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HOW EPSTEIN-BARR VIRUS "MANIPULATES" THE TUMORAL MICROENVIRONMENT IN HODGKIN LYMPHOMA?

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Abstract

The Epstein-Barr virus (EBV) is a gamma-herpesvirus that colonizes the B-cell system of its human host, allowing it to persist asymptomatically in the majority of the world's adult population. In most people primary infection goes unnoticed, whereas in a minority of individuals, primary infection results in infectious mononucleosis (IM), a benign condition that almost always resolves after several weeks or months. However, EBV is also causally linked with a number of malignancies, including B-cell lymphomas, such as classical Hodgkin lymphoma (cHL).

A proportion of patients with cHL harbor EBV within their tumor cells. Emerging evidence suggests that while EBV is able to subvert cellular processes to promote the growth and survival of HRS cells or their progenitors, mutations in key cell signalization pathways are probably required to do this when EBV is absent. The challenge is to unravel exactly how EBV and its latent genes contribute to the pathogenesis of cHL particularly with respect to how the virus co-operates with cellular genetic and epigenetic changes to drive transformation. It is hoped that the development of better in vitro and in vivo models of disease will reveal more fundamental aspects of EBV's role in Hodgkin lymphoma pathogenesis and pave the way for targeted therapies for patients with EBV-positive cHL.

Keywords: *Epstein-Barr virus, infectious mononucleosis, B-cell lymphomas, Hodgkin lymphoma.*

Internal Medicine

General Reviews

Abstract

Virusul Epstein-Barr (EBV) este un herpesvirus gamma care colonizează celulele B ale gazdei sale umane, ceea ce-i permite să persiste asimptomatic la majoritatea populației adulte. De obicei, infecția primară trece neobservată, în timp ce, la o minoritate de indivizi, infecția primară are ca rezultat mononucleoza infecțioasă (IM), o afecțiune benignă care aproape întotdeauna se remite după câteva săptămâni sau luni. Cu toate acestea, EBV este, de asemenea, legat cauzal de un număr de afecțiuni maligne, inclusiv limfoame cu celule B, cum ar fi limfomul Hodgkin clasic (cHL).

O proporție a pacienților cu cHL poartă EBV în interiorul celulelor tumorale. Dovezile emergente sugerează că, în timp ce EBV este capabil să submineze procesele celulare promovând creșterea și supraviețuirea celulelor HRS sau a progenitorilor lor, atunci când EBV este absent, sunt necesare probabil mutații în căile de semnalizare celulare cheie pentru a permite creșterea și dezvoltarea celulelor maligne. Provocarea este de a descoperi exact modul în care EBV și genele sale latente contribuie la patogeneza cHL, în special cu privire la modul în care interacțiunea dintre acțiunea virusului și modificările celulare genetice și epigenetice determină transformarea. Se speră că, prin dezvoltarea unor modele mai bune in vitro și in vivo ale bolii, se vor dezvălui aspectele fundamentale ale rolului EBV în patogeneza limfomului Hodgkin, deschizându-se calea pentru terapiile țintite pentru pacienții cu cHL pozitivi pentru EBV.

Cuvinte cheie: *virusul Epstein-Barr, mononucleoză infecțioasă, limfoame cu celule B, limfom Hodgkin.*

The Epstein-Barr virus (EBV) is a gamma-herpesvirus that colonizes the B-cell system of its human host, allowing it to persist asymptomatically in the majority of the world's adult population. In most people primary infection goes unnoticed, whereas in a minority of individuals, primary infection results in infectious mononucleosis (IM), a benign condition that almost always resolves after several weeks or months. However, EBV is also causally linked with a number of malignancies, including B-cell lymphomas, such as classical Hodgkin lymphoma (cHL).

The detection of raised antibody levels to EBV antigens in Hodgkin lymphoma patients compared with other lymphoma patients provided the first clues that EBV might be involved in the pathogenesis of cHL[1]. Furthermore, these raised levels were found to precede the development of cHL by

several years[2]. In 1974, two reports were published documenting a significantly increased risk of cHL in individuals with a prior history of IM[3,4].

Later, it was shown that a prior history of either self-reported or laboratory confirmed IM is associated with an increased risk of developing EBV-positive cHL, an association not observed for EBV-negative cHL[5,6].

In EBV-associated cHL, the viral genomes are found in monoclonal form indicating that infection of the tumor cells occurred prior to their clonal expansion[7]. Furthermore, EBV persists in Hodgkin-Reed-Sternberg (HRS) cells throughout the course of disease and in multiple sites of disease, suggesting that it is required for tumor maintenance in vivo[8].

The fraction of patients with cHL harboring the EBV genome in their tumor cells varies dramatically with factors such as age, gender, histological subtype, ethnicity and country of residence[9, 10]. Thus, EBV rates in cHL from North America and Europe vary between 20% and 50%, but much higher rates are observed in some underdeveloped countries[11,12].

EBV-positive rates have also been shown to be higher in males compared with females, in Asians and Hispanics compared with whites or blacks[9] and in South Asian children compared with non-South Asian children from the UK[13]. In developed countries, the proportion of cases with EBV is higher in older people and in children, especially in those under 10 years of age, whereas the lowest rates of EBV-positive disease are found in young adults[14, 15]. Thus, cHL might comprise three distinct entities: pediatric cHL (EBV-positive, mixed cellularity type), cHL of young adults (EBV-negative, nodular sclerosis type) and cHL of older adults (EBV-positive, mixed cellularity type) (14). EBV-positive cHL in older adults has been attributed to an age-related decline in EBV-specific immunity[14].

In contrast to some other forms of EBV-associated B-cell lymphoma, the incidence of EBV-positive cHL is only modestly increased in patients infected with the human immunodeficiency virus (HIV)[16, 17]. The incidence of cHL in HIV-positive patients has not fallen during the era of highly active antiretroviral therapy (HAART); indeed, some studies suggest cHL risk may be increased in the first few months following immune reconstitution on HAART[18, 19, 20]. These findings suggest that although defects in EBV-specific immunity contribute to the development of EBV-positive Hodgkin lymphoma, CD4+ T cells have a critical role in tumor development which is lost when CD4+ T-cell numbers fall below a critical threshold.

The incidence of EBV-positive cHL is dramatically increased in autoimmune lymphoproliferative syndrome (ALPS), providing another potential example in which a chronic immune stimulus could contribute to cHL development[21]. Most ALPS patients inherit mutations in the FAS gene leading to defective apoptosis and to higher than normal numbers of immature CD4-/CD8-, so-called 'double negative', T cells. These T cells are known to stimulate B-cell proliferation in ALPS patients which in turn may increase the risk of cHL development[22].

The non-tumor stroma of cHL is composed of T-cells, B-cells, macrophages, mast cells, eosinophils and fibroblasts, the composition of which appears to be important for patient outcomes[23, 24, 25, 26]. HRS cells can modify this reactive microenvironment by attracting certain cell types. For example, Reed-Sternberg cells secrete multiple chemokines such as CCL5 (RANTES), CCL17 (TARC), CCL20 and CCL22, which can recruit Th2

Internal Medicine

General Reviews

helper and FoxP3+ regulatory T cells[27,28,29,30]. HRS cells also activate fibroblasts to produce CCL11 (eotaxin) and CCL5 which further contribute to the attraction of eosinophils and regulatory T cells[31, 32]. In addition, HRS cells secrete multiple cytokines including IL5, IL9 and IL13, which influence the recruitment and proliferation of cells in the tumor infiltrate. In EBV-positive cases of cHL, LMP1 contributes to the recruitment and the modification of the tumor microenvironment by stimulating the production of many of these chemokines and cytokines in infected HRS cells[33,34,35].

While the tumor microenvironment supports the growth and survival of the HRS cells, it also contributes to the suppression of the host anti-EBV-specific immune responses.

EBV-positive cHL has more CD68- and CD163-expressing tumor-associated macrophages (TAMs) than EBV-negative cHL (36). Macrophage numbers are also strongly associated with inferior survival in newly diagnosed cHL patients, both in those treated with standard chemotherapy and in those having received autologous stem cell transplant[37].

Gene expression profiling of the tumor tissues of newly diagnosed cHL patients also showed that a gene expression signature of macrophage infiltration was associated with poor prognosis, a finding which was validated using immunohistochemistry to detect TAMs in an independent patient cohort[25]. The potential tumor-promoting functions of TAMs may also be explained by their ability to induce angiogenesis mediated by the secretion of soluble angiogenic factors, including VEGF[38, 39]. CD68+ and CD163+ TAMs are associated with increased micro-vessel density (MVD) in cHL (40, 41), and MVD correlates with poor outcome in cHL patients[42,43,44].

T-cell effector functions in cHL can be abrogated by the engagement of programmed cell death ligand-1 (PD-L1) expressed on HRS cells with its receptor, PD-1, on T cells resulting in functional exhaustion of the T cells[45,46]. The importance of this pathway in the microenvironment of cHL is exemplified by studies which show that many patients with relapsed or refractory cHL respond to PD-1 blockade therapy[47].

A proportion of patients with cHL harbor EBV within their tumor cells. Emerging evidence suggests that while EBV is able to subvert cellular processes to promote the growth and survival of HRS cells or their progenitors, mutations in key cell signalization pathways are probably required to do this when EBV is absent. The challenge is to unravel exactly how EBV and its latent genes contribute to the pathogenesis of cHL particularly with respect to how the virus co-operates with cellular genetic and epigenetic changes to drive

transformation. It is hoped that the development of better in vitro and in vivo models of disease will reveal more fundamental aspects of EBV's role in Hodgkin lymphoma pathogenesis and pave the way for targeted therapies for patients with EBV-positive cHL.

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