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Review article

In situ neoplasia in lymph node pathology

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ABSTRACT

In situ neoplasia represents the earliest form of malignant progression and is characterized by localization limited to the compartment corresponding to the cell of origin. Like other cancers, lymphoid neoplasms are considered to develop by multistep pathogenetic mechanisms. However, because of the circulating nature of lymphocytes, *in situ* lymphoid neoplasia may be difficult to identify histopathologically, and the compartment to which it is restricted may be physiological rather than strictly anatomical. The 2016 WHO classification of lymphoid neoplasms recognizes two *in situ* entities: *in situ* follicular neoplasia (ISFN) and *in situ* mantle cell neoplasia (ISMCN). This review summarizes the clinical features, histopathology, genetics, and differential diagnoses of these two entities, including distinction from both their overly malignant counterparts and a variety of reactive processes.

Introduction

A multistep pathway of tumorigenesis has been demonstrated and broadly accepted in cancers of various organs. Neoplastic cells increase their malignant potential by accumulating genetic alterations in the course of progression from their earliest form to that of invasive and/or disseminated disease. Specifically, the earliest form of cancer localized to its corresponding physical compartment is designated *in situ*, a Latin phrase meaning “on site” or “in position.” *In situ* neoplasms lack invasive growth, generally do not metastasize, and typically have a favorable prognosis when identified and treated at this early stage. Like other malignancies, increasing evidence suggests that lymphoid neoplasms also develop by multistep pathogenetic mechanisms. However, due to the circulating nature of the lymphocyte, most lymphomas are not anatomically contained even in their earliest stages. It has become appreciated, however, that a very early *in situ* form of lymphoid neoplasia may occur, found confined to the physiological compartment corresponding to its presumed cell of origin. Because of the lack of architectural effacement, these *in situ* lymphoid neoplasms often are difficult to identify histopathologically. Two such entities are recognized in the revised 4th edition of the World Health Organization (WHO) classification of lymphoid neoplasms: *in situ* follicular neoplasia (ISFN) and *in situ* mantle cell neoplasia (ISMCN).[1–3] Although ISFN and ISMCN were initially regarded as partial involvement by overt follicular lymphoma (FL) and mantle cell lymphoma (MCL), respectively, recent clinical and molecular studies have demonstrated that they represent a premalignant and/or early phase of lymphomagenesis that sometimes but not always progress to overt lymphomas. In this article, we review the clinical features, pathology, genetics, and differential diagnosis of ISFN and ISMCN (Table 1).

In Situ Follicular Neoplasia (ISFN)

Definition

ISFN is defined as a monoclonal proliferation of B cells with immunophenotypic and genetic features of FL but confined to the germinal centers of lymph nodes or other organs.[1,2] This pathological condition was initially described by Cong et al. in 2002,[4] and subsequently defined more strictly by Jegalian et al. in 2011.[5] It has been previously referred to by various names, including intrafollicular neoplasia/*in situ* FL (the 2008 WHO classification), *in situ* localization of FL, or FL-like B-cells of uncertain significance; however the new WHO classification uses the nomenclature ISFN because the diagnostic term “lymphoma” may be not be justified considering the unknown clinical impact of ISFN.[1]

Clinical features

The actual incidence of ISFN remains difficult to ascertain because of its subclinical nature. Three studies conducted immunohistochemical screening for ISFN in consecutive surgically-removed, otherwise

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Table 1
Clinical, pathological, and molecular features of ISFN and ISMCN.

	In situ follicular neoplasia (ISFN)	*In situ* mantle cell neoplasia (ISMCN)
Clinical features	Incidental finding	Incidental finding
Pathological features		
Architecture of lymphoid tissue	Reactive hyperplasia with preserved architecture[a]	Reactive hyperplasia with preserved architecture[a]
Germinal centers	Normal size, well defined, sharply demarcated	Normal
Cytology of germinal centers	May be monotonous with predominantly centrocytes	Normal
Follicular mantles	Normal	Well defined, normal configuration
Cytology of follicular mantles	Normal	Usually unremarkable
Perifollicular spread	Absent	Absent
Immunophenotype	CD20+, CD10+ (strong), BCL6+, BCL2+ (strong), Ki67 low (without normal polarization)	CD20+, Cyclin D1+, IgD+, CD5+/- (variable), Sox11 +/- (variable)
Molecular features	Clonal IG rearrangement, t(14;18)(q32;q21) (*IGH-BCL2*)	Clonal IG rearrangement, t(11;14) (*IGH-CCND1*)
Differential diagnosis	Follicular lymphoma (FL) including partial involvement by FL, reactive follicular hyperplasia	Mantle cell lymphoma (MCL) with a mantle zone pattern, reactive follicular hyperplasia

[a] Or another pathological process prompting evaluation.

Table 2
Reported prevalences of ISFN and ISMCN in unselected LN specimens.

	Patients with ISN		LN with ISN	
Reference	%	(n/N)	%	(n/N)
ISFN				
Henopp et al.	2.3	(3/132)	1.7	(22/1294)
Bermudez et al.	3.2	(11/341)	2.4	(27/1107)
Carvajal-Cuenca et al.	2.0	(2/100)	NA	
Total	2.8	(16/573)		
ISMCN				
Bermudez et al.	0.6	(2/341)	0.4	(4/1107)
Carvajal-Cuenca et al.	0.0	(0/100)	NA	
Adam et al.	0.0	(0/131)	0.0	(0/1292)
Total	0.35	(2/572)		

reactive lymph nodes, and identified ISFN in 2.3–3.2% of cases (Table 2).[6–8] Although examination of resected lymph nodes does not necessarily reflect incidence in the general population, these findings suggest that ISFN is more frequent than overt FL, which has an incidence of 2.6 per 100,000 population in Western countries,[9] and accordingly that ISFN does not necessarily undergo progression into FL. Similar to overt FL, ISFN usually affects middle-aged and older individuals. The mean age of patients with ISFN is in the fifth decade of life, and patients younger than 40 years are rare.[4–6,8,10] ISFN is generally identified incidentally in a lymph node resected for another reason or because of enlargement.[4–6,8,10] In a smaller number of cases, ISFN may be seen in secondary lymphoid follicles of extranodal organs including tonsil, thyroid, parotid gland, intestine, and spleen.[4,5,8,11] Of note, ISFN can involve more than one lymph node without progression to overt lymphoma; therefore multiple lesions do not exclude the diagnosis of ISFN.[6,8,10]

Histopathology

Although overt FL can be recognized by morphological observation alone, the diagnosis of ISFN requires immunohistochemistry. In lymph nodes with ISFN, the lymphoid follicles are often hyperplastic and the overall architecture of the lymph node is well preserved (Table 1; Fig. 1a).[10,12,13] Most follicles containing the neoplastic cells are well defined and appear normal or reactive, whereas rare neoplastic secondary follicles may be composed of mostly monotonous centrocytes and lack tangible body macrophages, similar to follicles seen in grade 1 FL (Fig. 1b,c). The appearance of the mantle zones is usually unremarkable, but may be sometimes show expansion or rarely attenuation.[13] The affected follicles are widely scattered in the lymph node and often not contiguous. Immunohistochemically, the neoplastic germinal center B cells in ISFN exhibit identical features to those of overt FL (Fig. 1d-h). They express pan-B cell markers such as CD20 and CD79a and the classical germinal center markers CD10 and BCL6. Sometimes, as also seen in some cases of overt FL, the neoplastic cells of ISFN express CD10 more intensely than normal or hyperplastic germinal center B cells. Most importantly, as a result of t(14;18)(q32;q21) corresponding to a chromosomal translocation involving the *BCL2* and *IGH* genes, BCL2 protein is uniformly overexpressed in the neoplastic germinal center B cells of ISFN. The aberrant BCL2 expression in germinal centers typically is more intense than the BCL2 expression in mantle zones and interfollicular areas.[12] The Ki-67 labeling index is usually low (10% or less) without the polarization seen in normal germinal centers, in which proliferating cells are concentrated in the dark zone.[14,15] The number of neoplastic B cells in affected germinal centers is varied: germinal centers may contain only a few BCL2-positive B cells, or may be nearly packed with neoplastic cells. By definition, the neoplastic B cells with CD10 and BCL2 are limited to the germinal centers[1,5,12] and remain within sharply outlined follicular dendritic cell meshworks.[14]

Genetics

The most characteristic and frequent genetic alteration in ISFN is t(14;18)(q32;q21), similar to that observed in conventional FL.[5] This translocation is considered the earliest event in follicular lymphomagenesis and is due to erroneous *V(D)J* recombination in the bone marrow.[16] This translocation juxtaposes the *BCL2* oncogene on chromosome 18q21 to the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14q32, causing aberrant expression of anti-apoptotic BCL2 protein. Although BCL2 is expressed in almost all normal B cell subsets, it is specifically down-regulated in normal germinal center B cells.[17] These cells undergo somatic hypermutation, generating random mutations in the immunoglobulin gene loci that alter B-cell receptor affinity to antigen, and decreased BCL2 expression is physiologically critical to ensure that only a limited number of B cells with enough affinity will escape apoptosis and be selected for further differentiation.[17] Because the vast majority of germinal center B cells lose antigen affinity as a result of somatic hypermutation and undergo apoptosis, t(14;18)-positive cells have a significant survival advantage in the germinal center through aberrant BCL2 expression. However, the pathobiology of FL with t(14;18) is not so straightforward, as highly sensitive PCR assays have identified t(14;18) cells in peripheral blood of otherwise healthy individuals as well as in benign lymphoid tissue.[18] The prevalence of t(14;18) cells in healthy individuals has been demonstrated to increase with age, smoking, and pesticide exposure,[18] and the prevalence in Asian (Japanese) individuals (10% to 20%) seems to be lower than in Caucasians (50% to 70%).[19–21] These observations demonstrate that *BCL2/IGH* alone is insufficient to develop overt FL. Although the clinical significance of t(14;18)-positive B cells in the healthy population remains incompletely understood, one recent study