The Molecular Basis for the Generation of Hodgkin and Reed-Sternberg Cells in Hodgkin’s Lymphoma

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Abstract
Hodgkin’s lymphoma (HL) is a lymphoid neoplasm with a low frequency of malignant tumor cells, known as Hodgkin and Reed-Sternberg (H-RS) cells, in a background of mixed cellular infiltrates. Despite extensive studies on H-RS cells, the molecular mechanisms of their growth and regulation have remained uncertain for a long period. Recently, constitutively activated nuclear factor-κB (NF-κB) was reported to be a unique and common characteristic of H-RS cells that prevents the cells from undergoing apoptosis. NF-κB triggers proliferation and provides a molecular basis for these cells’ aberrant growth and cytokine gene expression. In HL pathogenesis associated with Epstein-Barr virus infection, the activation of NF-κB is induced by viral latent membrane protein 1 (LMP1). Coupled with recent insights into the molecular mechanisms of activation of NF-κB signaling in H-RS cells, this review discusses a linkage between LMP1 and HL via CD99, which has recently been reported to be down-regulated by LMP1 through the NF-κB signaling pathway. This down-regulation leads to the generation of cells with H-RS phenotypes related to the clinical and histologic characteristics of HL. Int J Hematol. 2003;77:330-335.
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1. Introduction
Hodgkin’s lymphoma (HL) is morphologically characterized by a low occurrence of neoplastic mononuclear Hodgkin (H) and multinucleated Reed-Sternberg (RS) cells surrounded by abundant non-neoplastic infiltrates in the tumor tissue [1]. Since HL was first reported as a distinct clinical entity, it has become recognized as one of the most difficult lymphomas to study at the cellular as well as the molecular level because of the rarity of the malignant H-RS cells (less than 1% of the cells in an involved lymph node) and the presence of its histologically distinct subtypes [2]. In addition, because of the uncontrolled production of markers normally expressed in cells from different hematopoietic lineages, H-RS cells vary between B, T, and myeloid phenotypes. The Revised European American Lymphoma and World Health Organization classification recently distinguished lymphocyte predominance HL from classic HL that consists of 4 subtypes (lymphocyte-rich, nodular-sclerosing, mixed cellularity, and lymphocyte-depleted). This classification reflects the diversity in clinical presentation and behavior, morphology, phenotype, and molecular features of HL [3]. Despite apparent heterogeneity in their genotypes and phenotypes, individual H-RS cells show similar histopathologic features, such as the typical bizarre-looking morphology and the deregulation of various cytokines and growth factors, suggesting a common molecular mechanism in their generation. Recent advances in the comprehension of the etiology and pathogenesis of HL are noteworthy and have been made by studies of immunophenotyping, genotyping, and cytokine production of H-RS cells in tissue specimens or in cell lines derived from HL tissues. In particular, studies employing single-cell micromanipulation techniques with polymerase chain reaction amplification of RNA and genomic DNA have shed light on the identification of the cellular origin of H-RS cells, which has long been a matter of debate. A clonal rearrangement of the V, D, and J segments of the immunoglobulin heavy chain locus has been detected in the H-RS cells of most patients, and sequence
analyses of the rearranged VDJ regions from these H-RS cells have demonstrated a high load of somatic mutations, indicating that these cells had originated from germinal center B-lymphocytes or from B-cells at a later stage of differentiation in few exceptional cases [4-7]. In addition, micromanipulation of single cells also identified the recurrence of constitutive nuclear factor-κB (NF-κB) activity in H-RS cells, which is currently considered the common molecular defect that forms the basis for the occurrence of HL.

2. Constitutive Activation of the NF-κB Signaling Pathway in H-RS Cells

The NF-κB/Rel family of transcription factors mediates inducible gene expression in response to numerous pathogens and cytokines and is also known to regulate a wide variety of genes whose products play fundamental roles in inflammatory and immune responses. NF-κB consists of homodimers and heterodimers in a variety of gene products related to the v-Rel oncoprotein [8]. In most cells, NF-κB factors are sequestered in the cytoplasm with NF-κB inhibitor (IκB). Various cellular stimuli trigger the phosphorylation of IκB, which leads to ubiquitination and the subsequent degradation of IκB and the resultant release of NF-κB subunits into the nucleus [9-13].

The role of NF-κB in oncogenesis was first implicated in fatal lymphomas of birds induced by the v-rel gene and in human malignancies associated with rearranged and/or amplified genes encoding the NF-κB family factors [14]. The importance of NF-κB activation in HL was also demonstrated in cultured HL cell lines and primary H-RS cells, in which elevated NF-κB DNA-binding activities were consistently observed in the nucleus [15]. Immunohistochemical staining of biopsy samples of HL patients with an anti-RelA antibody confirmed abundant NF-κB–RelA activity in RS cells. Although c-Jun and JunB overexpression in tumor cells from all patients with classic HL was recently reported, the AP1 activities in these patients still showed synergy with NF-κB [16]. Furthermore, when a dominant negative IκBα that is not inductively degraded was overexpressed, Hodgkin cell lines showed decreases in nuclear NF-κB activity and proliferation rates and demonstrated an enhanced apoptosis [17]. The antiapoptotic effects of NF-κB have been documented in many other cell types, including mouse B-cells [18]. The presence H-RS cells with nonfunctional immunoglobulin genes that should have been negatively selected implies the rescue of the cells from apotosis by aberrant NF-κB activation. Thus, the roles of NF-κB as a direct requirement for human neoplastic disease suggest that the H-RS cells result from deregulated NF-κB expression.

The abnormal NF-κB activation in H-RS cells is associated with molecules closely linked to its signaling pathway, such as a mutated IκB, a constitutive activity of kinases upstream from IκB, or a modification of NF-κB, which renders NF-κB insensitive to inhibition by IκB. In fact, clonal deleterious somatic defects in the IκBα gene, such as a deletion or a point mutation, have been detected in some HL cell lines and H-RS cell cases and have resulted in the cells becoming functionally null for IκBα activity [19-21]. However, in other cases of H-RS cells that maintain wild-type IκBα alleles expressing IκBα protein, an activation of upstream factors leading to rapid degradation of IκBα, such as aberrant IκB kinase (IKK) or NF-κB inducing kinase (NIK) activities, appeared to be the reason for NF-κB activity in the nucleus [22].

3. Members of the Tumor Necrosis Factor Receptor Family and NF-κB Activation in H-RS Cells

Roles of some members of the tumor necrosis factor receptor (TNFR) family, such as CD30 and CD40, have long been suggested to be involved in the NF-κB activation of H-RS cells. Generally, the TNFRs transmit their signal through the direct recruitment of TNFR-associated factors (TRAFs) [23]. Although the molecular mechanisms by which TRAFs activate downstream effector proteins remain largely unknown, the current data suggest that TRAFs interact with different types of kinase, such as NIK, MAPK/extracellular response kinase, and transforming growth factor β-activated kinase, which are associated with IKKs leading to NF-κB activation [12,24,25]. Overexpression of CD30 in vitro has been demonstrated to activate NF-κB through self-aggregation and recruitment of TRAF2, TRAF5, and NIK in H-RS cells in a constitutive, ligand-independent way [26,27]. CD40-mediated signal transduction also activates NF-κB, and CD40-induced NF-κB activation has been demonstrated to be mediated by the proteolysis of TRAF3 in a Hodgkin cell line [28,29]. CD40 is expressed at significantly higher levels on HL cell lines than on other lymphoma cells, with a highly distinctive staining pattern irrespective of the antigenic phenotype or histologic subtype [30]. In both CD30 and CD40 cases, it was also suggested that their activation could be achieved by cross-linking their matching ligands on either the H-RS cell or on adjacent infiltrating lymphocytes [31,32].

Epstein-Barr virus (EBV) latent protein 1 (LMP1) is also a member of the TNFR superfamily, and constitutively activates NF-κB-mediated transcription, regardless of ligand stimulation of NF-κB. Because of its high frequency of occurrence (about 40%), LMP1 has also attracted considerable attention as a key molecule for activating NF-κB in H-RS cells from HL patients [33]. LMP1 is a viral pseudoreceptor, and the signaling regions of LMP1 have extensive functional homology to that of CD40, but LMP1 has no significant sequence homology with CD40. Although their signaling domains have been shown to be interchangeable, LMP1 and CD40 do not interact with exactly the same set of molecules, indicating that the signaling pathways may differ in some aspects.

4. EBV LMP1 Expression and NF-κB Activation in H-RS Cells

EBV, a ubiquitous human herpesvirus, has been reported to be associated with many human malignancies, such as HL, endemic Burkitt lymphoma, nasopharyngeal carcinoma, and posttransplantation lymphoproliferative disease. The EBV infection of resting B-cells in vitro leads to the establishment of immortalized lymphoblastoid cell lines. Among the EBV antigens that cooperate in the transformation of normal B-lymphocytes, LMP1 plays a key role. Expression of LMP1 induces the oncogenic transformation of established fibroblast cell lines and suppresses the senescence of mouse pri-