APOPTOSIS IN CLASSICAL HODGKIN LYMPHOMA - POSSIBLE SIGNIFICANCE OF THE MEASLES VIRUS

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Abstract

A review is presented on our conception of the trifaceted relationship between classical Hodgkin lymphoma, apoptosis modulation, as a lymphomagenesis avenue and the measles virus, as a possible apoptosis modulator in this malignancy. The well known association between this cancer and apoptosis is hereby modified to include an early phase of apoptosis resistance, which may or may not be followed by one of apoptosis stimulation. The present evidence on a putative role of the measles virus in the regulation of the apoptotic machinery in classical Hodgkin lymphoma is also reviewed.

Key Words: classical Hodgkin lymphoma; apoptosis; measles virus; TUNEL method

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Introduction

The significance of apoptosis in classical Hodgkin lymphoma (cHL) has been a topic of predilection for our research group soon after we started to investigate this peculiar malignancy (1, 2). The detection of apoptotic Hodgkin-Reed-Sternberg (HRS) cells on H&E was very difficult. By using the TUNEL method, it became evident in 61% of the 45 cases examined (1). A direct correlation between the number of HRS cells and the number of apoptotic HRS cells in 10 HPF was observed (2). An association between this malignant tumor and the expression of antigens and RNA of the measles virus (MV) was later demonstrated (3). Evidence of the expression of at least two MV antigens was found in 54.3% of the cHL patients. The demonstration of MV-RNA by RT-PCR was found in 7 of 28 ; and by *in situ* hybridization, MV-RNA was revealed in 10 of 28 cases. An attempt to summarize the complex relationships between cHL, apoptosis and the MV is now made.

Classical Hodgkin lymphoma and the apoptotic machinery

The consensual view regarding the role of apoptosis in Hodgkin-Reed-Sternberg (HRS) cells of classical Hodgkin lymphoma (cHL) is related with the tumor cells survival, due to apoptosis inhibition (4). As the HRS cells are germinal center B cells which have lost their ability to express the B-cell receptor on their cell membrane, they would have otherwise been subject to apoptosis (5).

A few years before the above hypothesis was released, morphological and electron-microscopic (EM) evidence of apoptosis in HRS cells had been demonstrated (1). A wide range of the apoptotic index in the tumor cells of this malignancy was shown by our group (2). In fact, apoptosis was found to be of prognostic significance in this tumor. By Kaplan-Meier analysis, an inverse association was found between an apoptotic index and survival (p=.05). In a multivariable logistic regression model, an elevated apoptotic index was correlated with a 3.27 risk of dying of Hodgkin lymphoma (OR=3.27; CI= .87-11.94).
These studies and a later revision of our data, employed morphological methods (H&E sections), the EM, and the TUNEL method (terminal deoxynucleotidyl transferase (TdT)- catalyzed deoxyuridinephosphate (dUTP)-nick end labeling assay, to investigate apoptosis. Immunohistochemical staining of apoptosis-related molecules (bcl-2, bax, bclx, mcl-1, cas3 and cas8), as well as p53,

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NFκB p65, NF-κB p52 and IκB, were further used for this purpose (6).

One of the goals of the later review was to confirm the significance of p65 and LMP/EBV in sustaining apoptosis resistance in cHL, as had been suggested by the above consensus hypothesis. However, p65 did not correlate with apoptosis arrest in our 217 cHL patients (6). This is in agreement with the finding of a preferred role for the alternative NF-κB pathway in cHL (7). Moreover, LMP/EBV was found to act as a pro-apoptotic factor, in contrast with the consensus position (6).

The measles virus in classical Hodgkin lymphoma

Another goal of this later review was to attempt to characterize a function for the measles virus (MV) in cHL (6). We had previously suggested an association between cHL and this virus (3). This followed the active search of an infectious agent, which might show the tendency to cause a common infection with a late exposure pattern (3). It appeared that measles disclosed several features in common with cHL: the MV is lymphotropic, reaching the human through the upper aerodigestive tract. In addition, measles is usually complicated by a cellular immune deficiency. It demonstrates, moreover, polykaryons in the infected tissues, which are however basically different from the HRS cells (8).

We have demonstrated by immunostaining, by RT-PCR and by *in situ* hybridization, the presence of MV proteins and MV RNA (3), in a proportion (about 50%, for the MV proteins) of our cHL patients. Until recently, a possible association between the measles virus and cHL was suggested, but we needed to sustain further this relationship and find a mechanism of action for the MV in cHL.

Such a link may have been found, when we demonstrated a statistically significant association between positive expression of anyone of several MV proteins and an apoptotic index above median (6).This finding implied that the MV favored increased apoptosis. If replicated and expanded, these data may represent the mechanistic confirmation of the relationship between the MV and cHL.

Role of apoptosis in viral infections

In this paragraph, the significance of apoptosis in a few viral infections is described. In some, apoptosis demonstrates a significant function thereof in the pathogenesis of the infection. In order to justify an effective influenza A virus proliferation, the infected host cell molecules related with apoptosis are

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manipulated by the virus (9). To promote its infection, the human papilloma virus has programmed a few molecular pathways to suppress apoptosis (10). Several antiapoptotic molecules in the viral kingdom underline their destructive significance for the infectious agent. One of the strategies to overcome this threat is by "backfiring the cell on itself": some viruses express proteins targeted by caspases. A possible consequences of the splitting of these proteins by the caspases is apoptosis arrest. Alternatively, increased or decreased viral proliferation may occur (11).

The human cytomegalovirus (HCMV) might be the closest mimic of the measles virus as described in cHL. Neither of the two viruses is considered as oncogenic. The concept of "oncomodulation", generated for HCMV, states that an infection caused by this virus, increases the chances for a malignant transformation. Although the significance of this hypothesis in a given patient is not known, statistical evidence thereof is becoming compelling. This may also be the case for the MV in the set up of cHL (12).

Conclusion

Apoptosis plays probably a critical role, to a variable extent in cHL. We have suggested that at the incipient stage of the malignant transformation, apoptosis arrest prevails, otherwise tumor cells would be rapidly eliminated (6). Later, a wide modulation of the apoptotic machinery, with some advantage to the extrinsic apoptotic pathway, will occur. In cHL, this modulation might be executed through the MV effect. Several limitations are found in our review, but probably one of major importance follows: the methods of determination of the apoptosis status are numerous and complex. Only part of them have been used in our research. Thus, we have not used for this purpose fluorescence microscopy, Annexin V staining, the Western blot and caspase activation assays (13).

Discussion

This short review has summarized the journey of our laboratory into the biology of cHL, which has lasted more than 15 years. It should be emphasized that the only two groups of scientist who have reacted to the presentation of our hypothesis on a relationship between cHL and the MV, have rejected our suggestions. We have nevertheless found in their response enough incentives to carry on with our line of research. That apoptosis plays an important function in cHL is most tenable. The question remains as to the

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nature of this function. Our compromise, as compared with the consensus hypothesis described above, must be the closest to reality. Thus, early in lymphomagenesis, apoptosis resistance is favored. However, thereafter, apoptosis may be arrested or upgraded, perhaps in conjunction with the level of MV expression.

By the same token, as other viruses have to cope with apoptosis, using all possible pathways in the host cells, we believe that the MV modulates the host apoptotic pathways primarily for its own purposes. While doing so, it may cause "oncomodulation" in the lymph nodes of cHL, thus contributing to the malignant transformation.

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Competing interests:

The author has declared that no competing interest exists.

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