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Evaluation of TERT mRNA expression using RNAscope®: A potential histopathologic diagnostic and prognostic tool

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Abstract

Background: Telomerase reverse transcriptase (*TERT)* activation has been shown to be an important cancer hallmark; the activation and expression of *TERT* has been documented in >90% of tumors and *TERT* activation has been touted as a prognostic marker in many cancers. However, there is currently no simple testing modality to detect TERT mRNA expression in surgical pathology specimens. In this study we aim to evaluate and validate the utility and reliability of the *TERT* RNAscope® in-situ hybridization (ISH) assay for the detection of TERT mRNA expression in formalin-fixed, paraffin embedded tissue.

Methods and Materials: RNAscope® detection for *TERT* was performed on a Leica Biosystems BOND III research staining robot using the Hs-TERT-O1 (ACD, 481968) probe. Twenty three samples containing 48 tissue types were assessed. *TERT* genomic alterations were determined by targeted next generation sequencing (NGS), while TERT mRNA expression was determined by both targeted RNA-sequencing and TERT RNAscope® and the results compared. Manual vs automated TERT expression quantification methodologies were evaluated for the ISH assay. The expression levels in normal vs. neoplastic tissues were also compared.

Results: The RNAscope® assay showed high TERT expression in neoplastic tissues, while most normal tissues have no or very low expression levels (p-value= 0.0001, AUC: 0.99). In addition, there was good correlation of TERT expression between the RNAscope® assay and RNA-sequencing. For RNAscope® quantification, manual calculation of TERT signal/cell ratio based on a count of 100 cells was superior compared to automated signal detection.

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Conclusion: TERT RNAscope® assay is a simple and reliable tool for the evaluation of TERT mRNA expression. TERT signal/cell ratio based on a count of 100 cells is a reproducible and accurate interpretation approach for evaluation of TERT expression.

Keywords

TERT; Telomerase; In situ hybridization; Cancer Diagnosis

1. Introduction

Telomeres are repetitive DNA regions located at the ends of chromosomes that form complexes with proteins and maintain the stability of the genome through multiple divisions. However, with aging and continued cellular division, these segments shorten leading to genomic instability and induction of senescence. Consequently, preserving telomere length is fundamental for tumor survival and immortalization [1]. The maintenance of telomere length is mainly achieved through reactivation of telomerase either through the telomerase reverse transcriptase catalytic subunit (*TERT*) or through alteration of the template subunit (TERC) [2]. In approximately 10% of tumors, alternative lengthening of telomeres occurs through mechanisms independent of telomerase [3].

Tumorigenesis requires neoplastic cells to acquire cancer hallmarks, one of which is enabling replicative immortality for which *TERT* activation is needed [4]. However, the role of *TERT* goes beyond telomere lengthening and enabling immortality. It has been shown that *TERT* is one of the main regulators of tumor progression as it exerts influence on cellular proliferation rate, resistance to apoptosis, and invasive properties of cancer. Furthermore, *TERT* appears to alter the metabolic and transcriptional landscape of cells and thus contributes to multiple hallmarks of cancer [5].

In fact, almost all tumors (>90%) have been shown to have increased expression of TERT to maintain telomere length which is essential for tumorigenesis and escape from senescence [6]. There are various mechanisms that lead to increased TERT expression; some tumors acquire *TERT* promoter mutations [7], while others acquire amplification of *TERT* gene locus on chromosome 5p15 [8], and yet others acquire *TERT* activation through *TERT* gene or promoter fusions [9]. However, alternative pathways can also lead to increased expression of TERT; for example, c-MYC has been shown to induce TERT expression [10], a mechanism that has been implicated in human papillomavirus (HPV) infected cells [11], although various other proteins can also be affected by the viral E6 protein leading to modified methylation of *TERT* promoter and subsequent increased expression of TERT [12].

Telomerase expression has been touted as a potential diagnostic and prognostic marker in tumors [6]. Various attempts have been made to develop an immunohistochemical assay to evaluate TERT status in formalin-fixed paraffin embedded (FFPE) tissue but to date, attempts using antibodies directed against TERT protein have had limited success and have shown poor correlation with *TERT* alterations or mRNA expression [13]. In this study we aim to evaluate and validate an mRNA in-situ hybridization brightfield assay (RNAscope assay) for TERT.

2. Methods and materials

2.1. Case selection

Twenty-three neoplastic and normal FFPE samples with known *TERT* promoter mutation and amplification status were selected; 5 samples contained only benign tissue (benign cervical tissue), 6 samples contained only neoplastic tissue and 12 samples contained a mixture of neoplastic and non-neoplastic tissue. The *TERT* gene alteration status was determined using a targeted hybrid exon-capture next-generation sequencing assay (MSK-IMPACT) in the neoplastic tissues, as previously described [14]. Neoplastic tissues were chosen to ensure representation of cases harboring *TERT* promoter mutations, *TERT* amplification and *TERT* gene or promoter fusion. *TERT* amplification was determined using the Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing (FACETS); copy number gains of more than 5 were considered as gene amplification [15]. A targeted RNA based sequencing assay (MSK-Fusion™) was employed to determine TERT mRNA expression level as well as *TERT* fusions in a subset of the samples (n = 9) [16]. TERT expression levels were defined using the number of reads of the TERT RNA probe normalized to the average number of reads of the assay's housekeeping genes *CHMP2A, GPI, RAB7A* and *VCP*.

2.2. RNAscope assay

RNAscope detection for TERT was performed on a Leica Biosystems BOND III research staining robot (Leica Biosystems), as follows: four micron thick FFPE sections were mounted on charged slides, which were subsequently placed onto a BOND III staining robot and stained using the ACD Bio BOND RNAscope Detection Reagents – Brown (ACD, 201000), as per the manufacturer's recommendations. Appropriate positive and negative control probes, UBC (ACD, 200178) and DapB (ACD, 200188), respectively, were used to determine that the optimal pre-treatment conditions were identified, which were found to be 15 min at 40 C with protease while heat-induced epitope retrieval was set at 15 mins at 95 C. The TERT probe used from the vendor was Hs-TERT-O1 (ACD, 481968) – a 40 base pairs long probe targeting the 1487 – 3825 region of the TERT gene – which was incubated for two hours at 40 C. slides were then counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene, and coverslipped. Each stained tissue was also interrogated with a positive control probe targeting common housekeeping gene UbC to help qualify the samples and a negative control probe targeting bacterial gene dapB gene to control for background noise.

2.3. Scoring and evaluation

The stained slides were evaluated and scored independently by two reviewers (AMB, EY). For scoring of the TERT mRNA expression in tissues, three approaches were employed. First, five 0.25 mm^2 areas with the highest amount of staining (as subjectively and qualitatively determined by the reviewers) for the TERT mRNA probe was selected and the number of brown dots within cells and the total number of cells within the area was counted and the ratio of signal to cell was calculated (Fig. 1); the highest attained number among the five areas was then used for comparisons.

Second, the areas of the tissue with highest TERT expression were selected and 100, 200, 300, 400 and 500 cells with the most TERT signals per nucleus were counted and the total number of TERT signal dots in these cells was also counted by the reviewers. Subsequently the average TERT signal per cell was calculated (Fig. 2).

Third, using scanned whole slides of the stained slides, after selection of five 0.25 mm^2 areas with highest TERT expression by reviewers, the ratio of DAPI (brown) colored pixels to the overall pixels within that area was calculated using the QuPath software [17] (Fig. 3).

For tissue sections containing various tissue components (e.g. carcinoma, benign epithelium, benign stroma, …), each tissue component on the slide was scored separately.

The scoring approaches were evaluated using ANOVA with post-hoc Tukey test comparing scores among malignant, benign and intraepithelial neoplasia. Multinomial regression analysis was also used to evaluate the discrimination capacity of TERT scoring approaches to differentiate between malignant, benign and dysplastic tissue. TERT scores were also compared between cases based on *TERT* gene alteration status (i.e. promoter mutation vs amplification vs fusion vs unknown vs normal). Interobserver reproducibility was determined by calculating the Pearson's R coefficient. Linear regression analysis was used to evaluate the correlation of the TERT scores with mRNA expression level as determined by the RNA sequencing assay for 9 cases for which TERT RNA expression data was available.

3. Results

In this study, 23 slides were stained using the TERT RNAscope® assay, which corresponded to 48 different tissue types. In total, 29 benign tissue samples, 3 dysplastic tissue samples and 16 malignant tissue samples were evaluated. The breakdown of the tissue sections evaluated is provided in Table 1.

All neoplastic tissue sections underwent NGS using the MSK-IMPACT assay to determine the somatic *TERT* status. As expected, the 3 intraepithelial neoplasias did not have any *TERT* alterations, whereas 14 of 16 malignant tissues harbored alterations in the *TERT* gene with *TERT* amplification being the most common (n = 7, 44%), followed by *TERT* promoter hotspot mutations (n = 6, 38%) and *TERT* gene fusion (n = 1, 6%). Two colonic adenocarcinomas did not show any genomic *TERT* alterations based on the targeted DNA or RNA sequencing assays.

Of the 16 malignant cases, 9 underwent testing using the Archer fusion assay and the quantitative TERT RNA expression was extracted, normalized against the housekeeping genes and converted to log-scale. The tested cases had variably increased TERT expression with the lowest expression seen in cancers lacking *TERT* genomic alteration, however, the numbers were too small for evaluation of statistically significant difference (Fig. 4-A).

Three different approaches were used to quantify TERT expression based on the TERT RNAscope® assay, including DAPI color pixel density ratio in a 0.25 mm^2 area of tissue (automated using QuPath software), signal to cell ratio in a 0.25 mm^2 area (manual counting

by two observers), and finally signal to cell ratio based on a set number of cells (manual counting by two observers). For the latter approach, the ratio was obtained in increments of 100 cells in all cases (i.e. signal to cell ratio based on 100, 200, 300, 400 and 500 counted cells) to establish the minimum number of cells that needs to be counted for determining TERT expression status. An ANOVA test followed by post-hoc pairwise comparison failed to show any statistically significant difference in the signal to cell ratio when counting different number of cells (p value: 0.187) and as a result hereafter we will only discuss the signal to cell ratio based on 100 cells along with the two other previously mentioned quantification approaches.

Using regression analysis, we tested to see whether TERT RNAscope® expression correlates with TERT expression as determined by the MSK-Fusion assay for the 9 samples that had the latter assay performed. Our results showed that a linear correlation exists between the two measures (p value = 0.039, $R^2 = 0.478$), further confirming the ability of the TERT RNAscope® assay to determine the *TERT* mRNA status in FFPE samples.

Comparison of the TERT expression based on TERT RNAscope® assay was made between neoplastic tissue with genomic *TERT* alterations identified by either the MSK-IMPACT or MSK-Fusion assays, benign tissue and neoplastic tissue without identifiable *TERT* genomic alterations. While all three TERT quantification approaches showed variations in TERT expression amongst the three tissue groups (Fig. 4-D, E, F), ANOVA analysis revealed that these differences were not statistically significant from each other, with the exception when quantification was performed using the signal to cell ratio based on 100 cells (p value: 0.0001). The average TERT signal per cell ratio based on 100 cells was 3.95 in the neoplastic tissues with *TERT* genomic alteration, 1.1 in neoplastic tissue without *TERT* genomic alteration, and 0.03 in benign tissue.

A breakdown of the various tissue types (Fig. 4-B) showed that benign tissue is generally negative for TERT expression, with the only exception being deep crypts of colonic mucosa where cells have low level TERT expression; these cells possibly represent colonic epithelial stem cells which have been shown to overexpress TERT [18].

Among intraepithelial lesions, the HPV associated lesions, including a case of endocervical HPV associated adenocarcinoma in situ and a separate case with high grade squamous intraepithelial lesion, showed relatively high TERT expression (4 and 1 TERT/Cell ratio based on 100 cells count respectively). In contrast, a case of differentiated vulvar intraepithelial neoplasia (dVIN) showed low level expression (0.25 TERT/Cell ratio based on 100 cells count) which was nonetheless significantly higher than benign squamous epithelium which only rarely had a cell expressing TERT (an average of 0.026 TERT/Cell ratio based on 100 cells count). Malignant tissue sections had invariably increased TERT expression levels, which was significantly higher than benign tissue (p value <0.0001). In fact, using the TERT RNAscope® expression alone, there is excellent delineation of non-neoplastic versus neoplastic tissue with a 0.99 area under curve (AUC) on ROC curve analysis and a TERT/cell ratio based on 100 cell-count of 0.448 corresponding to a sensitivity of 89.5% and specificity of 93.1%. Examples of TERT RNAscope® expression are shown in Figs. 5, 6 and 7.

Consistent with the NGS-based gene expression analysis (Fig. 4A), evaluation of TERT RNAscope® expression in malignant tissue showed that TERT expression is increased regardless of the *TERT* genomic alteration status, with no significant differences in TERT expression levels amongst the various *TERT* genomic alterations (p value=0.115) (Fig. 7-C). *TERT* gene amplification shows the highest degree of variability in TERT expression but the level of TERT mRNA expression does not appear to correlate with the total copy number of the *TERT* gene as determined by the FACETS algorithm (p value=0.430), however the lack of statistical significance may be due to small number of samples in the cohort.

Interobserver agreement was measured using Pearson's r correlation measure and showed good interobserver agreement for scoring using the TERT/cell ratio based on 100 cells count approach (Pearson's r = 0.81).

4. Discussion

TERT reactivation and expression is an essential milestone in many tumors; the neoplastic transformation of cells requires *TERT* to be activated either through genomic or epigenomic mechanisms. In fact, for many neoplasms, activation of *TERT* marks malignant transformation and/or acquisition of aggressive behavior and poor prognosis.

Among genomic mechanisms of *TERT* upregulation and reactivation, *TERT* promoter mutation is the most common; these alterations often involve G>A substitutions leading to formation of novel binding sites for E-twenty-six (ETS) transcription factor which causes upregulation of *TERT* transcription [19]. Alternatively, tumor cells can acquire *TERT* gene amplification. In some tumors, the transcriptional upregulation occurs through *TERT* gene or promoter fusion with other genes or promoter sequences; these fusion events often involve approximation of enhancer motifs of highly expressed genes to upstream of *TERT* gene leading to a robust increase in TERT expression and activity [9]. These alterations are often mutually exclusive, and tumors only need one of these alterations for upregulation and reactivation of *TERT* to occur [20].

TERT upregulation can also occur through epigenomic mechanisms including alteration in the methylation landscape of the upstream regulatory sequences of *TERT*; hypomethylation at specific CpG islands upstream of the transcriptional start site as well as hypermethylation within the promoter sequence have been correlated with increased *TERT* gene expression [12,21].

Irrespective of the underlying reactivation, the result is increased TERT mRNA expression, a neoplastic hallmark that is present in the majority of cancers and neoplastic processes. Thus, being able to document and quantify TERT expression is of high value in diagnostic pathology; such a tool can help in distinguishing non-neoplastic versus neoplastic processes (with exception of perhaps stem cell niches which have intrinsic TERT expression), serve as a marker of malignant transformation in some tumors and/or be a surrogate for prognosis in other tumors. We describe some examples for each of these applications below.

Hepatocellular carcinoma invariably has high TERT expression, and genomic *TERT* alterations are often present in these tumors. *TERT* promoter mutation has been shown to be

an early carcinogenetic event in liver and these alterations are often present in hepatocellular carcinoma and preneoplastic liver lesions including cirrhotic preneoplastic macronodules, and low-grade and high-grade dysplastic nodules. In addition, acquisition of *TERT* promoter mutation has been linked to malignant transformation of hepatocellular adenoma with *TERT* mutation status being touted as a possible distinguishing factor between adenoma and carcinoma in liver [22,23].

Thus, evaluation and quantification of TERT expression using the ISH probe can potentially serve as a surrogate of malignant transformation especially when dealing with small and difficult to interpret liver biopsies. In our cohort, we stained one case of hepatocellular carcinoma with the *TERT* ISH probe (Fig. 7-D, E, F) and subjected it to targeted RNA capture assay. As expected, high level of TERT mRNA expression was shown by both assays. TERT quantification may also serve as a prognostic marker in hepatocellular carcinoma [24].

TERT can also serve as a prognostic marker in tumors as well. For example, in thyroid carcinoma, *TERT* alterations are common and the frequency of *TERT* alterations in tumors is directly related to their aggressive behavior, i.e., *TERT* promoter mutations are shown in ~40% of poorly differentiated and anaplastic thyroid carcinomas versus 11% in papillary thyroid carcinoma and 17% in follicular thyroid carcinoma. *TERT* status has been proposed as a prognostic marker in thyroid carcinoma and this is a field in which TERT expression measurement using the ISH probe can potentially serve as a prognostic marker [25].

Squamous cell carcinoma often has *TERT* reactivation [26]; however, depending on the underlying etiology for development of the squamous cell carcinoma, different mechanisms will be utilized by the tumor to activate *TERT*. For example, in HPV independent squamous cell carcinoma of head and neck and vulva, *TERT* promoter mutations are the most common mechanism of *TERT* activation. Conversely, in HPV driven squamous cell carcinoma of cervix, vulva and head and neck, genomic alterations of *TERT* are uncommon; rather *TERT* is activated through epigenomic alterations as a direct result of HPV infection. Yet, regardless of the *TERT* activation mechanism, all squamous cell carcinomas and their neoplastic precursors have increased expression of TERT. As we have shown in this study, squamous cell carcinoma of the cervix (Fig. 8) and vulva have high expression of TERT (Fig. 5). Furthermore, both cervical high-grade squamous intraepithelial lesion and differentiated vulvar intraepithelial neoplasia (dVIN) have increased TERT expression (Fig. 4). In contrast, normal non-dysplastic squamous epithelium shows only very rare cells expressing TERT. As a result, TERT expression can be used as a surrogate for squamous precursor lesions; this would be especially helpful in diagnosis of dVIN, which is a notoriously difficult lesion to diagnose [27–30].

In addition, there have been studies that have suggested that TERT expression levels differ between low-grade and high-grade squamous intraepithelial lesions. Consequently, TERT expression may also be used in differentiating these lesions in difficult cases where other ancillary tests are not helpful [31,32].

Cutaneous melanoma is another malignant entity that has been shown to require TERT expression. In fact, in xenograft models it was shown that cutaneous melanoma requires *TERT* activation alongside a mitogenic driver (e.g. *NRAS* mutation), senescence evasion (e.g. *CDK4* mutation) and antiapoptotic alterations (e.g., *TP53* mutation) to acquire invasive properties; consequently, *TERT* alterations are categorized as immortalizing mutations that occur later in tumor evolution and allow for invasion and metastasis [33].

TERT activation is an important evolutionary landmark in cutaneous melanomas [34]; studies have shown that the majority (70%) of invasive melanomas have *TERT* mutations [35], while nevi or radial growth--phase melanomas rarely have *TERT* alterations [36]. On the other hand, recent studies have shown that *TERT* promoter mutations may be present at subclonal levels in precursor melanocytic lesions [37]. As such, a quantifiable biomarker such as the TERT RNAscope® may be better suited for difficult to classify melanocytic lesions compared to high-sensitivity sequencing assays, as it allows for the visualization of tissue level expression, as well as correlation with histomorphology. Among our samples we had a single case of invasive melanoma which showed remarkably high TERT expression levels (Fig. 7 – G, H, I) in all tumor cells, highlighting the possible utility of this assay. Furthermore, *TERT* alterations have been shown to be a significant predictor of adverse outcome in melanoma suggesting TERT expression may also serve as a prognostic tool in these tumors [38].

Our results show that the TERT RNAscope® assay is a reliable tool for the evaluation of TERT expression in formalin fixed paraffin embedded tissue as it correlates with TERT expression as determined by next generation sequencing assay. Interestingly, the RNAscope® assay shows that TERT expression can be variable throughout neoplastic tissue which may lead to underestimation of TERT expression by sequencing methodologies. Furthermore, the RNAscope® assay allows for morphology/expression correlation and can reliably distinguish neoplastic from non-neoplastic tissues, making this a valuable tool in diagnostic histopathology.

We also presented a relatively simple yet reliable method for documenting and quantifying TERT expression. In some instances, TERT mRNA expression by RNAscope® can be qualitatively interpreted since neoplastic tissue often has robust expression in contrast to the almost entirely negative expression in non-neoplastic tissue. However, quantification may be necessary in differentiating non-neoplastic from neoplastic tissue in some cases and we have shown that counting TERT signals in 100 cells with the highest TERT expression (as determined by a visual inspection and screening of the stained slide) is a reliable and reproducible approach in quantification of TERT expression: TERT/cell ratio based on 100 cells of more than 0.448 reliably separates neoplastic tissues from non-neoplastic tissues (AUC: 0.99). Cutoffs, however, would have to be adjusted based on specific use intended for TERT and it remains to be seen whether quantification of TERT expression is correlated with prognosis in various tumors. Additional studies are warranted to define these applications.

The main limitation of our study was the small size of the cohort; however, the main aim of this study was to serve as a proof of concept to show that TERT expression can be

adequately measured using the RNAscope® assay. Previous attempts at TERT RNA in-situ hybridization were made using custom designed probes with similar results to our cohort [39], however, the current methodology is promising in that it shows excellent sensitivity and specificity combined with standardized methodology and ease of utilization.

In summary, we have shown the utility of TERT mRNA expression analysis using the RNAscope® assay and we have also suggested a reproducible and simple quantification and interpretation approach for evaluation of TERT expression. The assay has potential to become a useful diagnostic tool in the arsenal of histopathologists; however, further work, including validation studies focusing on specific pathologic entities, is needed in order to establish the scope of the clinical utility of TERT expression analysis.

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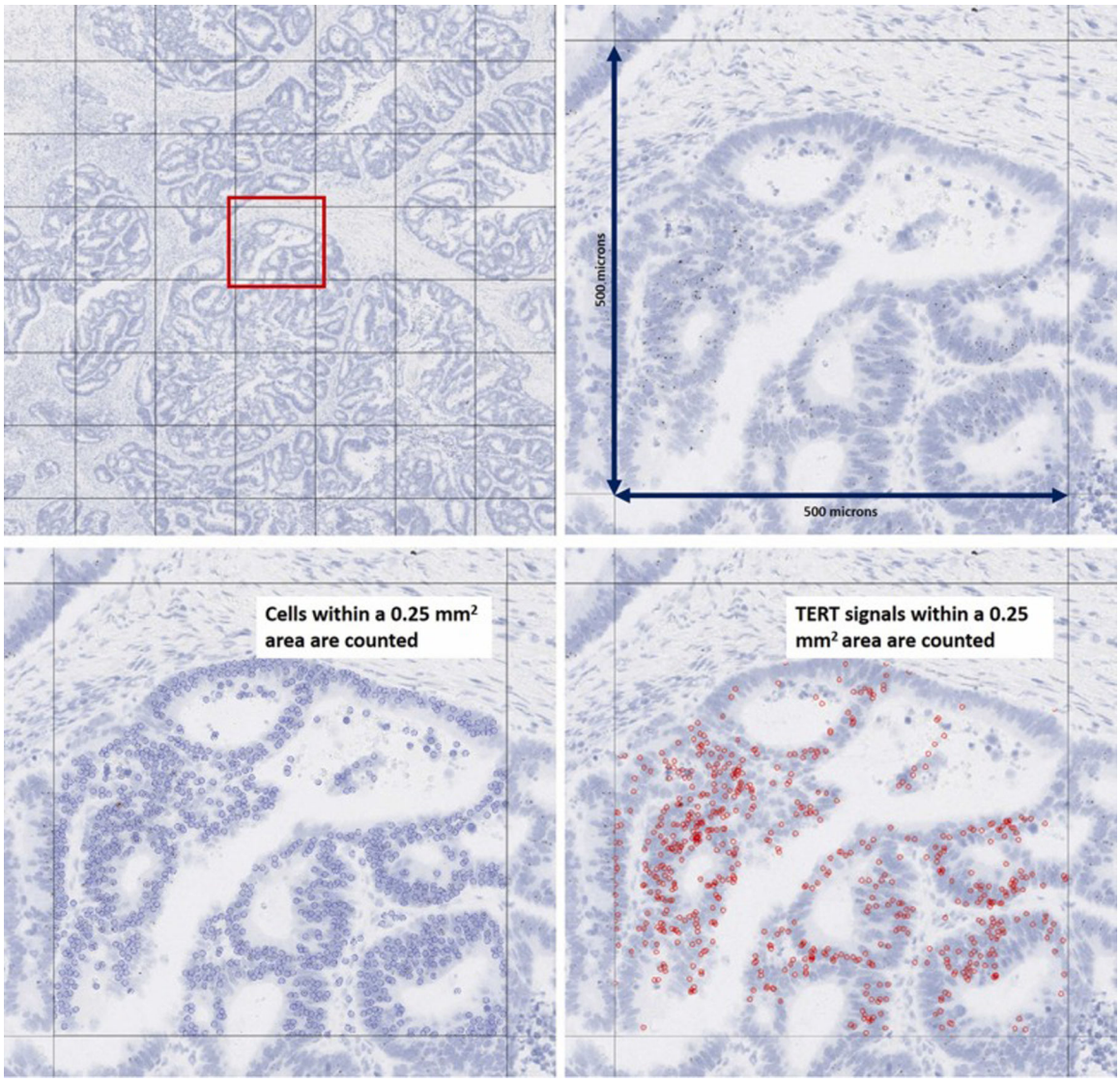

Fig. 1.

Approach 1 tested for the quantification of TERT ISH expression. In this first approach for quantifying *TERT* ISH expression, a *TERT* signal to cell ratio in a 0.25 mm^2 area was calculated.

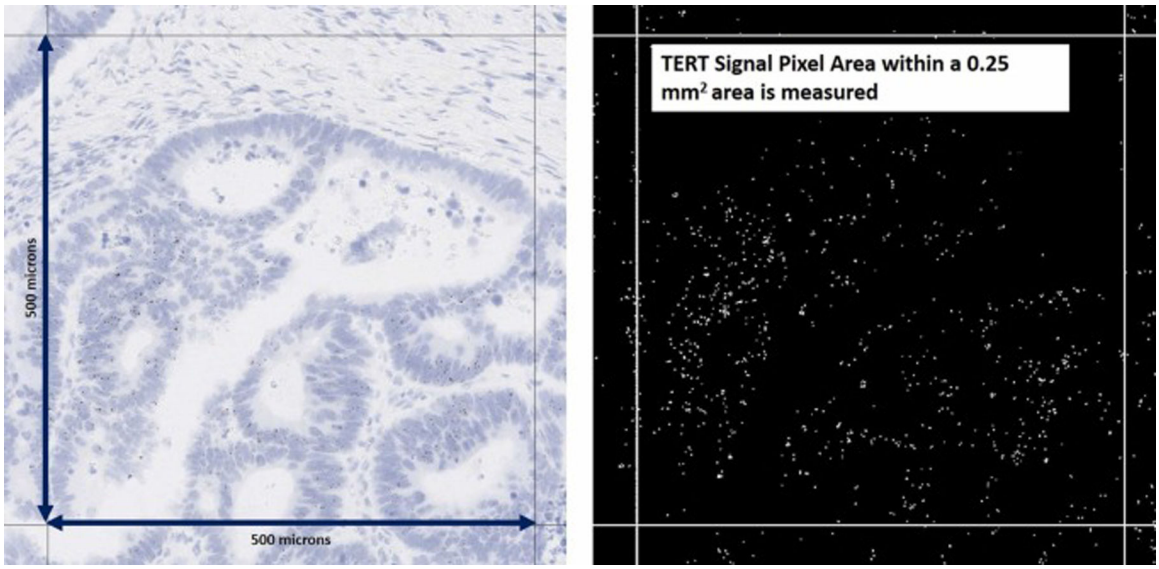

Fig. 2.

Approach 1 tested for the quantification of TERT ISH expression. In this second approach for quantifying *TERT* ISH expression, the number of *TERT* signals in a preset (e.g. 100, 200, 300, …) number of cells with highest *TERT* expression (here quantification is showed based on a 100-cell count) was counted and a signal to cell ratio calculated.

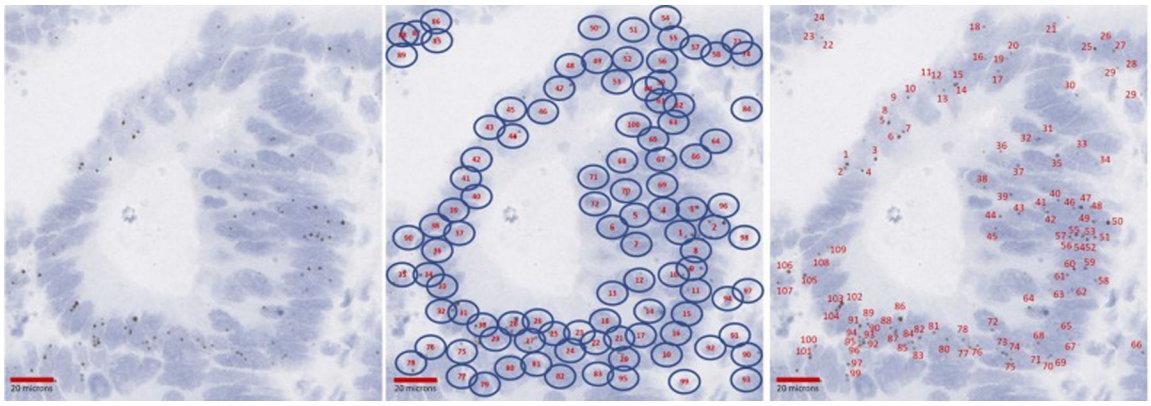

Fig. 3.

Approach 3 tested for the quantification of TERT ISH expression. In this third approach for quantifying *TERT* ISH expression computational tools of the QuPath software were employed to measure the *TERT* DAPI signal area within a 0.25 mm^2 area.

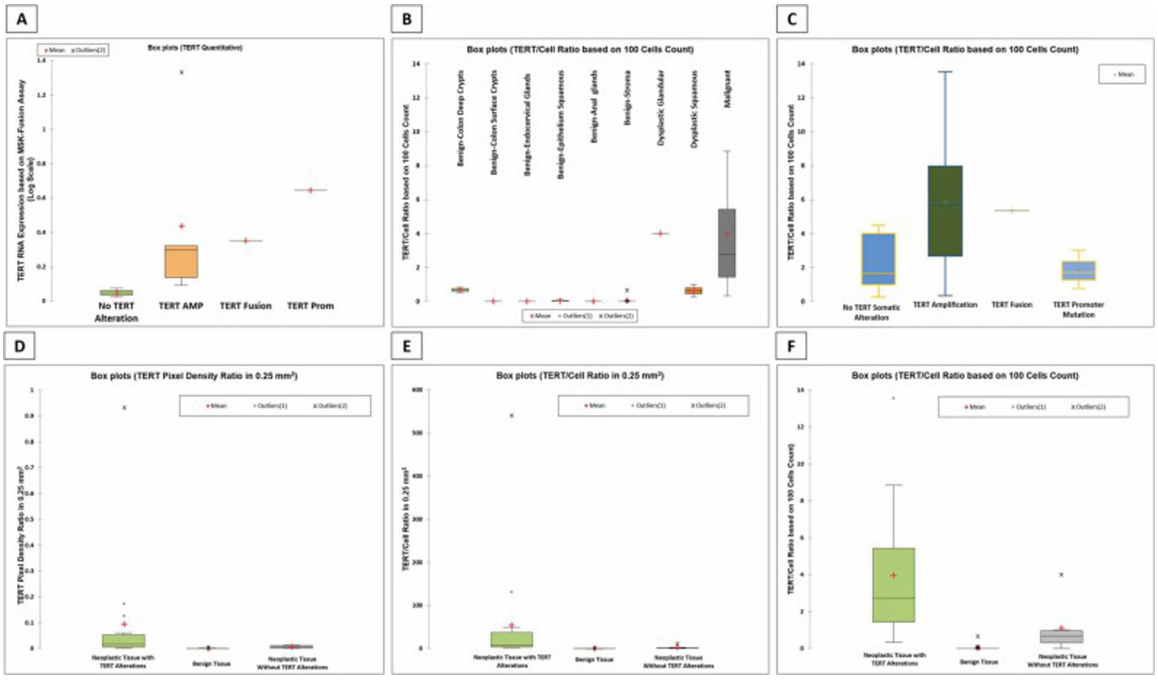

Fig. 4.

Boxplot showing *TERT* RNA expression levels in the 9 samples tested using the MSK-Fusion™ assay. The subcategories represent the somatic *TERT* alterations identified in the samples. (B) Boxplot showing *TERT* RNAscope® expression as quantified *TERT*/Cell ratio based on 100 cells count for different tissue types. (C) Boxplots showing *TERT* RNAscope® expression as quantified *TERT*/Cell ratio based on 100 cells count in malignant tissue and the corresponding *TERT* somatic alteration. (D – F) Boxplots depicting the *TERT* expression quantification using the *TERT* RNAscope® assay. Panel (D) shows quantification based on *TERT* pixel density ratio, panel (E) shows *TERT*/cell ratio in 0.25 mm^2 and panel (F) shows *TERT*/cell ratio based on 100 cells count. Note that the differences between the three tissue categories is best highlighted in the panel on the right.

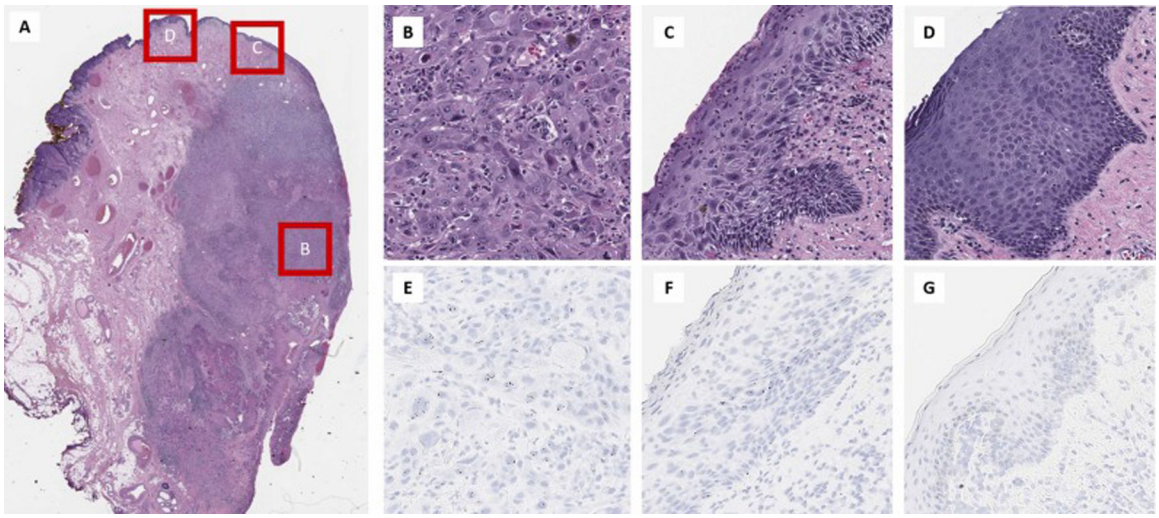

Fig. 5.

TERT RNAscope® expression in a case vulvar squamous cell carcinoma. (A) Low power magnification of the vulvar squamous carcinoma. The invasive squamous cell carcinoma component (B) has high *TERT* expression in the tumor cells (E), while the differentiated vulvar intraepithelial neoplasm component (C) has increased expression limited to the basal and parabasal cells (F). Normal squamous epithelium (D) however shows no *TERT* expression (G).

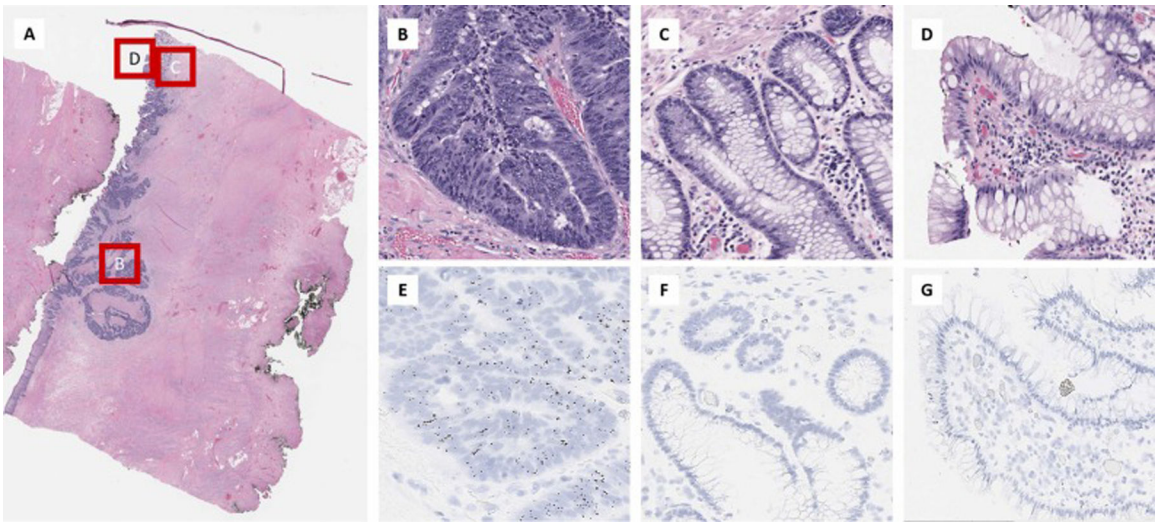

Fig. 6.

TERT RNAscope® expression in a case of colon adenocarcinoma. (A). Low power magnification of the colon adenocarcinoma. The invasive colon adenocarcinoma (B) has high *TERT* expression in the tumor cells (E) which is significantly different from normal colonic deep crypts in the same tissue section (C) which show slightly increased *TERT* expression (F) and superficial colonic epithelium (D) which shows no *TERT* expression (G).

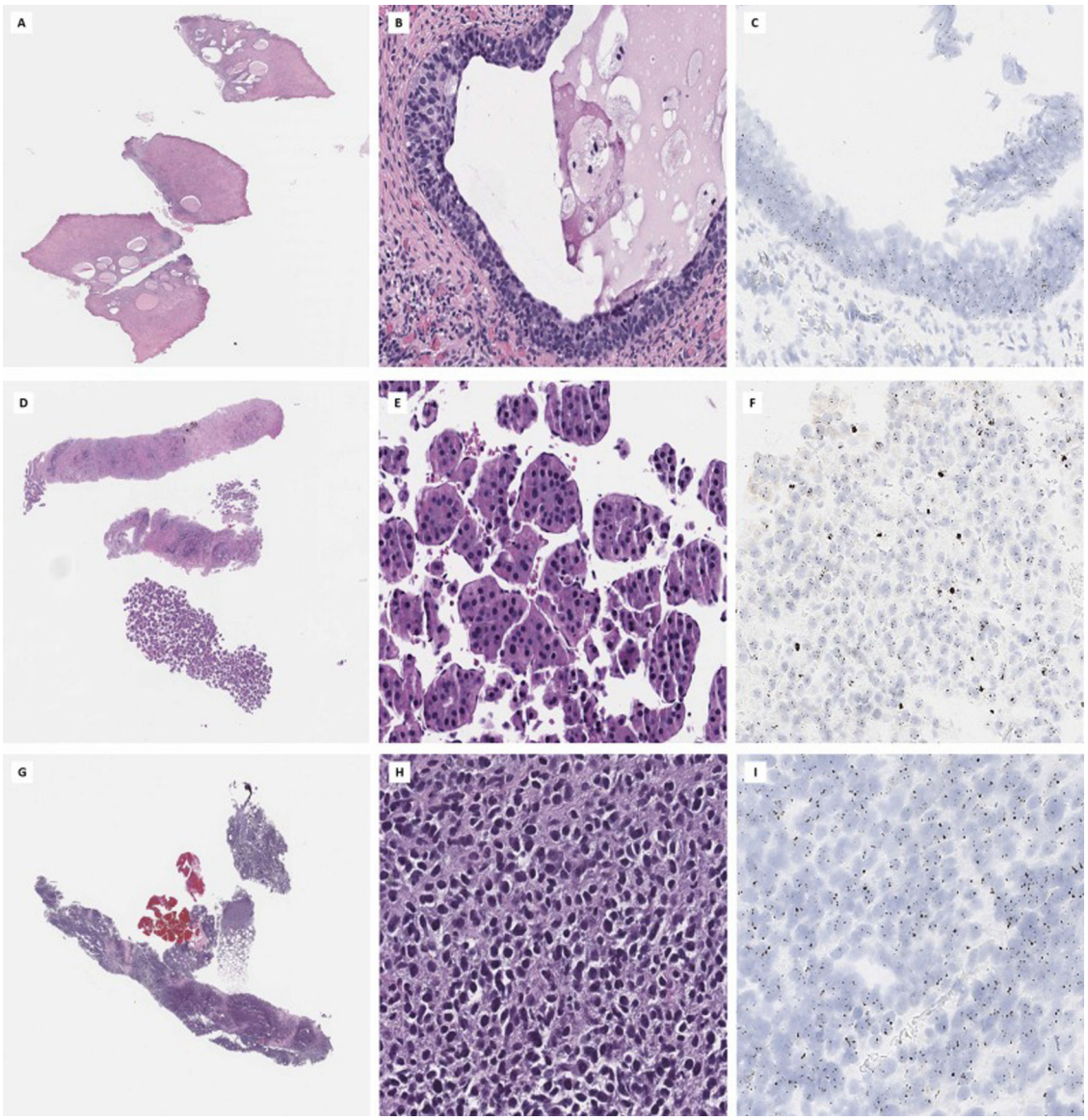

Fig. 7.

Neoplastic tissues show high levels of *TERT* RNAscope® expression. Examples of cervical adenocarcinoma in situ (A, B, C), hepatocellular carcinoma (D, E, F) and malignant melanoma (G, H, I) are shown, all of which have high levels of *TERT* expression by TERT ISH (right).

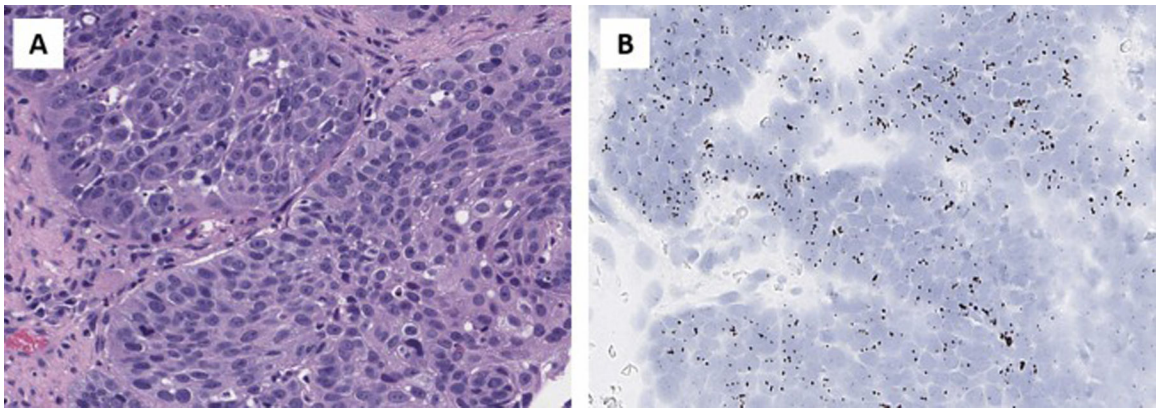

Fig. 8.

TERT ISH expression in HPV-associated cervical squamous cell carcinoma.

Table 1

Tissue types evaluated for *TERT* gene status and *TERT* RNAscope expression.

Tissue section type	Tissue section type detailed	Number of tissue sections	Somatic *TERT* alteration status	Median TERT/cell ratio based on 100 cells
Benign (N = 29)	Colon Deep Crypts	2	NA	0.675
	Colon Surface Crypts	2	NA	0.000
	Endocervical Glands	4	NA	0.000
	Squamous epithelium	6	NA	0.000
	Anal glandular epithelium	1	NA	0.000
	Stroma from benign and neoplastic samples	14	NA	0.000
Intraepithelial neoplasia (N = 3)	Endocervical adenocarcinoma in situ	1	None	4.000
	High-grade Squamous Intraepithelial Lesion (CIN3)	1	None	1.000
	Differentiated Vulvar Intraepithelial Neoplasia	1	Unknown	0.250
Malignant (N = 16)	Melanoma	1	*TERT* Amplification	4.048
	Dedifferentiated Liposarcoma	1	*TERT* Gene Fusion	5.350
	Desmoplastic Small Round Cell Tumor	1	*TERT* Amplification	13.550
	Osteosarcoma	1	*TERT* Amplification	7.091
	Myxoid Liposarcoma	1	*TERT* Promoter Mutation	0.750
	Uterine Serous Carcinoma	1	*TERT* Amplification	0.350
	Colon Adenocarcinoma	3	*TERT* Amplification [1], None [2]	1.65
	Vulvar Squamous Cell Carcinoma	5	*TERT* Promoter Mutation [5]	1.952
	Cervical Squamous Cell Carcinoma	1	*TERT* Amplification	6.423
	Hepatocellular Carcinoma	1	*TERT* Amplification	5.700
Total		48		