Original contribution

Fibroblast activation protein predicts prognosis in clear cell renal cell carcinoma ☆

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Summary: Clear cell renal cell carcinoma is a complex disease with only partial response to therapy and scarce reliable clinical parameters indicative of progression and survival. Fibroblast activation protein expression has been correlated with prognosis in several malignancies but never in renal cancer. We aim to analyze the immunohistochemical expression of fibroblast activation protein in 208 clear cell renal cell carcinomas and to evaluate its impact on the prognosis and survival. A positive cytoplasmic immunostaining of this protein in the stromal fibroblasts associated to cancer cells is associated with large tumor diameter (≥ 4 cm), high-grade (G3/4) tumors, and high-stage ($\geq$pT3) tumors. Fibroblast activation protein–positive cases had significantly shorter survivals after 5 ($P = .00015$), 10 ($P = .0000042$), and 15 ($P = .000043$) years of follow-up, with a hazard ratio of 0.31. Multivariate analysis showed that fibroblast activation protein ($P = .00117$) was stronger than grade and stage in predicting clinical aggressiveness in clear cell renal cell carcinoma. This study confirms the usefulness of fibroblast activation protein detection in the stromal fibroblast associated to cancer in clear cell renal cell carcinoma and adds a new immunohistochemical marker to predict clinical behavior in these patients.
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1. Introduction

Renal cancer is a common neoplasm and a leading cause of cancer death in Western countries, and its incidence is increasing in recent years with more than 62 000 new cases estimated in the United States in 2016 [1]. Clear cell renal

cell carcinoma (CCRCC) is by far the most common histological subtype, accounting for approximately for 75%-80% of the cases in most series [2]. CCRCC is a paradigmatic example of an intrinsically heterogeneous and aggressive neoplasm [3], and this fact explains the poor results obtained by modern personalized therapies. Actually, surgery remains nowadays as the only effective treatment in these patients.

Fibroblast activation protein–α (FAP) is a cell surface glycoprotein with dipeptidyl peptidase (DPPIV) and collagenolytic activity highly expressed on cancer-associated fibroblasts (CAFs) from several malignancies, including carcinomas of the oral cavity [4,5], esophagus [6], stomach [7], pancreas [8–10], colon [11], breast [12,13], ovary [14], endometrium [15], and lung [16], as well as in bone and soft tissue sarcomas [17,18] and in malignant melanoma [19]. Very recently, a study has shown that CAFs promote CCRCC progression in vitro [20], although the molecular basis of such activity remains unveiled. However, a clinical study analyzing the impact of FAP expression in CCRCC is still lacking.

The aim of this study was to evaluate the expression of FAP and its clinical significance in a retrospective series of 208 CCRCC.

2. Materials and methods

The authors declare that all the experiments carried out in this study comply with current Spanish and European Union legal regulations. Samples and data from patients included in this study were obtained from the medical records and archives of the pathology laboratory. All patients were informed about the potential use for research of their surgically resected tissues and accepted this eventuality by signing a specific document approved by the Ethical and Scientific Committees (CEIC 2015/060, CEIC-E PI2015101).

A total of 208 CCRCCs retrieved in 2 medical institutions were included in the study in a retrospective way. The series included 179 total nephrectomies and 29 partial nephrectomies. Follow-up was obtained from the clinical histories and was closed at December 31, 2014. Cases were reviewed by 2 pathologists (J. I. L., R. G.), who assigned Fuhrman grade [21] and 2010 American Joint Committee on Cancer stage [22] on hematoxylin and eosin sections from tumor samples obtained following standard protocols. Immunohistochemistry was previously performed using carbonic anhydrase IX (Epitomics, ref. code AC-0137RUO, dilution 1: 100), CK7 (Ventana, ref. code 790-4462, ready to use), and CD117 (Ventana, ref. code 790-2951, ready to use) to confirm as CCRCC those cases that were histologically unclear.

Tissue microarrays were performed for the evaluation of FAP expression. One core (2.5 mm in diameter) of well-preserved tumor tissue obtained at the front of invasion into the renal parenchyma was selected for tissue microarray in each case. FAP antibody (Abcam, ref. ab53066, dilution 1:70) was evaluated in the stromal fibroblasts adjacent to

neoplastic nests. Immunohistochemical stainings were performed in automated immunostainers (EnVision FLEX, Dako Autostainer Plus; Dako, Glostrup, Denmark, and BenchMark Ultra; Ventana Medical Systems, Tucson, AZ) following routine methods. Tris-EDTA was used for antigen retrieval in all cases. Negative controls were slides not exposed to the primary antibody, and these were incubated in phosphate-buffered saline and then processed under the same conditions as the test slides. The analysis was performed using a Nikon Eclipse 80i microscope (Tokyo, Japan).

The evaluated parameters were age, sex, Fuhrman grade, tumor diameter, staging, and FAP expression. A data-mining analysis was performed using Waikato Environment for Knowledge Analysis [23]. Multiple parameters were selected to obtain meaningful rules for the classification of the FAP-positive/-negative response. Also, an attribute selection turning out if the parameter was relevant or irrelevant to explain the data was performed. For such a purpose, different search methods were used such as the best-first, rank-search, or random-search algorithms.

R software was used for statistical analysis. Kaplan-Meier (KM) curves and log-rank P are displayed after performing a
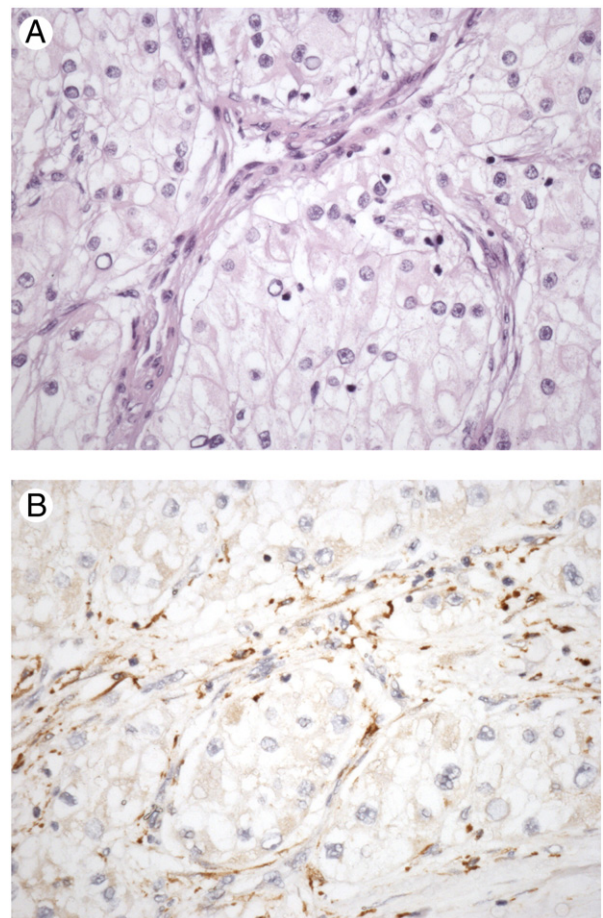
Fig. 1   Nested pattern of growth in CCRCC (A) with peripherally located stromal fibroblasts immunostained with FAP (B) (original magnification ×400).