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Review

Cell-secreted signals shape lymphoma identity

Annunziata Gloghini [a], Italia Bongarzone [b,*]

[a] Department of Diagnostic Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy
[b] Proteomics Laboratory, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

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ABSTRACT

Sequencing data show that both specific genes and a number of signaling pathways are recurrently mutated in various types of lymphoma. DNA sequencing analyses of lymphoma have identified several aberrations that might affect the interaction between malignant cells and the tumor microenvironment. Microenvironmental functions are essential to lymphoma; they provide survival and proliferation signals and license immune evasion. It is plausible that interventions that aim to destroy tumor–microenvironment interactions may improve responses to therapeutics. Accordingly, the identification of extrinsic factors and their downstream intracellular signaling targets has led to much progress in understanding tumor–microenvironment interactions. Lymphoma cells are differently influenced by cells' interactions with components of their microenvironment; these cell extrinsic factors include soluble and immobilized factors, the extracellular matrix, and signals presented by neighboring cells. Soluble factors, which are often cell-secreted autocrine and paracrine factors, comprise a significant fraction of targetable molecules. To begin to understand how intercellular communication is conducted in lymphoma, a first order of study is deciphering the soluble factors secreted by malignant cells and microenvironmental cells. These soluble factors are shed into the interstitial fluid in lymphoma and can be conveniently explored using mass spectrometry. Protein components can be detected and quantified, thus enabling the routine navigation of the soluble part of the microenvironment. Elucidating functional and signaling states affords a new paradigm for understanding cancer biology and devising new therapies. This review summarizes knowledge in this field and discusses the utility of studying tumor-secreted factors.

1. Introduction

Fundamental studies in recent years have demonstrated the possibility of using microarray technology to analyze DNA alterations and gene expression profiling in order to characterize a variety of lymphoma categories. The main contributions of DNA studies in lymphoid neoplasms include the demonstration of common chromosomal alterations across entities and the identification of genes and pathways targeted by the altered chromosomal regions. Gene expression studies in lymphomas have enhanced the molecular subgrouping of known entities (*i.e.*, identification of two major subgroups in the category of diffuse large B-cell lymphoma (DLBCL), germinal center B-cell-like DLBCL and activated B-cell DLBCL) and facilitated the recognition of new subtypes and categories of lymphoid neoplasms (the recognition of an intermediate category between Burkitt lymphoma and DLBCL), the identification of new biomarkers (ZAP70 in chronic lymphocytic leukemia [CLL]; SOX11 in mantle cell lymphoma) and the detection of oncogenic pathways with potential implications for targeted therapies (*i.e.*, NF-κB for activated B-cell DLBCL; the high expression of PDGFRα in peripheral T-cell lymphoma) (reviewed in Campo [1]). The application of high-throughput techniques to the study of different lymphoma types has yielded considerable progress regarding the most frequent B-cell mature neoplasms.

Advanced next-generation sequencing (NGS) technology has been applied to lymphoid neoplasms and has provided early insights into the mutational landscape of different lymphoid tumors. A list of consensus cancer genes can be found in the COSMIC database on the Sanger site (www.sanger.ac.uk/genetics/CGP/cosmic). Progress has been made in the diagnosis of multiple myeloma, CLL, mantle cell lymphoma, follicular lymphoma (FL), hairy cell leukemia, lymphoplasmacytic lymphoma, and splenic marginal zone lymphoma [2]. Sequencing technology

* Corresponding author at: Proteomics Laboratory, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, via Venezian 1, 20133 Milano, Italy. Tel.: +39 0223902219.
E-mail addresses: annunziata.gloghini@istitutotumori.mi.it (A. Gloghini), Italia.bongarzone@istitutotumori.mi.it (I. Bongarzone).

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has also provided important new findings for some cutaneous T-cell lymphomas with largely unknown molecular pathogenesis [3].

Although it is still a work in progress, the data generated thus far are helping to improve understanding of the molecular pathogenesis of lymphomas through the revelation of new mechanisms involving oncogenes and mutated genes. These studies have identified genetic alterations that promise to be immediately attractive for diagnosis and risk stratification, and provide a more solid rationale for therapeutic targeting of these tumors [4].

Sequencing data also show that specific genes, as well as a number of signaling pathways, are recurrently mutated in various types of lymphoma [2]. Mutations in genes that are already suspected of participating in B-cell lymphoma pathogenesis, such as those developed by BCR/Btk, PI3K, RAS/MAPK, TLR, or NF-κB, have been confirmed. Sequencing studies also show that other unsuspected pathways, such as those involved in chromatin remodeling events and the control of gene expression, are frequently mutated in almost every type of lymphoma (reviewed in [1,5–8]).

It has become increasingly evident that elements of the tumor microenvironment, far from being mere spectators or solely part of an antitumor inflammatory response of the host, participate in cancer pathogenesis and allow progression. Microenvironmental functions are essential to lymphoma; they provide survival and proliferation signals and license immune evasion. DNA sequencing analysis of lymphoma has identified several aberrations that potentially affect the interaction between malignant cells and the tumor microenvironment. Interventions that aim to destroy tumor–microenvironment interactions may improve the tumor response to therapeutics.

Although it has not yet been completely demonstrated, massive gene changes that occur in cancer cells and in the surrounding microenvironment are reciprocally interconnected, thus supporting a co-evolutionary dynamic process according to criteria of functional optimization. This process involves intercellular communication that occurs through direct cell–cell contact or through an indirect mode involving the release of biochemical signals and the binding of these signals to cellular receptors, such as cytokine release. Upon cytokine secretion, stromal cells produce proteases and angiogenic factors and promote the breakdown of the extracellular matrix (ECM), thereby producing the release of various ECM fragments (named matrikines or matricryptins) that are capable of regulating angiogenesis and tumor progression [9].

This review will analyze the benefits of generating a wealth of information about the soluble protein components of lymphoma's microenvironment. We attempt to assess the current status and challenges in the field by surveying recent discoveries and technical developments in this area.

2. The benefits of studying the soluble components in the lymphoma microenvironment

To begin to understand how intercellular communication is conducted in the context of cancer, a first order of study is deciphering the biochemical signals secreted by malignant cells in what is known as the tumor secretome (Fig. 1). In tumors, a plethora of cells, including fibroblasts, inflammatory cells such as macrophages, and endothelial cells, produce secreted factors that dictate the composition of the transcellular fluid compartment. The multiplicity and repertoire of these secreted factors affect the intercellular communication between tumor and stromal cells, which are accomplished through intercellular interactions; paracrine mechanisms involving growth factors, chemokines, and proteases; and extracellular vesicles (*i.e.*, exosomes and microvesicles).

Transcellular fluid coincides with the interstitial fluid that represents the physical and biochemical microenvironment of the tumor. The substances of the interstitium have important roles in the regulation of the immune response, inflammation, tumor growth, and metastasis. We denote this part of the microenvironment as the fluid portion of the tumor. Unlike other phases of the lymphoma microenvironment, such as the stroma or cellular elements, it has been the subject of little attention in lymphoma research thus far.

Although specific documentation regarding the biophysical consequences of the microenvironment is still lacking, an observational hallmark of many cancers is the corresponding increase in interstitial flow inside the tumor tissue. This type of microenvironment is both a biophysical and a biochemical barrier, thus determining therapeutic resistance [10]. From a biochemical point of view, the interstitial space contains proteins that are selectively expressed in lymphoma tissue. These proteins are expected to be more accessible than intracellular proteins, and might be informative regarding the treatment of relapsed and refractory lymphoma, whose resistance might be attributed (*e.g.*, in Hodgkin lymphoma [HL]) to relationships between Reed–Sternberg (RS) cells and various types of surrounding non-neoplastic cells. In fact, the expression of a variety of cytokines and chemokines by RS cells and lymphocyte-predominant (LP) cells is the driving force behind an abnormal immune response, perpetuated by additional factors secreted by reactive cells in the microenvironment that help maintain the inflammatory milieu [11]. Several studies have also demonstrated that enhancing the secretion of vascular endothelial growth factor (VEGF) and other mediators enhances angiogenesis in both HL and non-Hodgkin lymphoma (NHL) [12,13].

High-resolution mass spectrometry and bioinformatics analyses can help to deeply study the composition of the microenvironment, specifically of those soluble components included in interstitial space (Fig. 2). Using this methodology, proteins secreted from lymphoma cell lines have been studied in order to understand tumorigenic mechanisms and responses to stimuli, particularly in HL, in which crosstalk between RS cells and infiltrating cells is of crucial importance to the survival and growth of RS cells [14,15]. Thymus and activation-regulated chemokine (TARC)/CCL17 is highly expressed by malignant RS cells. High TARC levels combined with immune regulatory cytokines and chemokines contribute to the inflammatory HL microenvironment, promoting tumor initiation, maintenance, and progression [16]. Because it is secreted into the serum, TARC is used as biomarker.

Although not all secreted proteins can be considered tumor biomarkers, the knowledge of the molecular composition of tumor extracellular fluid and of the mechanisms associated with molecular representation may reveal new aspects of neoplastic transformation. Especially in lymphoma, this fluid has not been well studied; its study might provide a new perspective regarding the comprehension of crosstalk between tumor cells and their microenvironment.

Recent results indicate the importance of autophagy in the modification of interstitial fluid composition in lymphoma, along with drug sensitivity in leukemia [17]. This process reportedly acidifies the extracellular fluid, which in turn intensifies the release of lysosomal components, resulting in enhanced deformation of the microenvironment in favor of tumor progression [18]. Exocytosis combined with the acidic pH of extracellular fluid can cause profound failure in the normal immune functions that regulate the processes of tumor angiogenesis and vascular remodeling. Baginska et al. [19] described that autophagy can cause the degradation of natural killer cell-derived granzyme B, which compromises the ability of natural killer cells to eliminate tumor cells. Thus, the biochemical study of extracellular fluid could be informative regarding the details of many processes that may be involved in immunomodulation and interconnected pro-tumorigenic pathways.

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