Pathobiology of ALK-negative anaplastic large cell lymphoma

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Abstract

The authors revise the concept of ALK-negative anaplastic large cell lymphoma (ALCL) in the light of the recently updated WHO classification of Tumors of Hematopoietic and Lymphoid Tissues both on biological and clinical grounds. The main histological findings are illustrated as well as the phenotypic, molecular and clinical characteristics. Finally, the biological rationale for possible innovative targeted therapies is presented.

Introduction

Anaplastic large cell lymphoma (ALCL) is a peripheral T-cell lymphoma (PTCL), representing around 2-3% of all lymphoid neoplasms, according to the World Health Organization (WHO) estimates.[1,2] Originally described by Stein *et al.* in 1985,[3] it has undergone a series of revisions, which have led to a more restrictive definition of the process.[1,2,4,5] In particular, two different entities are recognized as systemic forms, the ALK+ and ALK- ALCL,[1,2,6] the former being characterized by the deregulated expression of chimeric proteins expressing the intracytoplasmic domain of the anaplastic lymphoma kinase (*ALK*) gene. Noteworthy, in the last edition of the WHO classification, ALK- ALCL was regarded as a provisional entity.[1,2] However, emerging evidences suggest it represent a distinctive condition.[7] On the other hand, differently from what initially reported by Stein *et al.*,[3] the cutaneous variant was recognized as a different disease.[8]

Morphology

According to the WHO classification, ALK- ALCL, like the ALK+ form, includes morphologic variants: common, giant cell-rich, lympho-histiocytic, and Hodgkin-like.[1,2,9,10] All morphological variants are characterized by a variable proportion of large hallmark cells with eccentric horse-shoe or kidney shaped nuclei). The giant cell-rich type is characterized by several multinucleated elements, often provided with Reed-Sternberg-like features and prominent intra-sinusoidal diffusion.[1,5,11,12] The lympho-histiocytic variant displays a marked variability of the neoplastic cell size that ranges from small to large. These are almost obscured by abundant reactive histiocytes with eccentric nuclei, a finding that can lead to a misdiagnosis of hyper-immune reaction.[1,2,9,13-15] Interestingly, transition from one histotype to the other is at times recorded within the same node (mixed variant) or in different nodes taken from the same patient at the time of diagnosis or in relapse: these modifications might correspond to intra-clonal modulation or different interaction between the tumor and micro-environment.[9,16] Finally, the so-called ALCL of the Hodgkin-like type deserves special attention.[17] It was originally described as a form of the tumor, presenting in young people with a bulky mediastinal mass and consisting of anaplastic cells arranged in nodules surrounded by sclerotic bands, as seen in nodular sclerosing Hodgkin's lymphoma (NSHL).[17] Following the introduction of the REAL Classification,[5] which regarded it as a provisional entity, such diagnosis was by no means also applied to cases of aggressive HL that could not be easily differentiated from ALCL, both on morphologic and phenotypic grounds.[5] This led to a diffuse skepticism on the existence of such variant: it was considered a basket more than an entity. However, although rare, *bona fide* examples of ALK- ALCL of the Hodgkin-like type can be encountered. These are characterized by homogeneous CD30-positivity, lack of CD15 and B-cell activator protein (BSAP), variable expression of T-cell antigens, CD45 and epithelial membrane antigen (EMA), Epstein-Barr virus (EBV) negativity and clonal TCR gene rearrangement.

Phenotype

Neoplastic cells of ALK- ALCL carry a distinctive phenotypic profile irrespectively of the histotype.[1,2,11,18] They regularly express CD30,[1-3,19] a glycoprotein of 120 kD carried by lymphoid elements following activation. At immunohistochemical analysis, the antibodies against CD30 produce different types of positivity: membrane-bound, dot-like in the Golgi area (corresponding to the accumulation of the 90 kD proteic precursor), and diffuse.[19] The first two patterns are exclusive of lymphoid elements with the exception of embryonic carcinoma,[19] while the third one can at times occur in a variety of malignant tumors other than lymphomas.[19] Therefore, the immunophenotypic diagnosis of ALCL should always be based on the application of a panel of antibodies, including reagents anti-cytokeratins, melanoma-associated antigens, CEA, and PLAP.[1,2] In 60-70% of cases, ALK- ALCL carries the EMA.[9,20] CD3 expression is appreciated in about half cases: it usually occurs at the cytoplasmic level as expected in activated cells.[9,21] CD3- tumors may carry CD2, CD5 and/or CD7.[9,11,21-23] The expression of CD4 and CD8 is variable.[9,11] Positivity for TIA-1, granzyme B and perforin is recorded in most nstances. About 20% of ALCL lack CD45 and/or express CD15.[9] Notably, BSAP (i.e. the PAX5 gene product) is absent:[24] its search represents a very useful tool for the differentiation of ALK- ALCL from HL and DLBCL, which are both BSAP-positive.[24] Finally, the search for EBV turns negative in ALK- ALCLs both by *in situ* hybridization (ISH) and immunohistochemistry: such negativity is regarded as one of the distinguishing features between ALCL and HL in controversial cases.[25]

Molecular genetics

Recently, by using a comparative genomic hybridization (CGH) platform, Salaverria *et al.*[26] identified chromosomal imbalances in 65% of ALK- cases, within a cohort of 31 tumors. In particular, gains of 1q and 6p21 were more frequently observed.[26] Interestingly, few recurrent chromosomal imbalances were found in common with ALK+ ALCL (gains of 7 and 6q and 13q losses), confirming that all ALCLs probably share common pathogenetic events (see below).

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As far as gene expression profiling (GEP) is concerned, Thompson *et al*.[27] initially demonstrated the ability of GEP to correctly distinguish between ALK+ and ALK- ALCL based on the analysis of their transcriptome and suggested that some pathogenetic mechanisms might be shared by these two entities, basing on the common expression of certain genes in both ALK+ and ALK- cases.

Subsequently, Lamant *et al*.[28] confirmed that the different morphological variants as well as ALK+ and ALK- ALCL could be distinguished based on the expression of specific genes. Specifically, ALK- ALCL were found to over-expression *CCR7, CNTFR, IL22* and *IL21*.

Our group then included some ALCL in a GEP study on PTCLs.[29] Interestingly, we found that ALCL can be roughly distinguished from other PTCLs irrespectively of the ALK status, confirming the idea of common pathogenetic events. Finally, Piva *et al*.[7] showed, that ALCL are molecularly distinct from PTCL/NOS. To this regard, grippingly, a predictive analysis allowed to identify 34 probe sets capable to distinguish ALCL from other PTCLs. Furthermore, it was possible to clearly differentiate ALK+ and ALK- cases according to their GEPs, basing on the expression of selected genes, including GAS1, an ALK dependent molecule.[7]

More recently, Eckerle *et al*.[23] studied isolated cells from ALCL cases. Interestingly, the analysis supported the derivation of ALCL from activated T cells, though it was not possible to identify a specific counterpart. Surprisingly, only few genes were differentially expressed between systemic and cutaneous ALCL despite their different clinical behavior, and between ALK- ALCL and classical Hodgkin lymphoma, despite their different cellular origin.[23]

Clinical behavior

ALK- ALCL is an aggressive lymphoma which frequently presents in advanced clinical stage (III-IV) with B-symptoms, and extra-nodal involvement, as other PTCLs do.[11,30] Bone marrow involvement is detected in up to 30% of cases, being a relevant prognostic feature.[31-33]

Importantly, ALCL display quite different clinical features depending on the expression or not of the ALK protein.[6,11,30,34-36] ALK- tumors are usually recorded among people aged 50-70,[6,11,30] and, most importantly, ALK+ has a significantly better outcome than ALK- ALCL. In particular, up to 90% of ALK+ ALCL achieve complete remission (CR) by adopting standard antracyclin-containing regimens, and 70-80% of patients were actually cured.[6,11,30,34-36] By contrast, only around 30-50% of ALK- cases obtain stable CR by the same therapies,[6,11,30] suggesting that more aggressive strategies including autologous or allogeneic bone-marrow/stem cell transplantation may be necessary.[37]

Noteworthy, the International T-cell lymphoma project have recently reported that ALK+ and ALK- ALCL seem to have a similar prognosis (in terms of both FFS and OS), when patients are stratified according to the clinical parameters (i.e. age and/or stage). Grippingly, this would suggest a prominent role for clinical factors in determining patients outcome, rather than for biological components.[6]

Notably, in the past years, it was suggested that the distinction between ALK- ALCL and PTCL/NOS was of limited clinical relevance, only age and the International Prognostic Index (IPI) being of prognostic relevance in these tumors.[38] Nevertheless, it was recently shown that clinical differences do exist between the two entities. In particular, in a large international study, a greater proportion of patients with poor performance status and B-symptoms, but a lower frequency of bone marrow invasion, splenic involvement and thrombocytopenia were observed in ALK- ALCL as compared to PTCL/NOS.[6] Indeed, in this study, ALK- ALCL showed an overall outcome significantly better than PTCL/NOS.[6]

Targeted therapy

Importantly, as tyrosine-kinase deregulation has been documented in several PTCLs,[29,39-41] it is conceivable that ALK- ALCL might present similar phenomenon as well, further studies being indeed warranted.

Finally, immunotherapy strategies could represent another possible therapeutic approaches. In particular, *in vitro* and *in vivo* studies showed that anti-CD30 antibodies induce apoptotic cell death and tumor regression in CD30+ lymphomas, including ALCL.[42-46] In addition, other studies have suggested that CD26 might represent a promising immunotherapeutic target.[47]

Conclusions

Although ALK- ALCL is still quoted as a provisional entity, increasing evidences, both biological and clinical, suggest that it actually represent a tumor with distinctive features.

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