Gene expression profiling of peripheral T-cell lymphoma: from bench to bedside

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Peripheral T-cell lymphomas (PTCLs) represent approximately 12% of lymphoid neoplasms (1). Their incidence varies among countries, being higher in HTLV-1 endemic areas, such as Southern Japan, Caribbean basin and some USA districts.[1] PTCLs are a heterogeneous group of tumors that can be roughly subdivided into specified and not otherwise specified (NOS) forms.[2] While the former correspond to distinct but rare entities, often occurring at extra-nodal sites, the latter represent the commonest type of T-cell lymphoma (40-50% of cases), followed by the angioimmunoblastic (AITL) and anaplastic large cell (ALCL) ones.

PTCLs have so far been the object of a limited number of gene expression profiling studies.[3-13] In particular, Tracey *et al.*,[13] Lamant *et al.*[5] and de Leval *et al.*[6] focused on specific topics: i.e. the gene expression profile (GEP) of mycosis fungoides, ALK$^+$ and ALK$^-$ ALCLs, and AITL, respectively. In contrast, Martinez-Delgado *et al.* and Ballester *et al.* analyzed large collections of PTCLs of the NOS, AITL and ALCL types.[3,4] However, their studies suffer of limitations that vary from the usage of chips with a restricted number of genes[3,4] to the lack of a reliable normal counterpart for comparison.[3] Martinez-Delgado *et al.* reported that PTCL/U corresponds to a heterogeneous group of tumors, whose GEP is difficult to interpret due to the significant amount of infiltrating reactive cells. According to these authors, the only clinically relevant information provided by GEP pertains the NF-kB gene expression level.[3] Ballester *et al.*[4] found that the GEP could discriminate among PTCLs of the U, AITL and ALCL types, although the former did not share a single profile. Using a multi-class predictor, the authors separated their cases into three molecular subgroups called U1, U2 and U3. The U1 gene expression signature included genes known to be associated with poor outcome in other tumors, such as CCND2. The U2 subgroup was associated with over-expression of genes involved in T-cell activation, including NFKB1 and BCL-2. The third group was mainly defined by the over-expression of genes involved in the IFN/JAK/STAT pathway and comprised most histiocyte-rich tumors. This finding suggests that the signatures recorded by Ballester *et al.* might be at least in part influenced by reactive components.

Recently, our Group[9] have published a GEP study based on the analysis of 28 PTCLs/NOS, all

corresponding to lymph node biopsies and containing an amount of neoplastic cells that exceeded the 70% value of the whole examined population. The m-RNA extracted from these cases was hybridised on the HG U133 2.0 Plus gene chip. The obtained results were compared with those of 6 AITLs, 6 ALCLs (2 ALK^+ and 4 ALK^-) and 20 samples of normal T-lymphocytes, purified from the peripheral blood and tonsil and corresponding to the main T-cell subsets ($CD4^+$, $CD8^+$, resting, and activated). This study significantly differs from most previous reports[3,13] in terms of methodology and selection criteria. In addition, it provides for the first time the rationale for possible targeted therapies in PTCL/NOS by offering clear evidence of their effectiveness *ex vivo*.

In particular, the GEP detected by Piccaluga *et al.*[9] indicates that PTCLs/NOS are distinct from normal T- and B-lymphocytes. Interestingly, GEP results suggested that PTCL/NOS is more closely related to activated rather than resting T-cells. As in normal mature T-lymphocytes, it was possible to identify two main subgroups of PTCL/NOS, with GEPs related to either CD4 or CD8 elements. Notably, this characteristic did not reflect the immunophenotype with regards to the expression of CD4 and CD8 molecules.

Besides histogenetic information, our study[9] provided several insights into the functional alterations of PTCL/NOS. A careful comparison of PTCL/NOS with the closest normal cellular counterparts revealed in fact the extensive deregulation of genes, which control functions that are typically damaged in malignant cells, such as matrix remodelling, cell adhesion, transcription regulation, proliferation, and apoptosis. In particular, our analysis might explain the dissemination pattern of PTCL/NOS, with frequent extra-nodal and bone marrow involvement and spread to peripheral blood,[1] by showing the up-regulation of *FN1*, *LAMB1*, *COL1A2*, *COL3A1*, *COL4A1*, *COL4A2*, and *COL12A1*, i.e. of genes which promote local invasion and metastasis in different types of human cancers.[14-16] In addition, it revealed the de-regulation of genes involved in apoptosis (e.g. *MOAP1*, *ING3*, *GADD45A* and *GADD45B*)[17-23] and chemo-resistance (such as *CYR61* and *NNMT*).[14-16,24-35]

Immunohistochemistry provided in situ validation of the genomic data by showing correspondence between m-RNA and protein expression, as seen, for example, with PDGFRα and BCL10. In addition, by comparison with normal tissues, immunohistochemistry allowed the identification of staining patterns corresponding to the synthesis of ectopic or paraphysiologic products by neoplastic cells. Finally, the phenotypic test highlighted the possibility that some of the results obtained by gene expression profiling may depend on non-neoplastic cellular components present in the analyzed sample, as seen for Caldesmon.

Interestingly, the regular detection of PDGFRα over-expression both at the m-RNA and protein levels, as well as its frequent phosphorylation, prompted us to design an ex vivo experiment aiming to test the sensitivity of PTCL/NOS cells to imatinib, a well-known PDGFRα inhibitor.[9,36] The results obtained were of interest, with about 50% cytotoxic effect seen at 48 hours with a 1 μmol concentration. Such rate became even higher (75%) with higher doses. Notably, imatinib exerted a limited effect on the viability of normal lymphocytes.

Finally, our group dedicated to GEP of AITL.[11] Interestingly, among other genes, we observed the consistent up-regulation of VEGF. The same finding had previously been reported by de Leval *et al.*[6] who had attributed it to the rich vascular component of the tumour. However, by immunohistochemistry on TMAs, we showed that neoplastic cells

strongly express both VEGF and its receptor KDR.[11] This fact suggests possible AITL sensitivity to anti-angiogenetic drugs, such as thalidomide and bevacizumab.

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