Specific von Hippel-Lindau Protein Expression of Clear Cell Renal Cell Carcinoma with “Immunogenic” Features*

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Human clear cell renal cell carcinoma (CCRCC) is characterized by specific von Hippel-Lindau gene alterations and “immunogenic” features. In the present study, the immunohistochemical expression of the von Hippel-Lindau gene protein (pVHL) was compared with the presence of major histocompatibility complex (MHC I-II), tumor infiltrating lymphocytes (TIL) and tumoral immune complexes (TIC) in CCRCC. Native tumor tissues of 132 RCC patients (95 with the common clear cell subtype), diagnosed according to the Heidelberg classification, were obtained for immunohistochemistry. Tumor stainings with pVHL, MHC I-II and tumor infiltrating lymphocytes (T and B lymphocytes, monocytes) were detected by immunoperoxidase methods using monoclonal antibodies. Tumoral immune complexes (IgG, IgA, IgM and C1q, C3 complement proteins) were visualized by fluorescent polyclonal antibodies. Immune stainings were semiquantitatively evaluated. Specificity and sensitivity of these markers in relation to the common histological subtype of RCC (CCRCC) were calculated. CCRCC was characterized by specific pVHL expression. At the same time, CCRCC was associated with constitutional MHC I-II expression and highly specific degree of TIL and TIC. It is concluded that specific pVHL expression of CCRCC is frequently associated with “immunogenic” features. Immunohistochemical analysis aims the initial tumor staging of RCC patients to achieve better patient selection for immunotherapy. However, the association of pVHL expression with the “immunogenic” CCRCC is statistically relevant, the mechanism and its clinical relevance in immunotherapy still remains to be tested. (Pathology Oncology Research Vol 7, No 1, 42–45, 2001)

Keywords: clear cell renal cell carcinoma, von Hippel-Lindau protein, tumor infiltrating lymphocytes, immune complexes

Introduction

Renal cell carcinoma (RCC) is a promising target for immunotherapy. The response rate should be enhanced by selecting patients expressing relevant tumor antigens with active antigen presentation through major histocompatibility complex (MHC I-II) and characterized by effective antitumor mechanisms (tumor infiltrating lymphocytes [TIL] and immune complexes [TIC]).2,13,16 MHC-restricted T lymphocytes with specific cytotoxic activity for autologous tumor cells persist in some RCC patients for years after removal of the primary tumor even in the absence of immunotherapy.9 Immunohistochemical studies proved a significant TIL infiltration of RCC by preoperative interferon-gamma (IFN-gamma) administration.3,15 The phenotype of TIL isolated from RCC patients is heterogenous (predominant MHC class I-restricted CD8+ T lymphocytes; few gamma/delta T cells, monocytes and NK cells).11 Activation markers (CD69, CD25, HLA-DR) are expressed on TIL, but autologous tumor lytic activity is impaired in most of the RCC patients.6 The contribution of cytokines and co-
stimulatory molecules in TIL-tumor cell interaction is under evaluation. The clinical relevance of intratumoral lymphocytic invasion is still controversial in interleukin-2 (IL-2) and TIL-based therapy. Routine histology does not improve on prediction of antitumor response in RCC. Some studies, however, found MHC-I expression, TIL and TIC exclusively in the clear cell subtype of RCC. This "immunogenic" subtype of RCC (the typical clear cell renal tumor of von Hippel-Lindau [VHL] syndrome and 75% of sporadic renal neoplasms) was separated from others on the basis of specific genetic alterations on chromosome 3p. Mutations of a single allele of the VHL gene are associated with deletion of the remaining functioning VHL allele ("loss of heterozygosity"). Recent development of monoclonal antibodies against VHL protein (pVHL) enabled the detection of tumoral pVHL expression by immunohistochemistry. Since pVHL is known as an antigen of RCC-associated paraneoplastic immune complex nephropathies, mutant pVHL can be a target antigen of antitumor immunity and specific immunotherapy.

In the present study pVHL expression and immunohistochemical markers accepted as tags of immunotherapy (MHC I or II, TIL and tumor immune complexes) were compared in 132 RCC patients.

Materials and Methods

Patients

Native tumor tissues with adjacent renal parenchyma were obtained from 132 resected kidneys of RCC patients. The histology according to the Heidelberg classification revealed a subtype distribution (common clear cell, 72%; sarcomatous, 5%; mixed, 6%; chromophobe, 3%; papillary, 7%; renal oncytoma, 7%) pathological grade (I-II, 73%; III-IV, 17%); stage (Std I-I, 68%; III, 24%; IV, 8%) and metastatic cases (22%) comparable with those in the literature.

Immunohistochemistry

Stainings was performed on acetone-fixed cryostat sections. For demonstration of TIC, fluorescent isothiocyanate (FITC)-labeled polyclonal rabbit antihuman IgG, IgA, IgM and complement (C1q, C3) antibodies (DAKO, diluted 1:10) were used. An immunoperoxidase method using a secondary biopolymer reagent (Envision kit, DAKO) detected pVHL (Pharmingen monoclonal IG32, 0.5 mg/ml, recognizing amino acids 1-213 of human pVHL). The ABC-immunoperoxidase method detected TIL (UCHL-I for "memory T lymphocytes", Mac387 for monocytes, CD45RB for B lymphocytes), the β-chain of MHC-I (β2-microglobulin) and HLA-DR chain of the MHC-II (DAKO monoclonals, dilution 1:50).

Evaluation of the immunohistochemistry and statistical methods

Immunohistochemical staining was graded microscopically by two independent observers as described before in detail. Briefly, tumor tissues of RCC cases with mild, focal or negative staining, were estimated as negative, in contrary to cases with moderate to strong diffuse positivity categorized as positive. Tumor infiltrating lymphocytes were counted in 20 microscopic fields of an 0.05 mm² eyepiece graticule at x200 magnification. Cases with marginal immune cell infiltration or intratumoral TIL below 100 cells/mm² were estimated as negative. In contrast, RCC cases with TIL present exclusively in the tumor area and exceeding 100 cells/mm² were positive. Specificity and sensitivity of these immunohistochemical markers in relation to the common clear cell histological subtype of RCC were calculated as follows:

Specificity = true negative cases / true negative + false positive cases x 100,
Sensitivity = true positive cases / true positive + false negative cases x 100.

As an example, by calculating the specificity and sensitivity of pVHL staining in relation to CCRCC histological subtype, all CCRCC cases stained positive with pVHL were "true positive", while CCRCCs with negative pVHL staining were estimated as "false negative". Renal cell carcinomas with non-CCRCC morphology, which exhibited negative pVHL staining, were estimated as "true negative", while the remaining cases with pVHL positivity, as "false positive". The specificity and sensitivity of the other markers were calculated similarly.

Results

Von Hippel-Lindau protein (pVHL) was exclusively present in RCC with clear cell morphology (CCRCC) (Table 1, specificity). Figure 1 presents the typical clear cell renal cell carcinoma (CCRCC).

<table>
<thead>
<tr>
<th>IHC staining</th>
<th>CCRCCs tested</th>
<th>specificity (%) to CCRCC</th>
<th>sensitivity (%) to CCRCC</th>
</tr>
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<tbody>
<tr>
<td>pVHL</td>
<td>68/95</td>
<td>100</td>
<td>91</td>
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<tr>
<td>MHC-I</td>
<td>55/76</td>
<td>25</td>
<td>91</td>
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<tr>
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<td>63/86</td>
<td>32</td>
<td>98</td>
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<td>95/132</td>
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<td>45</td>
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<tr>
<td>TIC</td>
<td>87/121</td>
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