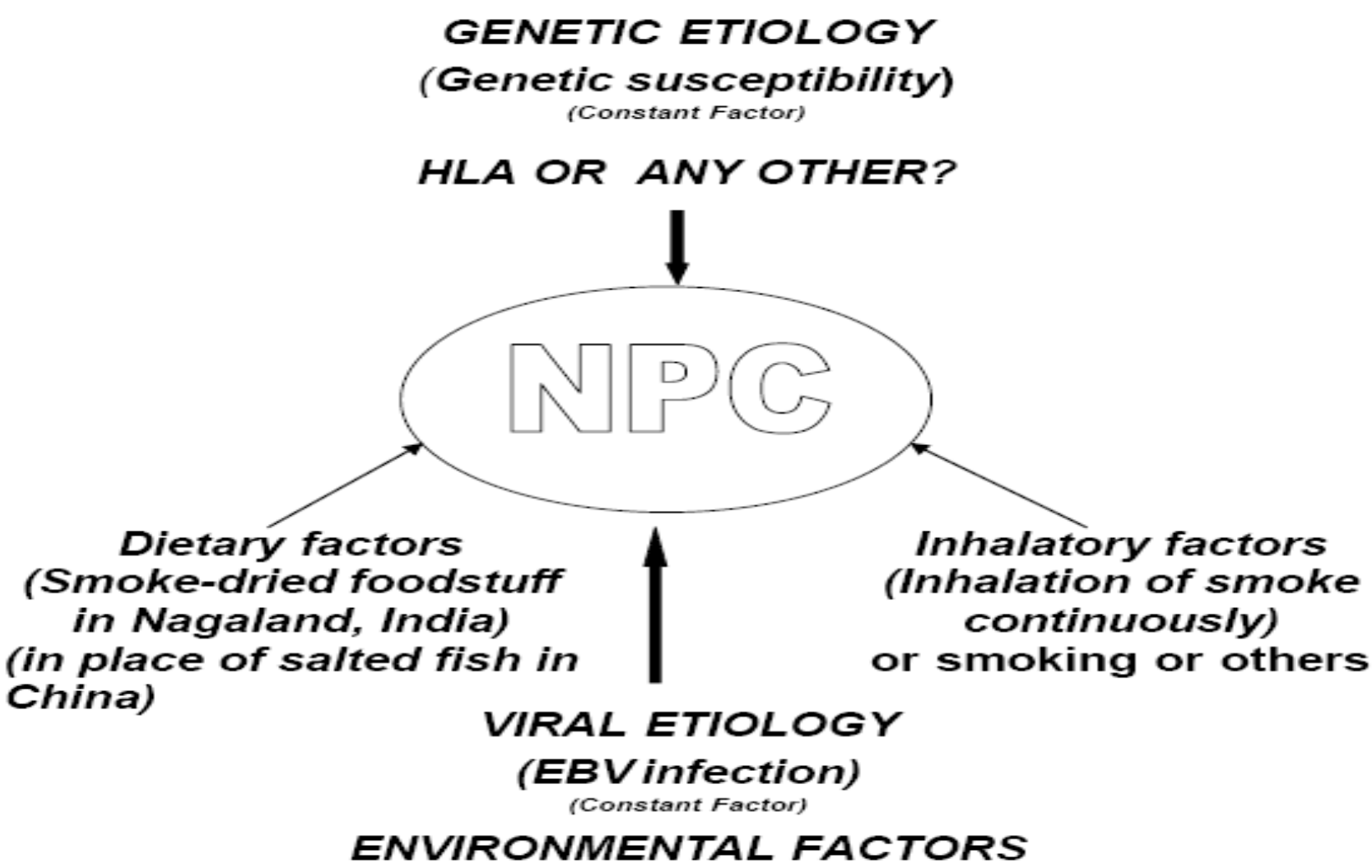


# *NPC – Etiology, **Environment***

- **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**

Vol.33, No.9 September, 2003




ICMR BULLETIN

# *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 | Decreased Risk |
|---|---|
| • HLA –A2 (Singapore) | HLA –A11 |
| • HLA A2-B38 | |
| • HLA A2-B16 | |
| • HLA B17 (**South China, Singapore and Malaysia**) | |
| • HLA –B14 | HLA –B13 |
| • HLA –B46 | HLA –B22 |

# *HLA&NPC*

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

- **HLA ~~ Susceptibility ~~ Prognosis~~ Survival**

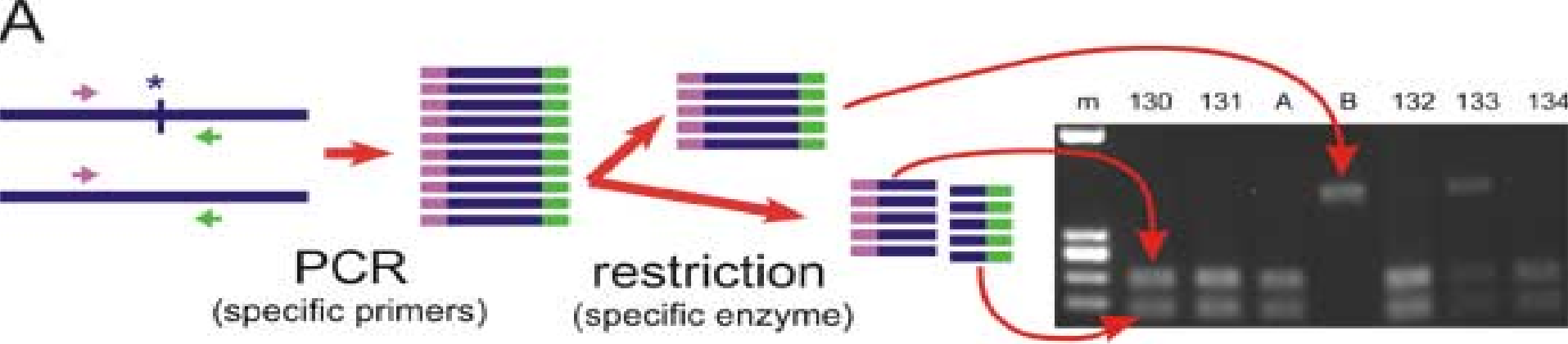
***Lu QL et al: Genetic susceptibility to Nasopharyngeal carcinoma within the HLA –A locus in Taiwanese. Int J cancer 103:745-751*


1. *Simons, M.J., Eds. G. de ET AL: The and Y. Ito. IARC Scientific Publication 20: 271, 1978.*
2. *Chan, S.H.ET AL: 1980. Ann Acad Med Singapore 9: 296, 1980*

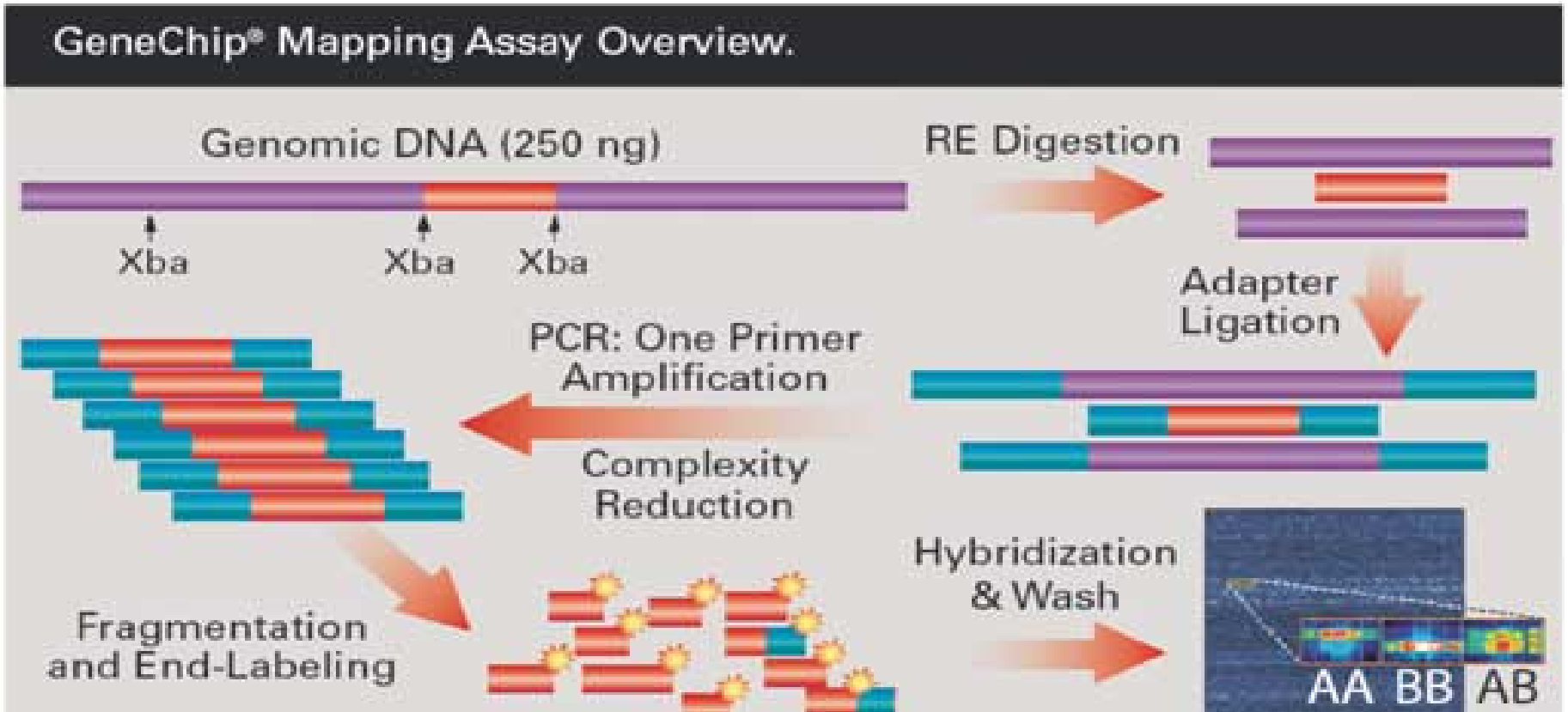
# *Nasopharyngeal Carcinoma, Genetic Susceptibility*

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**

A


PCR
(specific primers)
restriction
(specific enzyme)
m
130
131
A
B
132
133
134

B


GeneChip® Mapping Assay Overview.
Genomic DNA (250 ng)
Xba
Xba
Xba
RE Digestion
Adapter Ligation
PCR: One Primer Amplification
Complexity Reduction
Fragmentation and End-Labeling
Hybridization & Wash
AA
BB
AB

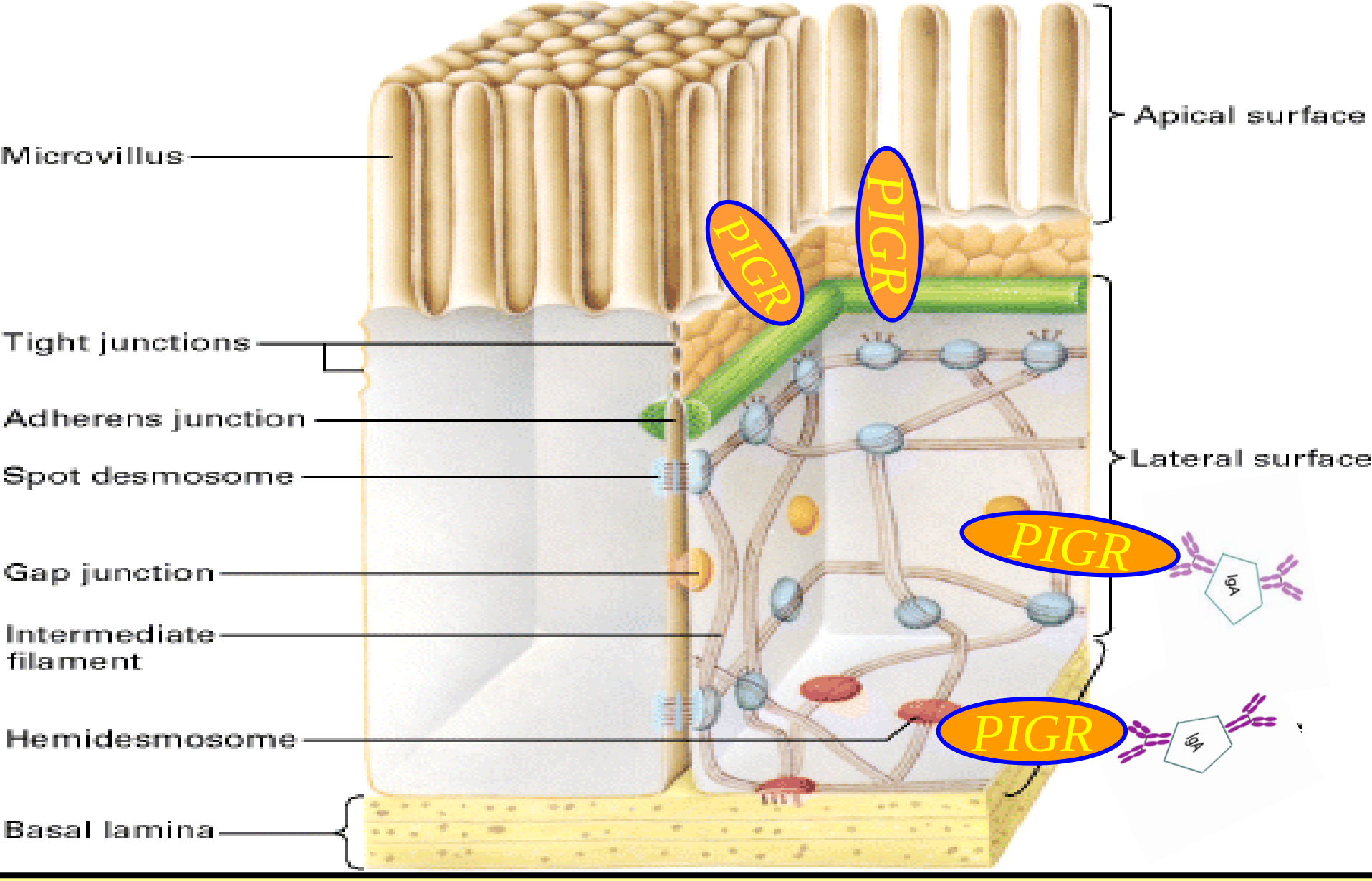
# *Single Nucleotide Polymorphism Cytochrome P540 2E1 (CYP2E1)*

- 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 (*)

*** Hildshieim A .et al : J Natl Cancer Inst 89:1207-1212; 1997**




Rungnapa Hirunsatit..et al: ***BMC Genetics* 2003, 4:3**
http://www.biomedcentral.com/1471-2156/4/3,2003

# *Polymeric Immunoglobulin Receptor (PIGR)*

- 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*IVS2-848 C→T**
  2. **Polymeric Immunoglobulin Receptor (*PIGR*)**
     - ✓ ***PIGR*1739 C→T**
     - ✓ ***PIGR*IVS3-156 G→T,**
     - ✓ ***PIGR*1093 G→A**

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R. Hirunsatit..et al: ***BMC Genetics* 2003, 4:3**
http://www.biomedcentral.com/1471-2156/4/3,2003

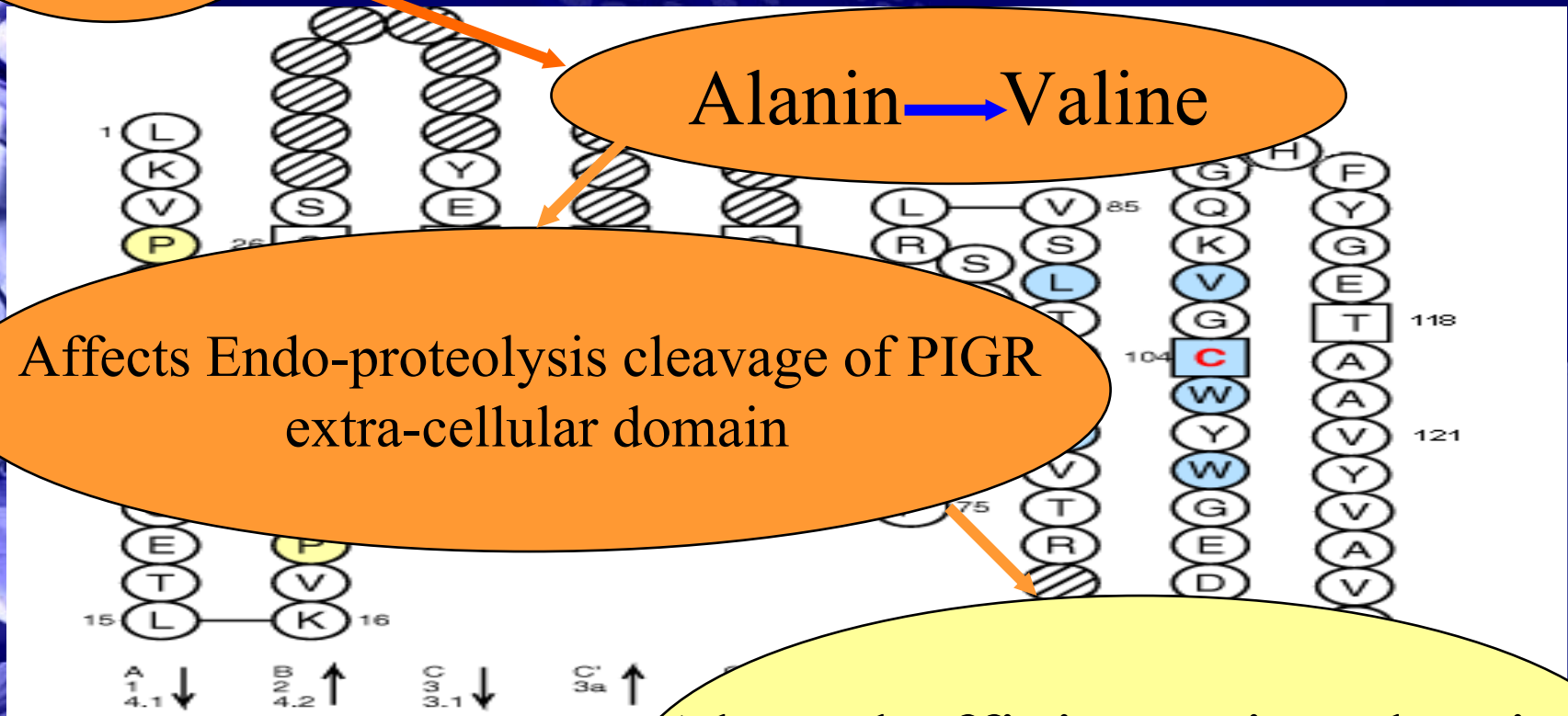
# *Polymeric Immunoglobulin Receptor (PIGR), Results:*

- Role of the nucleotide *PIGR*1739
  - ✓ ***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

# *Polymeric Immunoglobulin Receptor SNP, PIGR1739*

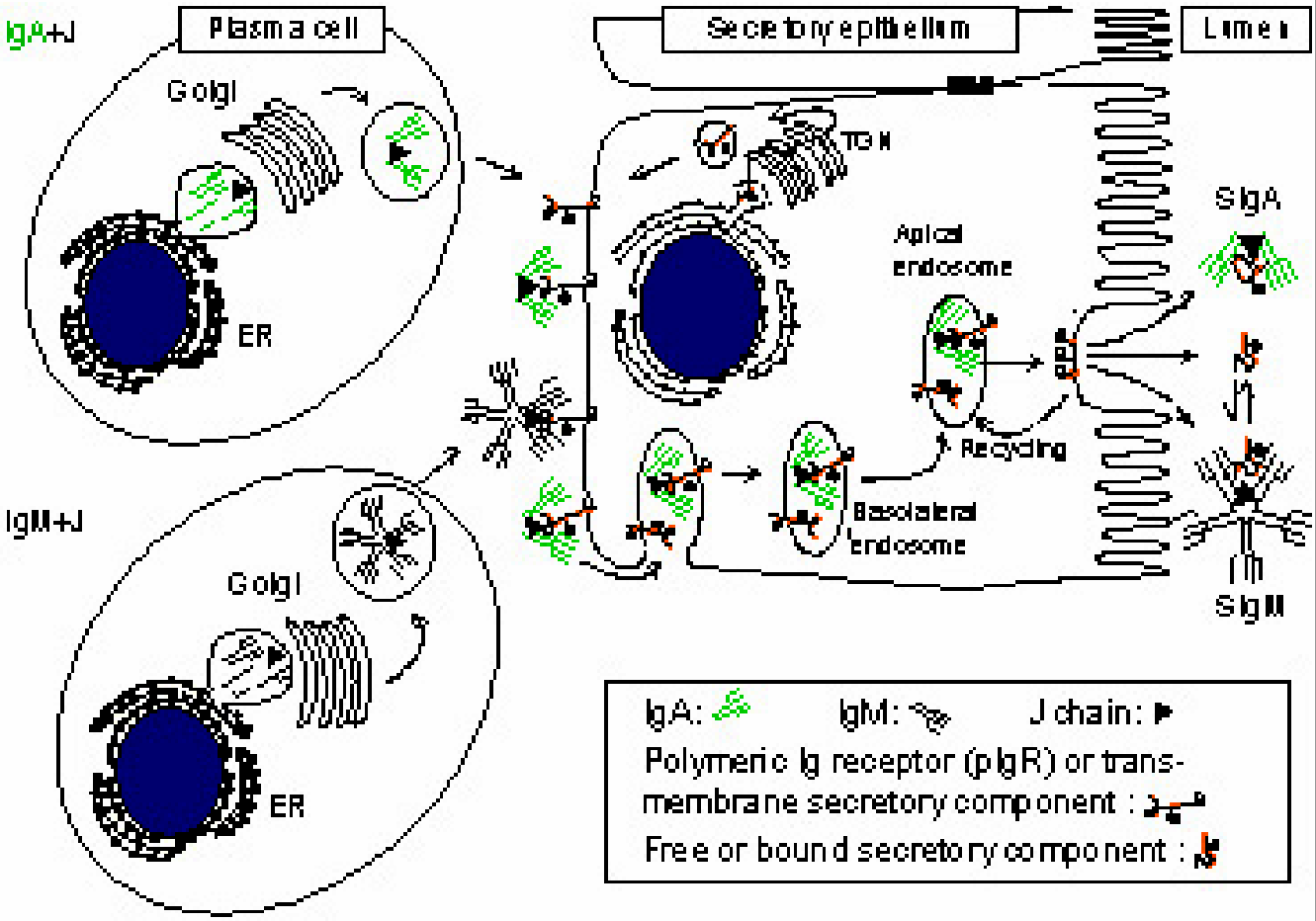
Alanin → Valine

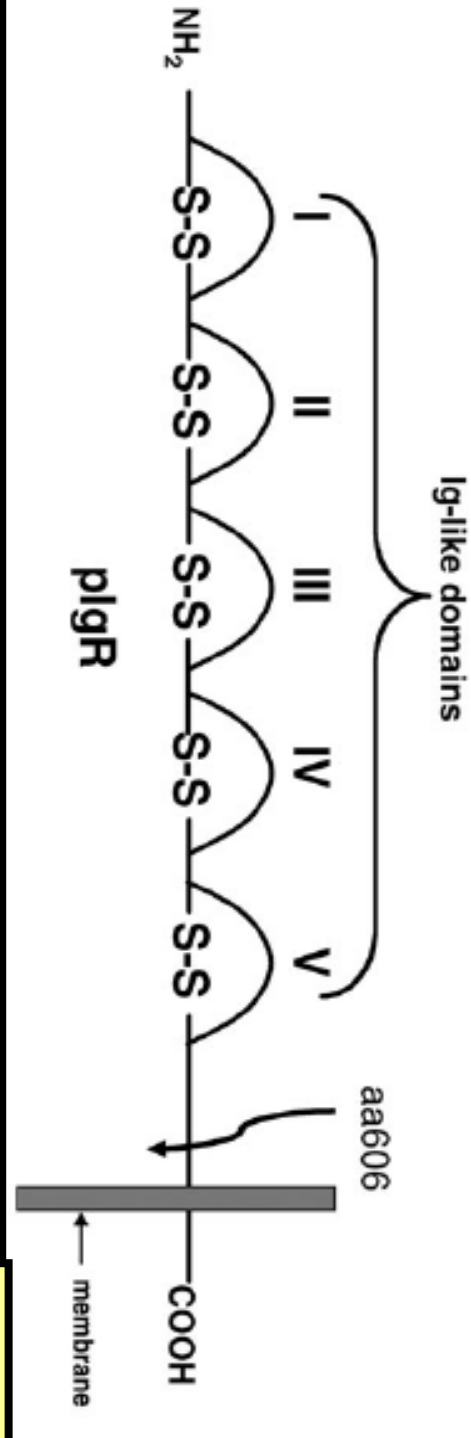


Affects Endo-proteolysis cleavage of PIGR extra-cellular domain

Altered efficiency in releasing IgA -EBV complex

**Rungnapa Hirunsatit.et al: http://www.biomedcentral.com/1471-2156/4/3**



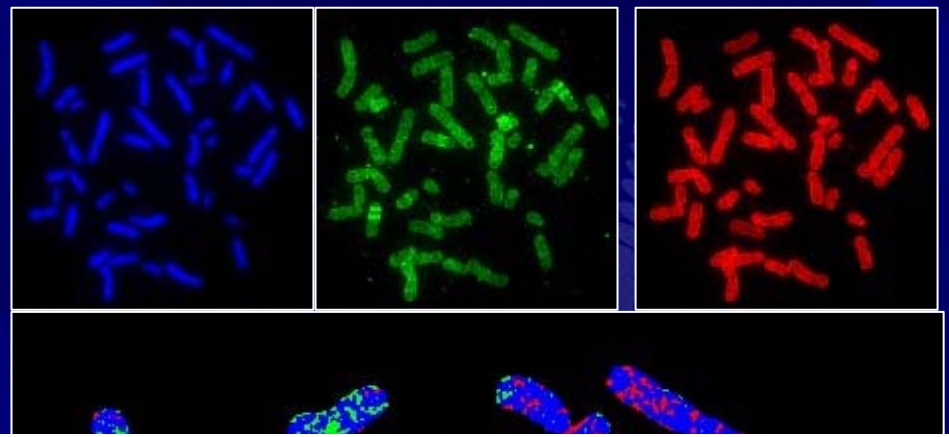

***Nature Reviews Molecular Cell Biology* 3, 944-956 (December 2002)| doi:10.1038/nrm972**

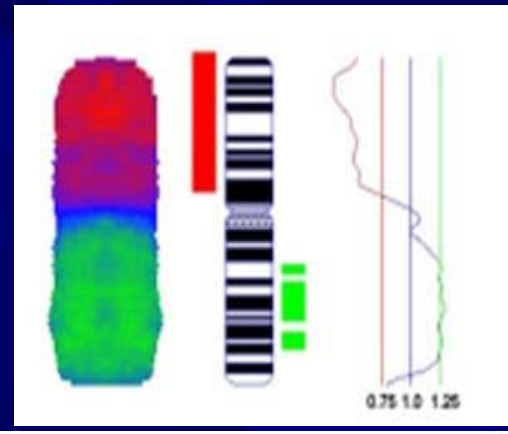
**Sanhita Gupta et al: Am J Respir Cell Mol Biol Vol 33. pp 363–370, 2005**

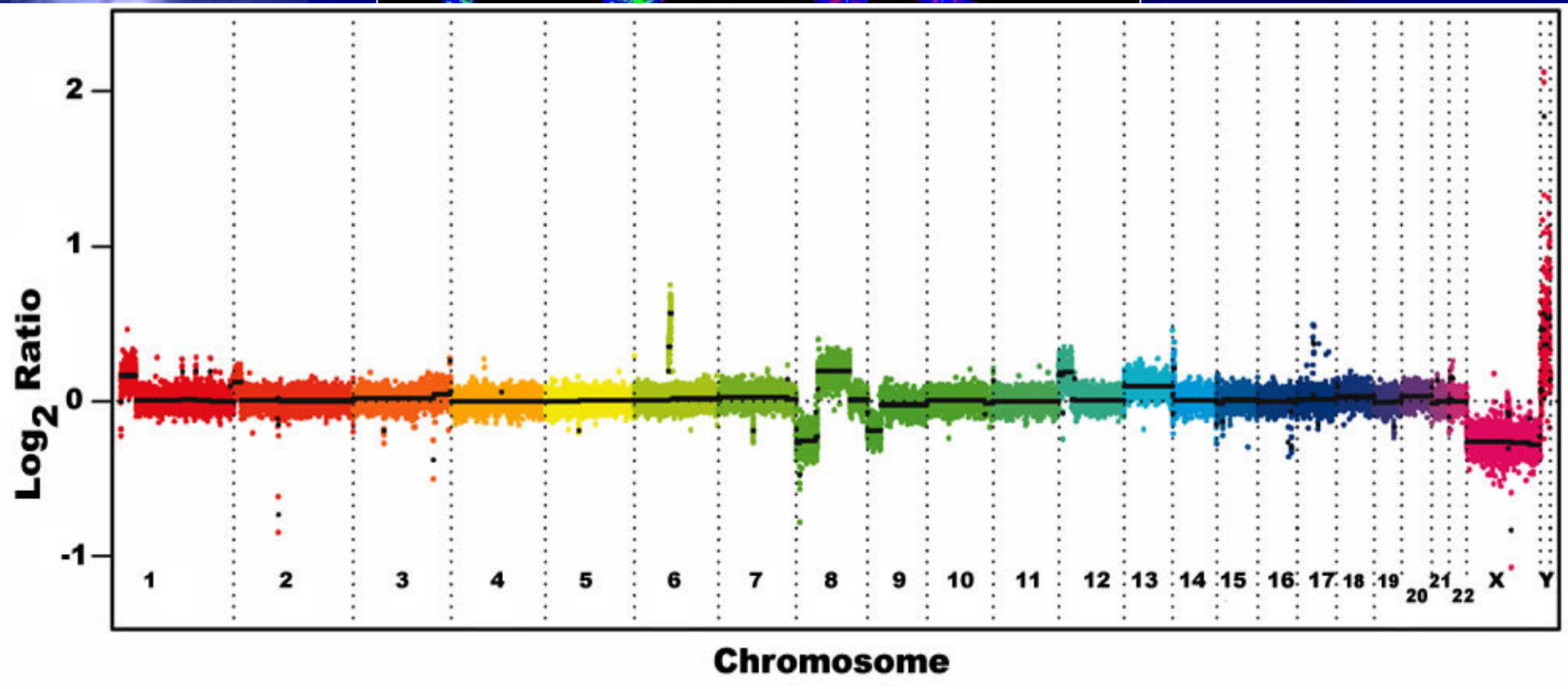
# *Nasopharyngeal Carcinoma, Histogenesis*

- Somatic genetics
  1. Cytogenetics
  2. Comparative Genomic Hybridization
  3. Molecular genetic alterations
  4. Expression profile/ Protiomics

# Comparative Genomic Hybridization





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# *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)

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- *1-Chan AS. et al: Int J Cancer 102:300-303, 2002*
- *2-Chan AS. et. Al: Cancer Res 60:5365-5370:2000*

# *Molecular Genetic Alterations*

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## 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**

[CANCER RESEARCH 61, 3877–3881, May 15, 2001]

*Advances in Brief*

# High Frequency of Promoter Hypermethylation of *RASSF1A* in Nasopharyngeal Carcinoma

Kwok-Wai Lo,[1] 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*

***Kwong J. et al:. Clin Cancer Res 8:131-137, 2002***

# *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

***Kwong J. et al: Clin Cancer Res 8:131-137, 2002***

Oncogene (2006) 25, 310–316

www.nature.com/onc

SHORT COMMUNICATION

# Identification of *RASSF1A* modulated genes in nasopharyngeal carcinoma

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

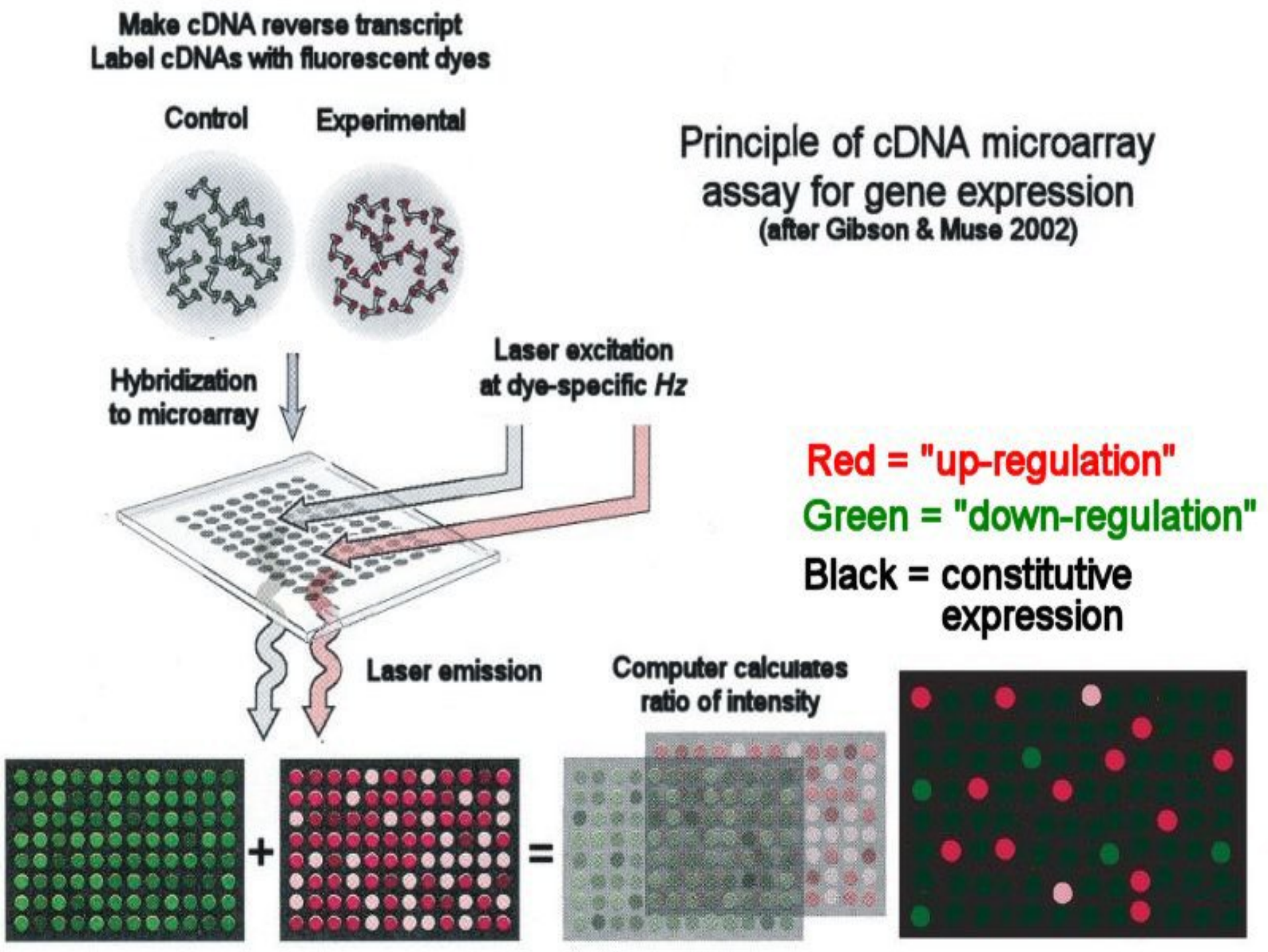
*[1]Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR; [2]Department of Chemical Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR; [3]Department of Anatomy, University of Hong Kong, Hong Kong SAR and [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.**

*L S-N Chow* ..et al: *Oncogene* (2006) **25,** 310–316.

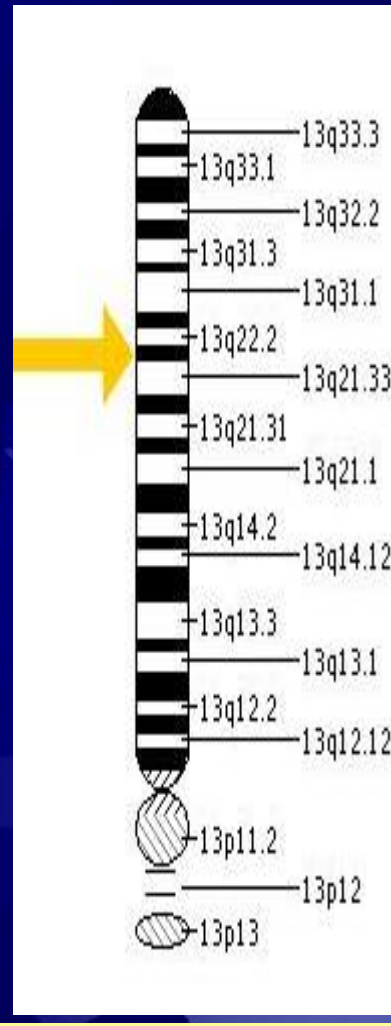
# Principle of cDNA microarray assay for gene expression

(after Gibson & Muse 2002)



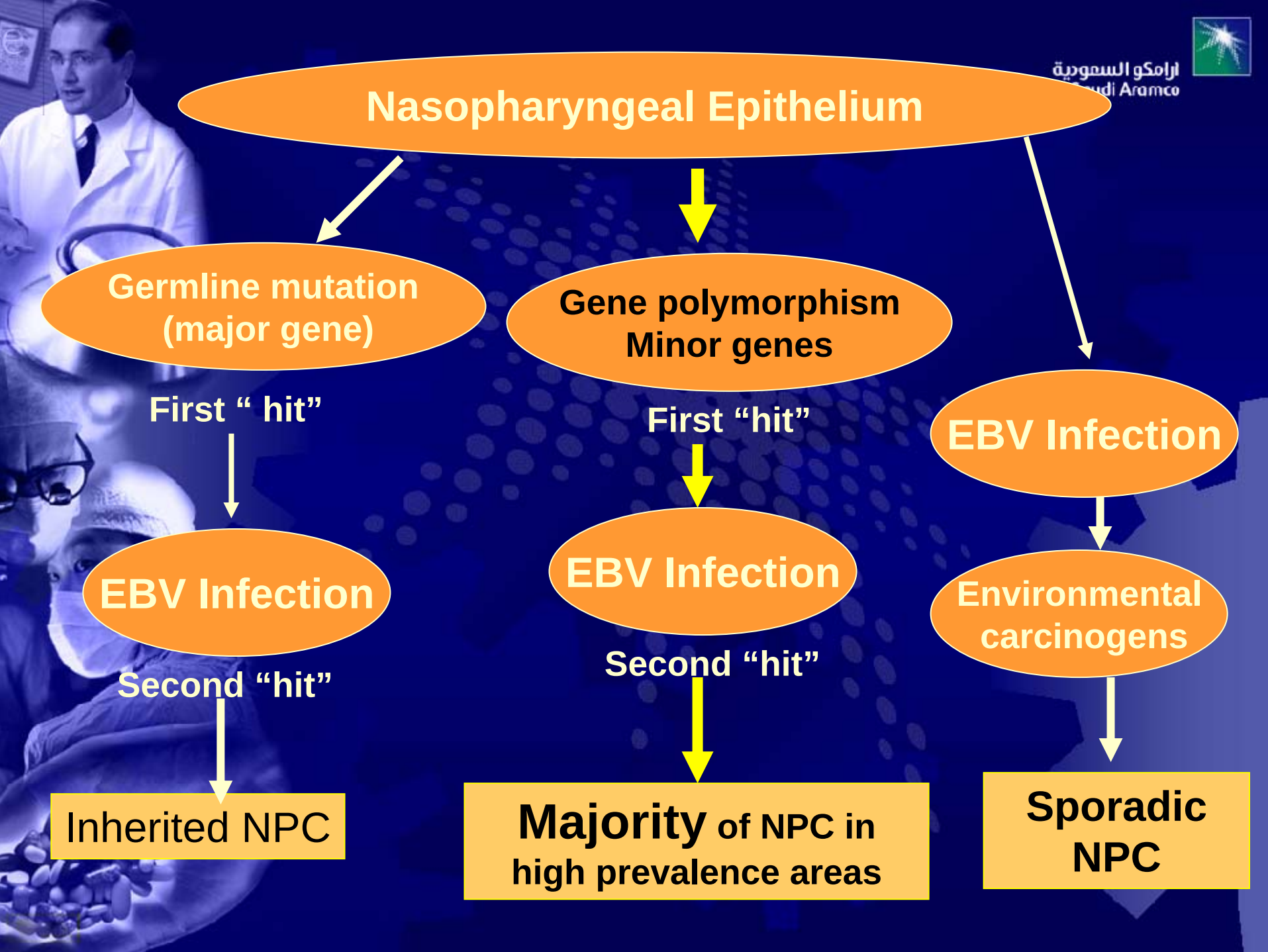
# *Molecular Genetic Alterations*

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

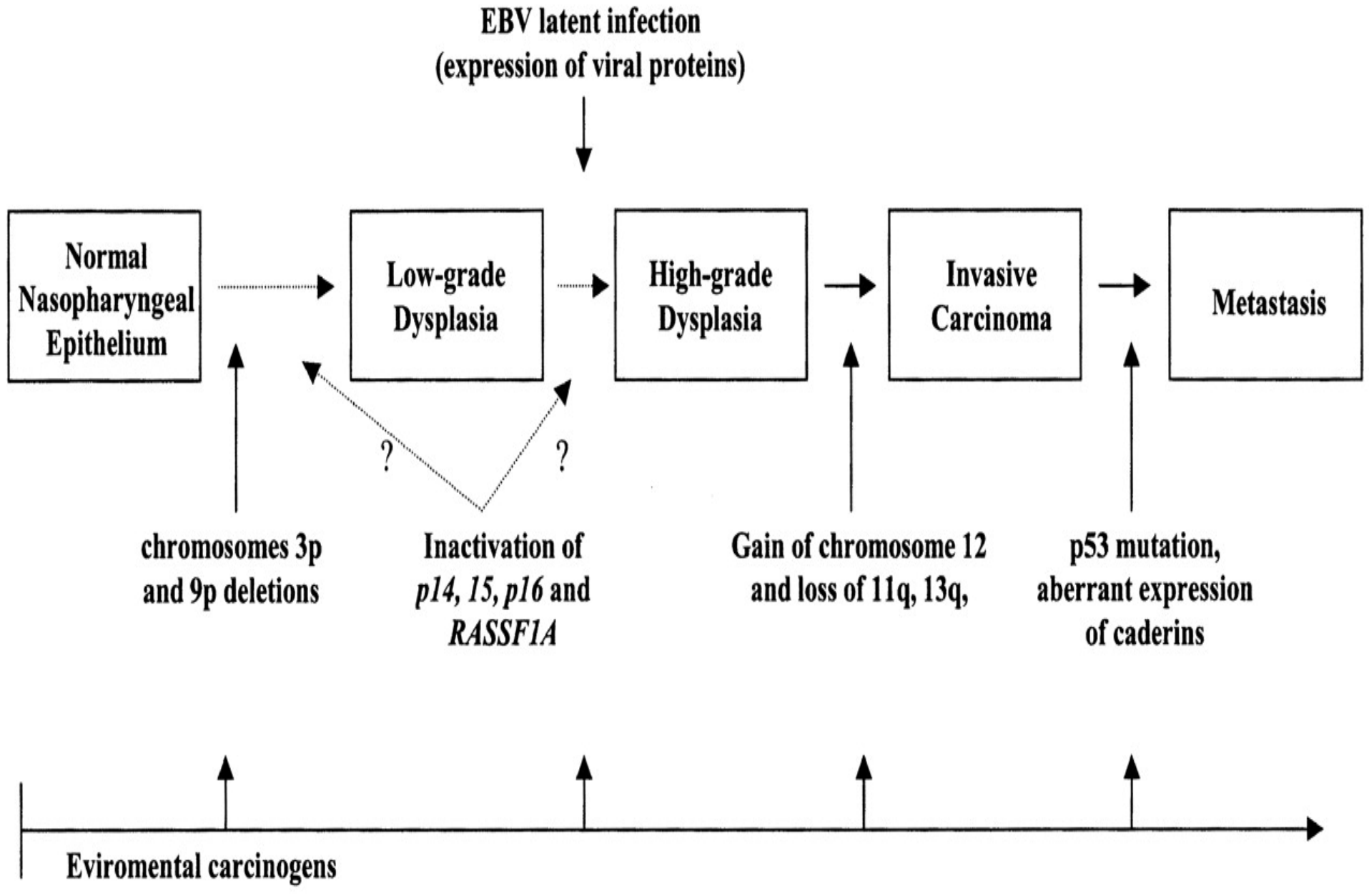




1. Kwong J. et al: Clin Cancer Research 8: 131-137, 2002
2. Lo KW et al: Int J Cancer : 98:651-655, 2002.


Nasopharyngeal Epithelium
Germline mutation (major gene)
Gene polymorphism Minor genes
EBV Infection
First " hit"
First "hit"
EBV Infection
EBV Infection
Environmental carcinogens
Second "hit"
Second "hit"
Inherited NPC
Majority of NPC in high prevalence areas
Sporadic NPC

# A.T.C. Chan, et al: *Annals of Oncology* 13:1007-1015, 2002



# *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.

# *Serum, Epstein Barr Virus & NPC*

- EBER-1 DNA in serum to monitor chemotherapeutic response. (1)

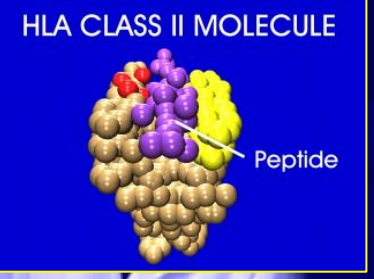
- The plasma EBV EBER-1 DNA load is proportionately related to the presence of NPC(2)


1. **Ngan RK, *Ann N Y Acad Sci* 2001, 945:73-79.**
2. **Tan EL, Looi LM: Singapore Med J 2006, 47:803-807**

# *Molecular ,Targeted Therapy, siRNA technique*

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


1. **Zhonghua Er Bi ; Yan Hou Tou Jing Wai Ke Za Zhi. 2005 May;40(5):347-51.**
2. **Weng DS, *Nan Fang Yi Ke Da Xue Xue Bao* 2006, 26:71-74.**
3. **Shi W; *Int J Cancer* 2006, 119:2467-2675.**
4. **Li G *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2005, 40:406-410.**

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# *Targeted, Immunotherapy*

- Boosting LMP2-specific CTL response

1. **Lee SP, *J Immunol* 2000; 165: 573–582.**
2. **A. T. C. Chan,*Annals of Oncology* 13:1007-1015, 2002**
3. **A.T.C. Chan , Annals of Oncology13:1007-1015, 2002**

# EBV, Vaccines

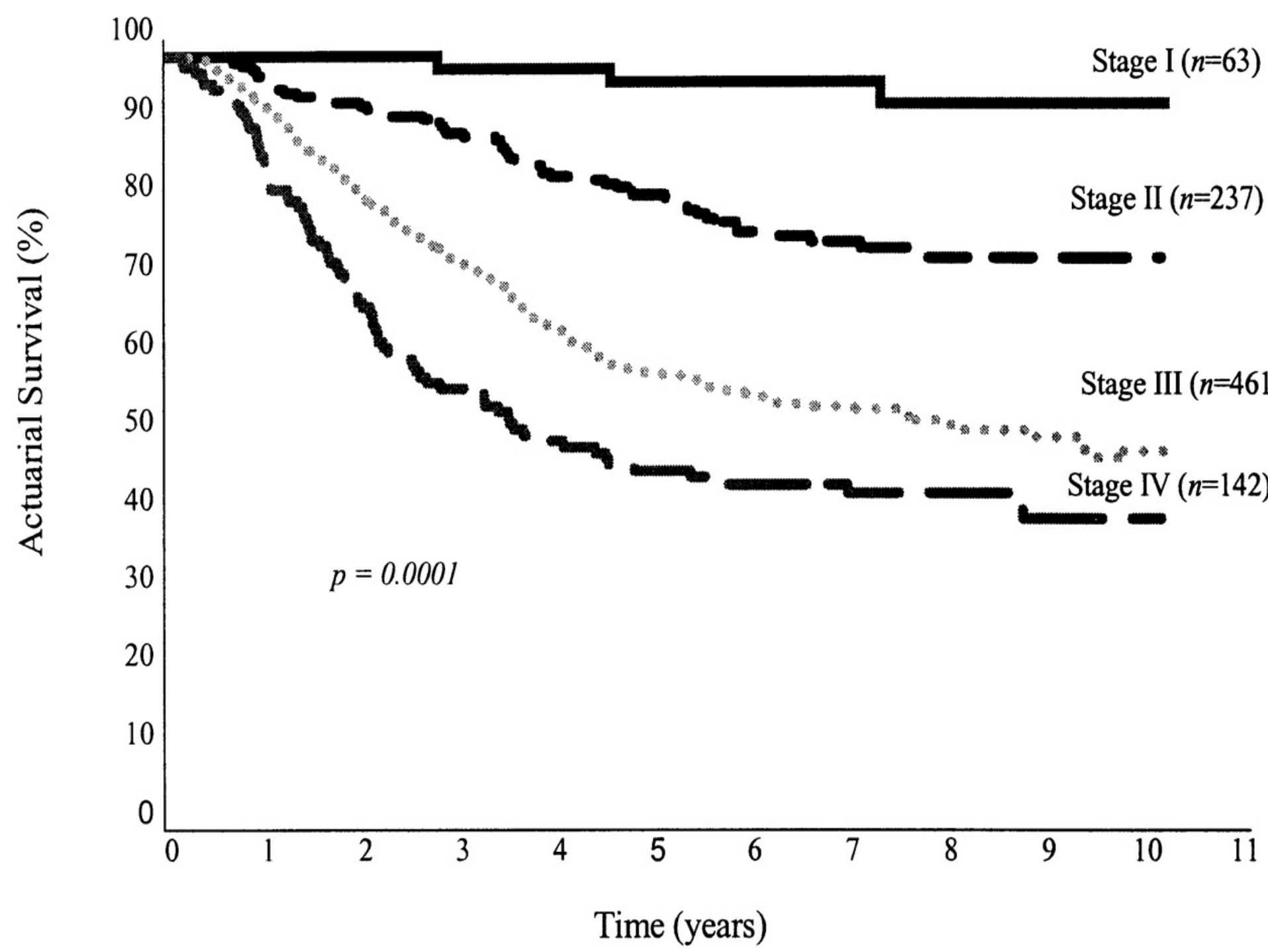
1. **Major virus surface glycoprotein GP 220/350**
   - MedImmune & GlaxoSmithKline (GSK)
   - Safe in humans but needs strong adjuvant
   - Clinical trials up to Phase 3

# EBV, Vaccines.

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.


100
90
80
70
60
50
40
30
20
10
0
Actuarial Survival (%)
Stage I (n=63)
Stage II (n=237)
Stage III (n=461
Stage IV (n=142)
p = 0.0001
0
1
2
3
4
5
6
7
8
9
10
11
Time (years)

# *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

**Lo YMD,.et al *Cancer Res* 2000; 60: 6878–6881**

EBV infection + genetic susceptibility = NPC

Environmental factors is more effective in genetically susceptible population

NPC incidence in KSA is similar to countries with moderately higher incidence

**Non-invasive NPC Dx:** EBV DNA load + *BARF1* glycoprotein mRNA on NP brushing

*LMP-1 is an* oncogene playing an essential role in cell transformation and metastasis.

**•HLA ~ Susceptibility ~ Prognosis~~ Survival**

## *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.*

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# *Incidence of EBER-1 in 140 NPC**

| Type | % |
|---|---|
| WHO I (N=5) | 80 |
| WHO II (N=73) | 97.3 |
| WHO III (N=62) | 96.8 |

Tsai S-T, et al, Cancer 77:231, 1996

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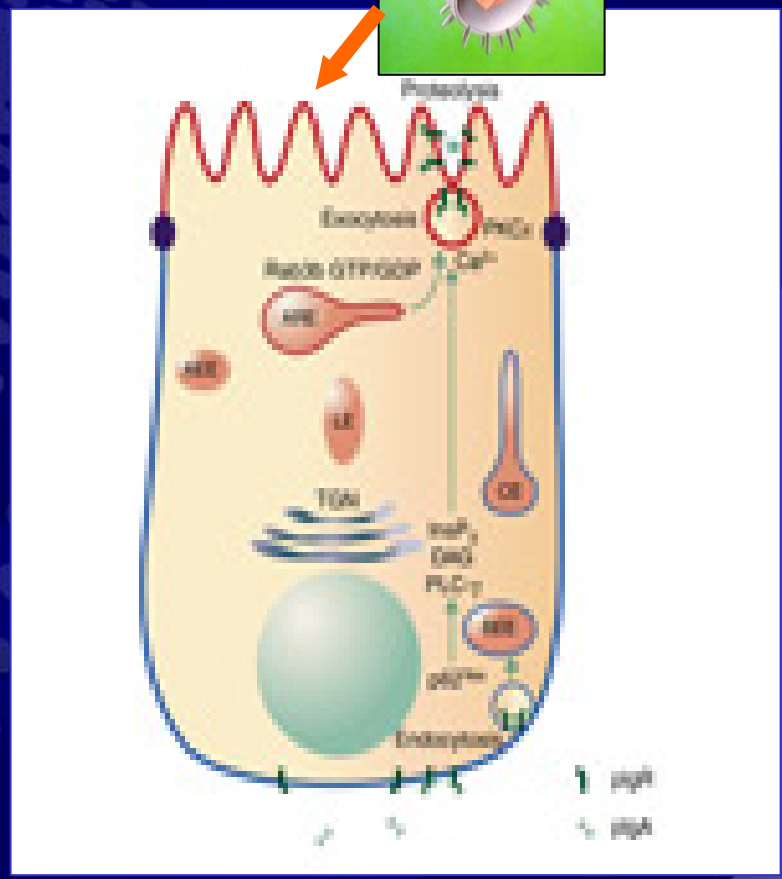
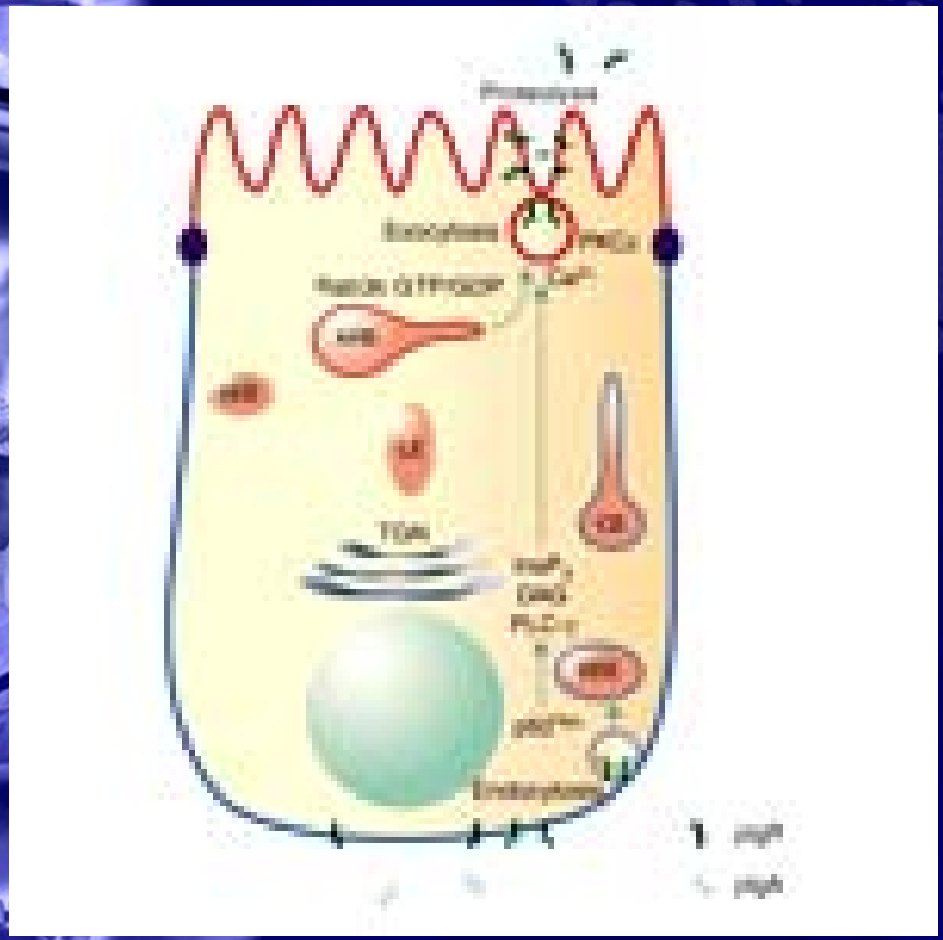
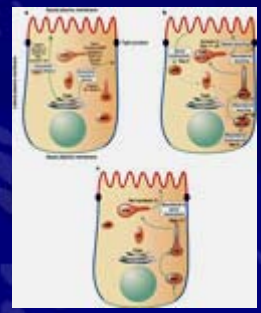
# *Expression of HER2 and C-KIT in nasopharyngeal carcinoma: implications for a new therapeutic approach.*

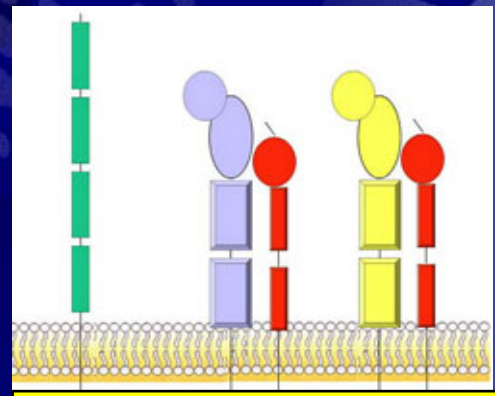
# 4- Polymeric Immunoglobulin Receptor (PIGR)

- 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 atypica 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.



# *NPC Etiology Conclusion*

- 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 world71.

# Nasopharyngeal carcinoma: molecular biomarker discovery and progress

William Chi-shing Cho*

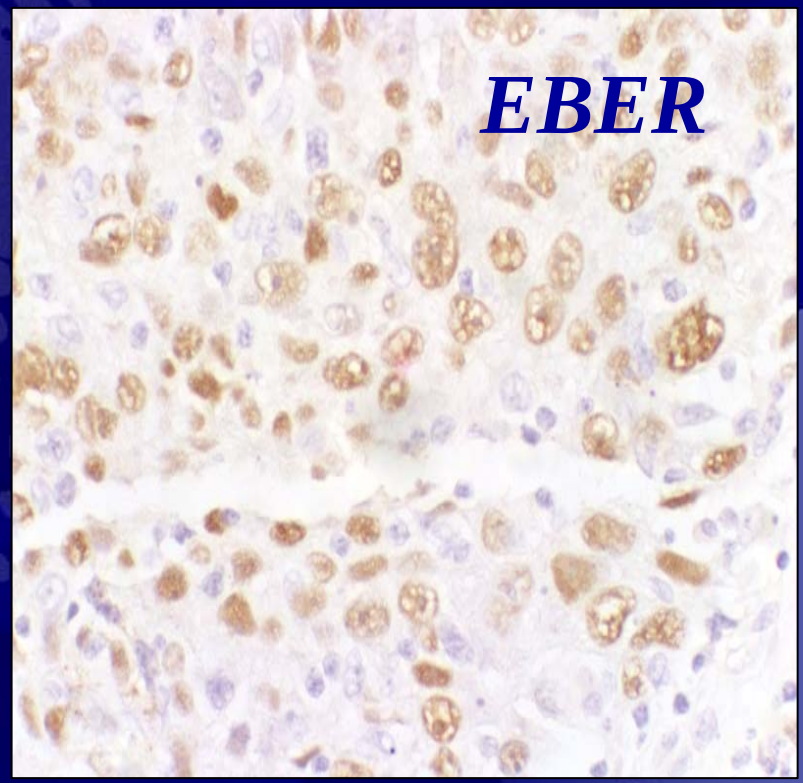
*Molecular Cancer* 2007, **6**:1

**Table 1: Biomarkers identified by proteomics technologies in nasopharyngeal carcinoma**

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

# *Epstein Barr Virus*

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



# *NPC & EBV*

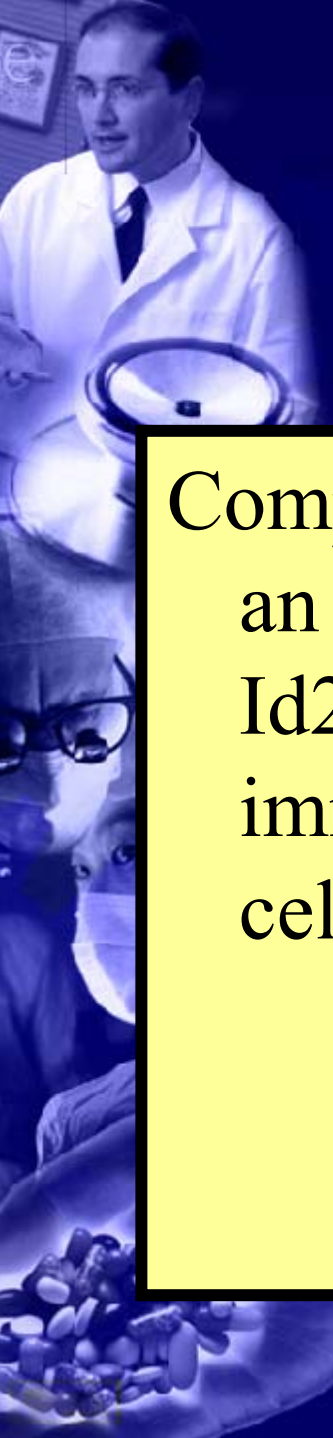
- Combination of salted fish and EBV was strongly associated with NPC, compared to EBV or salted fish alone.
- IgA-VCA was the most important predictor of NPC, followed by fish. (Zheng et al 60)

# *NPC – Treatment and Prognosis*

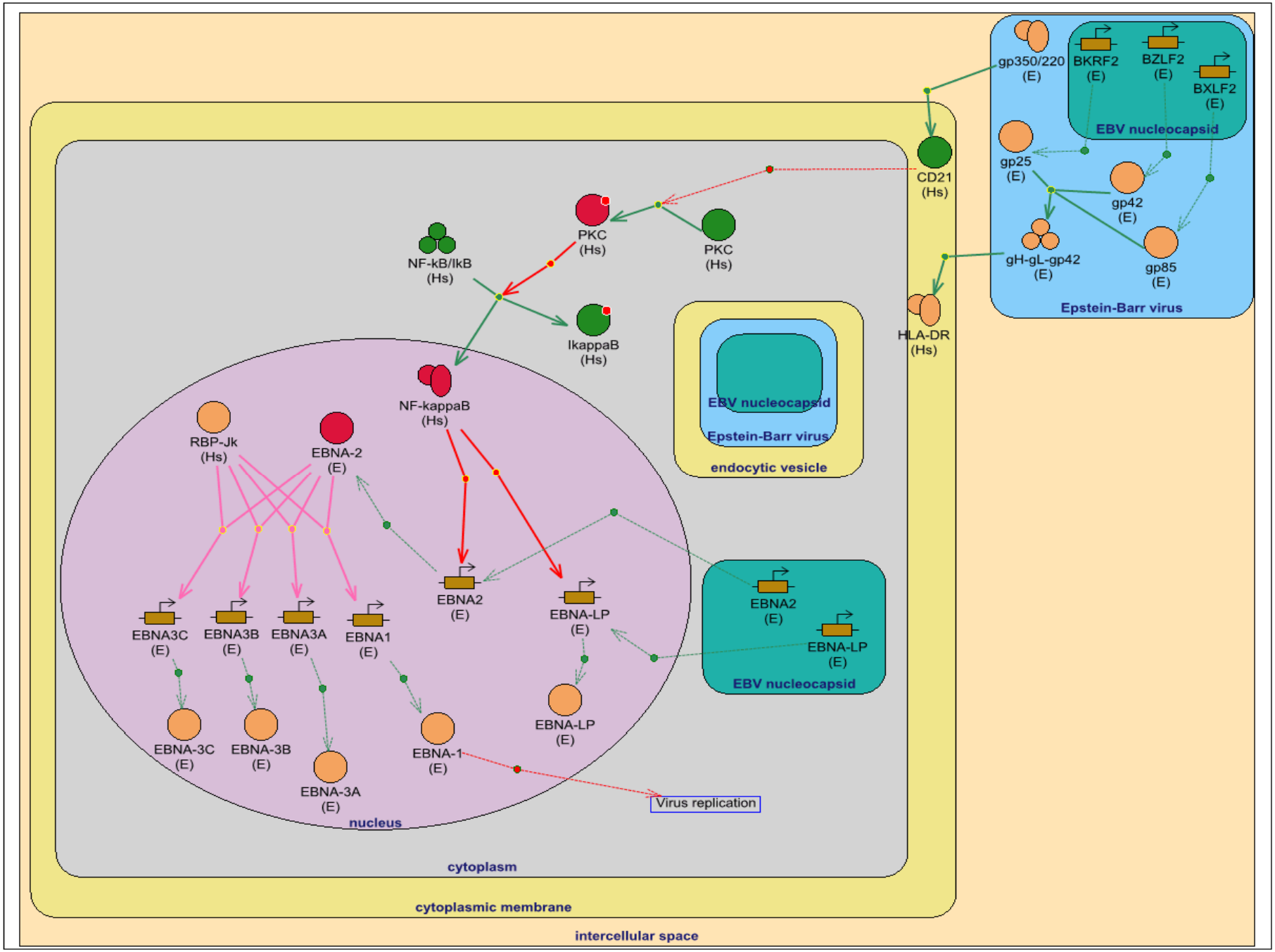
- Radiation
- May take up to 10 weeks for tumor to disappear histologically
- If post-treatment biopsy is still positive after 10 weeks, additional treatment needed
- 20-60% mets below clavicles (lungs, liver and bones)
- Over 90% of local and distant failures appear within 3 years of treatment

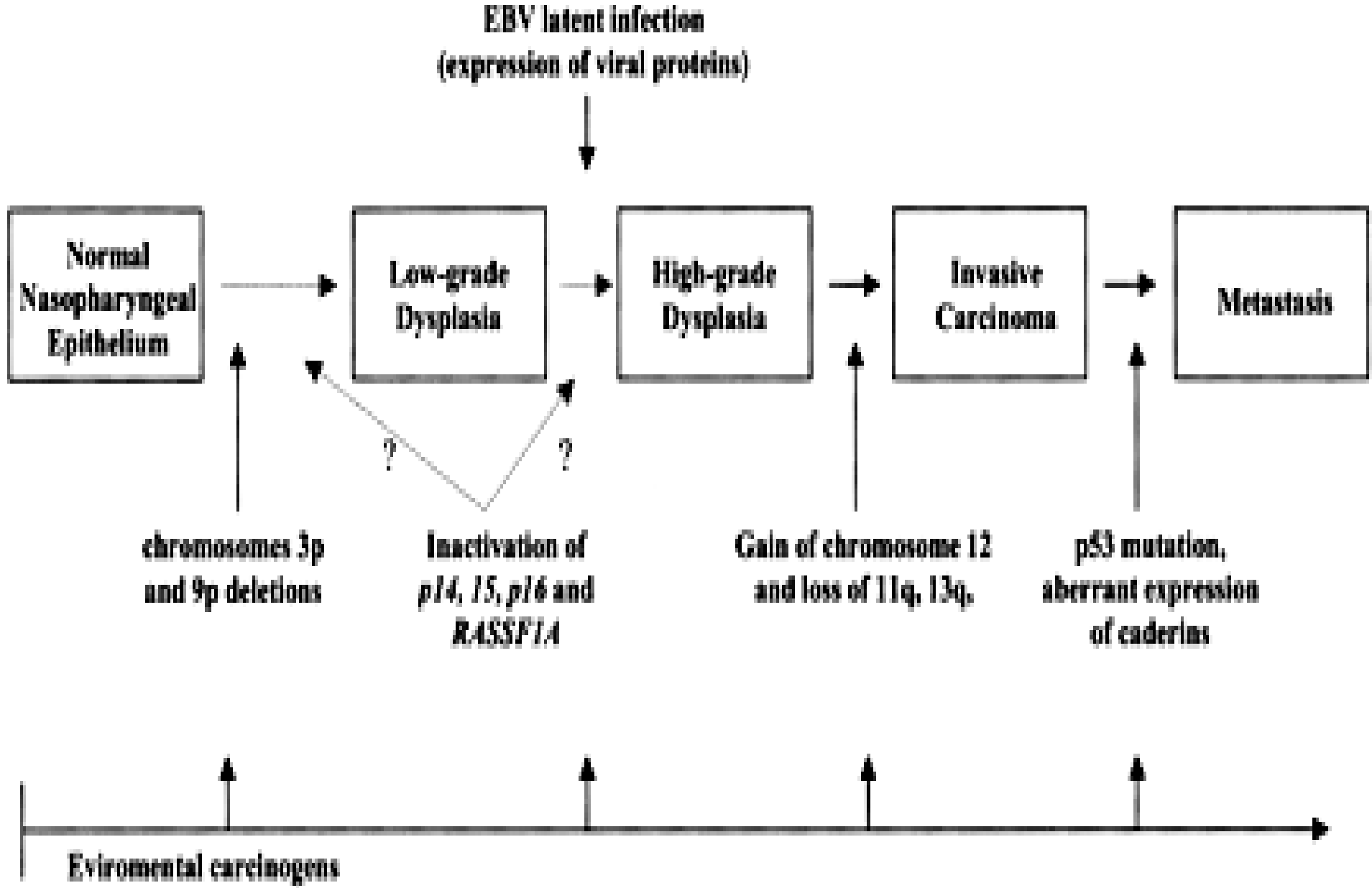
# *Multiple dysregulated pathways in nasopharyngeal carcinoma revealed by gene expression profiling.*

- 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


gp350/220
(E)
BKRF2
(E)
BZLF2
(E)
BXLF2
(E)
EBV nucleocapsid
gp25
(E)
gp42
(E)
gH-gL-gp42
(E)
gp85
(E)
Epstein-Barr virus
CD21
(Hs)
HLA-DR
(Hs)
PKC
(Hs)
PKC
(Hs)
NF-kB/IkB
(Hs)
IkappaB
(Hs)
EBV nucleocapsid
Epstein-Barr virus
endocytic vesicle
NF-kappaB
(Hs)
RBP-Jk
(Hs)
EBNA-2
(E)
EBNA3C
(E)
EBNA3B
(E)
EBNA3A
(E)
EBNA1
(E)
EBNA2
(E)
EBNA-LP
(E)
EBNA2
(E)
EBNA-LP
(E)
EBV nucleocapsid
EBNA-3C
(E)
EBNA-3B
(E)
EBNA-3A
(E)
EBNA-1
(E)
EBNA-LP
(E)
Virus replication
nucleus
cytoplasm
cytoplasmic membrane
intercellular space


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 caderins
Evironental 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.

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# *EBV-encoded* LMP-1 *& RNAi*

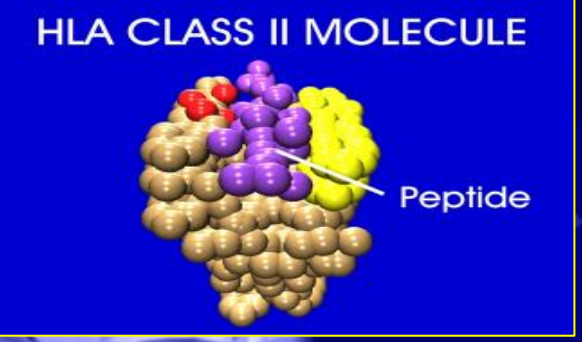
- EBV-encoded *LMP-1* was vulnerable to RNAi and selective inhibition of *LMP-1* had anti-proliferation effect on NPC cell.
- RNAi could be a powerful tool in further investigations of *LMP-1*
- A novel therapeutic strategy for associated NPC patients

# *[Effect of silencing epidermal growth factor receptor expression by RNA interference on the growth of nasopharyngeal carcinoma cell 5-8F]*

- 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.
- Am J Pathol 2001 Jul;159(1):27-33

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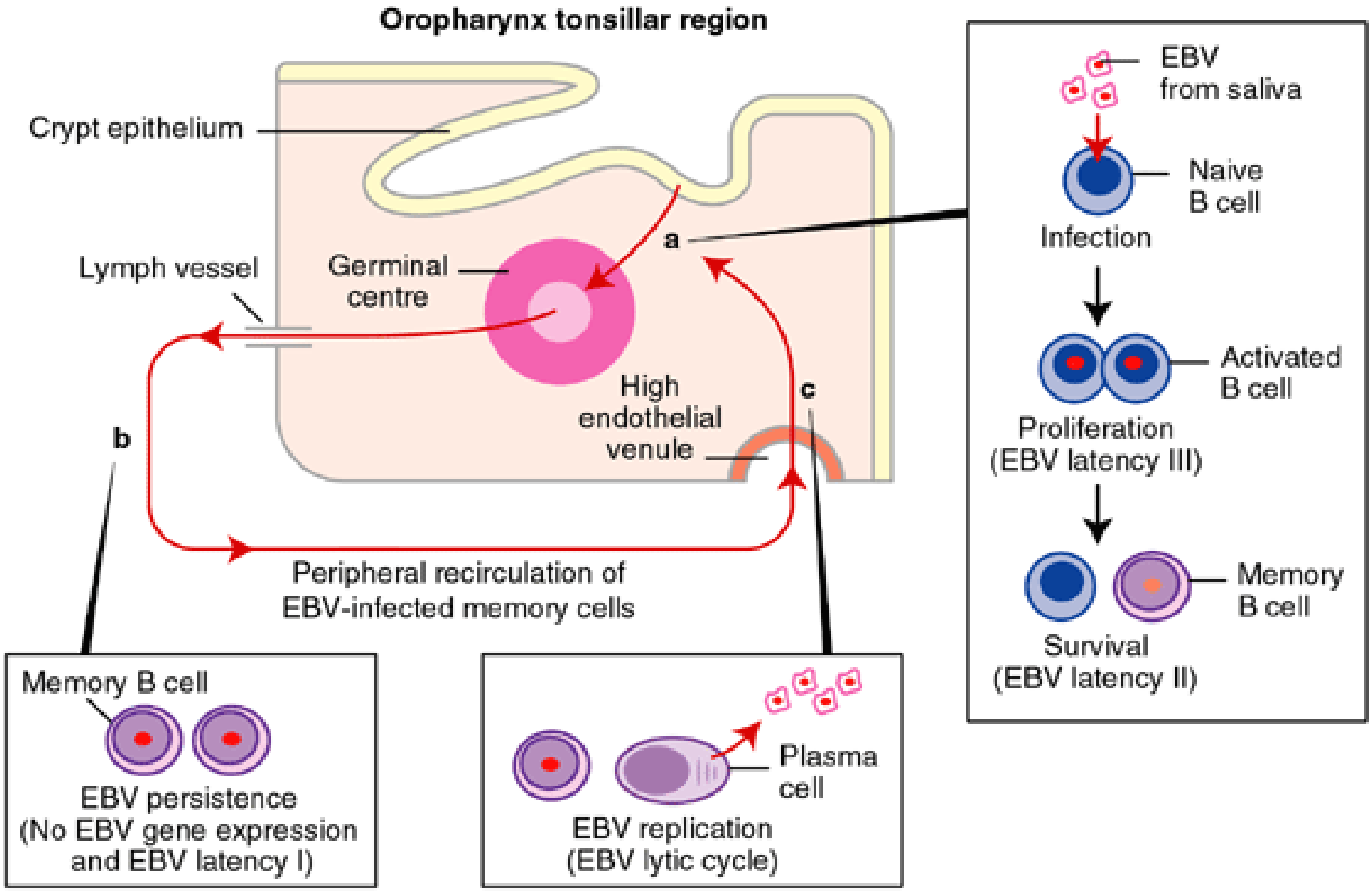
# *HLA & NPC*

- 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.T.C. Chan et al Annals of Oncology13:1007-1015, 2002 (Review)




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

ctgcctcatgggacctcactctgacaggctgacata
gcagtggtggccgtgatgaccacgtcccccaaactt
gcaacaggatacatggaggtggatggagaaatgtcc
agggtagttagtgaccatatgggggcaggaaaagat
tcatttggaagaaaatcagcagcatttgtttgtttg
ggggaacatccacgagcagattcatctggtggggac
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tcgtcgtcaccatcatcaccaccatcatcatcatca
tcatcatcatcatcatcatcatcaaattttatatat
taacgggcttccatgtgctggttcatcatgtgctta
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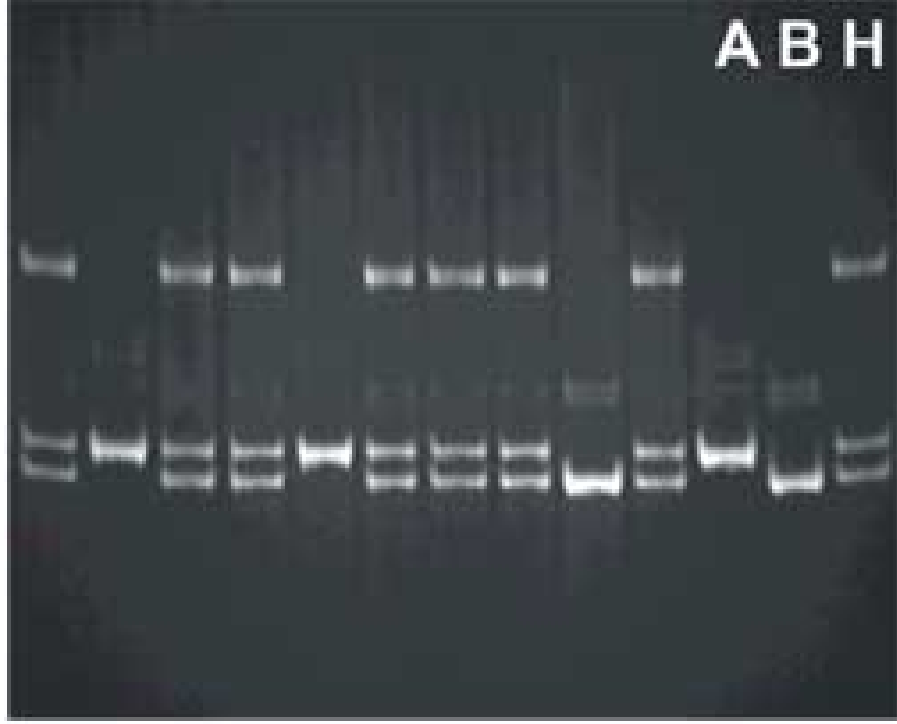


gctgcaggtcgactctagaggatccccccttggtta
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gcttccatgttcccagtcaccccatctttacttctc
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cacacacacacacacacacacacacacacacacaca
cacacacacactgttccatattccaaacttccaggt
ttccctttttgttgtatctaattttctatcaagtcta
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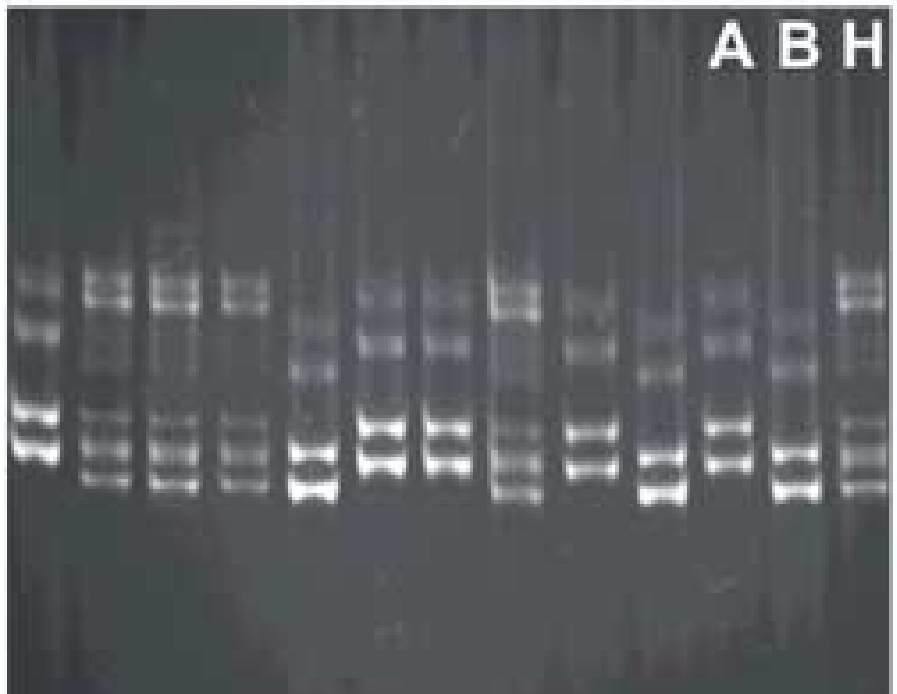
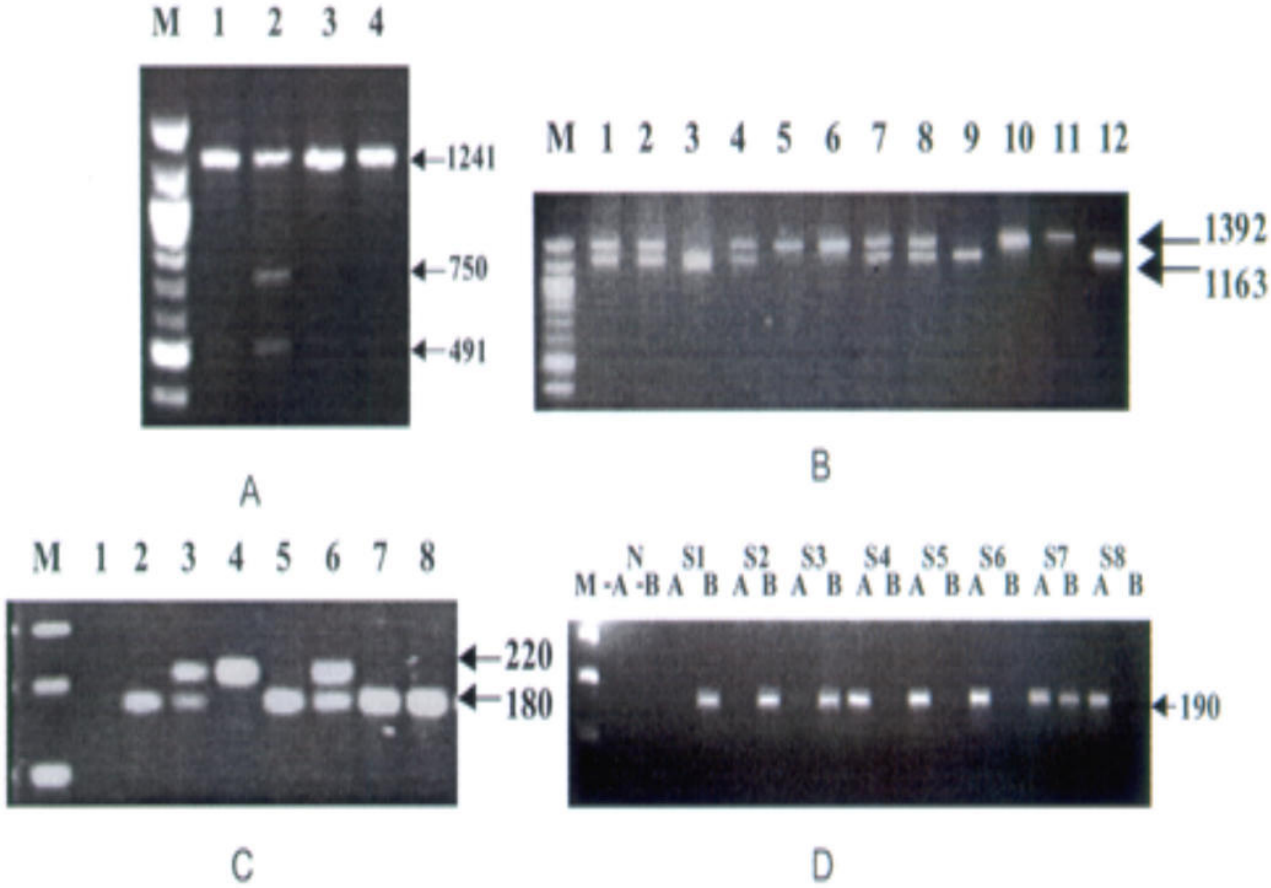


# *2- Polymorphism of Metabolic Enzymes GSTM1*

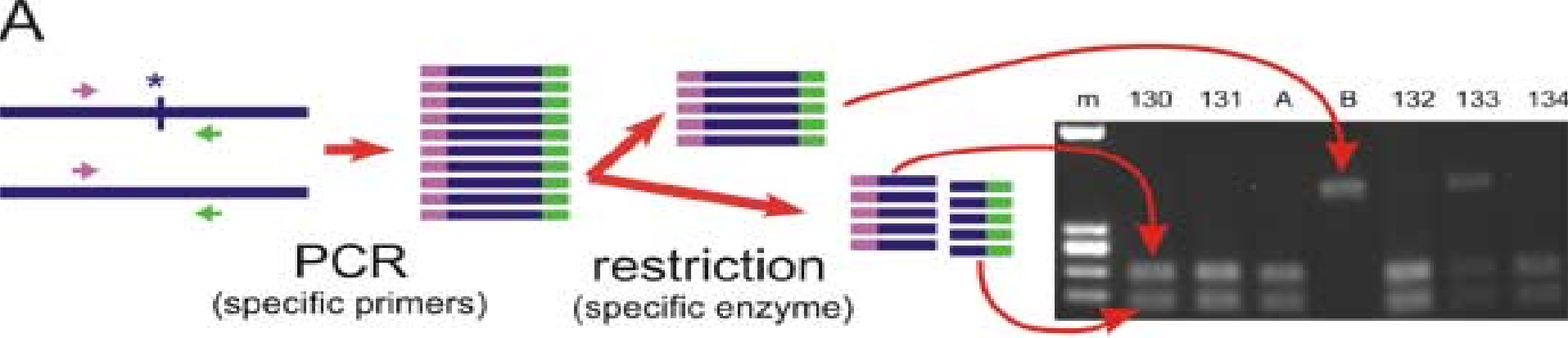
- Glutathione S- Transferase M1 Detoxifies benzopyrene and other carcinogens in tobacco smoke

Cheng Y J et al: Cancer Epidemiol Biomarkers Prev 12: 179-180

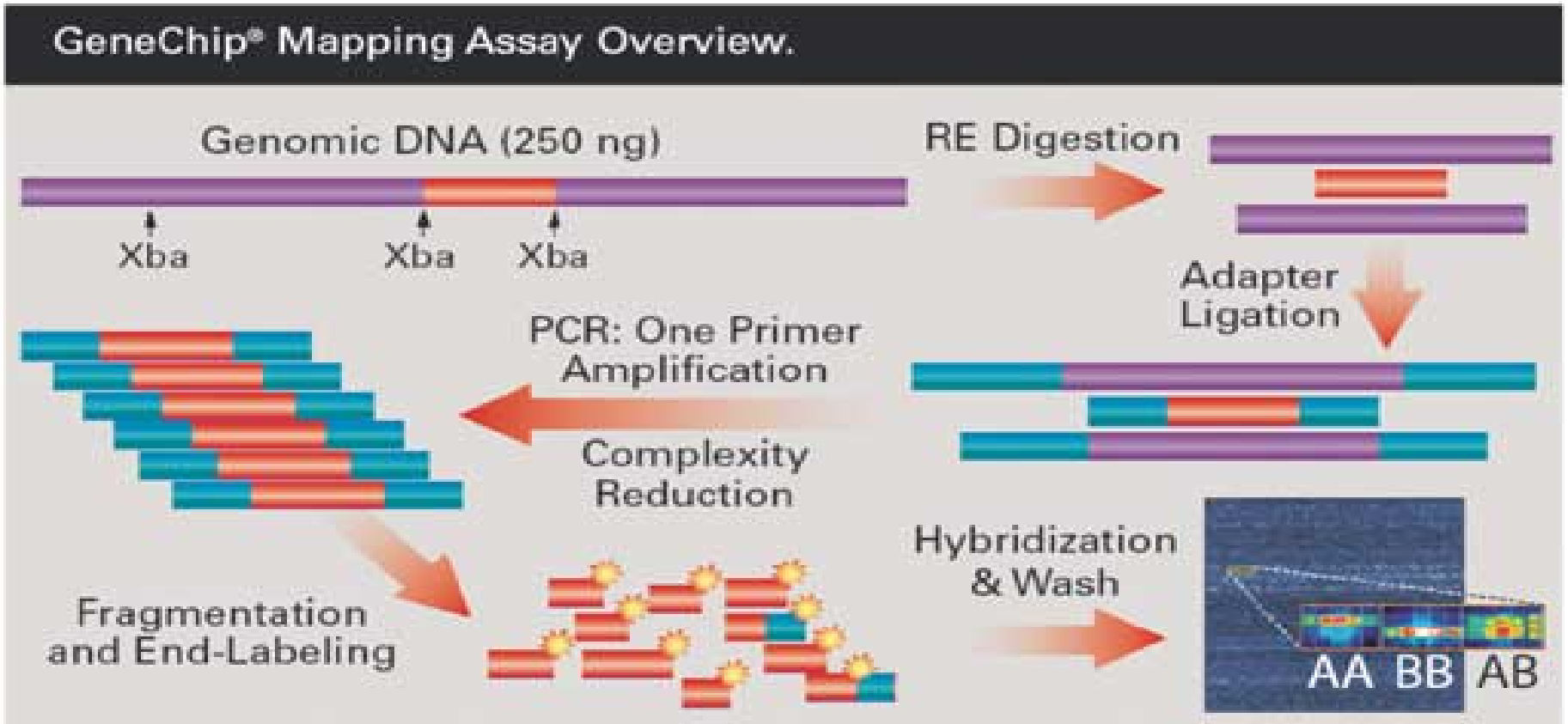



http://www.biomedcentral.com/1471-2156/4/3

A


PCR
(specific primers)
restriction
(specific enzyme)
m
130
131
A
B
132
133
134

B


GeneChip® Mapping Assay Overview.
Genomic DNA (250 ng)
Xba
Xba
Xba
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**

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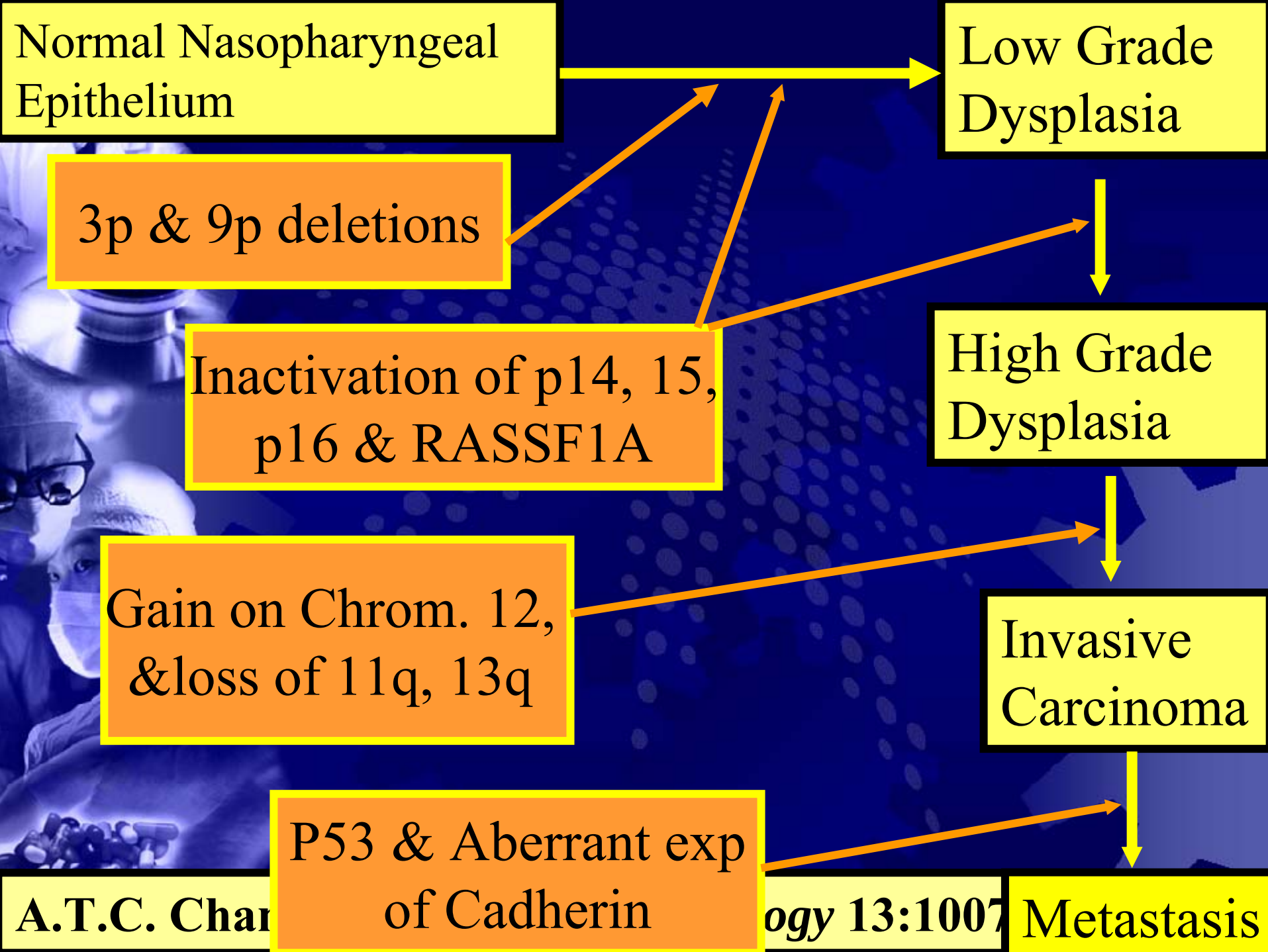
- Feng BJ et al: (2002) Nat Genet 31: 395-399

# *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

# *Prognosis and Molecular Markers, Cell free, Plasma EBV-DNA Levels*

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***
2. ***Chan ATC,. Proc Am Soc Clin Oncol 2001; 20***