Oral Oncology 84 (2018) 61–70

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Review

Cellular-based immunotherapy in Epstein-Barr virus induced nasopharyngeal cancer

Andrea Zhe Ern Lee[a], Louise Soo Yee Tan[a], Chwee Ming Lim[a,b,*]

[a] Department of Otolaryngology – Head and Neck Surgery, National University Health System, Singapore
[b] Department of Otolaryngology, National University of Singapore, Singapore

ARTICLE INFO

Keywords:
Nasopharyngeal carcinoma
Epstein-Barr virus
Immunotherapy
Cellular-based therapy
Vaccination
Head and neck cancer

ABSTRACT

Undifferentiated Nasopharyngeal carcinoma (NPC) is ubiquitously identified with the Epstein-Barr virus (EBV), making this cancer a suitable candidate for cellular-based immunotherapy (CBI) due to its expression of potentially targetable tumor-associated viral antigens. Various preclinical and clinical studies have explored the use of cytotoxic T cells (CTLs), tumor-infiltrating lymphocytes (TILs), natural killer (NK) cells, and dendritic cells (DCs) in the treatment of both refractory and locally advanced NPC with some success. Notably, immune-mediated antitumor effects were observed even in heavily pre-treated NPC patients, suggesting potential clinical benefit of CBI in this group of patients. These immune anti-tumor effects may be even more clinically evident when used as a first-line treatment, since there may not be an intense immunosuppressive environment which is typically encountered in refractory cancer patients. Additionally, CBI may exert an effect in priming the immune system and diminishing the cancer's acquired resistance to exert a more robust response to previously failed chemotherapy. Although these results are encouraging, further refinements of clinical protocols to boost anti-tumor response and benefit a larger subset of patients proved necessary. Herein, we aim to review the rational of developing CBI in EBV-induced NPC and summarize its current applications in clinical studies.

Introduction

Nasopharyngeal carcinoma (NPC) occurs worldwide with approximately 87,000 incident cases and 51,000 deaths annually, representing about 0.7% of the global cancer burden [1]. A distinct racial and geographical variation is evident with a higher incidence rate in both southern China and Southeast Asia. This geographic predisposition is suggestive of both genetic and environmental risk factors in its pathogenesis. For instance, several at risk *HLA* genes such as the *HLA-A* subtype; and exposure to nitrosamines and nitrosamine precursors in the diet have been reported as risk factors of developing NPC. [2–4]. Polymorphisms in certain genes, such as *CYP2E1*, *XRCC1,* and *hOGG1*, have also been associated with an altered risk of developing NPC [5,6]. For instance, CYP2E1 belongs to the cytochrome P450 family and is involved in the metabolic activation of nitrosamines and other pro-carcinogens into reactive intermediates capable of inducing DNA damage. Polymorphisms in *CYP2E1,* particularly the c2 variant (present in 20–25% of Asians as compared to less than 10% of Caucasians), results in a polymorphic base substitution which up-regulates *CYP2E1* expression [5]. Conversely, *XRCC1* and *hOGG1* are DNA repair genes which play a role in the base excision repair (BER) pathway, and polymorphic variants of both *XRCC1* (codon 194, 280 and 399) and *hOGG1* (codon 326) have demonstrated decreased capacity for repair of DNA damage caused by nitrosamine compounds and oxidative stress [6–8]. Taken together, an individual with polymorphism in all three genes may succumb to greater DNA damage, thus increasing the risk of developing NPC.

Due to its radiosensitive nature, radiotherapy is the primary treatment modality for all stages of non-metastatic NPC; with concurrent chemo-radiotherapy being administered for locally advanced cases. This treatment paradigm attained more than 85% chance of loco-regional control and 80% survival [9,10]. Despite positive clinical outcomes, a significant number of patients developed local regional failure (approximately 18%) or distant metastasis (approximately 17%),

Abbreviations: DC, dendritic cell; NK, natural killer cell; CTL, cytotoxic T lymphocyte/cell; NPC, nasopharyngeal carcinoma; HLA, human leukocyte antigen; TCR, T cell receptor; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor alpha; TNF-β, tumor necrosis factor beta; IL-12, interleukin-12; ADCC, antibody-dependent cellular cytotoxicity; Fc, fragment crystallizable; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1

* Corresponding author at: Department of Otolaryngology – Head and Neck Surgery, National University Health System (NUHS) Tower Block, 1E Kent Ridge Road, Level 7, Singapore 119228, Singapore.

E-mail addresses: andrea_lee@nuhs.edu.sg (A.Z.E. Lee), louise_sy_tan@nuhs.edu.sg (L.S.Y. Tan), chwee_ming_lim@nuhs.edu.sg (C.M. Lim).

https://doi.org/10.1016/j.oraloncology.2018.07.011
Received 7 April 2018; Received in revised form 6 July 2018; Accepted 18 July 2018

necessitating new treatment paradigms in this cancer [11]. Additionally, the close anatomical proximity of the nasopharynx to several critical structures including the brainstem, optic nerves, inner ears, and temporomandibular joint, precluded surgical salvage or radical re-radiation since severe radiation associated adverse effects like temporal lobe brain necrosis or carotid blow-out syndrome can occur [12,13]. Therefore, there is a critical need for novel treatment modalities so as to minimize treatment-associated complications and to improve current survival rates in this cancer.

Epstein-Barr virus (EBV) association with NPC

Close association between NPC and the Epstein-Barr virus (EBV) infection is well established with numerous studies reporting an elevated anti-EBV antibodies titers and the presence of EBV DNA in nearly all endemic undifferentiated variant of NPC [14,15]. EBV belongs to the γ-1 herpes virus family that infects more than 90% of the global population. In most individuals, infection occurs early during childhood and exists as lifelong asymptomatic infection which does not cause any pathological symptoms. Due to its ability to establish latency within the host cells, EBV has been causally associated with several human malignancies such as Burkitt's and Hodgkin's lymphoma, gastric carcinoma, and of particular interest in this review, NPC [16–18]. Viral latency is defined as the ability of the virus to remain dormant within the host cell without the production of infective viral particles; and upon appropriate trigger(s) retain the capacity to reactivate back into the lytic phase of replication in order to produce infectious viral progeny. During EBV latent infection, expression of the full viral genome is greatly restricted, thereby allowing only a few viral genes to be expressed so as to escape recognition by the host immune system and stay silent within the host cell. There are 3 latency patterns maintained by EBV-infected cells, each expressing a unique set of EBV-associated proteins and RNAs, including 6 nuclear proteins (Epstein-Barr nuclear antigens, EBNAs), 3 membrane proteins (latent membrane proteins, LMPs), and non-coding RNAs (EBV-encoded small RNAs, EBERs). These patterns of latency expression depend on the various stages leading from primary infection of naïve B lymphocytes to growth transformation of B cells and are associated with varying degrees of susceptibility to immunosurveillance. In type I latency which is associated with Burkitt's lymphoma, significant downregulation of EBV-associated proteins results in only EBNA-1 and EBERs being expressed, thereby allowing for escape of immune recognition [19,20] Type II latency expresses EBNA-1, LMPs, and EBERs, and confers intermediate immune surveillance to cancers associated with this pattern of latency, namely Hodgkin's lymphoma and NPC [21,22]. In type III latency, the full range of EBNAs, LMPs, and EBERs are expressed, and diseases associated with this latency pattern are observed only in the setting of immunodeficiency which includes post-transplantation lymphoproliferative diseases and in vitro establishment of lymphoblastoid cell lines (LCLs) [23,24].

In NPC, introduction of the EBV genome into the epithelial cells of the Waldeyer's ring, particularly the tonsils and the posterior epithelium of Rosenmuller's fossa, initiates the oncogenic process whereby EBV persists as a latent infection and occasionally reactivates into the lytic phase to release its virus progeny. A characteristic feature of this latent infection is epitomized by the presence of clonal circular EBV genome (episome) along with the expression of EBV type II latency genes in the early preinvasive dysplastic lesion, suggesting that viral infection began very early in the carcinogenic process, even before the expansion of the malignant cell clone [25]. It is unclear on how EBV gains entry into the epithelial cells of the nasopharynx since no EBV-compatible receptor has been found. Nevertheless, two proposed models attempt to explain this. One model suggests that EBV may exploit IgA-mediated endocytosis while another propose the use of a surface protein antigenically similar to CD21 receptor on B lymphocytes to gain access into nasopharyngeal cells [26,27]. Colonization of the memory B lymphocyte population via CD21 attachment also proved necessary for EBV to attain long-term persistence in vivo [28]. These infected epithelial cells and lymphocytes eventually express a set of HLA-restricted viral latent antigens on their surface membrane, allowing for potential targeting by CBI. Understanding the function of these EBV latent genes is therefore critical in establishing the course of progression from EBV infection to carcinogenesis, and could ultimately provide greater insights in developing novel diagnostic and therapeutic strategies.

EBV latent antigens

EBNA-1, LMP-1, and LMP-2A/B are among the EBV proteins expressed in NPC. While EBNA-1 is frequently expressed in all cases of undifferentiated NPC, expression of LMP-1 and LMP-2 is more heterogenous. EBNA-1 appears to be a dominant target for CD4+ T cells, due to the presence of a large glycine-alanine repeat domain within its protein sequence that prevents proteasomal breakdown of the molecule and its subsequent presentation by the HLA-class 1 pathway [29]. This reduces EBNA-1's visibility to CD8+ T cells [30]. Conversely, LMPs are generally less immunogenic as compared to EBNA-1 since it is more difficult to elicit both CD4+ and CD8+ T cells responses against LMPs. Nevertheless, the immune responses against LMP-2 proteins are more prominent than those against LMP-1, making LMP-2 a more relevant target for EBV-directed CBI [31,32].

EBNA-1

EBNA-1 is a critical homodimeric DNA-binding onco-protein that serves to maintain viral DNA in host cells via the tethering of EBV episomes to the host mitotic chromosomes, ensuring its stable persistence during latent infection. EBNA-1 localizes to the nucleus and forms a homodimer that binds with the EBV genome at an 18 bp palindromic sequence located in the latent origin of replication [33,34]. This cross linkage ensures the stable maintenance of EBV episomal DNA copy number and the proper segregation during cellular division, thereby contributing to malignant transformation [35]. It was also discovered that EBNA-1 acts as a transcription factor and enhances the expression of LMPs by up to 200-fold [36]. Additionally, EBNA-1 has been found to interact and alter various cellular pathways which likely contributes to malignant transformation [37,38]. Among them, it interacts with USP7 and confers a protective effect from apoptotic challenge on virus-infected cells by lowering p53 levels [39]. EBNA-1 also induces disruption of promyelocytic nuclear bodies (PML-NB) within NPC cells, leading to impaired DNA repair and increase genetic instability [40,41].

LMP-1

Similarly, LMP-1 behaves like a classical oncogene and represents a key transforming protein as evidenced by its in vitro transformation ability in rodent fibroblasts and B lymphocytes [42–44]. This is attributed to its ability to interact with the tumor necrosis factor receptor (TNFR) family-associated proteins, TNFR1 and CD40, which engage in multiple downstream signaling pathways that lead to several phenotypic and structural alterations in B lymphocytes as well as in epithelial cells [45]. These include but are not limited to anchorage-independent growth, inhibition of differentiation, enhanced motility, activation of NF-κB, and resistance to apoptosis [46–48]. LMP-1 also up-regulates epithelial-mesenchyme-transition (EMT), and contributes to the highly metastatic features of NPC [49]. Expression of LMP-1 is detected mostly in pre-invasive lesions, including dysplasia and carcinoma in situ, but not in advanced NPC, suggesting that its expression is essential for early, initiating event of NPC [25]. Although there has been identification of numerous CD8+ T cell epitopes in LMP-1, this protein's tendency to self-aggregate limits HLA-class I restricted LMP-1 epitope generation; and therefore facilitates immune evasion [31]. Several