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Original contribution

Quantitative evaluation of TP53 immunohistochemistry to predict gene mutations: lessons learnt from a series of colorectal carcinomas☆,☆☆

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Summary This study addressed if TP53 immunohistochemistry as a surrogate method for gene sequencing could be applied to colorectal carcinomas as successfully as recently reported for ovarian cancers. Sanger sequencing of the coding exons 2-11 of 87 tumors yielded a total of 65 mutations in 61 of the tumors. Immunohistochemistry was done with the Do-7 antibody. By a pattern recognition evaluation of immunohistochemistry, 44 cases were classified as "overexpressors" and 20 as having "wild-type" immunostaining; complete absence of or cytoplasmic immunostaining was seen in 9 and 4 cases, respectively. However, for 10 tumors, a confident distinction between overexpression and wild-type immunostaining was not possible ("indeterminates"). Quantitative analysis on digital images (i) using QuPath to determine the percentage of immunopositive cells and (ii) WEKA segmentation to obtain an index that quantified the intensities of tumor cells' nuclear immunostaining showed a continuous distribution of the data, explaining failure of assessment by pattern recognition in some cases. Quantitative data were then used to define cutoffs by receiver operator curve analysis, which allowed for predicting the mutational status of the *TP53* gene with sensitivities of 0.89 and 0.95 for the 2 methods, respectively, and specificities of 0.81 for both. In conclusion, by a dedicated approach, TP53 immunohistochemistry works well as a surrogate method for molecular studies. Considering the potential predictive role of *TP53* gene mutations in chemotherapy decisions, TP53 immunohistochemistry may be of value alongside with molecular gene studies, possibly even across different cancers.

1. Introduction

TP53 immunohistochemistry to detect gene mutations is a classical, albeit troubled surrogate for gene sequencing. Historically, researchers were aware of TP53 protein accumulation in cells lines harboring gene mutations immediately after discovery of the gene, soon followed by immunohistochemical studies demonstrating this in clinical tumor samples, colorectal cancer among them [1]. However, despite all efforts and testing of various antibodies, there stubbornly always was a fraction of false positives and false negatives sufficiently large to justify asking with a subtext of provocation: "Can we trust immunocytochemistry" [2], and for more than 2 decades since the situation has remained irksome.

In 2016, Köbel et al [3] published a carefully designed and executed study that correlated TP53 immunohistochemistry with gene mutations in a large series of ovarian cancers. By analysis of the whole *TP53* coding sequence and their flanking splice sites, optimized immunohistochemistry, and dedicated repeat sequencing of discordant cases, these authors were able

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to conclude their study with almost perfect sensitivity and specificity. This came as good news for practicing surgical pathologists because, contrary to other types of cancer, in diagnosing ovarian cancer, information on the *TP53* gene mutational status is desirable because it makes a very strong case for a high-grade serous cancer, a diagnosis that stratifies patients into unique clinical pathways but can be quite challenging by histology alone. However, although ovarian cancer is at the top of the list in this issue, considering the fundamental role of *TP53* in tumor biology, the mutational status of the gene may well be of clinical relevance in other types of cancer, too, including colorectal carcinoma, and as long as rapid gene sequencing at low cost is not universally available, a reliable immunohistochemical surrogate assay may be appreciated.

Physiologically, the TP53 protein is the main cell protection mechanisms against DNA damage, hence its well-known sobriquet "guardian of the genome" [4]. TP53 exerts this function as a tetrameric sequence-specific transcription factor, activating or repressing target genes that either induce DNA repair before the next round of DNA replication or tag defective cells to elimination via apoptosis [5]. In a very large proportion of different cancers, this important function is compromised by gene mutations that alter the DNA binding domain of the protein, many of which, in vitro, impart an aggressive and/or metastatic phenotype to tumor cells (dubbed gain-of-function mutations), whereas for a minority, simple loss of function is assumed (dubbed loss-of-function mutations) [6]. As regards colorectal cancer, from a practical point of view, such *TP53* gene mutations could, for example, interfere with chemotherapeutic regimes that target cancer cell DNA, potentially imparting a role for therapy prediction to the mutational state of the gene. However, given the high prevalence of gene mutations across different types of cancer, if TP53 immunohistochemistry performs reliably as a surrogate for molecular studies, it may have applications in quite a broad spectrum of different cancers and even precancerous conditions in the daily practice of surgical pathology.

The present study was conceived to test if the results reported by Köbel et al for ovarian cancer can be reproduced in a series of colorectal carcinomas. To reduce subjectivity in the evaluations of immunostains to a minimum, 2 freely available quantitative image analysis methods were tested in addition to the "pattern recognition" approach.

2. Materials and methods

2.1. Case selection and molecular studies

Study cases were selected from our database of primary colorectal carcinoma patients operated in the years 2000 to 2016. Prior written informed consent was obtained from all patients, and all procedures were approved by the Ethics Committee of Rostock University (ref. II HV 43/2004). No patient included in this study had received neoadjuvant treatment. All specimens included in this collection were processed and reported as previously published [7]. The series included 87 tumors, 50, 17, 13, and 7 of which were of the sporadic standard type, CpG island methylator phenotype, sporadic microsatellite–unstable type, and Lynch-associated, respectively.

Genomic DNA was extracted from tumor tissue harvested immediately after surgery and stored at −80°C after snap-freezing in liquid nitrogen until use. Initially, frozen sections stained with hematoxylin and eosin were prepared to ascertain that the tumor was well represented, and 15 to 20 subsequent sections (20-μm thickness each) were then digested in 180 μL lysis buffer containing proteinase K (200 μg/mL) at 56°C overnight for DNA extraction by means of the NucleoSpin Tissue Kit (Macherey-Nagel, Dueren, Germany). Alternatively, DNA was extracted from tissue sections taken from archived paraffin blocks. In these cases, sections were viewed through a stereo microscope compared with a hematoxylin and eosin–stained scout slide, and tumor tissue was dissected with a sterile scalpel blade. DNA was isolated by proteinase K digestion (400 μL digestion solution at 10 mM Tris, 0.1 mM EDTA [pH 8.0], and 0.5% Tween-20 and 40 μL proteinase K solution, 20 mg/mL). The samples were incubated at 56°C for up to 16 hours and then processed using the Wizard DNA Clean-up System (Promega, Madison, WI) according to the manufacturer's instructions. DNA content was measured using the QuantiFluor ONE dsDNA System (Promega). For Sanger sequencing, exons 2-11 of the *TP53* gene were amplified by polymerase chain reaction (PCR; primer sequences are in Supplementary Table 1). Exons 2-4 and 9-11 were amplified as previously described (http://www.p53.iarc.fr; [8]), and exons 5-8 were amplified as follows: 25 μL reaction mixtures at 0.2 μL MyTaq polymerase with 5 μL 5× PCR buffer (Bioline, Luckenwalde, Germany), 1 μM of each primer set, and 50 ng of template DNA. PCR reactions were started at 95°C for 60 seconds, followed by 35 cycles at 95°C for 15 seconds, 58°C for 15 seconds, and 72°C for 10 seconds. PCR products were purified with alkaline phosphatase (Thermo Scientific, Dreieich, Germany) and exonuclease I (Thermo Scientific). Subsequently, sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) with each pair of forward and reverse primers, followed by analysis on a 3500 Genetic Analyser (Applied Biosystems). The sequence data were compared with reference sequences (*TP53*: ENSG00000141510) using SeqScape Software v2.7 (Applied Biosystems).

2.2. TP53 immunohistochemistry and quantitative evaluations

TP53 immunohistochemistry was done with the Do-7 mouse monoclonal antibody (Dako, Glostrup, Denmark) at 1:100 dilution. A tissue microarray was constructed with 2 punches taken from the archived paraffin blocks (1-mm core diameter). Four-micrometer sections were used for automated immunohistochemical reactions (Dako autolink 48) with 3,3′-diaminobenzidine (DAB) as a chromogen after heat-induced

antigen retrieval. Repeat immunohistochemical reactions with whole tissue sections were done in selected cases as specified in the Results section.

Quantitative evaluations of TP53 immunohistochemistry were made on digital microphotographs. For this, digital images were obtained from representative areas of the tumors (32 bit RGB images, 3384 × 2708 pixels, area of 1.267 mm^2). For each image, tumor areas were separated from the stroma by manually drawing lines around neoplastic glands with the computer mouse and clearing stroma areas from the images. Quantitative evaluations were then made with these digital images by 2 different approaches:

(1) Determination of the percentage of tumor cell nuclei positive for the TP53 immunoreaction. This was done as implemented with the QuPath software developed by Dr Pete Bankhead and generously made available to the biomedical community as freeware [9].

(2) Calculation of the mean optical density (MOD) of immunopositive nuclei in relation to all tumor cell nuclei. This was done using Fiji plugins (freeware available at http://rsb.info.nih.gov/nih-image) as follows: first, DAB color deconvolution was done, yielding an image with TP53-immunopositive tumor cell nuclei of varying intensities (0-255) as listed in the corresponding histogram that was exported into an Excel table. Second, the total area covered by the tumor cell nuclei was determined by applying the trainable WEKA segmentation classifier to an 8-bit version of the image. WEKA segmentation is another generous contribution to the biomedical community [10] that, by application of machine learning algorithms, allows for an almost perfect delineation of tumor cell nuclei in digital microphotographs and their area measurement. Setting a cutoff for immunopositive pixels in the DAB histograms at 140, the MOD was then calculated by the histogram data and the WEKA segmentation data according to the formula.

$$\mathrm{MOD} = 255 \times i_{255} + 254 \times i_{254} + \ldots + 140 \times i_{140} \,/\, i_{\text{tumor cell nuclei}}$$

where $i_{255} - i_{140}$ indicates the number of pixels in the histograms at the indexed intensities, and $i_{\text{tumor cell nuclei}}$ is the number of pixels representing tumor cell nuclei. Contrary to determining percentages of immunopositive nuclei, which implies application of a cutoff, the MOD thus obtained dispenses with positive versus negative decisions per nucleus, but rather is based on immunostaining intensities on a continuous scale.

2.3. Statistical evaluations

Receiver operator curve (ROC) analysis was done with the Statistical Package for the Social Sciences (SPSS version 13.0; Chicago, IL).

3. Results

A total of 65 *TP53* gene mutations classified as "damaging" and/or "disease causing" by PolyPhen or MutationTaster algorithms, respectively, were found in 61 of the 87 colorectal carcinomas (4 tumors harboring 2 mutations each; Table). As expected, most of these mutations (n = 50; 77.0%) were nonsynonymous missense mutations in the DNA binding domain of the gene. In addition, there were 7 frameshift mutations, 4 stop-gain or nonsense mutations, 1 splice-site mutation, and 1 mutation found in the 3′-untranslated region. Five *TP53* gene mutations observed in this series are not on record in the COSMIC database. A synoptic presentation of the gene mutations is found in Fig. 1. The complete molecular and immunohistochemical data are listed in detail in Supplementary Table 2; results of PolyPhen and MutationTaster algorithms as well as the COSMIC database status of the different *TP53* gene mutations are given in Supplementary Table 3.

In a qualitative "at-a-glance" evaluation of TP53 immunohistochemistries with sections from the tissue microarray, we observed the expected patterns of expressions, which in 65 of the 87 cases allowed for classification with confidence: unequivocal strong nuclear immunostaining of most tumor cells ("overexpressors"); faint to moderate staining of a minority of the tumor cells ("wild types"); complete absence of immunostaining ("completely negatives"), alongside of some immunostaining of stroma cell nuclei as internal control; and

Table TP53 immunohistochemistry results (overexpression based on MOD evaluation) in relation to gene mutations

Mutation type	Overexpressor (MOD ≥ 20)	Completely negative	Cytoplasmic	Wild type (MOD < 20)
Missense	43	1	0	2
Missense (2×)	2	0	0	0
Missense + stop-gain/nonsense	0	1	1	0
Frameshift	1	3	2	1
Stop-gain/nonsense	1	1	0	0
Splice-site	0	1	0	0
3′UTR	1	0	0	0
No mutation detected	2	2	1	21

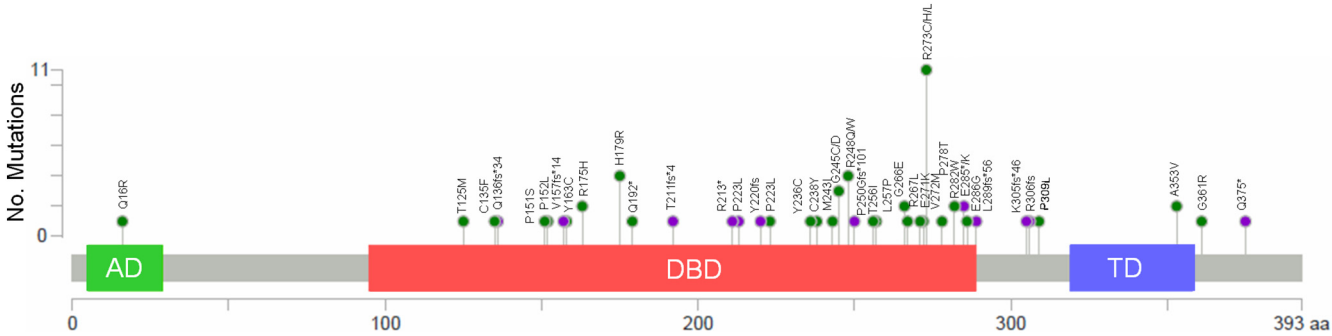

Fig. 1 Frequency and positions of *TP53* gene mutations. In this "lollipop" plot, nonsynonomous missense mutations are depicted in green and truncation or frameshift mutations are in violet. AD, activation domain; DBD, DNA binding domain; TD, tetramerization domain.

cytoplasmic (and some nuclear) immunostaining of tumor cells ("cytoplasmics"). However, there remained 12 tumors for which assessments remained difficult ("indeterminates"). Repeat immunohistochemical reactions with whole tissue sections were done for the indeterminate cases as well as for the completely negative cases and the tumors with cytoplasmic immunostaining, which confirmed the initial assessments in all cases, except for 2 tumors from the indeterminate class that showed a curiously clonal pattern of overexpression that was missed in the tissue punches taken for the tissue microarray and moved them into the group of overexpressors. Thus, by pattern classification of TP53 immunohistochemistry, we finalized with 44 overexpressors, 20 wild types, 9 with complete absence of immunostaining, 4 cytoplasmics, and 10

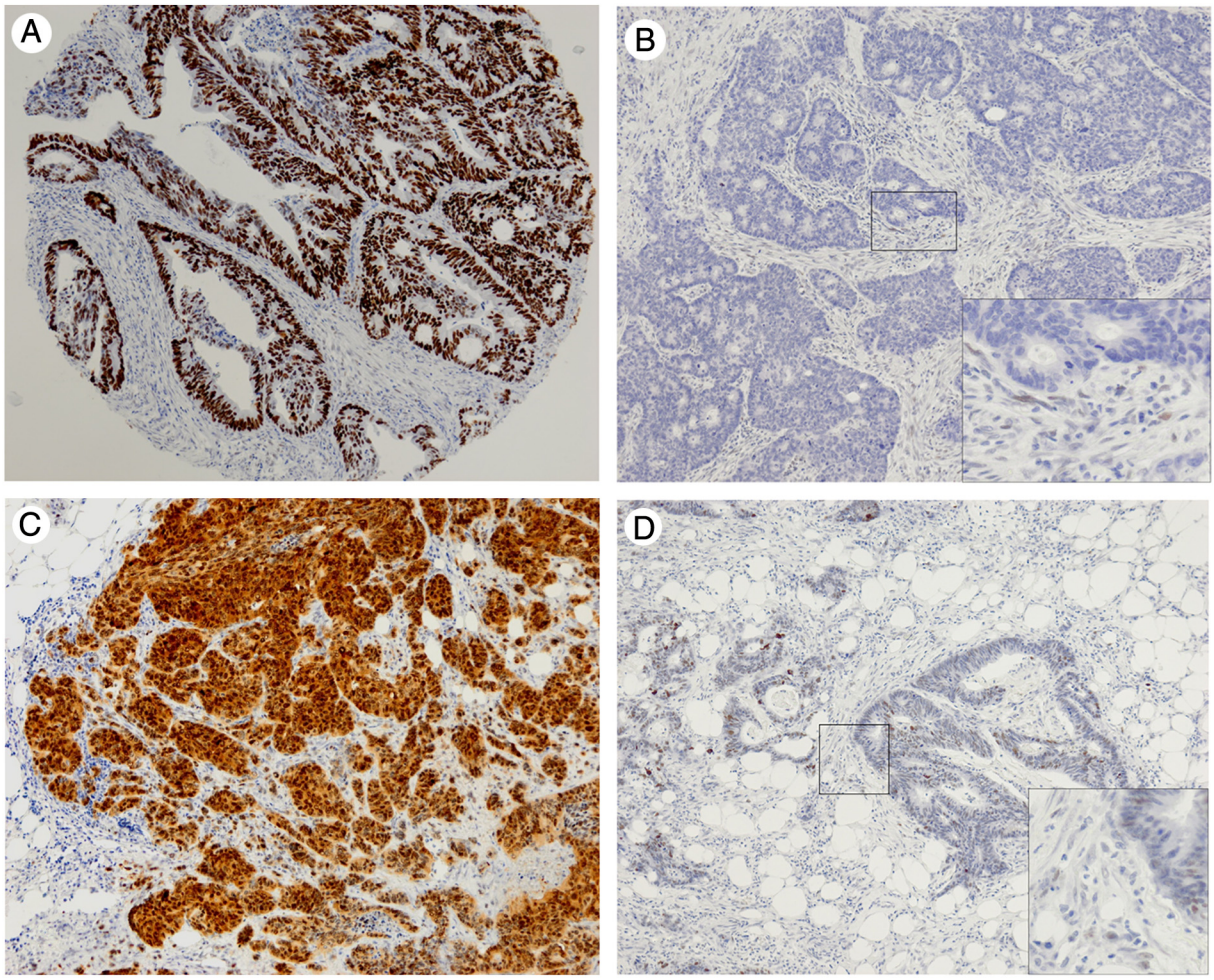

Fig. 2 Examples of aberrant (mutation-type) TP53 immunostaining patterns, viz. overexpression (A), complete absence (B), and cytoplasmic staining (C). D, An example of wild-type TP53 immunohistochemistry is shown. Note weak, albeit appreciable immunostaining of stromal cells (magnified in the insets in panels B and D).

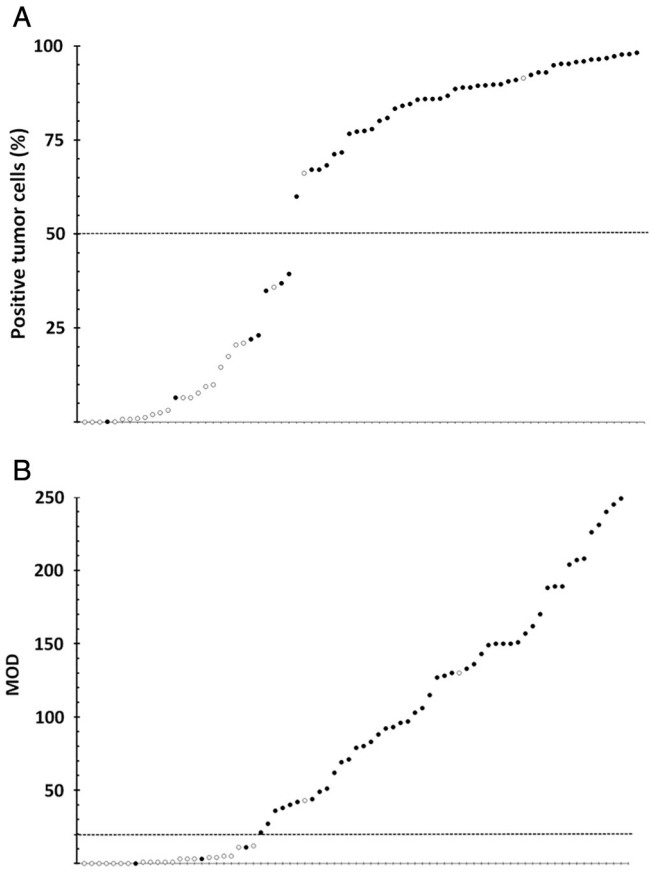

Fig. 3 Quantitative TP53 immunohistochemistry data of QuPath evaluations (A) and evaluations by WEKA segmentation and calculation of MODs (B). Closed circles are for tumors with *TP53* gene mutations, whereas tumors plotted with open circles are without a demonstrable mutation.

indeterminates. Representative images of the TP53 immunostaining patterns are given in Fig. 2 (also see Supplementary Fig. 1).

To explore if the issue of indeterminate cases could be overcome by quantification, overexpressors, wild types, and indeterminates were evaluated by QuPath and WEKA as described in Materials and methods. The results of these evaluations are plotted in Fig. 3. QuPath evaluations could be made rapidly and with confidence, although by setting thresholds for immunopositive cells, there entered a minor subjective component into the procedure. By contrast, WEKA segmentation was a more complex and time-consuming method of evaluation that, however, worked remarkably well and virtually excluded observer-related factors. Plotting the data (Fig. 3) showed that percentages of positive tumor cells (QuPath method) and MOD values (WEKA method) were distributed on a continuous scale. ROCs were then generated, which related these immunohistochemistry data to the *TP53* gene mutational status (Fig. 4). Based on the ROCs, cutoffs for quantitative TP53 immunohistochemistry results indicative of a gene mutation were set: for classification by percentage of positive tumor cells at least 50% (QuPath method), which

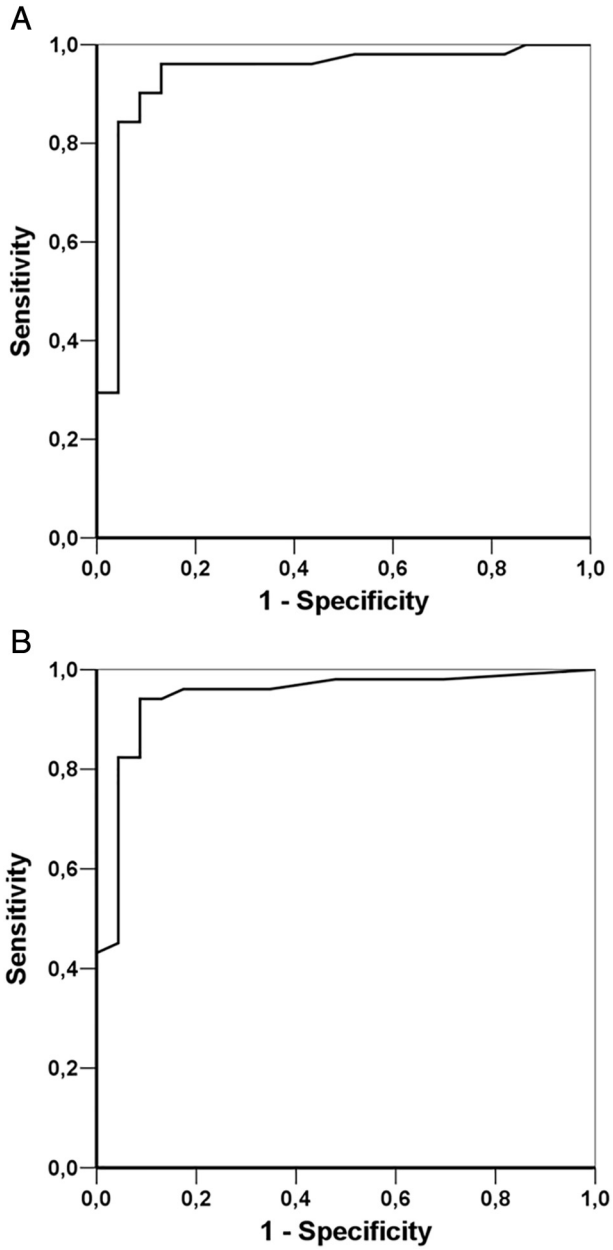

Fig. 4 ROC analysis for TP53 immunohistochemistry as a surrogate for mutational analysis by QuPath (A) and MOD (B) evaluations. AUCs were 0.937 and 0.945, respectively. Cutoffs were set at 50% or higher for evaluations by percentage of positive tumor cell nuclei (A) and MOD of 20 or greater (B).

resulted in 7 false-negative cases (ie, <50% tumor cell nuclei positive by immunohistochemistry but *TP53* gene mutation) and 2 false-positive cases (ie, ≥50% tumor cell nuclei positive but no *TP53* gene mutation detected); by MOD classification (WEKA method), there were 3 false-negative cases and 2 false-positive cases (see Supplementary Table 2 for details).

Relating the *TP53* gene mutational status to TP53 immunohistochemistry in the completely negative cases and those with cytoplasmic immunostaining led to the following results (see Supplementary Table 2): first, *TP53* gene mutations were

found in 7 of the 9 tumors completely negative by immunohistochemistry, and most of these mutations were of less common types, namely, frameshift, stop-gain/nonsense, and splice-site mutations (3, 2, and 1, respectively); there were only 2 nonsynonymous missense mutations, one of which was combined with a stop-gain/nonsense mutation. Second, 3 of the 4 tumors with cytoplasmic TP53 immunostaining harbored a gene mutation, frameshift mutations in all these cases.

To exclude the possibility that a *TP53* gene mutation in the false-positive case may have escaped detection by gene sequencing, we obtained step sections from these tumors immediately adjacent to the immunohistochemistry sections, dissected tumor tissue under a stereomicroscope, and repeated sequencing of all *TP53* exons with DNA extracted from this tissue. However, *TP53* gene mutations were not found, confirming the results from the initial tests. Furthermore, to exclude that tumor heterogeneity could be the cause for false negatives, tumor tissue dissected from step sections obtained from the paraffin blocks used for immunohistochemistry was used for a second round of *TP53* gene sequencing. Again, the initial results were confirmed.

Sensitivities and specificities of detecting *TP53* gene mutations by TP53 immunhistochemistry were as follows: (i) relating any aberrant TP53 immunostaining (ie, overexpression by ≥50% of tumor cells positive by QuPath evaluation, or complete absence, or cytoplasmic staining) to the TP53 mutational status of the gene yielded a sensitivity of 0.89 and a specificity of 0.81, and (ii) using a MOD of at least 20 as determined by the WEKA method instead yielded a sensitivity of 0.95 and a specificity of 0.81 (Supplementary Table 4).

4. Discussion

In this study, we tested TP53 immunohistochemistry as a surrogate technique for gene sequencing in a series of colorectal carcinomas. There have been repeated attempts at this in more than 2 decades, but the upshot has been quite disappointing and earned TP53 immunohistochemistry a reputation of being unreliable and of limited usefulness. Indeed, specificities reported in the studies that were carried out with colorectal carcinomas were not very satisfying (range, 0.4-0.78) [11-15].

However, at least in the field of ovarian cancer, the perspective has brightened significantly since the recent study by Köbel et al [3], who were able to assign the *TP53* gene mutational status of 251 ovarian cancers by TP53 immunohistochemistry with a final sensitivity and specificity of 0.96 and 1.00, respectively. As compared with previous studies, these authors tackled the problem by a more comprehensive strategy and with dogged determination: 3 different antibodies were tested in comparison (Do-7, Do-1, and E26; Do-7 performing best), all coding exons and the flanking regions of the *TP53* genes were sequenced, and if immunohistochemical and molecular data did not match, repeat immunohistochemistry (on whole tissue sections as compared with the initial immunohistochemistries on a tissue microarray) as well as repeat sequencing with independent DNA samples was done (9.16% of their cases).

Stimulated by such fine results, our study was modeled on the strategy by Köbel et al: we used Do-7 immunohistochemistry, sequenced all coding exons of the *TP53* gene and their flanking regions, and tried to resolve discordant cases by repeating immunohistochemistry and gene sequencing. In evaluating our immunostains, initially we followed the recommendation that this best be pattern-based, distinguishing between wild-type staining, on one hand, and nuclear overexpression, cytoplasmic staining, or complete absence of immunoreactive tumor cell nuclei as indicative of gene mutations, on the other hand. In this, as pointed out by Köbel et al, it is of paramount importance to titrate the immunoreaction to weak staining of some stromal cells as an internal control, a prerequisite for scoring complete absence. Heeding this and going about the evaluations by pattern recognition, we could confidently assign most cases in 1 of these 4 classes. Nevertheless, there remained a fraction of 10 cases in which a distinction between overexpression and wild-type pattern could not be made with confidence, despite repeat immunohistochemistry, and this is not a nugatory proportion. We also attempted to overcome this by titrating the immunoreactions, but without convincing success, because this led to reactive-type immunostaining of tumor cell nuclei or loss of immunopositive stromal cells in the previously completely negative cases, which as controls had been included along with the equivocal cases.

Thus, the shortcomings incurred by "eyballing" led us to attempt a quantitative approach. We applied freely available image analysis software (i) to determine the percentage of immunopositive tumor cell nuclei and (ii) to obtain an index (the MOD), which integrates immunostaining density across all tumor cell nuclei. The first method was carried out using QuPath and the second by WEKA segmentation, a plugin included with Fiji. By both methods, subjectivity is significantly reduced or completely avoided, respectively: as regards evaluations by QuPath, there remained the step of setting a DAB threshold to define immunopositive cells, but beyond that, counting was automatized, and determining MODs completely dispensed with setting a threshold for individual tumor cell nuclei. Both methods worked very well. On the upside of QuPath, evaluations could be done with very little effort, and data were handled rapidly by the PC, whereas WEKA segmentation, as a downside of this method, required case-by-case classifier training, which was laborious, and computations were much slower. Conversely, on the upside of WEKA segmentation, tumor cell nuclei could be delineated almost to perfection, whereas less precision was a QuPath downside (as became clear when "toggeling" the negative and positive annotations of the tumor cells in the outputs on the computer screen provided by the program). Basically, both methods yielded analogous results that, when visualized in a plot (Fig. 3), clearly show that "indeterminates," indeed, are intrinsic to the data and not due to observer limitations: data are distributed on a continuous scale, and there cannot be

found a "natural" cutoff that segregates tumors as immunohistochemically positive versus negative. In other words, any immunohistochemical TP53 surrogate method is blemished by a "gray zone," which, however, could be minimized by our quantitative evaluations. Using cutoffs determined with the aid of ROCs (≥50% for QuPath and MOD ≥20 for WEKA), we ended up with sensitivities and specificities below the results reported by Köbel et al for ovarian cancer, but better than the results reported for other colorectal carcinoma series.

To exclude technical issues as an explanation as far as possible, we repeated TP53 immunohistochemistry, using whole tissue sections, and DNA sequencing of tumor tissue that was microdissected from areas corresponding to those evaluated by immunohistochemistry. We may also point out that collection of the resection specimens followed standardized procedures, which included transportation to the Institute of Pathology immediately after surgical removal, and standardized formalin fixation [16]. Settling upon the MOD method, which works best for determining *TP53* overexpression as the standard, discordant cases then were as follows: (i) in the group of false negatives, cases 54, 71, and 84 were classified as wild type by TP53 immunohistochemistry, whereas gene sequencing revealed a mutation. In case 54, a T211fs*4 was found, which may well explain failure of detection by immunohistochemistry. However, for cases 71 and 84, the mutations were nonsynonymous missense mutation, and there is not any obvious explanation why there should not be overexpression by immunohistochemistry. Hypothetically, there may be nonsynonymous missense mutations that do not result in *TP53* overexpression, but to our knowledge, there are not any data on record suggesting this for the E271K (case 84) and Y236C (case 71) mutations found here, although conversely, we are not aware of reports linking these mutations to overexpression. We may add that TP53 immunohistochemistry with a tumor xenograft [17] derived from case 84 (which, as expected, retained the gene mutation) yielded exactly the same result as the primary tumor. Unfortunately, a xenograft was not available for case 71. (ii) In the group of false positives, there were 2 overexpressors, 2 completely negatives, and 1 tumor with cytoplasmic staining for which a *TP53* gene mutation could not be detected. Undeniably, technical shortcomings of the analyses remain a possible explantation, despite the repeat analyses and microdissection of DNA to exclude this as specified above. However, tumor biological explanations are also conceivable, though hypothetical and not in any way addressed by our investigation, among which are postranslational modifications that could have an effect on protein degradation or nuclear export/import, genetic or epigenetic changes of the protein degradation machinery, or even intronic gene mutations.

Doubtlessly, the *TP53* mutational status presently is not pivotal in colorectal carcinoma pathology, as opposed to its importance in the field of ovarian cancer where its role for diagnosing high-grade serous ovarian cancer is undebated. Nevertheless, TP53 immunohistochemistry may be of value for surgical pathologists (and clinicians) when dealing with colorectal carcinomas for the following reason. As is well appreciated by previous studies, most *TP53* gene mutations in colorectal carcinoma and other types of cancer are nonsynonymous missense mutations that target the DNA binding domain in a dominant-negative fashion when a mutated protein is included in the tetramer. Importantly, because TP53 is implicated in cells' response to DNA damage and because most cytotoxic chemotherapies used against colorectal carcinoma in one way or another work by DNA interference, the *TP53* mutational status of colorectal carcinomas may well have a role to play in predicting effects of adjuvant or palliative chemotherapy. Indeed, reduced effectiveness of adjuvant 5-FU–based chemotherapy in patients with *TP53* mutated cancers has been reported in clinicopathological studies [18,19]. Thus, conceivably, demonstrating a *TP53* gene mutation in a patient's colorectal carcinoma may stratify him or her to more aggressive chemotherapy than traditional 5-FU. Although predictive effects may not depend on the overall mutational status but rather on specific gene mutations [20], which would have to be determined by gene sequencing, TP53 immunohistochemistry still could be of value in this setting: presuming that *TP53* gene sequencing (eg, by next-generation sequencing techniques) were available broadly at reasonable costs and routinely applied for therapy prediction, there still would remain false negatives by molecular studies (roughly 10%, as documented in the study by Köbel et al) that would come to attention by a reliable TP53 immunohistochemical assay (fast and cheap at that), and such discordant cases would have to be subjected to a second round of molecular analysis. Thus, TP53 immunohistochemistry would have a well-deserved place as a complementary method.

However, a case can be made for taking a broader perspective, to look at other types of cancer and on precancerous conditions, too. As regards other cancers, an analogous role of *TP53* gene mutations for therapy predictions as proposed for colorectal carcinoma can be envisioned. This may encompass a wide-ranging spectrum, extending from different carcinomas to leukemias, sarcomas, and even gliomas. Furthermore, every surgical pathologist from time to time is confronted with biopsies where it is difficult to render a cancer diagnosis. For example, true-cut needle biopsies taken to ascertain a pancreatic cancer before (palliative) chemotherapy can be quite a challenge because neoplastic glands are sparse, and deciding upon (or against) a *TP53* gene mutation in the cells in question by immunohistochemistry with confidence can be quite helpful. As regards precancerous conditions, demonstrating a *TP53* gene mutation by immunohistochemistry in squamous epithelia (eg, of the oral cavity, or the glans penis), urothelium, Barrett mucosa, or colonocytes in chronic inflammatory bowel disease may aid in histologic assessments. Furthermore, the proportion of tumor cells or dysplastic cells in this setting often is below the detection threshold even of next-generation sequencing techniques, which adds merit to an immunohistochemical approach.

Taken together, although we were not able to reproduce the almost perfect results reported by Köbel et al for ovarian

cancer, nevertheless, in our series of colorectal carcinomas, TP53 immunohistochemistry as a surrogate for gene sequencing performed better than its reputation. As long as its limitations are kept in mind, TP53 immunohistochemistry seems to be useful in the field of colorectal carcinoma pathology and, likely, also across cancers and when dealing with precancerous conditions.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.012.

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