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Original contribution

Expression of stem-cell markers OCT-4 and CD133: important prognostic factors in papillary renal cell carcinoma

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Summary Except for tumor stage and histologic subtype, the prognostic factors of papillary renal cell carcinoma remain controversial. To the best of our knowledge, the prognostic significance of the expression of stem cell markers, OCT-4 and CD133, has not yet been studied in papillary renal cell carcinoma. Expressions of OCT-4 and CD133 were examined immunohistochemically in a tissue microarray construct generated from 119 cases of papillary renal cell carcinoma, collected from November 1996 to December 2008, and then the results were correlated with the clinicopathologic findings. OCT-4 was expressed at the nuclei of tumor cells in 26 cases (22%). The high expression of OCT-4 with a cut-off value of 12.5%, was associated with frequent microscopic lymphovascular invasion and poor disease-specific survival. CD133 was expressed in the apicolateral cell membrane of tumor cells in 21 cases (17.8%) with a cut-off value of 5%. The CD133 expression was correlated with small tumor size and lack of microscopic lymphovascular invasion, and it tended to be associated with a low Fuhrman nuclear grade and prolonged disease-specific survival. On multivariate analysis, tumor stage, histologic subtype, and OCT-4 expression, but not CD133 expression, were independent prognostic factors for disease-specific survival. OCT-4-expressing and CD133-nonexpressing papillary renal cell carcinoma showed the shortest disease-specific survival. These results showed that the expression of stem cell markers, OCT-4 and CD133, may serve, respectively, as a poor and favorable prognostic marker, in papillary renal cell carcinoma.

1. Introduction

Papillary renal cell carcinoma (pRCC) accounts for 10% to 15% of all renal tumors and constitutes the second most frequent form of renal cell carcinoma (RCC) in adults followed by clear cell RCC (ccRCC) [1]. There are 2

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prognostic factors widely used in pRCC. One is the TNM stage and the other is the histologic subtype proposed by Delahunt and Eble [2]. However, in pRCC patients exhibiting similar clinical and pathological features, only limited information is currently available for predicting the clinical outcomes and providing guidance for making the optimal therapeutic decision.

The cancer stem cell (CSC) model has become the center of growing attention. According to the CSC model, a tumor is composed of a heterogeneous cell population, among which a small proportion of cells, so-called CSCs, have the ability to initiate tumors because of their self-renewal capacity giving rise to CSC themselves and the multipotency generating the bulk of more differentiated tumor cells [3]. A high proportion of CSCs indicates a poor prognosis in various malignant tumors including carcinomas of the endometrium and colon [4,5]. It has recently been shown that CSCs are highly resistant to conventional chemo-/radiation therapy and that the molecular imprints of stemness might serve as molecular predictors of the cancer therapy outcome [6].

OCT-4 is a member of the POU-domain transcription factor family and its expression is normally confined to pluripotent cells of a developing embryo [7]. In malignant tumors, OCT-4 expression has been observed not only in germ cell tumors such as seminoma/germinoma and embryonal carcinoma but also in carcinomas of various organs including the urinary bladder, breast, oral cavity, lung, liver, and stomach [8]. In malignant kidney tumors, one previous study reported that OCT-4 expression was not present in ccRCC, but no study has been performed on pRCC [9].

CD133 (prominin-1), a pentaspan membrane glycoprotein, was initially identified as a cell surface antigen specific to hematopoietic stem cells but is currently widely used as a stem cell surface marker of various normal and neoplastic human tissue including the brain, skin, prostate, pancreas, liver, lung, stomach, uterus, and kidney [4,10–12]. In the normal kidney, CD133-expressing cells are shown to have stem cell characteristics such as self renewal, multipotency, and participation in kidney repair [13]. However, there are only a limited number of studies regarding CD133 expression in RCC, particularly for pRCC [14].

In an attempt to identify the prognostic significance of CSC markers in pRCC, we examined the immunohistochemical expressions of OCT-4 and CD133 in 119 cases of pRCC and then correlated the results with the clinicopathologic findings.

2. Materials and methods

2.1. Patients and clinical data

This retrospective study initially included consecutive 139 pRCC patients who had undergone nephrectomy at Asan Medical Center from November 1996 to December 2008. Among these patients, 10 were excluded because their glass slides and paraffin blocks were not available for the slide review and tissue microarray construction. Five patients were excluded because the ccRCC component was mixed with pRCC, as seen on the slide review. Five additional cases were excluded because they were reassessed as renal carcinomas associated with Xp 11.2 translocations/*TFE3* gene fusions on the basis of the histologic features and immunostaining for TFE3. Finally, 119 pure pRCC cases were included in this study. Patients' clinical information including age, sex, diagnosis date, follow-up data, and radiological findings was obtained from our electronic medical records. Survival data were retrieved from the Department of Medical Records at Asan Medical Center and from the National Statistical Office of the Republic of Korea. The tumor stage was assigned according to the American Joint Committee on Cancer staging system, 7th edition [15].

2.2. Pathology evaluation of the pRCC cases

The histological findings were reviewed for diagnostic reassessment and histologic subtyping according to the 2004 World Health Organization Tumor Classification and Delahunt and Eble, respectively [2]. Each tumor was graded according to the Fuhrman nuclear grading system and the nucleolar grading system [16,17]. Microscopic lymphovascular invasion and resection margin status were also evaluated.

2.3. Tissue microarray construction

Tissue microarray (TMA) blocks composed of 1-mm-diameter cores were generated from the formalin-fixed, paraffin-embedded tissue blocks of primary pRCCs using a manual tissue microarrayer (Quick-RAY, Unitma, Seoul, Republic of Korea). Three representative cores of the tumor for each case were included in the TMA blocks.

2.4. Double immunohistochemical staining

The primary antibodies used in this study were OCT-4 (1:200 dilution, Oct-3/4(C-10):sc-5279, Santa Cruz Biotechnology, Santa Cruz, CA) and CD133 (1:50 dilution, AC133, Miltenyi Biotec, Auburn, CA). Double immunostaining was feasible as OCT-4 is expressed in the nucleus and CD133 displays distinct cell membrane staining. Double immunostaining for OCT-4 and CD133 was performed manually using the Bond Polymer Intense Detection System (Leica Microsystems, Wetzlar, Germany), as previously described but with minor modifications [18]. The anti-OCT-4 antibody was applied for the first 15 minutes, followed by incubation with the anti-CD133 antibody for another 15 minutes at room temperature. Sections were then treated with post-primary and polymer reagents. Diaminobenzidine was used as a