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VII. The role of the microenvironment in lymphoid cancers

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introduction

Much of the focus of cancer biology has for many years been aimed at understanding the genetic alterations harbored by neoplastic cells that distinguished these cells from their normal counterparts. Lymphoid cancer B cells deregulate pathways resulting from underlying genetic alterations that include cell cycle perturbations (e.g. MYC in Burkitt lymphoma, CCND1 in mantle cell lymphoma), anti-apoptotic signaling (e.g. BCL2 expression in follicular lymphoma), blocks in terminal differentiation that hold these cells at a stage of differentiation conducive to uninhibited growth [e.g. BCL6 upregulation and *PRDM1* mutations in diffuse large B cell lymphoma (DLBCL)] and constitutive activation of intracellular signaling pathways that promote tumor cell growth (e.g. activation of NF-κB pathway signaling in the ABC subtype of DLBCL). More recently, a growing appreciation of the contribution of the tumor microenvironment to the pathogenesis of lymphoid malignancies has gained significant momentum, in part a result of gene expression profiling (GEP) of whole tissues [1, 2]. The tumoral microenvironment encompasses the accessory and immune-related cells in the immediate vicinity of the tumor cells that engage in crosstalk and cell-to-cell contact with the neoplastic cells, typically providing a nurturing milieu by providing nutrients, new blood vessel formation and fostering immune privilege. The microenvironment may fluctuate between anatomic sites in a given B-cell lymphoma with some sites seemingly creating a niche that promotes acquired drug resistance and cancer stem cell maintenance. Understanding the role of the microenvironment in lymphoid malignancies has also fostered renewed interest in the development of biological agents that act by disrupting the growth-promoting interactions or crosstalk between neoplastic and non-neoplastic cells, resulting in a potential paradigm shift in therapeutic strategies away from standard chemotherapy alone. In the future cytotoxic agents might be combined with agents that interfere with this crosstalk, either inhibiting the growth-promoting effects of specific cytokines or promoting cell killing by adjacent but transiently ineffective tumor-infiltrating lymphocytes. Although the microenvironment appears to have some role in virtually all lymphoid cancers, this review will focus on a brief discussion of follicular lymphoma (FL), DLBCL, Hodgkin lymphoma (HL) and conclude with a description of a novel recurrent genetic event that may explain immune privilege in a related entity, primary mediastinal large B-cell lymphoma (PMBCL).

follicular lymphoma

FL represents the most common indolent non-Hodgkin lymphoma in North America and Europe [3]. It is characterized by a diverse spectrum of clinical outcome with median overall survival in the range of 10 years. In contrast to DLBCL where standard of care treatment is defined by R-CHOP, there is no agreement on optimal initial therapy for FL patients. Recently released data from the PRIMA trial will undoubtedly inform on this question, but it remains unclear whether these data will be practice changing [4]. As a result of marked clinical heterogeneity and lack of uniform therapy in FL, the interpretation of studies aimed at understanding the prognostic significance of the microenvironment is seriously hampered. Comprehensive correlative science studies focused on phase III clinical trials are desparately needed in order to clarify the role of the microenvironment in the biology and outcome prediction for FL patients. Until such time, we can only reliably draw limited conclusions from those published studies where the treatment variable is held constant [5–11]. Such studies in FL represent only a handful of the published literature, yet even these reveal conflicting findings, almost certainly the result of specific therapies that differentially impact the non-neoplastic cells. For example, the study of microvessel density (MVD) by Koster et al. [5] found a correlation of increased MVD with favorable outcome in FL patients treated with CVP and interferon (IFN) α2b followed by maintenance IFN, while Farinha et al. [8] found the complete opposite result in a larger number of FL patients treated with BP-VACOP and involved-field radiotherapy. Might this difference in the prognostic impact of MVD be the result of IFN in the study by Koster? IFN is known to have some anti-angiogenic effects that might explain why cases with increased MVD might have differentially benefited in the study by Koster et al. [5].

The therapies used to treat FL are differentially visited upon both the neoplastic B cells and the immune cells in the tumoral microenvironment including subsets of T cells, benign B cells, antigen presenting cells including follicular dendritic cells and other stromal cells (fibroblastic reticular cells, endothelial cells, etc.) found within the lymph node niche. Distinct differences in

the number and distribution of non-neoplastic immune cells in FL biopsies at diagnosis represent true biological differences [9, 12]. Why some patients have many intrafollicular CD4+ T cells while others show a predominance of interfollicular CD4+ T cells suggests marked heterogeneity that likely reflects meaningful biology. However, interpreting these differences across the spectrum of therapies becomes very difficult, if not impossible. Several studies have shown that increased numbers of macrophages in diagnostic biopsies of FL were associated with inferior survival [9, 10]. After the addition of rituximab (R) to chemotherapy, the prognostic impact of macrophages either disappeared (Canioni et al. [10]) or in fact was associated with a survival advantage (Taskinen et al. [11]). These are precisely the issues that preclude us generating consistent hypotheses regarding the role of the microenvironment in FL, suggesting that progress will only be realized when studies are performed using large clinical cohorts of uniformly treated patients given R-chemotherapy-containing regimens.

Similarly, the role of the microenvironment in histologic transformation of FL can only be resolved in patients treated uniformly and biopsies obtained at the time of transformation are mandated [7, 8]. Finally, functional studies based on *in vitro* analysis of clinical samples suggest very different hypotheses than those put forward in some of the published literature based on immunohistochemistry (IHC) alone [13–16]. Future reviewers of the literature will need to hold authors more accountable for reconciling these disparate findings. More studies that use cell enrichment techniques coupled with sophisticated molecular analysis (functional phosphor-flow studies, GEP, etc.) are needed to arrive at a unifying hypothesis and understanding of the contribution of the microenvironment to the prognosis and pathogenesis of FL [17, 18]. The role of IHC cannot be under estimated however, as architectural distribution of the non-neoplastic cells may also be an important factor in determining prognosis.

diffuse large B-cell lymphoma

DLBCL represents a clinically, morphologically and biologically heterogeneous disease with several morphological variants and specific subtypes recognized in the 2008 WHO classification [19]. Even within the category of 'DLBCL-not otherwise specified' there is a wide spectrum of admixed non-neoplastic cells that comprise the tumoral microenvironment, which includes benign B cells (the other tumor-infiltrating lymphocyte), subsets of T cells including regulatory T cells, Th1 cells, Th2 cells, Th17 cells, natural killer cells, antigen presenting cells, stromal elements and vascular endothelial cells to name but a few [20]. These cells vary in content, distribution and almost certainly function within a given tumor and may fluctuate over time in individual patients [21]. Although DLBCL was largely considered a genetically complex tumor with autonomous growth, it is clear that signals or crosstalk between the neoplastic large B cells and the microenvironment is an integral and important aspect of the biology. Emerging clinical data regarding new biological agents that appear to target the microenvironment (lenalidomide, agonist anti-CD137 antibodies, etc.), some showing single-agent efficacy in DLBCL, even in the refractory setting, suggest that disrupting the crosstalk may become a viable treatment paradigm in the upfront setting [22].

Clues to the importance of the microenvironment in DLBCL became apparent following the first large GEP studies by Rosenwald and colleagues [23]. These authors found that a 'lymph node' signature and class II HLA gene expression were contributors to an outcome predictor in DLBCL patients treated with CHOP-like chemotherapy. The functional role of the lymph node signature could not be fully elucidated at that time, but IHC correlates of MHC class II loss were shown to be prognostic in DLBCL and related lymphoma subtypes. Down-regulation of HLA-DR on tumor cells appeared to be one mechanism of immune escape, at least for a small subset of cases [24–26].

The LLMPP group later studied 233 *de novo* cases of DLBCL treated with R-CHOP using GEP and was able to confirm the continued importance of cell-of-origin (GCB versus ABC) distinctions and refined the role of the microenvironment in DLBCL pathogenesis [2]. The whole-section GEP data were complemented by data derived from magnetic bead separations of CD19+ B cells versus CD19Neg cells from DLBCL biopsies that convincingly showed that two new signatures, stromal-1 and stromal-2 were clearly derived from non-neoplastic CD19Neg cells in the tumor microenvironment. The stromal-1 signature conferred a favorable outcome and revealed genes suggesting extracellular matrix deposition and macrophages/monocytes +/– myeloid-derived cells. Specific genes could be shown to be co-expressed by CD68+ macrophages using fluorescence microscopy, including MMP-9 and SPARC. Subsequent studies using SPARC IHC have confirmed these findings [27]. The stromal-2 gene signature was associated with inferior survival and revealed genes involved in endothelial cell biology and adipocyte function. Although the latter gene signature requires further study, the stromal-2 signature was correlated with MVD, which revealed that DLBCL cases with increased small blood vessels had inferior overall and progression-free survival. In aggregate, these data firmly establish an important role for non-neoplastic cells in the pathogenesis of DLBCL and suggest that treatment approaches that target both the malignant B cells and the non-neoplastic cells in the microenvironment may improve the efficacy of standard of care treatments such as R-CHOP in selected patients with DLBCL.

classical Hodgkin lymphoma

Classic Hodgkin lymphoma (cHL) comprises several different histological variants, but nodular sclerosing (NSHL) and mixed cellularity (MCHL) account for >95% of the cases. cHL represents a paradigm of tumor cell–microenvironment interactions, as the neoplastic Hodgkin Reed–Sternberg (HRS) cells typically represent <1% of the total infiltrate in lymph node biopsies. The dominant cellular populations are made up of a mixture of inflammatory cells and immune cells. The HRS cells and several of the inflammatory cells in the microenvironment create and sustain this milieu by secreting a number of cytokines and chemokines that allow the malignant HRS cells to fully develop their immunophenotype and at the same time evade immune cell attack. The resultant

microenvironment is one that fosters immune privilege allowing the HRS cells to thrive in a Th2 milieu. The HRS cells produce a number of factors, including galectin-1, PD-L1, CSF-1, TARC and others that help to create this cellular milieu, leading to the recruitment of macrophages and regulatory T cells that further contribute to immune privilege. A recent integrative analysis revealed that cases of cHL harbor HRS cells with copy number gains of 9p24 that include the PD-L1 and PD-L2 loci in addition to JAK2 [28]. Green et al. [28] have shown that cases with increased copy number of PD-L1 are correlated with the same cases that overexpress the mRNA and protein, providing one explanation for fostering immune privilege. Moreover, increased expression of JAK2 helps stave off apoptosis and also leads directly to up-regulated expression of PD-L1.

A number of GEP studies have been performed using whole biopsy sections from cHL biopsies to largely explore the contribution of the microenvironment to outcome prediction in cHL [29–32]. Most studies have found consistent findings that implicate macrophages, benign B cells and specific T-cell subsets as important players in predicting the success of primary therapy in patients with cHL. The largest study to date by Steidl and colleagues [32], analyzed 130 cases of cHL (virtually all NSHL) including 38 primary treatment failures and 92 treatment successes. The analysis of the GEP data revealed a number of gene signatures correlated with the two treatment classes (success versus failure), including several macrophage signatures associated with treatment failure. These authors then validated this result using an unrelated clinical cohort and a different approach based on CD68 IHC. The findings validated and showed that increased CD68+ macrophages at the time of diagnosis were associated with primary treatment failure and interestingly, were also associated with failure following salvage therapy. These data have now been subsequently confirmed in a large number of independent datasets [33]. These results become of particular interest as they suggest that targeting the microenvironment might be a useful strategy in the treatment of selected patients with cHL. More importantly, the introduction of biological treatments, including those that target the HRS cells (e.g. SGN-35) combined with agents that can disrupt the crosstalk with the non-neoplastic cells in the microenvironment (e.g. lenalidomide) might represent important new strategies in HL therapeutics that would likely lessen the burden of long-term treatment-related sequelae that continue to haunt the survivors of cHL and their physicians.

primary mediastinal large B-cell lymphoma

PMBCL is an uncommon lymphoma closely related to cHL, particularly NSHL. Both diseases affect younger patients and arise in the anterior mediastinum, often presenting as large, frequently localized masses. Unlike many B-cell lymphomas that are characterized by recurrent translocations (e.g. MYC gene in Burkitt's lymphoma, BCL2 gene in FL and GCB-type DLBCL, CCND1 gene in mantle cell lymphoma and BCL6 enriched in the ABC-type of DLBCL), PMBCL has not been associated with a known recurrent translocation until recently. Steidl and colleagues [34] used next-generation sequencing approaches to study two HL cell lines (KM-H2 and L428) by RNAseq and found several novel fusions, one including CIITA, the master regulator of MHC class II expression (HLA-DR). The consistent partner in the translocations was CIITA on chromosome 16p13, leading to loss of one allele, as the majority of the breaks occur in the first intron and thus disrupt most of the coding sequence of this large gene. These investigators went on to study clinical cases of cHL and PMBCL. Using FISH studies they could show that CIITA rearrangements occurred in 15% of cHL patients, but importantly, were also found in 38% of PMBCLs. Using RACE they identified partners of the CIITA fusion in PMBCL and discovered that although the fusion partners were promiscuous, half involved PD-L1 and PD-L2 on chromosome 9p24, leading to in-frame fusions and deregulated overexpression of these PD-1 ligands. A number of functional studies were performed lending support to the hypothesis that these translocations result in a microenvironmental 'double-whammy', whereby CIITA loss through a possible dominant-negative effect lead to down-regulation of HLA-DR by the tumor cells and thus escape from immunosurveillance, while the fusion partner in half of the cases leads to overexpression of the ligands for PD-1 on T cells and induces T-cell exhaustion in the tumoral microenvironment. These findings establish an unprecedented oncogenic mechanism whereby a recurrent genetic event and resultant fusion impact immune privilege through both sides of a translocation. Importantly, very recent phase I clinical trials in non-lymphoid cancers suggest that overexpression of PD-L1 or PD-L2 could be targeted with specific PD-1 receptor neutralizing antibodies or molecules that interfere with PD-1 receptor signaling in cases that harbor the specific fusion event or overexpress PD-L1 or PD-L2 as a result of copy number gain [28, 35]. This strategy would fulfill the promise of a diagnostic test (FISH testing) matched to a targeted, personalized therapy that would hopefully improve outcomes in PMBCL patients.

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