Original contribution

IMP3 as a supplemental diagnostic marker for Hodgkin lymphoma

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Summary Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is ubiquitously expressed in embryos, mediating organogenesis, RNA trafficking, and cell growth, and is generally down-regulated in adult tissue. However, IMP3 has recently been shown to be overexpressed in some malignant epithelial neoplasms and to be a useful diagnostic and/or prognostic biomarker for several carcinomas. To determine whether IMP3 might also be an accurate biomarker of Hodgkin lymphoma, we examined 81 Hodgkin lymphomas for immunoreactivity to IMP3 as compared to commonly used markers such as CD30, CD15, PAX5, and MUM1. Consequently, in 98.8% (80/81) of Hodgkin lymphomas, the malignant Hodgkin and Reed-Sternberg cells were selectively reactive for IMP3, with 72.8% (59/81) of the tumors showing strong, diffuse cytoplasmic staining. Positive staining of the Hodgkin lymphomas was also seen for CD30 (82.7%, 67/81), CD15 (65.4%, 53/81), PAX5 (84.0%, 68/81), and MUM1 (85.2% (69/81), but significantly fewer cells showed strong staining intensity for CD30 (32.1%, 26/81), CD15 (17.3%, 14/81), PAX5 (12.3%, 10/81), and MUM1 (29.6%, 24/81). Furthermore, the IMP3 staining was selectively restricted to Hodgkin and Reed-Sternberg cells, with a clearly negative background, and complementary to CD30 staining. Our findings show that IMP3 may be a useful diagnostic marker of Hodgkin lymphoma, helping to improve diagnostic accuracy for this malignancy. © 2013 Elsevier Inc. All rights reserved.

1. Introduction

Hodgkin lymphoma has emerged as one of the most frequently diagnosed lymphomas in Western populations. This malignant disease manifests most often in early adulthood. Fortunately, early diagnosis and standard treatment (chemotherapy, radiotherapy, and/or surgery) is associated with a high likelihood of remission and long-term survival [1-4]. Based on the morphology and immunophenotype of the tumor cells, Hodgkin lymphoma is currently classified into nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and 4 subsets of classical Hodgkin lymphoma (CHL)– nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted. The malignant cells of Hodgkin lymphoma are known as Hodgkin and Reed-Sternberg (HRS) cells, which usually account for <1% of the tumor tissue and reside in a complex admixture of cells with an inflammatory background. While the origin of HRS cells remains controversial, most studies indicate that they may arise in a germinal center B (GCB)
cells [5-8], but some suggest that HRS cells may also be
derived from T lymphocytes [9,10].

Differential diagnosis of Hodgkin lymphoma is based
upon histopathological exclusion of epithelioid sarcomas,
undifferentiated carcinomas, proliferative lesions of lym-
phoid tissue, and the non-Hodgkin lymphomas, especially
diffuse large B-cell lymphomas, natural killer/T-cell lym-
phomas, and anaplastic large cell lymphomas [1,11].
Nonetheless, detection of HRS cells (via morphological
analysis and immunophenotyping) is considered a key
diagnostic feature of Hodgkin lymphoma. The immunohis-
tochemical profile plays an important role in routine
diagnostic practice for Hodgkin lymphoma. Currently, HRS
cells are identified by positive immunostaining with some
commonly used biomarkers: CD30 (also known as Ber-H2),
CD15 (Leu-M1), PAX5 (BSAP), and MUM1 (IRF4)
[1,12-16]. However, the available antibodies targeting these
putative HRS markers are not adequate to provide an absolute
diagnosis for all cases. Identification of additional immuno-
reactive markers is necessary to improve diagnostic accuracy.

IMP3 is a member of the family of insulin-like growth
factor II mRNA-binding proteins (IMPs) that comprises
IMP1, IMP2, and IMP3. The IMP family members have
important physiologic roles in the early stages of embryo-
genesis, mediating RNA trafficking and stabilization to
regulate cell growth and migration [17-19]. However, this
oncofetal protein also appears to have a carcinogenic role.
IMP3 overexpression has been detected in many epithelial
malignancies, including bladder, liver, breast, pancreas,
lung, colon, prostate, ovary, kidney, and some soft tissue
sarcomas. As such, IMP3 has been proposed as a diagnostic
biomarker for some epithelial malignancies [20-27].

Since very few studies in the published literature have
investigated the expression of IMP3 in lymphoid neoplasms,
this study was designed to evaluate the immunohistochem-
ic expression profile of IMP3 in 81 Hodgkin lymphoma
specimens, resected from 10 NLPHL and 71 CHL patients.
Comparison of the IMP3 staining results against simulta-
neous staining for CD30, CD15, PAX5, and MUM1
suggests that IMP3 may be a sensitive, clinically useful
diagnostic marker of Hodgkin lymphoma.

2. Materials and methods

2.1. Case selection

A series of paraffin-embedded specimens from Hodgkin
lymphoma patients (50 males, 31 females with a median age
of 30 years, range 5-79 years) who underwent surgical
 Treatment between 2009 and 2012 were included in this
study. These 81 cases included 10 NLPHL and 71 CHL (27
nodular sclerosis, 19 mixed cellularity, 23 lymphocyte-rich,
and 2 lymphocyte-depleted) patients, diagnosed and classi-
fied according to the World Health Organization criteria [1].

Control specimens were obtained from patients with T-cell-
rich large-B-cell lymphoma (TCRLBCL; n = 5) and
infectious mononucleosis (n = 7). In addition, 15 reactive
lymph nodes were also collected as control specimens. All
cases had hematoxylin and eosin–stained tissues available for
review.

2.2. Immunohistochemistry

The primary antibodies used in this study are presented in
Table 1. The immunohistochemical analysis was carried out
as follows, with triplicate washes in 0.01 mol/L phosphate-
buffered saline performed between each step: first, 4-μm
sections freshly cut from paraffin-embedded blocks were
dewaxed by xylene and rehydrated in a graded ethanol series.
Second, the sections were immersed in 3% hydrogen
peroxide (to block endogenous peroxidase activity) and
subjected to heat-induced epitope retrieval by boiling in 0.01
mol/L citrate buffer (pH 6.0) in a steam pressure cooker (full
pressure for 4 min). Third, the sections were incubated with
the respective primary antibodies (90 min at room temper-
ature) and immunoreactivity was detected with diaminoben-
zidine substrate using the EnVision+ Detection Kit (Dako,
Carpinteria, CA).

Two pathologists (T.Z. and HP.T.) working independent-
ly and blinded to the clinical data carried out semi-
quantitative evaluation of immunohistochemical staining
on the unidentified samples using a 4-tiered system. Staining
was scored as positive if at least 10% of the tumor cells were
immunoreactive, and then scored as weak (1+), moderate
(2+), or strong (3+) according to staining intensity.

3. Results

The immunohistochemical staining results for all 81
Hodgkin lymphomas are summarized in Table 2 (IMP3
according to tumor type) and Table 3 (IMP3 and other
antibodies in total samples).

3.1. IMP3 protein expression in Hodgkin lymphoma

Nearly all (98.8%, 80/81) of the Hodgkin lymphoma
specimens were positive for IMP3 immunostaining, and the
majority (72.8%, 59/81) showed strong staining intensity.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Supplier</th>
<th>Clone No.</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP3</td>
<td>Dako</td>
<td>69.1</td>
<td>1:100</td>
</tr>
<tr>
<td>CD30</td>
<td>Leica, Newcastle, UK</td>
<td>1G12</td>
<td>1:50</td>
</tr>
<tr>
<td>CD15</td>
<td>Leica</td>
<td>BY87</td>
<td>1:50</td>
</tr>
<tr>
<td>PAX5</td>
<td>Invitrogen, Camarillo, CA</td>
<td>ZP007</td>
<td>1:100</td>
</tr>
<tr>
<td>MUM1</td>
<td>Dako</td>
<td>MUM1P</td>
<td>1:100</td>
</tr>
</tbody>
</table>
When the specimens were evaluated according to Hodgkin lymphoma type, a similar staining trend was found for the CHL and NLPHL specimens. In CHL, with nearly all (98.6%, 70/71) specimens showing IMP3-positive immunostaining of HRS cells (Fig. A). Of those, the majority (73.2%, 52/71) showed positive staining in a membrane pattern with accentuation of dot-like staining in the Golgi region of cytoplasm in the HRS cells. Strong staining intensity was observed for CD30 in 36.6% (26/71) of CHL specimens, for CD15 in 19.7% (14/71) of the specimens, for PAX5 in only 12.7% (9/71), and for MUM1 in 32.4% (23/71).

All 10 NLPHL specimens showed uniform negativity for both CD15 and CD30, while approximately half showed positivity for PAX5 (60.0%, 6/10) or MUM1 (40.0%, 4/10) which included one specimen with strong staining intensity for both PAX5 and MUM1.

The percentage of positive staining for the various markers among the total 81 Hodgkin lymphoma specimens was: 82.7% (67/81) for CD30, 65.4% (53/81) for CD15, 84.0% (68/81) for PAX5, and 85.2% (69/81) for MUM1. In addition, less than half of the immunostained cells showed strong staining intensity for CD30 (32.1%, 26/81), for CD15 (17.3%, 14/81), for PAX5 (12.3%, 10/81), and for MUM1 (29.6%, 24/81).

When the IMP3 expression in HRS cells was compared to these diagnostic markers, we were intrigued to find that IMP3 and CD30 expressions may be complementary for pathological diagnosis of Hodgkin lymphoma. For example, five CHL specimens did not show double-positivity for IMP3 and CD30, but did show positivity for either IMP3 (n = 4) or CD30 (n = 1). Therefore, when immunostaining was performed for both IMP3 and CD30, all 71 CHL cases were detected. For the NLPHL specimens, IMP3 immunostaining may be more useful as a diagnostic marker to overcome the lack of CD15 and CD30 expression.

### 4. Discussion

Accurate diagnosis of Hodgkin lymphoma is essential for initiating a timely and appropriate treatment strategy, thereby improving patient prognosis and survival [1,28]. While the morphologic and immunohistochemical features of HRS cells are the current standard of diagnosis, no single biomarker has yet been identified for use as a specific target of clinical tests. The current panel of markers, including CD15, CD30, PAX5, MUM1, and some other lymphocytic markers such as CD3 and CD79a [11-16], help to identify HRS cells, but some limitations exist and interpretation of the results remains somewhat subjective. For example, differential diagnosis is confounded by the fact that anaplastic large cell lymphomas also expresses CD30 and some B cell lymphomas, such as large B cell lymphoma with Hodgkin features, express CD15 [29]. Thus, there is a need for identifying more biomarkers that may help to improve the immunohistochemical diagnosis of Hodgkin lymphoma.
IMP3 was originally identified in pancreatic cancer tissues and cell lines and was published under the designation of “K-homology domain-containing protein overexpressed in cancer” prior to being recognized as an insulin-like growth factor II family member [17-19]. Interest in its role in malignant neoplasms has grown in recent years. Indeed, IMP3 overexpression has been detected in many malignancies, and many studies have indicated its clinical utility as a diagnostic biomarker [20-23]. Moreover, the overexpression of IMP3 has been shown to correlate with aggressive biological behavior of several epithelial cancers, suggesting its potential to act as a prognostic biomarker as well [24-27].

Little is known about the roles of IMP3 in neoplasms of the hematopoietic and lymphoid systems. King et al. [30] investigated the IMP3 overexpression in neoplastic lymphoid tissues and showed an intriguing differential expression profile among the various types of lymphomas, with 100% of Hodgkin lymphomas showing the overexpression and 20% less of diffuse large B-cell lymphomas, Burkitt’s lymphomas, and follicular lymphomas showing the overexpression. Hartmann et al [31] investigated the functional roles of IMP3 overexpression in mantle cell lymphomas and discovered a pro-proliferative function. Since no studies to date had specifically investigated the potential diagnostic or prognostic value of IMP3 for lymphomas, we used immunohistochemical analysis to evaluate 81 Hodgkin lymphoma specimens and determine the utility of IMP3 to supplement the results of the other commonly used biomarkers.

Almost all of the HRS cells in the Hodgkin lymphoma specimens showed selective immunostaining for IMP3, and the majority of those cells had strong staining intensity that was easily recognizable. Additionally, the obvious IMP3-negative results detected for the other background cells further helped to make the identification.

Table 3 Different HRS markers’ expression in Hodgkin lymphomas (n = 81)

<table>
<thead>
<tr>
<th>Markers</th>
<th>Negative staining</th>
<th>Positive immunostaining intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>CD30</td>
<td>14*</td>
<td>11</td>
</tr>
<tr>
<td>CD15</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>PAX5</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>MUM1</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

* All 10 cases of NLPHL were negative for both CD30 and CD15.

However, we also determined that IMP3 is not a specific marker of Hodgkin lymphoma. The other lymphoid proliferative lesions such as reactive lymph nodes, infectious mononucleosis-infected tissue, and TCRLBCL also showed IMP3 expression in GCB cells, interfollicular immunoblasts, and malignant large B cells, respectively. This finding, however, is consistent with previous data reported in the literature that suggests HRS cells may be derived from B lymphocytes.

Lastly, when we compared the expression of IMP3 to that of other commonly used markers of Hodgkin lymphoma we found that IMP3 has the highest positivity for HRS cells among CD30, CD15, PAX5, and MUM1. Thus, while not a specific marker of Hodgkin lymphoma, IMP3 may act as a useful supplemental marker to the established panel of diagnostic biomarkers.

The strong diffuse cytoplasmic immunostaining of IMP3 in HRS cells, contrasting with the absolute nonreactivity (with the occasional exception of the rudimental germinal centers) of complex background non-HRS cells, will substantially facilitate the ability to identify HRS cells in clinical analysis of Hodgkin lymphoma. Both CD30 and CD15 are typically detected in the membrane and Golgi region of HRS cells, but this expression pattern leads to their immunostaining being less conspicuous than that of IMP3. Other limitations of CD15 and CD30 detection are their negative reactivities in NLPHL specimens and the fact that CD15 is also detected (although rarely) in reactive B and T lymphocytes [32]; both of these limitations may be at least partially addressed by using supplemental immunostaining for IMP3. The other commonly used markers of Hodgkin lymphomas, PAX5 and MUM1, have similar limitations for diagnosis; although their immunostaining occurs in an adequate amount of HRS cells, the immunostaining of PAX5 is actually stronger in background B cells and that of MUM1 is equivalent in activated B cells, ultimately complicating observer interpretation.

While IMP3 appears to be promising as a supplemental marker for HRS cells, it is also likely to act as a good complement to CD30, in particular [1,11]. We noted that a detection strategy using IMP3 and CD30 is more appropriate to detect all Hodgkin lymphomas than use of either marker alone.

In summary, the study described herein has indicated several potential advantages to using IMP3 as a novel immunohistochemical marker for diagnosis of Hodgkin lymphoma: (a) its relatively higher expression, evidenced by a stronger staining intensity, compared to the other traditional Hodgkin lymphoma markers; (b) its ability to differentiate HRS cells against a complex background of non-HRS cells; and (c) its putative complementary role with...
CD30, especially in CD30-negative NLPHL specimens. Even when the non-specific expression of IMP3 in various lymphoid proliferative lesions is considered, this marker may still represent a useful supplemental marker for helping to identify HRS cells in clinical testing. It is important to note, however, that further studies are required to determine whether IMP3 plays a mechanistic role in the generation and/or development of Hodgkin lymphoma will likely benefit its development as a molecular target of diagnostic and prognostic testing as well as possible therapeutic strategies.

**Acknowledgments**

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**References**