Journal of Biomedical Sciences | ISSN: 2960-9356
Research Article Peer-review, Open Access
World of Medicine: Journal of Biomedical Sciences Vol .2 No. 9 (2025)
https://wom.semanticjournals.org/index.php/biomed

Comparative TERF2 Gene Activity and TRF2 Protein-Pathway Profiling in Human Liver and Gastric Tumors: A Tissue-Based Diagnostic Biomarker Study of Tumor Development

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Abstract: Cells that are cancerous need to keep their chromosome ends intact while surviving replication stress, DNA damage response remodeling, and continued proliferation. Telomere protection is becoming more and more recognized as an important factor in tumor development. Protecting the ends of chromosomes is what TERF2 does. It creates the shelterin protein TRF2, which may change gene-regulatory and microenvironmental processes outside of telomeres. Unfortunately, there aren't many direct comparisons between human tissues that include TERF2 gene activity, TRF2 protein expression, pathway behavior, and diagnostic modeling in liver and stomach tumors. Using protocol-defined criteria, this observational comparative molecular pathology study looked at 100 original human tumor samples, 50 of which were liver tumors and 50 of which were stomach tumors the study was conducted in March 2025 and concluded in June 2025. Cases that were recurring, metastatic, previously treated, poor-quality, or linked with a chronic disease were not included. DNA damage response and proliferation pathway behavior was studied using γ-H2AX, p-ATM, p53, and Ki-67 marker profiling. TERF2 mRNA levels were measured by RT-qPCR, and nuclear TRF2 protein levels were quantified by immunohistochemistry. There was no significant difference between the two groups in terms of nuclear TRF2 H-scores, but there was a difference between stomach and liver tumors in terms of TERF2 relative expression (median $2^{-\Delta Ct}$, 0.120 vs. 0.084; p =.0037). TRF2 H-score and TERF2 expression were linked (Spearman ρ =.588; p <.001), and aggressive tumors had higher TRF2 H-score and pathway score. According to the results of a combination TERF2/TRF2/pathway model, there was good internal diagnostic differentiation for aggressive tumor-development trait (AUC = 0.916; bootstrap-corrected AUC = 0.907). Additionally, these results show that TERF2/TRF2 pathway profiling is a promising tissue-based diagnostic indicator of tumor-development biology that needs to be confirmed by other scientists before it can be used in patients.

Keywords: TERF2; TRF2; liver tumors; gastric tumors; biomarker; telomere biology

1. Introduction

The growth of a tumor is a complex biological process with many steps. Cancer cells learn how to multiply even when genomic stress, changes in DNA damage-response signaling, unstable telomeres, and selective pressures from the tissue microenvironment are present. This process requires cancer cells to balance two opposing forces: making changes to their genomes that cause clonal evolution while also keeping enough chromosome integrity to stay alive and keep copying. Mistaking unprotected chromosome ends for DNA double-strand breaks can cause the chromosomes to rearrange, end-to-end fusions, senescence, or cell death. Part of this balance is telomere failure. Meanwhile, tumor cells that protect the structure of telomeres while lowering checkpoint barriers might be able to grow faster when there is genetic instability and replication stress. This means that telomere-protection biology is a very important way to study how tumors grow, how aggressive diseases act, and how tissues can show biomarker markers.

The shelterin complex is a group of proteins that protects telomeres, which are nucleoprotein structures found at the ends of chromosomes. This complex can tell the difference between natural chromosome ends and DNA breaks. Shelterin manages the length of telomeres, the formation of T-loops, the replication of telomeric DNA, and the activation of the DNA damage response at the ends of chromosomes [1]. A key protein needed for protecting chromosome ends is encoded by TERF2 within this complex. Its name is telomeric repeat-binding factor 2. TRF2 helps keep the structure of telomere loops stable and stops telomeres from activating ataxia telangiectasia mutated kinase-mediated DNA damage signaling. This stops chromosome ends from being mistakenly recognized as double-strand breaks [1], [2]. Changing or not controlling TRF2 can change telomere stability, DNA damage response control, replication stress tolerance, and genomic continuity. These are all things that are connected to tumor growth and malignant adaptation [3], [4], [5] but can also go wrong.

TRF2/TERF2 is important for more than just keeping telomeres long at the genetic level in cancer. To protect telomeric regions, deal with replication stress, manage DNA repair, and change pathways that help tumor growth, researchers have found that TRF2 [6], [7]. An experiment with cancer cells showed that blocking TRF2 function can lead to telomere problems, senescence, ferroptosis, autophagic death, or apoptosis. This suggests that tumor cells may need to keep TRF2 activity high in order to survive chromosomal stress [8]. New studies on cancer also show that TERF2/TRF2 dysregulation may be important for many types of tumors and may affect how cancer cells grow, survive, get around the immune system, communicate with blood vessels, and respond to treatment [9], [10], [11]. Importantly, TRF2 may also work in ways other than telomere protection. For example, it may link to G-quadruplex-containing regulatory regions and change gene expression programs in ways other than the usual way of protecting telomeres [12], [13], [14]. The results back up the idea that TERF2/TRF2 should be looked at not only as a molecule connected to telomeres but also as a regulator of tumor biology that is linked to a pathway.

TERF2 needs to be looked at at the transcript level in order to be used as a diagnostic biomarker. The amount of mRNA may show that genes are active, but it doesn't always show what's going on with functional proteins, where they are in the nucleus, which pathways are being used, or how tumor cells act. For TRF2 proteins to do their job of protecting the ends of nuclear chromosomes and interacting with telomeric and extra-telomeric chromatin, they need to be present in the right amounts and locations. Besides that, differences between transcripts and proteins may happen because of protein stability, microRNA-mediated effects, altered degradation, or pressures on pathways that are specific to the tumor environment. For that reason, it is more biologically sound to use markers for DNA damage response and proliferation along with the activity of the TERF2 gene and the expression of the TRF2 protein to make a diagnosis based on tissue than just gene expression alone. If you want to find possible biomarker patterns that can tell the difference between different types of tumor growth rather than being used as separate diagnostic tests, this is the best way to do it.

To directly compare liver and stomach tumors is scientifically sound because both are major cancer causes in the world and both grow in solid epithelial tumor environments that are marked by genomic stress, proliferative remodeling, and microenvironmental adaptation [15]. No matter what, these tumors are very different in how they affect the stroma, metabolic forces, inflammation backgrounds, and organ biology. It's possible for oxidative stress, telomere loss, capillary invasion, and genetic instability to

affect how a liver tumor grows in an organ that has a lot of metabolic activity and blood flow. Cell biology and liver cancers are linked, according to Cao [16] and Qiu [17]. Unlike other types of cancer, stomach cancer starts in glandular mucous epithelium, which can change its cells, invade deeper, send signals about DNA damage, and affect the ecosystem. Pancreatic and stomach cancers are also linked to TERF2, and in models of stomach cancer, decreasing its activity has useful results [18]. Therefore, comparing liver and stomach tumors might show if TERF2/TRF2 activity acts as a common cancer-related process or as a tumor-development marker that depends on the organ.

There is more and more evidence that TERF2/TRF2 is involved in cancer biology, but most of the studies that have been done so far have only looked at one type of tumor, cell line models, pan-cancer bioinformatic associations, or mechanistic intervention systems. We still need to do direct comparisons between human tissues that include TERF2 gene activity, TRF2 protein expression, DNA damage response and proliferation pathway behavior, as well as diagnostic modeling in a single tissue-based biomarker framework. Combining these things is important because differential expression is not the only thing that makes a biomarker useful in molecular disease. Along with being able to accurately locate the tissue, being able to tell the difference between different diagnoses, and having biological similarities with how tumors grow. Because of this, a mixed TERF2/TRF2 pathway map might tell us more about how aggressive tumors work than just measuring the transcripts or proteins. Before it can be used on people, though, it still needs to be carefully read and confirmed by other experts. The point of this study was to look at how TERF2 gene activity and TRF2 protein-pathway behavior change in human liver and stomach tumors. They also wanted to see if combining TERF2 and TRF2 molecular pathway profiling could be used to tell how a tumor is growing and how aggressive it is.

2. Materials and Methods

2.1 Study design, setting, and ethical approval

TERF2 gene activity, TRF2 protein expression, and TRF2-associated pathway behavior were looked at in primary liver and stomach tumors in this observational, comparative, human tissue-based molecular pathology and diagnostic biomarker study. Within the study, there were 100 samples of human tumors, with 50 being primary liver tumors and 50 primary gastric tumors. Anonymized original tumor biospecimens were taken from qualified patients who were having a diagnostic biopsy or surgical tumor sampling. We only looked at primary tumor tissues so that metastatic biology, molecular selection related to recurrence, and treatment-related changes in RNA or protein expression wouldn't confuse the results.

Human biospecimen research at the school oversaw the study's ethical behavior, and informed consent or consent withdrawal methods were used as required by local rules for tissue-based analysis that did not reveal the identity of the subjects. Followed the Declaration of Helsinki and any national rules that apply to research using human biospecimens. Study design and writing followed the guidelines from STROBE-ME for molecular epidemiology reporting, REMARK for tumor marker studies, STARD for diagnostic accuracy studies, and MIQE for quantitative PCR transparency.

2.2 Patient and sample eligibility criteria

Eligible cases were those whose primary liver or stomach tumors had been confirmed by histopathology and for whom enough tumor tissue was available for RNA- and protein-based molecular analysis. To be eligible, the specimen had to be well preserved, have enough tumor cells left after a pathological review, and have at least the following clinical and pathological information available: the tumor's histological diagnosis, its grade, stage, or invasion category; it also had to show vascular invasion, necrosis, and lymph node involvement for stomach tumors when data was available. People who had tumor tissue taken before chemotherapy, radiotherapy, targeted therapy, immunotherapy, or any other type of anti-cancer treatment were included. This kept changes in TERF2/TRF2 expression and DNA damage-response markers to a minimum.

Cases weren't included if the tumors came back, spread to the liver or stomach from another original site, or weren't well kept enough for genetic study. According to the study protocol, tumors that were caused by clearly documented chronic diseases were not included. Patients with known chronic hepatitis, cirrhosis, alcohol-related liver disease, drug-induced liver damage, long-term exposure to hepatotoxic medications, or a history of drug abuse were not eligible for liver tumors. The presence of

documented chronic gastric inflammatory disease was a protocol-level exclusion for gastric tumors. These other conditions were not included: predominant tissue necrosis, poor formalin fixation, not enough tumor cells, RNA or protein material that had been broken down, and not enough tissue volume for comparing genes, proteins, and pathways. Age and gender were not used as matching factors, but they were kept for informational purposes and used in sensitivity studies when they were available.

2.3 Histopathological classification and tumor-development variables

Before the molecular tests were done, all cases were looked at by qualified pathologists who used hematoxylin and eosin-stained sections. In histopathology, the classification included the type of tumor, its histology diagnosis, its histological grade, its stage or spread category, and any harmful pathological traits that were important. Variables that were recorded for stomach tumors included the depth of invasion, the involvement of lymph nodes when known, the invasion of blood vessels and nerves when known, necrosis, and the number of cells in the tumor. We kept track of the histological tumor type, grade, vascular invasion, microvascular invasion, capsule invasion, necrosis, and tumor cellularity percentage for liver tumors. RNA and protein measurements could be better understood with the help of information about the type of tissue preservation, the quality of the specimens, and how well they were fixed.

A trait for tumor growth and ferocity was set up front to help with diagnosis models. It was decided that a tumor was high-development or aggressive if it had at least one of several known bad pathological features. These could be a high histological grade, an advanced stage or invasion category, vascular invasion, necrosis, lymph node involvement (if present), microvascular invasion or capsule invasion in liver tumors (if present), or other bad pathological criteria set by the protocol. Cases that didn't have these traits were labeled as lower-development or non-aggressive so that they could be compared. If specific abnormal traits weren't available, the case was kept for studies that didn't need that variable, and missing values were reported instead of being assumed without a good reason.

2.4 TERF2 gene activity measurement by RT-qPCR

The activity of the TERF2 gene was measured using a reverse-transcription quantitative polymerase chain reaction and tissue that was high in tumors. For each case, typical tumor areas were found on matched histological sections. When needed, macrodissection was used to increase the number of cells in the tumor and decrease the amount of dead, stromal, inflammatory, or non-neoplastic tissue that got mixed in. We got the total RNA by using a standard column-based RNA extraction method that works with clinical tumor tissue samples. We checked the amount and purity of RNA using spectrophotometry with A260/A280 and A260/A230 ratios. We also checked the integrity of the RNA using an electropherogram-based RNA integrity number or another similar RNA-quality test. RT-qPCR analysis did not include samples that did not meet certain RNA quality standards.

From the same amount of RNA in each sample, complementary DNA was made using a tried-and-true reverse-transcription process and standard reaction conditions. Real-time RT-qPCR on a measured quantitative PCR device was used to measure the amount of TERF2 mRNA. In Supplementary Table S1, you can find the TERF2 forward and reverse primer sequences, the reference gene primer sequences, amplicon details, and the qPCR cycling conditions. We used at least two steady housekeeping genes, like GAPDH, ACTB, RPLP0, or other known internal standards. Reference-gene stability was checked in tumor samples from the stomach and liver before they were normalized. There were no-template controls, reverse-transcription negative controls, and inter-run calibrators used for all three reactions. Primer efficiency was found using standard models, and it was only accepted if it was within certain ranges of efficiency. The $2^{-\Delta Ct}$ method was used to figure out the relative TERF2 expression for a comparison of only tumors. The RT-qPCR methods followed the reporting rules set by MIQE.

2.5 TRF2 protein expression by immunohistochemistry

TRF2 protein levels were checked using immunohistochemistry on formalin-fixed, paraffin-embedded tumor slices. Sections 3–4 μm thick were put on charged slides, deparaffinized, rehydrated, and heated to retrieve epitopes using a proven retrieval buffer and standard temperature-time conditions. Incubation of slides with a validated anti-TRF2 primary antibody using an optimized dilution and detection protocol blocked the body's own peroxidase activity. Additionally, Supplementary Table S2 lists the antibody clone, seller, catalog number, dilution, recovery settings, and confirmation source. Negative controls were made by not using the primary antibody or using an isotype-matched control. Positive controls

included tissue that was known to express nuclear TRF2.

The main scoring area was nuclear marking in living tumor cells because TRF2 is connected to protecting chromosome ends and playing a role in chromatin-related or nuclear telomeric activities. While cytoplasmic staining was seen, it was recorded for description purposes only and did not replace nuclear interpretation. TRF2 expression was measured using the H-score method, which is as follows: H-score = º Pi × i, where Pi is the number of stained tumor cells at each intensity level and i is the intensity of the staining from 0 to 3. The final H-score was between 0 and 300. Two pathologists scored each case separately, without knowing the tumor type, TERF2 mRNA results, or pathway-marker results. Interobserver agreement was measured with the intraclass correlation coefficient for continuous H-scores or kappa statistics for high/low classification. We compared digital nuclear staining scores with pathologist-derived scores to see how well the scores could be repeated when digital pathology quantification was available.

2.6 TRF2 protein-pathway tracking

To find out if TRF2 levels and locations were connected to DNA damage response and tumor growth, the protein pathway activity of TRF2 was studied. Multiplex immunofluorescence or confocal immunofluorescence was used on representative tumor sections to find the best way to track pathways. This method included TRF2, γ-H2AX, p-ATM, p53, Ki-67, and DAPI nuclear counterstaining methods. These cells were chosen because they show TRF2 nuclear protein expression, DNA damage signaling, ATM pathway activation, p53 pathway alteration, and cell proliferation all in the same tissue. A measured fluorescence or confocal microscope tool was used to take pictures. Standardized digital image-analysis processes were then used to do quantitative analysis after the nuclei of live tumor cells were separated.

To find out how strong a marker was, how many tumor cells it detected, and where in the tumor it was located, we looked at specific areas and left out areas that were necrotic, had tissue folds, were bleeding, or were not representative of the whole sample. The closeness of TRF2 to DNA damage-response signals was described briefly and only included when there was enough image data to do so. A panel of immunohistochemical markers, such as TRF2, γ-H2AX, p53, and Ki-67, was used instead if multiplex immunofluorescence wasn't available. Standardized marker scores were made from semiquantitative or digital image measuring. From the standard TRF2 protein score, the DNA damage-response marker score, the p53 category, and the Ki-67 proliferative index, a TRF2-pathway activation score was established. Instead of showing a possible path, the score was meant to link the amount of TRF2 protein and where it is located in the nucleus to quantitative aspects of tumor growth biology.

2.7 Diagnostic and statistical analysis

Data analysis using validated statistical software for biomedical data was done. The software had routines for non-parametric testing, logistic regression, ROC analysis, and bootstrap internal validation. Summarized continuous factors with mean and standard deviation or median and interquartile range, based on how they were distributed. Categorical variables were summed up using percentages and frequencies. We checked for normality by using the Shapiro–Wilk test and looking at histograms or Q–Q plots manually. We compared TERF2 mRNA expression, TRF2 H-score, pathway-marker scores, and TRF2-pathway activation score between liver and stomach tumors using the independent-samples t-test or the Mann–Whitney U test, depending on whether the data was normally distributed or not. These were compared using either the chi-square test or Fisher's exact test, depending on what was needed.

The connection between TERF2 mRNA expression and TRF2 nuclear H-score was studied using Spearman correlation. The links between TERF2/TRF2 markers and the aggressive tumor-development phenotype were studied with both one-variable and multiple-variable logistic regression. With 100 people as a group, diagnosis modeling was kept simple so that it could be understood physically. The main model contained TERF2 mRNA, TRF2 nuclear H-score, and one pathway marker or pathway score chosen based on its biological relevance and data completeness. This was done without using complicated machine-learning models or unstable train/test splitting.

Utilizing receiver operating characteristic analysis, we tested how well tissue-based diagnostic discrimination could separate aggressive tumor-development phenotype. A lot of information was given about TERF2 mRNA, TRF2 H-score, the combined TERF2/TRF2/pathway model, including the area under the curve, 95% confidence intervals, sensitivity, specificity, positive predictive value, negative

predictive value, and accuracy. The optimism-corrected diagnostic score was estimated using bootstrap resampling for internal confirmation. Multiple route markers were tested, and false finding correction was used. Two-sided alpha levels of.05 were used to determine statistical significance. Effect sizes and confidence intervals were given more weight than p-value analysis alone.

3. Results

3.1 Clinicopathological and biospecimen characteristics

The collection that was looked at had 100 valid original human tumor samples, with 50 being liver tumors and 50 being stomach tumors the study was conducted in March 2025 and concluded in June 2025 . Histologically, all cases in the stomach were called gastric adenocarcinoma and all cases in the liver were called hepatocellular cancer. The grade breakdown was pretty much the same across all tumor types. There were 9 grade 1 cases, 20 grade 2 cases, and 21 grade 3 cases in liver tumors, and 12 grade 1 cases, 22 grade 2 cases, and 16 grade 3 cases in stomach tumors. The stage distribution also showed that both early and advanced stages were represented. In 31 liver tumors and 25 gastric tumors, vascular invasion was seen. In the same group, aggressive tumor development was seen in 31 liver tumors and 30 gastric tumors. People's age and sex were only written down in a general way. The average age in the liver group was 60 years, and the average age in the gastric group was 63 years. The quality of the RNA was good enough for further study, with median RIN values of 7.45 in liver tumors and 7.25 in stomach tumors. In liver tumors, the median cell density was 73.0%, while in stomach tumors, it was 69.0%.

Table 1. Clinicopathological and biospecimen characteristics of liver and gastric tumor samples

Characteristic	**Liver tumors (n = 50)**	**Gastric tumors (n = 50)**
Histological diagnosis	Hepatocellular carcinoma (100%)	Gastric adenocarcinoma (100%)
Sex, male/female	31/19	27/23
Median age, years (IQR)	60.0 (54.2–65.8)	63.0 (55.2–66.8)
Grade 1, n	9	12
Grade 2, n	20	22
Grade 3, n	21	16
Stage I/II/III/IV, n	4/20/19/7	9/13/18/10
Vascular invasion present, n	31	25
Aggressive tumor-development phenotype, n	31	30
Median necrosis, % (IQR)	23.0 (18.0–26.8)	24.0 (18.5–30.5)
Median tumor cellularity, % (IQR)	73.0 (65.2–77.8)	69.0 (66.0–75.0)
Median RNA integrity number (RIN) (IQR)	7.5 (6.8–7.9)	7.2 (6.8–7.9)
Median RNA A260/A280 (IQR)	1.9 (1.9–2.0)	1.9 (1.9–2.0)
Eligible samples analyzed, n	50	50

3.2 TERF2 gene activity differs between liver and gastric tumors

The activity of the TERF2 gene was different in liver and stomach tumors compared to other organs. The relative expression of TERF2 was higher in stomach tumors than in liver tumors. The median 2^-ΔCt value was 0.120 in stomach tumors compared to 0.084 in liver tumors. The non-parametric test (Mann–Whitney U = 828.0, p =.0037) showed that this difference was significant. This means that TERF2 transcriptional activity was not the same in the two types of tumors. TERF2 expression went up at all stages of clinical growth when looked at by tumor grade. The median expression level was lowest in grade 1 tumors, while it was highest in grades 2–3 tumors (Kruskal–Wallis p =.0175). Also, TERF2 expression levels were higher in aggressive tumors compared to non-aggressive tumors, with median

values of 0.111 versus 0.082 (p =.0014). We used Spearman ρ =.354 and p =.0003 to find a link between TERF2 expression and the tumor-development score. This shows that there is more than one link between having more TERF2 gene activity and bad abnormal development. The results show that TERF2 mRNA may help in telling the difference between different types of tumors based on their growth pattern. However, because of its small effect size, examining it at the transcript level alone probably won't give us a full picture of the functional biomarker profile.

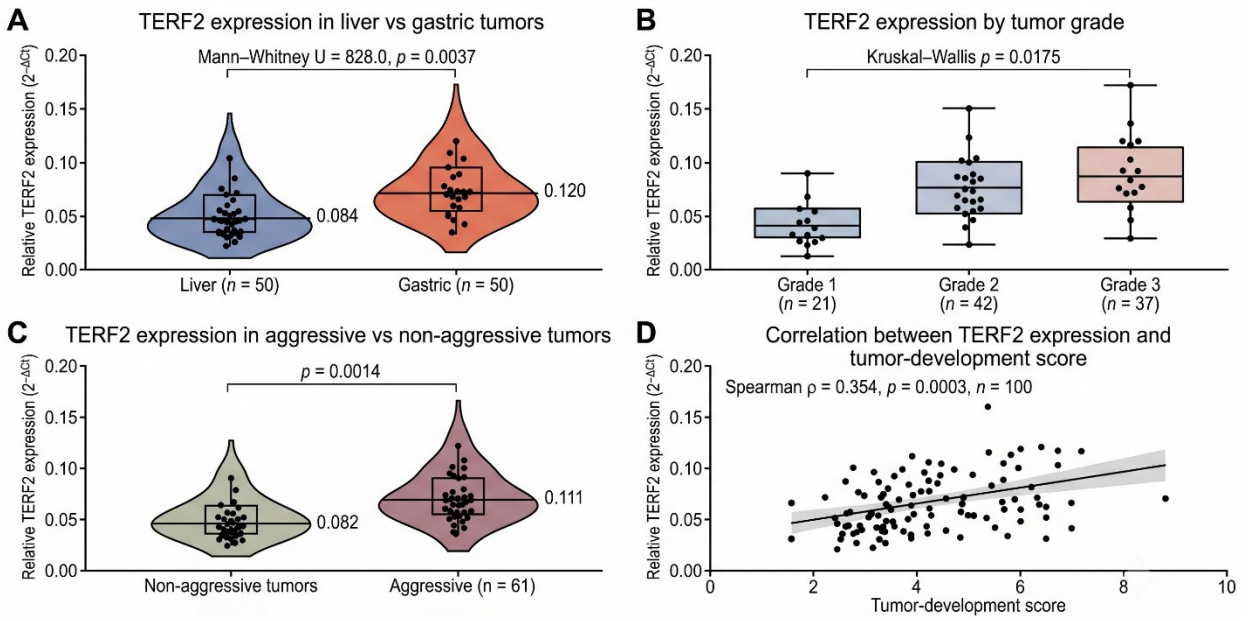


Figure 1. TERF2 gene activity in liver and gastric tumors. (A) Comparison of relative TERF2 expression (2^-ΔCt) between liver tumors (n = 50) and gastric tumors (n = 50). (B) TERF2 expression across tumor grades. (C) TERF2 expression in non-aggressive and aggressive tumor phenotypes. (D) Correlation between TERF2 expression and tumor-development score. Numeric labels indicate median values where shown, and statistical annotations reflect the reported study results.

3.3 TRF2 protein expression and nuclear accumulation

TRF2 protein expression was mostly seen as nuclear marking in living tumor cells, and the amount was measured using the nuclear H-score. There was no statistically significant difference between the organ groups in terms of the median TRF2 H-score (197.5 for liver tumors and 195.5 for stomach tumors; Mann–Whitney U = 1094.5, p =.285). While TRF2 protein levels did not clearly separate liver tumors from stomach tumors as well as TERF2 mRNA did, they did strongly correlate with factors related to tumor growth. The median H-scores for aggressive tumors were 204.0, while the median H-scores for non-aggressive tumors were 176.0 (p <.001). There was a significant increase in the TRF2 H-score with higher histological grade (p <.001) and stage or invasion group (p =.0005). Tumors that had vascular invasion had higher TRF2 H-scores than tumors that did not have vascular invasion. It was also more common for aggressive tumors to be TRF2-high—59 of 61 aggressive tumors were TRF2-high compared to 24 of 39 non-aggressive tumors (Fisher's exact p <.001). Also, tumors that were high in TRF2 had higher levels of necrosis, Ki-67, the tumor-development score, and the TRF2-pathway score.

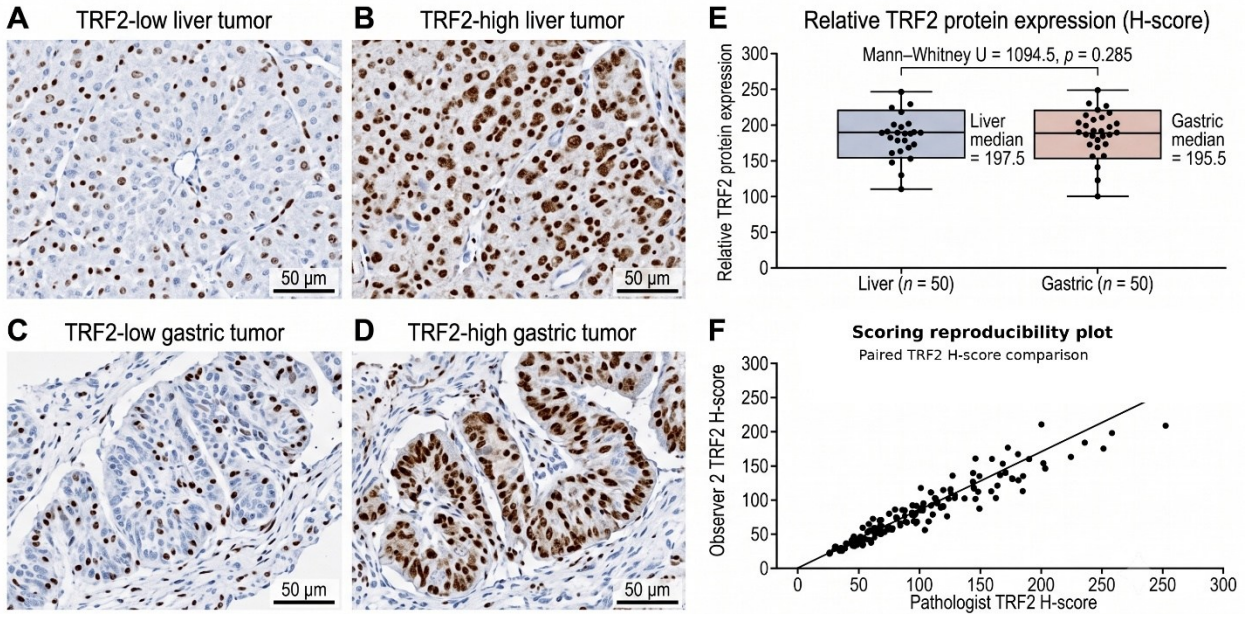


Figure 2. TRF2 protein expression by immunohistochemistry. (A) Representative TRF2-low liver tumor. (B) Representative TRF2-high liver tumor. (C) Representative TRF2-low gastric tumor. (D) Representative TRF2-high gastric tumor. (E) Quantitative comparison of nuclear TRF2 H-scores between liver and gastric tumors. (F) Paired scoring reproducibility plot for TRF2 H-score assessment. Statistical annotations reflect the reported study results.

Table 2. TRF2 protein expression and association with tumor-development variables

Comparison	Result	p value
TRF2 H-score, liver vs gastric tumors (median)	197.5 vs 195.5	.285
TRF2 H-score, aggressive vs non-aggressive tumors (median)	204.0 vs 176.0	< .001
TRF2-high classification, aggressive tumors	59/61 (96.7%)	< .001†
TRF2-high classification, non-aggressive tumors	24/39 (61.5%)	—
Association with histological grade	—	< .001
Association with stage/invasion category	—	.0005
Association with vascular invasion	Higher TRF2 H-score in invasion-positive tumors (median 202.0 vs 184.0)	< .001

3.4 Concordance between TERF2 gene activity and TRF2 protein expression

The levels of TERF2 mRNA and nuclear TRF2 protein expression were mostly similar, but not completely. The relative expression of TERF2 was linked to the H-score of TRF2 in the whole group (Spearman ρ =.588, p <.001). The strength of this link varied depending on the type of tumor. It was stronger for liver tumors (ρ =.704, p <.001) than for stomach tumors (ρ =.464, p =.0007). This trend shows that higher TERF2 gene activity usually led to higher levels of TRF2 protein, but this wasn't always the case. There were times when transcripts and proteins did not match up. This supports the idea that the amount of TERF2 mRNA present may not be the only thing that changes how TRF2 proteins behave. These include keeping proteins stable, keeping nuclei in place, the pressure inside the tumor, or feedback at the pathway level. So, looking at the proteins added biologically useful details that

gene expression alone could not fully reveal.

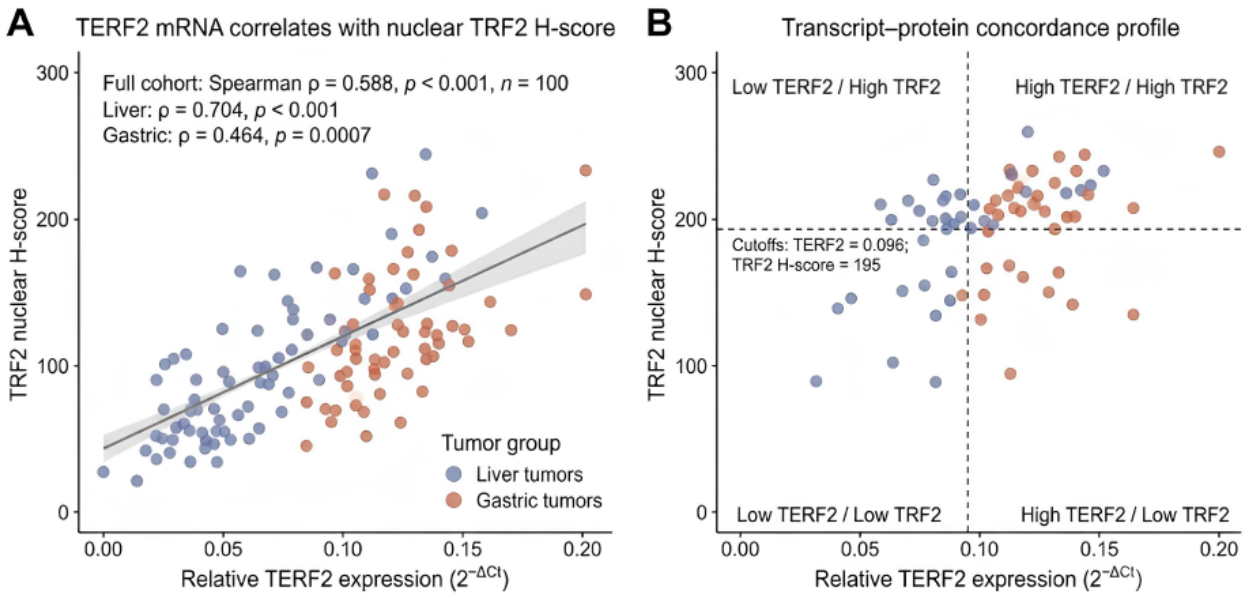


Figure 3A–B. TERF2/TRF2 concordance and pathway localization. (A) Scatter plot showing the relationship between TERF2 relative mRNA expression (2^-ΔCt) and nuclear TRF2 H-score across liver and gastric tumor samples. (B) Transcript-protein concordance plot using TERF2 mRNA and TRF2 H-score cutoffs to visualize concordant and discordant TERF2/TRF2 profiles. Statistical annotations reflect the reported study results.

3.5 TRF2 pathway behavior in tumor-development progression

The TRF2-pathway analysis showed that aggressive tumor activity was linked to changes in markers for DNA damage response and growth. When looking at median TRF2-pathway activity scores, they were 58.4 for aggressive tumors and 44.1 for non-aggressive tumors ($p < .001$). The strength of this link was greater than the difference between organ groups, since total pathway scores were the same for liver and stomach cancers. Different aggressiveness phenotypes also caused different pathway markers. It was found that aggressive tumors had higher γ-H2AX H-scores, p-ATM H-scores, and p53 H-scores than non-aggressive tumors. In aggressive tumors, the median Ki-67 level was 63.0%, while in non-aggressive tumors, it was 46.0%. The median γ-H2AX H-score was 160.0 versus 122.0, respectively. The mean p-ATM H-score for aggressive tumors was 166.0, while it was only 113.0 for non-aggressive tumors. The results show that TRF2-high aggressive tumors were not just marked by higher levels of telomere-associated proteins, but also by a wider protein pathway pattern that included DNA damage response remodeling and cell proliferation. The heatmap of standardized TRF2, γ-H2AX, p53, and Ki-67 scores helped group aggressive tumors by pathway intensity, and the TRF2-pathway activation score gave a full picture of how tumors grow.

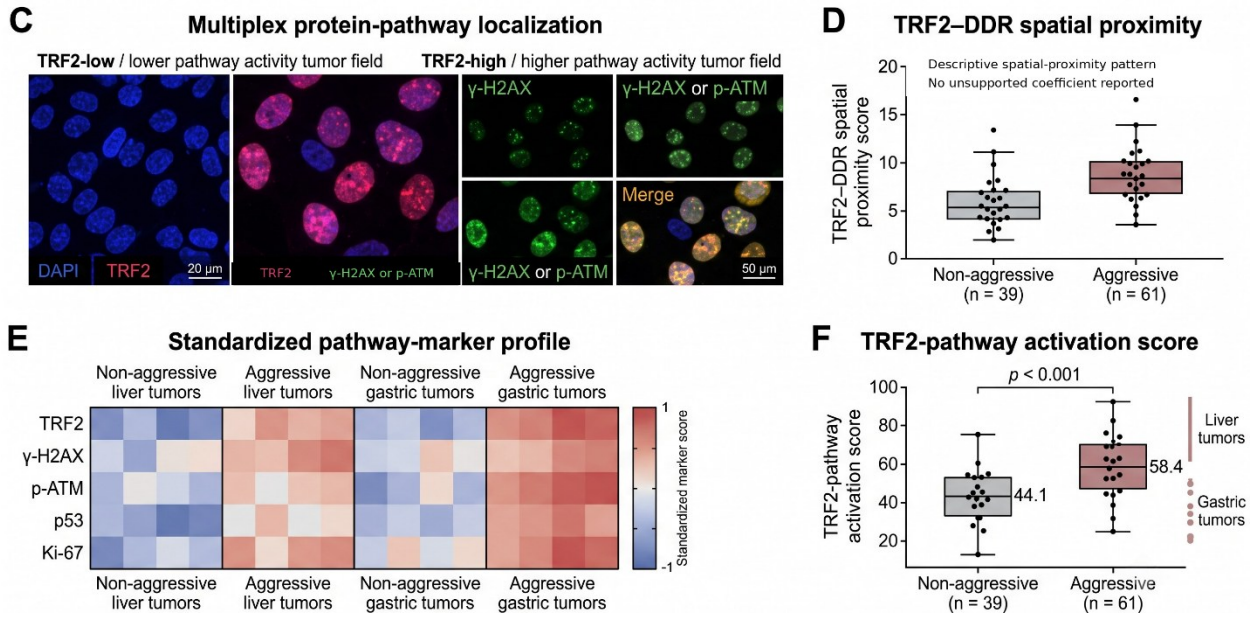


Figure 3C–F. TERF2/TRF2 concordance and protein-pathway tracking. (C) Multiplex protein-pathway localization showing TRF2 with DNA damage-response markers and nuclear counterstaining. (D) TRF2–DNA damage-response spatial proximity pattern. (E) Heatmap of standardized TRF2, γ-H2AX, p-ATM, p53, and Ki-67 scores across tumor-development groups. (F) TRF2-pathway activation score in non-aggressive and aggressive tumor phenotypes. Statistical annotations reflect the reported study results.

3.6 Diagnostic performance of TERF2/TRF2 pathway profiling

Diagnostic modeling was used to see if TERF2/TRF2 profiling could tell the difference between tissue samples that display aggressive tumor growth phenotype. AUC of 0.691 and a bootstrap 95% CI of 0.576–0.794 for TERF2 mRNA alone showed modest differentiation. Using 0.096 as the cutoff for relative TERF2 expression showed that it was 68.9% sensitive, 64.1% specific, 75.0% positive, 56.8% negative, and 67.0% overall accurate. For example, the TRF2 nuclear H-score did better, with an AUC of 0.885 and a bootstrap 95% CI of 0.811–0.948. It was 83.0% accurate, 87.2% specific, 90.7% positive predictive value, 73.9% negative predictive value, and 80.3% sensitive at an H-score cutoff of 195. Combining the TERF2/TRF2/pathway model gave the best overall tissue-based diagnostic separation, with an AUC of 0.916 and a bootstrap-corrected AUC of 0.907. The combined model's bootstrap 95% CI was between 0.867 and 0.972. The levels of sensitivity, specificity, PPV, NPV, and accuracy were all 83.0% at the chosen probability cutoff. Analysis of the calibration showed a Brier score of 0.111. These findings support the combined profile as a possible tissue-based diagnostic indicator of aggressive tumor development biology, rather than a complete clinical diagnostic test on its own.

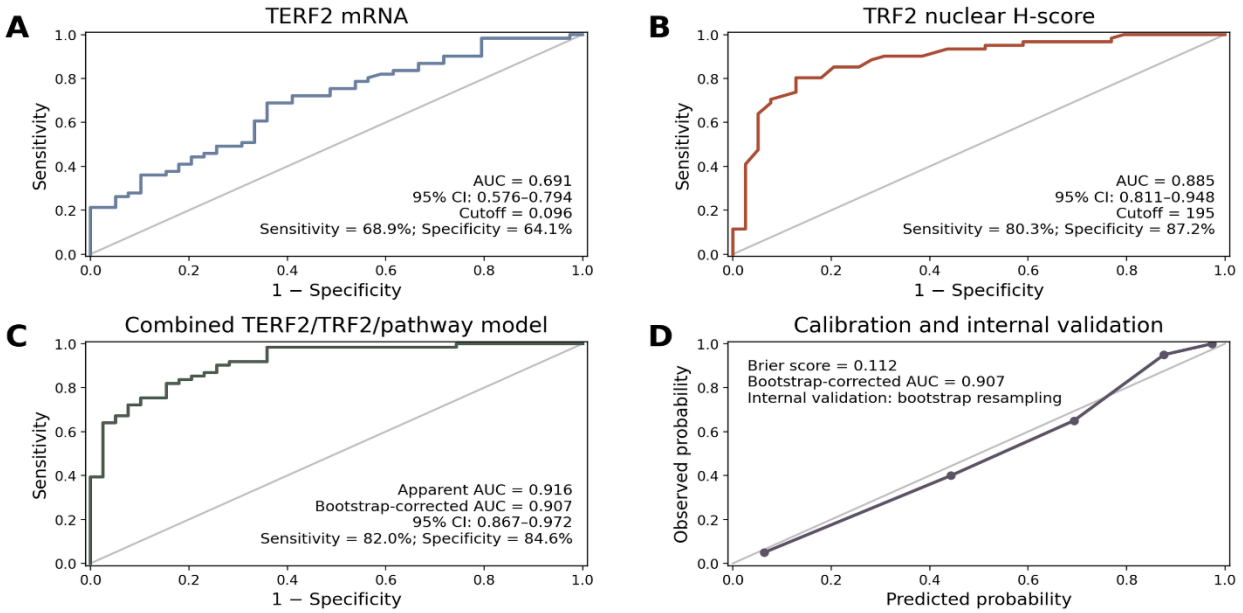


Figure 4. Diagnostic performance of TERF2/TRF2 pathway profiling. (A) Receiver operating characteristic curve for TERF2 mRNA alone. (B) Receiver operating characteristic curve for TRF2 nuclear H-score alone. (C) Receiver operating characteristic curve for the combined TERF2/TRF2/pathway model. (D) Calibration plot and internal validation summary for the combined model. Statistical annotations reflect the reported study results.

Table 3. Diagnostic performance of TERF2/TRF2 markers and combined pathway score

Marker	AUC (95% CI)	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
TERF2 mRNA ($2^{-}\Delta Ct$)	0.691 (0.576–0.794)	0.096	68.9%	64.1%	75.0%	56.8%	67.0%
TRF2 nuclear H-score	0.885 (0.811–0.948)	195	80.3%	87.2%	90.7%	73.9%	83.0%
Combined TERF2/TRF2/pathway model	0.916 apparent; 0.907 bootstrap-corrected (0.867–0.972)	Model-derived probability threshold	82.0%	84.6%	89.3%	75.0%	83.0%

3.7 Robustness and sensitivity analyses

TERF2 mRNA was still linked to an aggressive tumor type when age, sex, and tumor type were taken into account in multivariable logistic regression (95% CI, 1.47–4.61; p =.001). The chances ratio increased by 2.60 for every standard deviation. With an odds ratio of 8.31 for every standard deviation increase (95% CI, 3.29–20.97; p <.001), the TRF2 H-score showed a stronger adjusted association. The pathway score was the most important independent marker in the combined model (OR = 11.89; 95% CI, 2.91–48.64; p <.001). After pathway integration, TERF2 mRNA and TRF2 H-score were weakened.

Table 4. Multivariable logistic regression for aggressive tumor phenotype

Model / Variable	Adjusted OR (per SD increase)	95% CI	p value
TERF2 mRNA, modeled alone (adjusted for age, sex, tumor group)	2.60	1.47–4.61	.001
TRF2 H-score, modeled alone (adjusted for age, sex, tumor group)	8.31	3.29–20.97	< .001
Combined model — TRF2-pathway score	11.89	2.91–48.64	< .001

4. Discussion

4.1 Principal findings

The results of this study show how the TERF2 gene works, how much TRF2 protein is present, and how the TRF2-related pathways behave in separate liver and stomach tumors. The main finding is that TERF2 transcriptional activity was different between the two tumor groups. Gastric tumors had higher median TERF2 expression than liver tumors. However, the amount of nuclear TRF2 protein was higher in both groups and did not show the same level of compartmentalization between organs. You shouldn't use TERF2 mRNA and TRF2 protein as interchangeable biomarker readouts based on this transcript-protein pattern. Findings showed that TRF2 protein production was more closely connected to some factors that determine how quickly a tumor grows than TERF2 mRNA alone. These included grade, invasion-related variables, necrosis, proliferation activity, and the general trait of aggressiveness. Researchers found a positive but not perfect link between TERF2 mRNA and TRF2 H-score. Also, this shows that regulation likely happens after transcription and depends on the tissue being studied, which supports the idea that there is a biologically plausible link between the two. Importantly, the TERF2/TRF2/pathway model was better at telling the difference between aggressive tumor types based on DNA than either TERF2 mRNA or TRF2 protein by itself. Overall, these results support the idea that profiling genes, proteins, and pathways together might produce a more useful candidate biomarker signature than measuring only one level. But these results aren't enough to prove TRF2 as a clinical diagnostic test on their own; more study needs to be done to confirm them.

4.2 Biological interpretation of TERF2/TRF2 dysregulation

The molecular meaning of these results comes from how important TRF2 is for keeping chromosome ends safe. The TRF2 gene encodes a shelterin protein that stops ATM-dependent DNA damage signaling from going off at chromosome ends. This keeps telomeres from being mistaken for DNA double-strand breaks. TRF2 helps keep chromosomes whole during repeated cell division by supporting telomere design and lowering the damage signals that telomeres send. On the other hand, these functions that keep cancer from spreading may become less effective because tumor cells that are facing a lot of replication stress and genomic instability may benefit from systems that help them keep their ability to divide [2], [3].

This model works for the link between high amounts of TRF2 protein and fast tumor growth. It's possible that tumors can handle chromosomal stress, avoid telomere crisis, and keep the cell cycle going better when they have a lot of nuclear TRF2. Furthermore, research has shown that stopping TRF2 can lead to telomere problems, senescence, ferroptosis, autophagic death, and apoptosis in cancer cells [8]. Because of this, higher levels of TRF2 in aggressive tumors might not just mean that the protein is overexpressed; they could be a sign of an adaptive telomere-protection state that helps cancer cells stay alive when genomic pressure is put on them.

With the results of the route markers, this view is even stronger. There may be a link between protecting telomeres and faster cell growth in tumors that are high in TRF2 and high in Ki-67. Some cancers that have changing γ-H2AX or p-ATM patterns and are high in TRF2 may show how telomere preservation

and DNA damage-response modification work together. As you try to figure out the direction of this link, you should be careful because global DNA damage markers show both telomeric and non-telomeric stress. Changes in the tumor-suppressor pathway activity may happen along with telomere-protective adaptation in cancers that are high in TRF2 and have abnormal p53 expression. Additionally, this supports the idea that TRF2 is not only a structural telomere protein but also a part of a larger plan that helps tumors grow. We think that extra-telomeric TRF2 activity may also play a bigger part in this. This includes managing relationships with G-quadruplex-containing promoter regions and cancer regulatory networks [12], [13], [14].

4.3 Organ-specific comparison between liver and gastric tumors

Because liver and stomach tumors are not the same, TERF2/TRF2 biology may not work as a single signal for all cancers. The levels of TERF2 mRNA expression were higher in stomach tumors, while the levels of TRF2 nuclear protein expression were higher in both types of tumors. It's possible that the setting of the organ has a bigger effect on transcriptional activity than on protein buildup. It's also possible that factors other than the amount of mRNA present control the stability and survival of TRF2 proteins in the nucleus. Newly found information from both pan-cancer and gastric cancer shows how TERF2 instability changes the way gastric tumors grow and behave. Ma [18], for instance, found that the functional effects of TERF2 knockdown in models of stomach cancer are important. Adding to this idea, the new tissue-based study shows that stomach tumors may have a larger TERF2 signal, while strong trait differentiation depends more on protein and pathway-level readouts.

It's possible that metabolic stress, oxidative injury, vascular invasion, replication pressure, and chromosomal instability in hepatocellular carcinoma will change how TRF2 is used in liver tumors. Scientists Cao [16] and Qiu [17] say that hepatocellular carcinoma biology is connected to control of telomeres, and different types of liver cancer cells have been treated with TRF2-targeting methods. People who had chronic hepatitis, cirrhosis, diseases related to alcohol or drugs, or exposure to hepatotoxins were not included in this group of people with tumors. This was done so that people with chronic liver diseases would not change the results. This is a little more proof that the TERF2/TRF2 patterns seen are linked to the biology of the tumor, but it also means that the results are less useful for most liver cancer patients because many of them get tumors in livers that are already badly damaged.

TERF2/TRF2 activity in stomach tumors may show how stresses change glandular epithelial cells, the replacement of mucosa, the depth of invasion, DNA damage signals, and the remodeling of the local microenvironment. This means that TERF2/TRF2 might not only be a general cancer marker; it might also help in diagnosing cancer depending on the type of tissue, the way the tumor is built, and the main way that genetic instability happens.

4.4 Diagnostic value of combined gene-protein-pathway profiling

This study adds to the toolbox of diagnostics by measuring the TERF2 transcript. Finding the differences between those situations was easy when TERF2 mRNA, TRF2 nuclear protein levels, and pathway marker activity were all looked at together. A biologically coherent multimodal signature may work better than a single analyte in molecular pathology biomarker logic because it shows where cells are, how proteins are translated or stabilized, and how functional pathways are activated. Researchers discovered that TERF2 mRNA wasn't very good at telling the difference between types of violent tumor growth. More accurately, the TERF2/TRF2/pathway model told the difference better than the TRF2 H-score alone.

These results suggest that measuring proteins and pathways is important to understand what TERF2 activity means for function. Nuclear TRF2 labeling is a test that finds a lot of proteins in a portion of tumor cells that is important for telomere and chromatin biology. TRF2 expression is linked to visible tumor activity by adding markers for DNA damage response and growth. The overall number is not a screening test or a guaranteed way to identify a tumor, but it can help you figure out how a tumor is growing based on tissue.

Diagnostic results should be carefully interpreted by medical professionals. Although AUC, sensitivity, specificity, positive predictive value, negative predictive value, calibration, and bootstrap-corrected performance are all useful pieces of internal data, they do not show clinical usefulness by themselves. Individual internal validation lowers people's optimism, but it doesn't take the place of independent external validation. Testing the model on larger groups of people with a variety of tumors, matching

non-tumor tissues, and a mix of tumor subtypes is needed for it to really be useful in therapeutic settings. Knowing this, the current findings should be seen as proof of a possible biomarker signature that needs more research.

4.5 Strengths

Rather than just using cell lines or computer simulations, real human tumor material was used. Next, using the same way of research, it directly compared cancers in the stomach and liver. That allowed researchers to see TERF2/TRF2 patterns that are unique to every organ. Third, it checked both TERF2 mRNA and TRF2 protein, which improved our understanding of signals that are only transcripts. Fourth, using nuclear TRF2 scores, masked clinical assessment, and pathway-level markers helped scientists grasp the issue at the molecular level more clearly. In addition to finding changes in expression, the study also connected genetic results to factors that were already known to affect tumor growth and aggression. There were also fewer biological and technical issues that could have led to problems by not including people who had previously been treated for cancer, had recurrent or metastatic tumors, chronic disease-associated tumors, a history of drug abuse, or had poor-quality tissue.

4.6 Implications and future validation

The TERF2/TRF2 route marker needs to be tested in future with bigger groups of people from more than one center. For these groups, there should be standard ways to process tissue, matched samples of tumor and nearby healthy tissue, checks for other diseases that aren't cancer, and follow-up that is based on survival. Different sets of data for liver and stomach cancer could be used to check for repeatability, and functional models could show if TRF2 directly impacts the aggressive pathway or displays how the tumor responds to excessive stress. Add tests of liquid biopsy, moving tumor material, or exosomal biomarkers to see if TERF2/TRF2-related signals can be used for things other than tissue disease. It's important to keep in mind that the signature is still just a suggestion and not a fully tested way to diagnose anything.

5. Conclusion

Screening for both TERF2 and TRF2 together is a good way to see how liver and stomach cancers grow from a biological point of view. The TERF2 gene activity was different in different organs. However, the levels of the nuclear TRF2 protein and the actions of pathways connected to TRF2 were more closely linked to violent clinical traits. These findings back up the idea that studying proteins and pathways might help with diagnosis more than just measuring TERF2 mRNA. The reason for this is that TRF2's function is based on the number of nuclear proteins that are present, how well they protect telomeres, how they work with DNA damage responses, and the situation of the cell proliferation pathway. It was found that the TERF2/TRF2/pathway signature worked better than a single marker evaluation to tell the difference between aggressive tumor-development phenotypes in tissue samples. It seems like it could be useful as a biomarker profile in molecular pathology because of this. The results should not be seen as proof that TRF2 can be used as a medical test on its own, though. It is important to be careful about how this study is understood because it only looked at tumors, had a small sample size, wasn't checked by anyone else, and didn't include any follow-up data on life. Larger international studies with matched local tissue, disease-free controls, independent evaluation groups, and outcome data need to be done before it can be used in real life. In general, studying the TERF2/TRF2 pathway might help in figuring out the biology of aggressive tumors, but this needs to be tested fully in the real world.

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