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Immunohistochemistry as a tool for screening rare renal cancers

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Recent additions of succinate dehydrogenase (SDH) and (Hereditary Leiomyomatosis and Renal Cell Cancer, HLRCC, associated) fumarate hydratase (FH)-deficient renal cell carcinomas (RCC) to the 2016 WHO Classification of Renal Tumours (1) have highlighted an evolving need for the distinction between renal cancer subtypes. Differences in molecular characterisation, clinical phenotypes, and therapeutic responses (1–3) further corroborates this paradigm shift and strongly points towards the development of subtype-specific management (3). SDH and FH-deficient RCCs are rare tumours strongly associated with hereditary neoplastic syndromes and early-onset(2, 4, 5). FH-deficient RCCs are highly aggressive tumours associated with poor patient prognosis(2, 3, 6), whereas SDH-deficient RCCs are phenotypically more variable, but also known to exhibit aggressive disease behaviour (7–9). Whilst localised RCCs (regardless of subtype) are usually managed surgically (5, 7, 10), in advanced/ metastatic RCCs where systemic therapies are the mainstay of management (7, 10), the majority of clinical trials that provide the basis for these guidelines are centred around clear cell RCC (11). Limited and/or no data is available to guide management of metastatic SDH-deficient RCC and, in particular, FH-deficient RCC (5, 7). Furthermore, there is a lack of consensus as to the follow-up regime of patients with resected sporadic RCCs, and little evidence on how to observe SDH- or FH-deficient RCCs (7). Therefore, logic prevails on the strong emphasis placed on improving the accurate detection and diagnosis of these rare subtypes (9, 12–16) to discern the phenotypic features more comprehensively and improve patient stratification in the evolving era of precision-based medicine.

Inactivating mutations rendering SDH and FH enzyme activity absent lead to the accumulation of succinate and fumarate respectively, which are *bona fide* oncometabolites that have pro-oncogenic capabilities such as the induction of protein modifications,

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Conflict of interests

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Ethical statement:

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

aggressive tumour phenotypes, and epigenetic modulation (17, 18). In particular, fumarate accumulation was shown to bind reactive thiol residues of proteins inducing a post-translational modification called succination (15, 19). Of note, succinated proteins can be detected using antibodies against succinated cysteines residues (anti-2SC). In the manuscript recently published in Human Pathology, Gupta and colleagues capitalise on this unique oncometabolite-associated trait and used immunohistochemistry (IHC) to probe SDHA/SDHB and FH/2SC for the detection of SDH and FH-deficient RCCs in a large historical panel of resected renal tumour subtypes (n=1009).

The authors used qualitative scoring (i.e. positive (+) or negative (-) comparative to an internal positive control) for SDHA, SDHB, and FH staining. Positive 2SC (2SC+) staining was determined based on a previous study that correlated an intensity rating and staining pattern of 2SC with molecular analyses of *FH* mutations in RCC tissues (12). Tissue sections with abnormal IHC staining underwent pathological review using the WHO 2016 classification and cases were clinically correlated with patient records. Overall, three cases of SDH-deficient RCCs (SDHA+/SDHB-) were identified, and were all tumours originally diagnosed as oncocytomas (1.1%, n=273). Retrospective review of these tumours identified cardinal histological features in keeping with SDH-deficient RCC. Clinically, these patients presented with localised tumours (stage pT2 or lower) with no disease recurrence or adverse outcomes reported on follow up. Four cases of FH-deficient RCC were identified in a subset of tumours stained for FH/2SC (n=730). Two cases were identified in the papillary RCC cohort (0.5%, n=400), and 2 cases in the unclassified RCC cohort (4.4%, n=46). Characteristic absence of FH staining coupled with positive 2SC staining (FH-/2SC+) was reported in 3 cases, whereas one case (papillary RCC) exhibited retained FH expression with positive 2SC staining (FH+/2SC+). These tumours exhibited a highly aggressive phenotype with three patients developing metastatic disease, and in all four cases proved fatal. Unfortunately, confirmatory *SDH/FH* genetic analyses were not provided in this study.

This study reiterates the rarity of SDH- and FH-deficient RCC subtypes, and underlines some of the important caveats related to aberrant/ indeterminate IHC staining and morphological heterogeneity of these rare subtypes. Although all 3 cases of SDH-deficient RCCs were detected in the oncocytoma cohort, the original diagnosis of these cases occurred between 1970–2012, before the addition of SDH-deficient RCC to the WHO 2016 Classification (1). It is likely that as awareness of these rare subtypes gains traction, the identification of the associated cardinal morphological features will lead to increased detection and diagnosis, which would be in keeping with this study's pathological review reporting classic morphology consistent with SDH-deficient RCC in all 3 cases. Although it is possible that detecting classic morphological SDH-deficient RCC may be sufficient without IHC, the presence of variant histology has been previously reported for SDH-deficient RCC (8, 20). In addition, this study identified 2 cases of FH-deficient RCCs of unclassified morphology. Both cases had highly aggressive disease behaviour in keeping with FH-deficient RCC. One patient had early onset of disease (22 years old) and in both cases, presented with locally advanced disease with subsequent metastatic spread, suggesting the clinical phenotype may remain consistent despite variant histology.

A limitation to this study is the lack of confirmatory molecular analyses to substantiate the authors' claims. In the case of aberrant FH+/2SC+ staining, the authors suggested the possibility of dysfunctional FH protein in FH-deficient RCC, accounting for the retained FH expression on IHC. This hypothesis was backed by the authors reporting a separate case of a HLRCC patient harbouring a *FH* germline mutation exhibiting FH+/2SC+ staining on IHC. However, previous similar studies coupled with molecular analyses were able to identify cases of aberrant FH+/2SC+ staining with wildtype *FH* expression, as well as with *FH* mutations (14, 21). We agree with the authors in that concurrent use of 2SC with FH staining is essential for the detection of FH-deficient RCCs, furthermore, aberrant staining patterns crucially need to be validated with *FH* molecular analysis before confirmation of disease. Of note, degradation of tissue specimens in this study posed a major issue in performing crucial molecular analyses and highlights a need for better technical methods in ensuring suitable sample preservation for multiple lines of testing. Other metabolic markers that exploit these unique oncometabolite-associated properties also show promise in detecting FH-deficient RCC. In pre-clinical models of FH loss, consistently elevated levels of urinary argininosuccinate as a result of fumarate-induced urea cycle metabolic reprogramming (22), as well as observed cellular accumulation of fumarate, 2SC and succinated proteins such as succinic-GSH (23, 24), marks their potential as biomarkers for the detection of FH-deficient RCCs in tumour tissue and bodily fluids. A combination of these inexpensive, relatively straightforward detection methods that are highly specific to FH-deficiency may by the way forwards for improving detection and diagnosis of this aggressive subtype.

Overall, this study by Gupta and colleagues highlights the potential of IHC to be used as an adjunctive tool in the diagnosis of rare RCC subtypes, which is invaluable given the evidence for these rare subtypes to masquerade behind variant histology. Given that IHC is routinely established in clinical practice to assist the diagnosis of renal tumours (25), it would be relatively straightforward to add SDHA/B and FH/2SC staining to the IHC panel for clinical use to improve detection of these rare and aggressive subtypes. As highlighted, the ramifications of optimising detection of these subtypes are multi-fold. Detection will affect patients presenting with both localised and advanced disease, it will improve our knowledge of these poorly understood cohorts, and as both RCC subtypes are strongly associated with hereditary syndromes, it will have important implications for genetic testing for the patient and their relatives. In particular, diagnosis of aggressive FH-deficient RCC at renal biopsy or resection of a localised tumour will improve stratification of patients into more intensive follow-up regimes (5), whereas in the advanced setting, it will enable stratification of patients into appropriate clinical trials that will ultimately enable data to be gathered for improving systemic and targeted therapies as well as guidelines for these cohorts, which would be in keeping with the climate directed towards subtype specific management. However, with a very low incidence of SDH- and FH-deficient RCCs detected in this and previous similar studies (13, 14, 16, 21), the question to debate is whether or not it would be logical and/or cost-effective to screen the entire RCC cohort to detect a few cases of SDH- or FH-deficient RCC. Perhaps, it is in these cases that physicians and pathologists use their clinical acumen in aligning variant histology with a suspicious clinical history e.g. family or personal history of associated cancer phenotypes, early onset of

disease, clinically aggressive phenotype etc, that may really serve in increasing the detection of these tumours within variant RCC subtypes.

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