Background: To achieve greater understanding of the epidemiology, pathogenesis, molecular oncology, diagnostic, and therapeutic aspects of nasopharyngeal cancer (NPC), an international meeting was held in June 2005, Toronto, Canada.

Results: Further insights were obtained into the role of EBV in NPC development, with its diverse effects ranging from proliferative signals via NF-κB, to immunesuppression, to angiogenic gene regulation. Subsequently, multiple pathways are dysregulated in NPC as revealed by expression array analyses, including apoptosis, integrin, and B-catenin cascades. Advances have been made in the diagnosis and monitoring of NPC, using transoral brushings and plasma levels of EBV transcripts, which may not directly correlate with the number of circulating tumor cells, but is nevertheless informative in predicting and tracking disease response. Many novel therapies have promising results, particularly in the areas of immunotherapies, and the exploration of molecularly targeted approaches such as cetuximab or histone deacetylase inhibitors.

Conclusions: The results from large randomized trials and meta-analyses have consistently demonstrated the benefit of concurrent chemotherapy with curative radiation therapy, but at a cost of greater acute and late-tissue toxicities. Further advances are required to achieve an improved understanding on the inter-relationship between environmental and genetic determinants in NPC development, to reduce the global burden of this disease. At the same time, novel therapeutic approaches are necessary to increase curability of NPC, but with reduced long-term toxicities. © 2007 Elsevier Inc.

Nasopharyngeal cancer, Epstein-Barr Virus, Epidemiology, Clinical trials, Radiation therapy, Expression arrays, Chemotherapy.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a unique malignant head/neck cancer with distinct clinical, demographic, and geographic features from other head and neck epithelial malignancies. First, NPC patients are ~1 decade younger than patients with squamous cell head and neck cancers. Second, there are geographic regions in the world where NPC is endemic, such as in Southeast Asia, or northern Africa, where the annual incidence can be up to 1/4000 (1). Third, NPC has a unique biology with its intimate association with the Epstein-Barr Virus (EBV), whereby its genome is identified in 80% of cases worldwide (2, 3), yet whose complex role in NPC development remains to be clearly elucidated. Conventional treatment for NPC comprises of curative radiation therapy (RT), with or without chemotherapy (CT). The 5-year overall survival rates for patients with locally advanced diseases are still hovering around 55–60%, underscoring significant opportunities for improvement (4).

Given the global distribution of NPC and the broad range of research expertise spanning this disease, an East-West Symposium was held with the purpose to promote exchange of information, and increase international collaborations, scientific and Organizing Committee (Jean Bourhis, Pierre Busson, Anthony Chan, Marilyns Corbex, Jamel Daoud, Anne Lee, Tamamasa Ooka, Nancy Raad-Traub), and enthusiastic participation from our international faculty (Kian Ang, Paul Farrell, Lindsey Hutt-Fletcher, Shannon Kenney, Rajiv Khanna, Jaap Middeldorp, Kenzo Takada, Lawrence Young, Yi-Xin Zeng), and local faculty (Andrew Bayley, Bernard Cummings, Ralph Gilbert, Lee Manchul, David Payne, Jolie Ringash, Lillian Siu, John Waldron). Conflict of interest: none.

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with the ultimate objective to reducing the global burden, and improving outcome for NPC patients. This event took place in June 2005 in Toronto, Canada sponsored by UICC, the University of Toronto, Canadian Institutes of Health Research, the National Cancer Institute of Canada, and members of industry. This meeting was also dedicated to the memory of Dr. Dolly Huang, a pioneering and dedicated researcher in NPC, whose seminal work has contributed significant insight into NP carcinogenesis (5). The current paper summarizes the key observations reported from this Symposium.

**Epidemiology**

Preliminary data were presented from a detailed epidemiologic study conducted on 1200 NPC cases and controls in northern Africa (Morocco, Algeria, and Tunisia), to determine life-style and environmental factors, which might be associated with NPC development (6). After adjusting for confounding variables, preliminary observations noted that NPC was associated with poor socio-economic conditions such as living in crowded quarters, co-habitation with animals, and household fumes. These findings are similar to those previously reported for Asian studies (1), thereby providing the necessary data to develop a framework for prevention programs to reduce the global burden of this cancer.

A large EBV serology study conducted on 669 Inuit children in Greenland demonstrated a high prevalence of VCA-IgA positivity in early years (less than age 4), with 60% being serology positive in the age 5–11 year group. This serology positivity was not related to any environmental determinants or triggers, suggesting a strong genetic regulation in EBV immune response in this population, which might also predispose such patients to developing EBV-associated malignancies in later life (7).

The first full-length EBV genome in NPC patients was deposited in GenBank (8) denoted as the GD1 (Guangdong strain 1), which differed from the previously deposited EBV strain from B95.8 (9), originally derived from a patient with infectious mononucleosis. The entire sequence of GD1 was 171,656 bp in length, with significant variations compared to the B95.8 strain, including 43 deletions, 44 insertions, and 1,413 point mutations. This strain is highly prevalent, being identified in >90% of Cantonese patients with NPC, thereby providing a critical tool with which to derive further insights into the contribution of EBV to NP carcinogenesis.

**EBV and NPC development**

The complexities of EBV in NPC never fail to intrigue Epstein-Barr virologists. How EBV gains entry into epithelial cells remains to be completely understood, but the core function of gH (a conserved glycoprotein common to all herpes viruses) fusion with B-lymphocytes and epithelial cells appear to be the same, but is activated differentially, and likely requires the presence of a putative co-receptor, when EBV infects epithelial cells (10). The gp42 protein, which is required for B-cell entry, appears to be antagonistic for epithelial entry, suggesting that the virus has evolved mechanisms to modulate gp42 levels to facilitate viral shuttling between the 2 cell types. It was also observed that saliva enhances EBV entry into epithelial cells, but inhibits entry into B-cells, although the components, which are responsible for this effect, remain unknown.

Among its many latently expressed genes, EBNA1 interacts with EBP2 (as in Fig. 1), regulated by aurora family kinases during mitotic segregation (11). EBNA1 also binds the deubiquitinating enzyme USP7, which attaches and stabilizes p53 in response to DNA damage. EBNA1 was shown to block the USP7-p53 interaction, which enables cells to avoid apoptosis under experimental conditions (12). This might therefore allow EBV infected cells to survive and accumulate DNA damage, thereby facilitating malignant transformation. LMP2A and 2B inhibit basal and IFN-stimulated STAT/IRF activity, thereby blocking cellular anti-viral response, which has implications for EBV persistence in epithelial cells (Fig 1) (13, 14). Finally, EBV-encoded RNA’s (EBER) promotes growth of malignant cells, mediated through at least IGF-1 (Fig 1) (15).

Using heteroduplex tracking assay technique, distinct LMP1 polymorphisms can be evaluated (16); different LMP isoforms are differentially associated with the ability to escape recognition by cytotoxic T-lymphocytes (CTL), which in turn contribute to EBV-associated diseases, and might also facilitate EBV persistence (Fig 1). The EBV in C666-1 cells (17), heretofore the classical EBV-positive NPC model, appears to have a viral gene expression pattern distinct from that conventionally reported in NPC, but more consistent with humoral immune response observed in NPC patients, underscoring the importance in closely examining EBV gene expression in NPC (18).

A new membrane-based binding partner for LMP1 has been identified as galectin 9, a β-galactoside binding lectin and Hodgkin tumor antigen (19). Galectin-9 expression is abundantly expressed in NPC biopsy specimens, but absent from Burkitt lymphoma, and likely mediates LMP1 trafficking and signaling. The uncertain role of BARF1 in NPC was clarified by the demonstration that it was a rapidly secreted protein, which might explain its abundance at the transcriptional level, yet uncommonly detected in primary NPC tissue samples using immunoblotting (20). Its expression is independent of the presence of other EBV lytic genes, suggesting that it is latently expressed, and thereby might contribute to NP carcinogenesis.

Another interesting observation relates to the potential role of lytic EBV genes, such as BZLF1, which is observed to promote angiogenic growth. This process is mediated at least via increased VEGF translation or secretion, suggesting that lytically infected cells could contribute to growth of EBV-associated malignancies by enhancing angiogenesis (Fig. 1) (21). This possibility is also supported by the observation that tumor development in mice was slower in the absence of BZLF1.
Molecular translational studies in NPC

Results from expression array studies were presented, demonstrating multiply dysregulated pathways, including apoptosis, Wnt/B-catenin, and integrin signalings, once NPC develops (22). When EBV transcripts along with expression arrays were measured, EBNA1 was identified to be consistently expressed, associated with inhibition of genes involved in antigen processing and presentation, further strengthening the role of EBV genes (EBNA1 and LMP1) in NPC development by evading immune recognition.

The RASSF1A tumor suppressor gene is inactivated in the vast majority of human NPC specimens via promoter methylation (23). In an effort to understand the pathways affected by silencing of RASSF1A, expression array studies of transfected NPC cell lines demonstrated changes in multiple processes including transcription, signal transduction, cell adhesion, and RNA processing. RASSF1A was found to repress Id2, which negatively regulate cell differentiation via TGF-B signaling, strengthening its putative tumor suppressor role whereby its loss leads to cellular proliferation and failure to differentiation (24).

Pre-clinical therapeutic studies in NPC

Several pre-clinical studies were presented, using anti-sense oligonucleotide (ASO) approaches (25), such as ASO sequences targeting LMP1 or EBNA1, which decrease the respective protein levels, and in turn reduced growth of C15 tumors by 43% (26). Similarly, ASO targeting Bcl-2 induced apoptosis both in vitro and in vivo, and caused significant delays in tumor re-growth, demonstrated in 2 NPC models (C15 and C666-1) particularly when combined with local tumor radiation therapy (27).

The class of statin drugs, which block the conversion of HMG-CoA to mevalonate in the cholesterol synthesis pathway, can induce apoptosis and inhibit growth of several human cancer models, including EBV-positive lymphomas (28). Specifically, Simvastatin has been shown to dissociate LMP1 from membrane rafts in EBV-transformed B cells (28), hence this compound was evaluated in NPC cells (19). Simvastatin caused significant cytotoxicity to NPC cells, but this toxicity was independent of LMP1, suggesting that Simvastatin might be a potentially useful therapeutic agent for NPC, although its mechanism of action was distinct between NPC and lymphoblastoid cells.

An intriguing strategy was described whereby an adenoviral vaccine was designed which encoded for multiple HLA class I-restricted CTL epitopes from LMP1 and LMP2 covalently linked to glycine-alanine repeats deleted for EBNA1 as a polyepitope vaccine (29, 30). Immunization with this polyepitope consistently generated strong EBNA1 and LMP-specific CTL responses in mice, and successfully reduced growth of NPC models. These results demonstrate that such an adenoviral polyepitope vaccine could be an excellent tool for the induction of protective CTL response directed towards multiple LMP CTL epitopes prevalent in different ethnic groups where EBV-associated malignancies are endemic.

An alternate vaccine approach was described whereby
LMP1 and LMP2-specific T cells could be activated in EBV positive patients using an adenoviral vector mediating expression of inactive LMP1 or LMP2 constructs (dLMP1-I-LMP2) (31, 32). The adv5F35 vector was utilized to preferentially transduce peripheral blood mononuclear cells. Indeed, this strategy successfully induced LMP1 and LMP2-specific T cell responses in the majority of donors. An alternative monoclonal antibody approach was also described, targeting CD70, a surface marker expressed on EBV positive malignancies such as lymphomas, but absent from the majority of normal T and B lymphocytes (33), thereby providing a selective strategy targeting malignant tissues.

**Diagnostics in NPC**

The diagnostic value of circulating EBV DNA and anti-EBV IgG and IgA levels were investigated in 149 Indonesian NPC patients (34). This group observed that the majority of circulating EBV DNA was fragmented, based on a much higher detection rate of EBV DNA when shorter primer sets were utilized for the real-time experiments. Using a 213-bp EBV EBNA1 sequence, 72.5% of patients were positive with 29.5% having levels exceeding a previously determined clinical cutoff value (COV) for healthy carriers. In contrast, with a shorter 99-bp polymerase chain reaction (PCR) primer sequence, these numbers increased to 85.9% of patients being positive; 60.4% had levels above the COV, resulting in a significantly higher detection rate ($\alpha < 0.0001$). These values did not correlate with either serology or BARF1 mRNA in the serum, which was used as a surrogate for burden of circulating tumor cells. These data are consistent with those previously reported (35, 36), suggesting that plasma EBV DNA likely result from NPC cells undergoing apoptosis, which also fragmented the EBV DNA contained within the cancer cells. Despite the lack of a direct correlation of whole blood EBV EBNA1 with serology, the predictive and prognostic values of plasma EBV DNA have been clearly documented by several other groups (37–39).

The issue was raised as to whether an assay developed to measure lytic EBV gene expression, such as BRLF1 or BARF1, which are exclusively expressed in NPC, but not in EBV-associated lymphomas, might provide greater sensitivity in diagnosis and detection of NPC, particularly in northern Africa, where both NPC and EBV-positive lymphomas are endemic (40).

A highly promising screening method for early diagnosis of NPC was presented, by combining a unique transoral biopsy brush with a PCR-based EBV quantitation method (NP Screen®). This technique was evaluated in a multi-institutional study, involving centers in Hong Kong and Toronto, Canada. A total of 272 specimens were obtained, correlated with biopsy results. Among the 69 newly diagnosed NPC patients, 68 had a positive brushing, resulting in a 100% PPV, and a 99.7% NPV. This indicates that this NP Screen® technology has significant potential as an effective screening tool in regions where NPC is endemic. The utility of such nasal brushings is further supported by a recent report on high detection rates of 86% and 74% for EBNA1 DNA and BARF1 mRNA respectively, on 78 NPC patients from Indonesia (41).

**Clinical treatment developments**

The preliminary results of the recently completed NPC-9902 trial of patients with locally advanced T3–4 NPC patients was reported, which comprised of 189 participants in a 2×2 factorial design of accelerated or conventional fractionated radiation therapy (RT), with or without concurrent chemotherapy. The regimens were very similar to the design of NPC-9901, targeting NPC patients with nodal disease (42). The highest 3-year freedom from relapse (FFR) survival rate of 92% was observed for patients treated with accelerated RT with concurrent chemotherapy, compared to 61–74% for patients in the other 3 arms of this trial ($\alpha = 0.04$). However, similar to the NPC-9901 data, this improved survival was associated with an increased incidence of late tissue toxicities of 31% (for all patients who received chemotherapy), vs. 10–24% for patients treated with RT only. The most common irreversible complication was deafness.

These data from Hong Kong are consistent with a meta-analysis comprising of 1753 participants in 8 randomized NPC trials, which demonstrated an absolute overall survival benefit by 6% at 5 years (from 56% to 62%), with the addition of chemotherapy to RT (43). The 5-year absolute event-free survival was improved by 10%, from 42% to 52%. This improvement in overall and event-free survival was primarily observed among the patients who were treated with concurrent chemotherapy with RT.

Several different molecular or immunotherapy approaches are currently being evaluated for NPC patients. These include a phase II trial examining the combination of cetuximab with carboplatin in 60 NPC patients with either recurrent or metastatic disease (44). Overall response was observed in 7 patients (12%), with Grade 3 or 4 toxicities occurring in 31 (52%), demonstrating that this combinatorial regimen has clinical activity, with acceptable safety profile among a group of heavily pre-treated patients. Alternative molecularly targeted therapies could include azacytidine, which de-methylates the silenced promoters of tumor suppressor genes, a common epigenetic event in NPC (23). The lead compound in the class of histone deacetylase inhibitors (HDACI) is suberoylanilide hydroxamic acid (SAHA), which can selectively alter the transcription of cancer-relevant genes. Phase I and II clinical trials are currently conducted for patients with both hematologic and solid tumors (45), which might also have a role in NPC (46).

The results of a phase I/II clinical trial on an immunotherapy approach was reported (47), whereby EBV-specific CTLs were generated in 11 NPC patients, and then re-infused in a dose-escalating manner ($2\times10^{7}$–$1\times10^{8}$ cells/m2). This regimen was well tolerated, and clinical responses were observed in 4/7 patients with measurable disease. Future work will investigate the feasibility of further increasing the...
Innovations along technical RT delivery approaches were also reported, including delivering full-dose (76 Gy) RT using IMRT (intensity-modulated RT) to 50 NPC patients with Stage III & IV disease (48). Concurrent chemotherapy (5-FU and cisplatin) was delivered to 34 patients. The 2-year loco-regional control, distant metastasis-free, and disease-free survival rates were 95.7%, 94.2%, and 93.1%, respectively. Only 1 patient died from neutropenia (CT-related), although interestingly, 2 patients subsequently developed carotid aneurysms, likely related to RT. Hence, IMRT is feasible and tolerable, even when combined with CT.

Two groups reported on quality of life assessments in NPC patients treated with RT, with or without CT. Both studies reported that in the short-term (within 6 months of treatment completion), the quality of life in the combined treatment group appeared worse than when treated with RT alone. However, with longer follow-up (12 months post-treatment completion), there was no discernible difference in quality of life scores between the 2 patient groups (42).

REFERENCES


CONCLUSION

This 2 day East-West Symposium successfully brought together an international group of researchers dedicated to better understanding and improving the outcome of EBV-associated NPC. The global burden of this disease is significant, and measures to improve the socio-economic environment of endemic regions should reduce the incidence of this disease, although the intriguing role of genetic determinants viz susceptibility and host response to EBV infection remains to be clearly elucidated. The myriad of molecular and biologic consequences of EBV latent and lytic gene expressions continue to be slowly unraveled, and immunotherapy approaches among alternative molecularly targeted therapies will in time, contribute to improve outcome of NPC patients. The standard therapies of concurrent chemotherapy with curative RT will achieve respectable cure rates, but strategies need to be developed to reduce the long-term toxicities of this combinatorial treatment regimen, which would likely include both RT technical and biologic innovations.


