Human Pathology (2015) **46**, 820–826

ELSEVIER

Human PATHOLOGY

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Original contribution

Succinate dehydrogenase B: a new prognostic biomarker in clear cell renal cell carcinoma☆

Kristine M. Cornejo MD[a,b,1], Min Lu MD, PhD[a,b,c,1], Ping Yang MD, PhD[a,b,d], Shulin Wu MD[a,b], Chao Cai MD[e,f], Wei-de Zhong MD[e], Aria Olumi MD[f], Robert H. Young MD[a,b], Chin-Lee Wu MD, PhD[a,b,f,*]

[a]*James Homer Wright Pathology Laboratories, Massachusetts General Hospital, Boston, MA*
[b]*Department of Pathology, Harvard Medical School, Boston, MA*
[c]*Department of Pathology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, People's Republic of China*
[d]*Department of Pathology, Sun Yat-sen University Cancer Center, Guangzhou, People's Republic of China*
[e]*Department of Urology, Guangzhou First Municipal People's Hospital, Guangzhou Medical College, Guangzhou, People's Republic of China*
[f]*Department of Urology, Massachusetts General Hospital, Boston, MA*

Received 22 October 2014; revised 24 February 2015; accepted 26 February 2015

Keywords:
Renal cell carcinoma;
Clear cell;
Succinate dehydrogenase B;
Immunohistochemistry;
Oncocytoma;
Prognosis

Summary Succinate dehydrogenase B (SDHB) is a mitochondrial enzyme complex subunit. Loss of SDHB protein expression has been found to correlate with *SDHx* gene mutations. Little is known about its expression in subtypes of renal cell carcinoma (RCC) and whether it is a prognostic indicator. Four hundred fifty renal epithelial neoplasms were analyzed for SDHB, comprising clear cell RCC (CCRCC) (n = 240), papillary RCC (n = 84), chromophobe RCC (n = 49), renal oncocytoma (n = 47), clear cell papillary RCC (CCPRCC) (n = 19), and von Hippel-Lindau (VHL)–associated CCPRCC-like tumors (n = 11). Succinate dehydrogenase B expression was graded based upon staining intensity using a 4-tiered system (0-3+), in which 3+ was strongest and complete absence was 0. Neoplasms were further categorized based upon staining extent into SDHB weak (1+-2+) and strong (3+). Succinate dehydrogenase B was strongly preserved in 131 (55%) of 240 CCRCCs, 84 (100%) of 84 papillary RCCs, 49 (100%) of 49 chromophobe RCCs, 1 (5%) of 19 CCPRCC, 5 (45%) of 11 VHL-associated CCPRCC-like tumors, and 47 (100%) of 47 renal oncocytomas. The remaining 109 CCRCCs, 18 CCPRCCs, and 6 VHL-associated CCPRCC-like tumors had weak but preserved SDHB. Succinate dehydrogenase B expression in CCRCCs with high International Society of Urological Pathology nucleolar grade (G3-G4) correlated significantly with survival (log-rank, $P = .0004$). Succinate dehydrogenase B is variably expressed in RCCs with clear cell morphology and strongly preserved in most other neoplasms. Therefore, weak staining, particularly in clear neoplasms, should not be misinterpreted as negative. Finally, SDHB expression in CCRCCs with high nucleolar grade (G3-G4) is significantly associated with survival, indicating it may be both a diagnostic and prognostic marker in RCC.

☆ Disclosures: This study was supported by the National Institutes of Health grant CA120964 to C.-L. Wu.
* Corresponding author. Massachusetts General Hospital, Department of Pathology, Warren Building 225, 55 Fruit Street, Boston, MA 02114.
E-mail address: cwu2@partners.org (C. -L. Wu).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.humpath.2015.02.013
0046-8177/

1. Introduction

Succinate dehydrogenase (SDH)/mitochondrial complex II is a key respiratory enzyme located in the inner mitochondrial membrane that links the Krebs tricarboxylic acid cycle to oxidative phosphorylation [1]. The SDH complex, composed of 5 proteins (SDHA, SDHB, SDHC, SDHD, and SDHAF2), are encoded by 5 nuclear genes known as *SDHx* complex genes. Mutations in *SDHx* genes are associated with the development of hereditary pheochromocytoma/paraganglioma syndrome and, less commonly, gastrointestinal stromal tumors and renal cell carcinoma (RCC) [2-4]. A mutation or inactivation in any *SDHx* gene results in the degradation of the SDH complex and is associated with negative succinate dehydrogenase B (SDHB) protein expression [2,3,5].

Recently, we described negative SDHB immunohistochemical staining in papillary RCCs (PRCCs) associated with tuberous sclerosis complex (TSC). Tuberous sclerosis complex is an autosomal dominant genetic disorder that can affect in varying degrees nearly every organ system, including the brain, skin, heart, lungs, and kidneys. A mutation in either *TSC1* (chromosome 9q34), encoding the protein hamartin, or *TSC2* (chromosome 16p13), encoding the protein tuberin, can be identified. These proteins act together as tumor suppressors and are components of the mTOR (mammalian target of rapamycin) signaling pathway [6]. These tumors have been uniquely found to be negative for SDHB protein expression, despite being associated with *TSC* gene mutations and not *SDHx* gene mutations [7]. Tuberous sclerosis complex–associated PRCCs contain overlapping morphological features with clear cell (tubulo) PRCCs (CCPRCCs) and von Hippel-Lindau (VHL)–associated CCPRCC-like tumors because they also contain papillary architecture lined by cells with clear cytoplasm. Immunohistochemically, they were found to be uniquely negative for SDHB in addition to being positive for CD10, CK7, and CAIX, making them distinct [7].

Succinate dehydrogenase B immunohistochemical staining has not been well documented in renal epithelial neoplasms, with most studies focusing on *SDHx* mutation–associated RCC. In addition, SDHB expression has not been studied as a possible independent prognostic biomarker in RCC. To better characterize SDHB protein expression in renal epithelial neoplasms, we performed immunohistochemical analysis on a total of 450 renal tumors. We found that SDHB protein expression was preserved in all renal epithelial neoplasms analyzed, albeit in varying intensities, as described below. In addition, we tried to validate the prognostic significance of SDHB expression in clear cell RCC (CCRCC).

2. Materials and methods

2.1. Case selection

A total of 420 surgically resected renal epithelial tumors were selected from the case archives of the department of pathology at the Massachusetts General Hospital from 1992 to 2003. The patient demographics and follow-up data were obtained from the medical records with approval of the institutional review board. The gross findings, including the laterality, number, and size of the tumors, were noted from the pathology reports.

Histologic subtypes of RCCs were reviewed and reclassified according to the International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia and evaluated for the ISUP nucleolar grade [8,9]. All 420 tumors contained remaining formalin-fixed, paraffin-embedded tissue in which 4-μm sections of each tumor were obtained for immunohistochemical analysis and tissue microarray (TMA) studies.

2.2. Tissue microarray

Microarrays of renal epithelial neoplasms (n = 420) were constructed from CCRCC (n = 240), PRCC (n = 84), chromophobe RCC (ChRCC) (n = 49), renal oncocytoma (RO) (n = 47), and normal kidney tissue taken adjacent to tumor (n = 15). Four 1.0-mm or 0.6-mm-diameter cores were obtained from paraffin-embedded tissue for each tumor tested.

2.3. Clear cell papillary renal cell carcinoma

Hematoxylin and eosin–stained slides including RCCs selected by TMA together with RCCs resected from 2004 to 2012 at Massachusetts General Hospital were reviewed for morphological features of CCPRCC. One representative paraffin block from each case was selected for immunohistochemical staining. The combination of characteristic morphological features and immunohistochemical staining resulted in a total of 19 CCPRCCs.

2.4. VHL-associated CCPRCC-like tumors

A total of 11 VHL-associated CCPRCC-like tumors were included in this study. The diagnosis of VHL disease was based either on a known family history or a combination of tumors diagnostic of VHL disease including capillary hemangioblastoma of the central nervous system or retina plus another VHL-associated extrarenal tumor.

2.5. Immunohistochemical staining

Series of 4-μm sections were cut from formalin-fixed, paraffin-embedded tissue blocks, and SDHB immunohistochemistry was performed (Abcam Inc, Cambridge, MA; clone 21A11AE7; 1:1000 dilution). Succinate dehydrogenase B immunostaining was first performed on TMA samples of renal epithelial neoplasms. For confirmation, SDHB immunohistochemistry was performed on one whole slide in 50 randomly selected cases from SDHB-weak CCRCC on TMA. Non-neoplastic renal parenchyma was