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INVITED COMMENTARY

You won't believe this old test … that does cheap single-cell mutation detection†

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†Invited commentary for Khalique *et al.* Optimised ARID1A immunohistochemistry is an accurate predictor of *ARID1A* mutational status in gynaecological cancer. *J Pathol Clin Res* 2018; **4**: 154–166.

Abstract

Detecting mutations in single cells from cancer specimens is now a major area of translational research. In a recent article in this journal, Khalique *et al* validated an immunohistochemistry assay for ARID1A that reliably identifies loss of function mutations in single cells in tissue sections. This work exemplifies best practice for developing and orthogonally validating immunohistochemical assays to provide clearly interpretable mutational results with spatial context.

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The reputation of immunohistochemistry assays (IHC) as biomarkers for precision medicine is not the best. There is an impression in some quarters that interpreting different shades of brown is like reading tea leaves. Clinical IHC has been around for decades [1]—so why do we have such a jaundiced view of its value for clinical biomarkers? Incomprehensibly, there is still wide use of low-specificity antibodies and poorly standardized assays, resulting in variable biological and clinical signals. Over 20 000 publications using IHC are published each year and the majority should probably have never seen the light of day [2]. By contrast, in a recent article in this journal Khalique and colleagues demonstrate what is needed to turn research-grade IHC into a clinically useful assay for detecting mutations in *ARID1A* [3].

Their study rigorously validates three different ARID1A (AT-rich interactive domain-containing protein 1A; also called BAF250a) antibodies using an automated, standardized assay against the gold standard of next-generation sequencing to detect *ARID1A* loss of function mutations. Staining from all three antibodies shows (almost) perfect agreement with very high concordance between loss of ARID1A expression and detection of predicted loss of function *ARID1A* mutations.

This finding is of immediate clinical relevance for diagnostic questions such as distinguishing clear cell and endometrioid histotypes of ovarian or endometrial carcinomas from histotypes in which ARID1A alterations do not occur [4,5]. While the prognostic value of *ARID1A* mutation is controversial, even contradictory, this well validated assay now enables large-scale studies to definitively test prognostic utility [6]. Looking forward, there is increasing clinical interest in the development of synthetic-lethal treatments with loss of function of *ARID1A* mutations across a broad spectrum of tumors [7–9]. The results from Khalique and colleagues now provide a robust biomarker for clinical trial inclusion and potentially a predictive biomarker for future therapies.

The history of *ARID1A* as a cancer-relevant gene is relatively short. In 2010, two groups simultaneously

identified recurrent *ARID1A* mutations in slightly less than half of endometriosis-associated clear cell [10,11] and endometrioid ovarian carcinomas [10]. Since then, *ARID1A* mutations have been detected in many cancer types including endometrial endometrioid and clear cell carcinomas, urinary bladder carcinomas, Hodgkin lymphoma, gastroesophageal, colorectal and hepatobiliary carcinomas among others [12]. *ARID1A* encodes one component of the SWI/SNF chromatin remodeling complex that facilitates target-specific DNA binding and regulation of transcription by repositioning nucleosomes [6]. Cancers with alterations in the SWI/SNF chromatin remodeling complex are not sensitive to conventional chemotherapy and have distinct clinical features [13,14]. Co-inactivation of ARID1A and its paralog ARID1B give rise to particularly aggressive dedifferentiated endometrial and ovarian endometrioid carcinomas [15] and mutations in another SWI/SNF component *SMARCA4* causes lethal small cell carcinoma of the ovary [16–19]. ARID1A-targeted therapies are now in clinical development with trials focusing on cancers with loss of function *ARID1A* mutations (NCT02059265, NCT03297424; see https://clinicaltrials.gov/).

Although DNA sequencing is the gold standard for detecting *ARID1A* mutations, nonsequencing-based assays still have important clinical utility. Firstly, next-generation sequencing of *ARID1A* has been difficult owing to its large size and high GC content in exon 1. Secondly, the implementation of an IHC based test (once properly validated) has fewer barriers to implementation as the infrastructure required is inexpensive and has ISO accreditation in most pathology centers.

The origins of IHC can be traced back to the 1930s [20,21] but wider clinical adoption had to wait until the 1980s, enabled by commercial-scale production of monoclonal antibodies and other refinements allowing use of formalin-fixed paraffin embedded tissue [1]. Publications from the 1990s then showed that IHC could be used to identify mutant p53 [22]. However, it was not until very recently that an IHC assay could provide near-perfect accuracy in predicting *TP53* mutation status [23]. Advancements have depended on 3 main factors: first, significantly improved sensitivity and specificity by using higher affinity rabbit monoclonal antibodies and newer polymer-based detection systems with increased signal amplification. Second, robust standardization of experimental conditions from improved automated staining platforms. Third, improvements in interpretation by mandating use of normal cells as intrinsic controls for judging loss of protein expression [23]. This has enabled sophisticated interpretation of abnormal p53 staining that maps the different classes of *TP53* mutations to their functional consequences (Table 1).

Table 1. Correlation of p53 immunohistochemical phenotype with *TP53* genotype

p53 staining pattern	Interpretation	*TP53* mutation status
Nuclear staining in variable distribution and intensity	Wild type pattern	Wild type*
Diffuse strong overexpression in virtually all tumor cells	Mutant	Nonsynonymous/missense mutation
Complete absence with retained internal control	Mutant	Loss of function mutations including indels, stopgains and splicing mutations
Cytoplasmic (uncommon)	Mutant	Loss of function mutation disrupting nuclear localization domain

*with the exception of truncating or splicing *TP53* mutations that may result in wild type IHC staining observed in 2–4% of tubo-ovarian high-grade serous carcinomas.

There are limitations to the ARID1A IHC assay from Khalique and colleagues that should be kept in mind. First, while most *ARID1A* mutations are loss of function mutations, there are a number of recurrent missense mutations that may impair ARID1A function, but do not appear to change protein expression. For a clinical trial, using only IHC could result in contamination of ARID1A-competent trial populations. The extent to which IHC could miss deleterious *ARID1A* missense mutations is unknown and requires further study.

Second, ARID1A loss can be subclonal, indicating a later mutational event during tumor evolution. Temporal modeling of endometrial endometrioid carcinomas showed that only a portion of *ARID1A* mutations were truncal [24]. Subclonal *ARID1A* mutations are common in mismatch repair deficient (MMR-D) cancers because *ARID1A* has numerous short mononucleotide repeats that have a high chance of slippage errors (Figure 1) [25,26]. In such cases, convergent evolution of different *ARID1A* alterations resulting in phenotypically indistinguishable but mechanistically distinct loss of ARID1A protein can occur [24].

Subclonal loss may also be critical in identifying premalignant lesions. In the example of endometriosis, malignant transformation is rare and no biomarkers exist to distinguish "premalignant" endometriosis from the much more common chronic disease. A handful of studies have implicated subclonal loss of ARID1A immunoreactivity in endometriosis without co-existing cancer (i.e. loss in only a subset of endometriosis glands and/or loss limited to contiguous epithelium

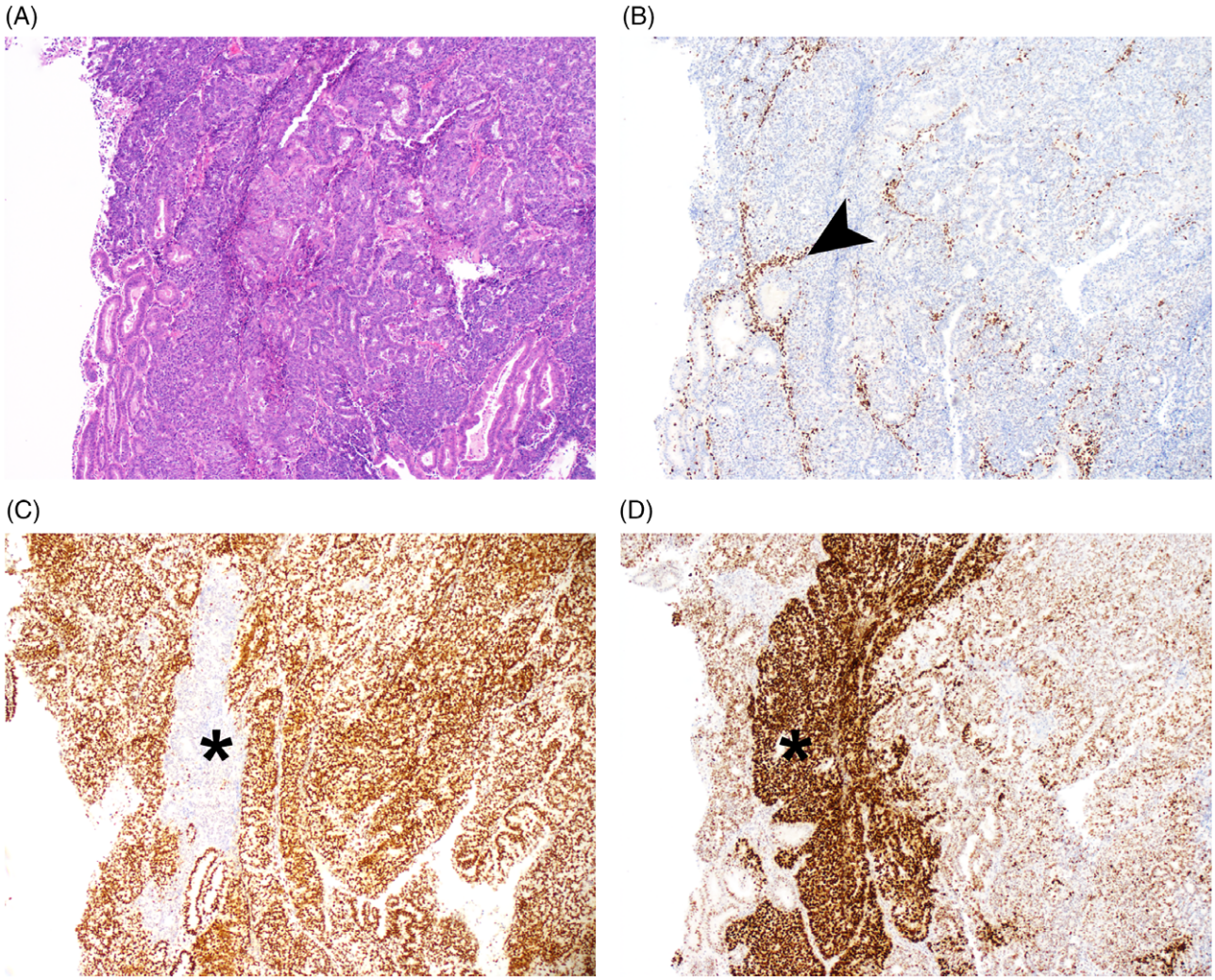

Figure 1. (A) H&E photomicrograph of endometrial endometrioid carcinoma, grade 3; (B) absence of MSH6 suggesting mismatch repair deficiency (additional MSH2 absence not shown). The arrow shows positive internal control stromal staining; (C) small focus of subclonal ARID1A loss (asterisk); (D) focus of p53 overexpression overlying but extending beyond the ARID1A focus (asterisk). Taken together these panels suggest a prototypical somatic mismatch repair deficiency resulting in subclonal *TP53* missense alteration, and subsequent *ARID1A* loss of function mutation.

within a gland) [27–29]—although validation of these findings with orthogonal sequencing data is very limited [30]. Now that we have high quality IHC tools, there is certainly sufficient justification for larger scale investigation of ARID1A loss (and mutation) in endometriosis, particularly if cohorts can be identified where progression to cancer has occurred. A clear understanding of how to interpret heterogeneous/subclonal ARID1A staining will undoubtedly accelerate such studies.

Finally, the mechanisms explaining total loss of ARID1A protein are still elusive. Unlike *TP53*, loss of heterozygosity at the *ARID1A* locus has not been widely reported, and at least for clear cell and endometrioid ovarian carcinomas, does not appear to be common [31,32]. In the cohort by Khalique and colleagues 8/45 (18%) cases harbored more than one *ARID1A* mutation and similar rates (6/27; 22%) have previously been reported [32]. Systematic analyses are required to validate whether (or when) both alleles are affected and to rule out alternative possibilities, such as the presence of two co-existing subclones carrying unique alterations.

Despite these caveats, the report from Khalique and colleagues suggests that ARID1A IHC can reliably detect loss of function mutations and is ready to be accepted as a clinical-grade test. A categorical scoring system should now be agreed upon to distinguish

between normal retained/present ARID1A expression and abnormal/absent expression, and whether this is complete or subclonal. As for p53 IHC, ARID1A IHC has the potential to achieve near-single-cell resolution for detecting mutations whilst also providing detailed information about the spatial context of mutated cells. IHC based prediction of *ARID1A* mutation now joins a small and exclusive group of well-validated and clinically useful predictive IHC biomarkers.

Author contributions statement

MK, MSA and JDB conceptualized the commentary. MK wrote the initial draft, MSA and JDB contributed to subsequent versions and all authors approved the final version.

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