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RESEARCH ARTICLE

STAT3, but not Ki-67, expression is independently associated with tumor progression in colorectal carcinoma: A comparative immunohistochemical biomarker analysis

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Abstract

The insidious progression of colorectal carcinoma (CRC) at early stages often hampers timely screening and diagnosis, resulting in delayed intervention and poor clinical outcomes. Ki-67 and Signal Transducer and Activator of Transcription 3 (STAT3) have been widely studied for their roles in tumor proliferation and survival. However, comparative studies assessing their clinicopathological relevance in CRC progression remain limited, and their relative contributions to tumor aggressiveness are unclear. This study aimed to evaluate the clinicopathological significance and association of Ki-67 and STAT3 expression with tumor aggressiveness, focusing on their roles in tumor infiltration and size as indicators of CRC progression. Thirty-eight formalin-fixed, paraffin-embedded (FFPE) CRC tissue specimens were collected from patients undergoing surgical resection for this cross-sectional study. Ki-67 and STAT3 expression were assessed using immunohistochemical (IHC) staining. Clinicopathological data were retrieved from medical records. Protein expression was quantified using inverted mean gray values via digital image analysis. Ki-67 and STAT3 expression levels significantly correlated with tumor infiltration (Ki-67: $r = 0.322$, $p = 0.049$; STAT3: $r = 0.429$, $p = 0.007$) and size (Ki-67: $r = 0.425$, $p = 0.008$; STAT3: $r = 0.452$, $p = 0.004$). No significant correlation was found between Ki-67 and STAT3 ($r = 0.225$, $p = 0.175$). Only STAT3 was independently associated with infiltration (OR per 1-standard deviation: 4.11, $p = 0.043$) and size (OR per 1-SD: 3.41, $p = 0.047$). While both markers are associated with CRC progression, only STAT3 shows an independent association with tumor aggressiveness, underscoring its role beyond cell proliferation and suggesting broader involvement in tumor invasion and progression pathways.

1. INTRODUCTION

Colorectal carcinoma (CRC) represents a significant global health burden, ranking third in terms of incidence and second in mortality among all cancers worldwide, with approximately 2.17 million new cases and 1.09 million deaths reported annually as of 2019 (1). The global incidence of CRC is projected to increase by approximately 63% by 2040 compared to 2020 (2). The incidence of CRC is markedly higher in males compared to females, and geographical differences suggest that lifestyle, environmental, and genetic factors are key contributors to its development (3,4). CRC pathogenesis typically progresses from adenomatous polyps through a series of genetic and epigenetic alterations that drive dysplasia and malignant transformation (5,6). While conventional treatment modalities—including surgery, chemotherapy, and

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radiotherapy—remain the cornerstone of therapy, recurrence and resistance underscore the need for improved prognostic and predictive biomarkers (5,7). Recently, the exploration of molecular markers involved in cell proliferation, immune modulation, and inflammation has gained attention to enhance early detection, prognostication, and individualized therapy (8,9).

Among the molecular biomarkers studied, Ki-67 and Signal Transducer and Activator of Transcription 3 (STAT3) are of particular interest due to their involvement in tumor biology. Ki-67 is a nuclear protein associated with cell proliferation, widely used to assess tumor aggressiveness across various malignancies, including CRC (10,11). Elevated Ki-67 expression has been correlated with increased tumor grade, metastasis, and poor therapeutic response in CRC patients, although the findings remain heterogeneous (10,12). Conversely, STAT3 is a transcription factor activated by inflammatory cytokines, such as IL-6, which regulates genes involved in proliferation, angiogenesis, and anti-apoptotic mechanisms (7,13). Constitutive activation of STAT3 has been observed in CRC tissues, particularly in cancer stem-like cells and at advanced stages of tumor progression, suggesting its role in promoting tumor growth and therapy resistance (3,8,14).

Despite their overlapping functions in tumorigenesis, comparative studies evaluating Ki-67 and STAT3 simultaneously within the same patient cohort are scarce. Moreover, the relative contribution of each marker to tumor progression and whether they act as independent predictors remains poorly defined (7,8,13). Clarifying this could have significant implications for patient stratification, prognostication, and selecting patients who might benefit from targeted therapies against the STAT3 pathway.

Therefore, this study aimed to compare Ki-67 and STAT3 expression in CRC tissues directly to evaluate their independent associations with clinicopathological indicators of tumor aggressiveness. By performing a comparative immunohistochemical analysis, we sought to determine whether these biomarkers function as independent indicators of tumor progression, thereby addressing a critical gap in CRC biomarker research.

2. MATERIALS AND METHODS

2.1. Study Design and Sample Collection

This cross-sectional observational study enrolled 38 patients with primary non-mucinous colorectal adenocarcinoma who underwent surgical resection at Dr. Soetomo General Academic Hospital, Surabaya, between 2021 and 2022. The required sample size was calculated using an a priori analysis (linear multiple regression: fixed model, single regression coefficient) in G*Power 3.1 software (Düsseldorf, Germany) with an effect size $f^2 = 0.3$, $\alpha = 0.05$, and a target power of 0.9. The inclusion and exclusion criteria have been presented earlier (4). Briefly, the patients should not have received neoadjuvant chemotherapy and should not have been diagnosed with colon mucinous adenocarcinoma. Formalin-fixed paraffin-embedded (FFPE) CRC specimens that met the inclusion criteria were identified and randomized using a computer-generated random number list to minimize selection bias before further analysis. Tumor differentiation was graded according to the World Health Organization (WHO) 2010 criteria. Tumor infiltration was determined according to the 8th edition of the American Joint Committee on Cancer (AJCC). This study protocol was approved by the Ethical Review Committees of Dr. Soetomo General Hospital, Surabaya (Reference No. 0461/LOE/301.4/V/2021).

2.2. Slide Preparation and IHC Staining

Each FFPE specimen was sliced into 5 µm-thick sections and stained with hematoxylin and eosin. Following deparaffinization and rehydration, the sections were microwaved for antigen retrieval using citrate buffer (pH 6.0) at 750 W for 10 minutes, followed by 350 W for 15 minutes. After 20 minutes of cooling at room temperature, the sections were treated with hydrogen peroxide for 10-15 minutes, followed by two washes with buffer. Then, the sections were incubated with anti-Ki-67 monoclonal antibody (1:500, Clone 3B7, Cat. No. AN005400L, Elabscience, Texas, USA) or anti-STAT3 monoclonal antibody (1:150, Clone 4E3, Cat. No. E-AB-22119, Elabscience, Texas, USA) for 60 minutes at room temperature. Subsequently, the slides were treated with biotinylated goat anti-polyvalent secondary antibody (Cat. No. E-AB-1097, Elabscience, Texas, USA) for 10 minutes, followed by streptavidin-horseradish peroxidase (HRP) (Cat. No. 434323, Thermo Fisher Scientific, Waltham, USA) for an additional 10 minutes. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) substrate solution (40 µL chromogen in 2 mL substrate) for 15 minutes, counterstained with hematoxylin, and meticulously evaluated under light microscopy in ten randomly selected high-power fields (400×) by two independent pathologists.

2.3. Digital IHC Image Analysis

Protein expressions of Ki-67 and STAT3 were visualized using a light microscope (Olympus CX31; Olympus, Tokyo, Japan) equipped with a digital camera (Olympus DP21) at a fixed optical magnification of 400× (field area: 0.0876 mm^2 per high-power field [HPF]). For each slide, five randomly non-overlapping HPFs were systematically selected. IHC slides were assessed and captured by two board-certified pathologists, regardless of tumor status, using a predefined grid-based scanning pattern. IHC-stained slide images were analyzed using FIJI (ImageJ) image processing software (version 1.54f; NIH, Bethesda, MD, USA). Protein expression was measured semi-quantitatively using the inverted mean gray value to

represent relative intensity (arbitrary unit) as previously described (15). In brief, deconvoluted DAB staining images were chosen, binarized using automatic thresholding, and inverted to obtain black and white images. These preprocessed images were then measured as mean gray value (scale 0-255). No specific size or circularity filters were applied, as the analysis aimed to capture overall staining intensity rather than individual cellular structures. Although this approach may include minimal artifacts, consistent thresholding across all groups minimized bias, and the uniform application of the method preserved comparability of intensity measurements. The image size analyzed was 1600 × 1200 pixels. In addition to mean gray value (FIJI/ImageJ), we scored nuclear staining using an H-score on pathologist-annotated ROIs at the invasive front; agreement between metrics and inter-observer variability were assessed.

2.4. Statistical Analysis

Data are presented as the mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism (version 9.5.0; San Diego, CA, USA). Clinicopathological variables were dichotomized to facilitate statistical analysis and interpretation. Age was split at 55 years to balance the distribution of samples, while other markers—including tumor infiltration depth (pT1–2 vs. pT3–4), tumor size (<5 cm vs. ≥5 cm), tumor differentiation, and lymph node status—followed thresholds commonly used in prior colorectal cancer studies (16–18). These categorizations enable comparability with existing literature, ensure sufficient group sizes for analysis, and facilitate a meaningful interpretation of associations with biomarker expression. An independent two-tailed t-test was performed to compare these two groups. Univariate correlation analysis was performed using Pearson's correlation analysis. Multivariate correlations were analyzed using point-biserial correlation analysis and visualized as a heatmap generated with ChiPlot (https://www.chiplot.online/). Univariate and multivariate logistic regression were conducted to assess the independent association of Ki-67 and STAT3 with tumor progression. Both biomarkers were reciprocally adjusted (each biomarker was analyzed as the primary independent variable while controlling for the other) with tumor infiltration and size as dependent variables. Multicollinearity was assessed by the variance inflation factor (VIF), with all values <5 indicating acceptable collinearity. Events per variable (EPV) was also calculated to evaluate the stability of the multivariate regression analysis. The Odds ratio (OR) of from the multivariate analysis was presented as per a 1-SD increment (19). Data is considered statistically significant if the p-value < 0.05.

3. RESULTS AND DISCUSSION

STAT3 and Ki-67, two biomarkers extensively studied for CRC, are believed to be involved in tumor proliferation and progression. STAT3 regulates oncogenic signaling by inflammatory mechanisms, cell survival, and angiogenesis, while Ki-67 is a nuclear protein associated with cell proliferation (10,20). The prognostic significance of these markers in relation to tumor behavior remains a topic of debata. In our study, we found that STAT3 expression is independently associated with deeper tumor invasion and larger tumor size in CRC patients, while Ki-67 expression was not significantly associated with either of these tumor progression parameters. These findings suggest that STAT3 may play a more direct role in the molecular processes of tumor progression, likely through chronic inflammation and epithelial-mesenchymal transition (EMT), rather than simply promoting proliferative activity (21). The disparity in prognostic value between STAT3 and Ki-67 underscores the need to understand the functional role of these markers beyond proliferation in evaluation of biomarkers for colorectal cancer, which could have significant implications for the diagnosis and treatment of this disease.

3.1. Clinicopathologic Features of The Study Participants

During the study, 64 CRC patients underwent colon resection at Dr. Soetomo General Academic Hospital. Among them, 38 samples met the inclusion criteria and were included in the study. The clinicopathological features of the patients are summarized in Table 1. In this study, there was no specific gender dominance in CRC cases, with a sex ratio (male: female) of 1:1. The age distribution shows that patients aged 50-64 years had the highest CRC incidence (44.7%); the mean age was 54.84 ± 12.51 years old, with females (53,47 ± 11.51) generally comparable with males (56.21 ± 13.62, p = 0.507).

CRC incidence in this analysis showed no significant gender predominance, with a ratio of 1:1 between females and males, as also reported in our previous study (4). This contrasts with some previous references to a slight predominance of males among CRC cases, which may reflect population and lifestyle-specific determinants (22). Distribution by age showed that the majority of CRC cases occurred in individuals aged 50–64. There was no significant age difference between male and female patients, suggesting that disease onset or diagnosis occurs at a similar age across sexes. This finding differs from a previous study involving 185,967 patients in Germany, where females were older than males at the time of diagnosis (23). Nevertheless, acknowledging potential sex-related biological or lifestyle factors remains important for guiding future early detection and screening strategies (24).

Table 1. Baseline clinicopathological features of the study participants.

Characteristics	Value
Gender, n (%)	
Male	19 (50)
Female	19 (50)
Age (year), n (%)	
0-49	12 (31.6)
50-64	17 (44.7)
65+	9 (23.7)
Tumor differentiation, n (%)	
Well-differentiated	19 (50)
Moderately differentiated	16 (42.1)
Poorly differentiated	3 (7.9)
Tumor infiltration, n (%)	
pT1 (limited to the submucosa)	1 (2.6)
pT2 (infiltrate muscularis propria)	8 (21.1)
pT3 (infiltrate muscularis propria into pericolorectal tissues)	22 (57.9)
pT4 (infiltrate visceral peritoneum or other organs)	7 (18.4)
Nodal involvement, n (%)	
pN0 (no regional lymph node metastases)	18 (47.4)
pN1 (metastases in 1-3 regional lymph nodes)	12 (31.5)
pN2 (metastases in >3 regional lymph nodes)	8 (21.1)
Tumor size, n (%)	
0-3 cm	3 (7.9)
3-5 cm	11 (28.9)
>5 cm	24 (63.2)
Angioinvasion, n (%)	
Negative	29 (76.3)
Positive	9 (23.7)
Tumor morphology, n (%)	
Polypoid	30 (78.9)
Non-polypoid	8 (21.1)
Expression as relative intensity, mean ± SD	
Ki-67	139.2 ± 21.57
STAT3	154.0 ± 19.76

pT, pathological tumor infiltration; pN, pathological nodal involvement; SD, standard deviation.

Pathological examination of tumor differentiation (grade) shows that 50% of the patients were well-differentiated, while only 7.5% were poorly differentiated. The majority of patients presented with tumors that had extended into the peri colorectal tissues, classified as pT3 (57.9%). This deep infiltration was accompanied by nodal metastases (pN1–pN2, 52.6%) and larger tumor size (>5 cm, 63.2%). Interestingly, angioinvasion is present in only 23.7% of the samples, and non-polypoid tumors are found in 21.1%. Ki-67 IHC staining shows a relative intensity of 139.2 ± 21.57, while the STAT3 of 154.0 ± 19.76 mean gray value (MGV). Representative IHC staining of Ki-67 and STAT3 is shown in Figure 1A.

3.2. Ki-67 and STAT3 Measurements Show High Consistency, but Notable Discrepancies

Our study employed a rigorous methodology. Two blinded observers evaluated Ki-67 and STAT3 IHC expression, and inter-observer variation was assessed using the intraclass correlation coefficient (ICC). Both markers demonstrated high ICC values (0.929 and 0.802, $p < 0.001$, respectively), indicating very good agreement in ranking samples (Table 2). To further explore systematic bias, Bland–Altman plots were generated (Figure 2). For Ki-67, the mean bias was 1.52 with 95% limits of agreement ranging from –14.67 to +17.72, while for STAT3, the mean bias was –4.78 with limits of agreement from –29.18 to 19.62. These wide limits of agreement highlight that, although observers were consistent in relative ranking (as reflected in the ICC), their absolute quantification of individual samples could differ considerably. This distinction is crucial as the high ICC supports reliability in group-level comparisons, but wide Bland–Altman limits indicate caution when interpreting absolute expression levels for a single case.

While our study revealed consistent overall measurements, individual sample assessments showed considerable variability (Figure 2), which may be attributed to differences in digital image thresholding or subjective interpretation during quantification (25). We chose mean gray value (MGV) analysis using FIJI/ImageJ because it provides a continuous, objective measure of staining intensity across the entire tissue section, avoiding the categorical limitations of traditional scoring systems such as H-score or percentage of positive nuclei. To further reduce inter-slide variability, color deconvolution and background normalization were applied prior to analysis, ensuring that differences in staining intensity actual true biological variation rather than technical artifacts. Nonetheless, some degree of variability remains inevitable

given the heterogeneous nature of tumor tissue and staining artifacts. Therefore, while IHC quantification using MGV is a valuable tool for assessing biomarker expression, our findings are most reliable when interpreted in a comparative or group-based context rather than as absolute measures in individual samples. The potential impact of our findings on future research is significant, inspiring further exploration and refinement of IHC quantification methods.

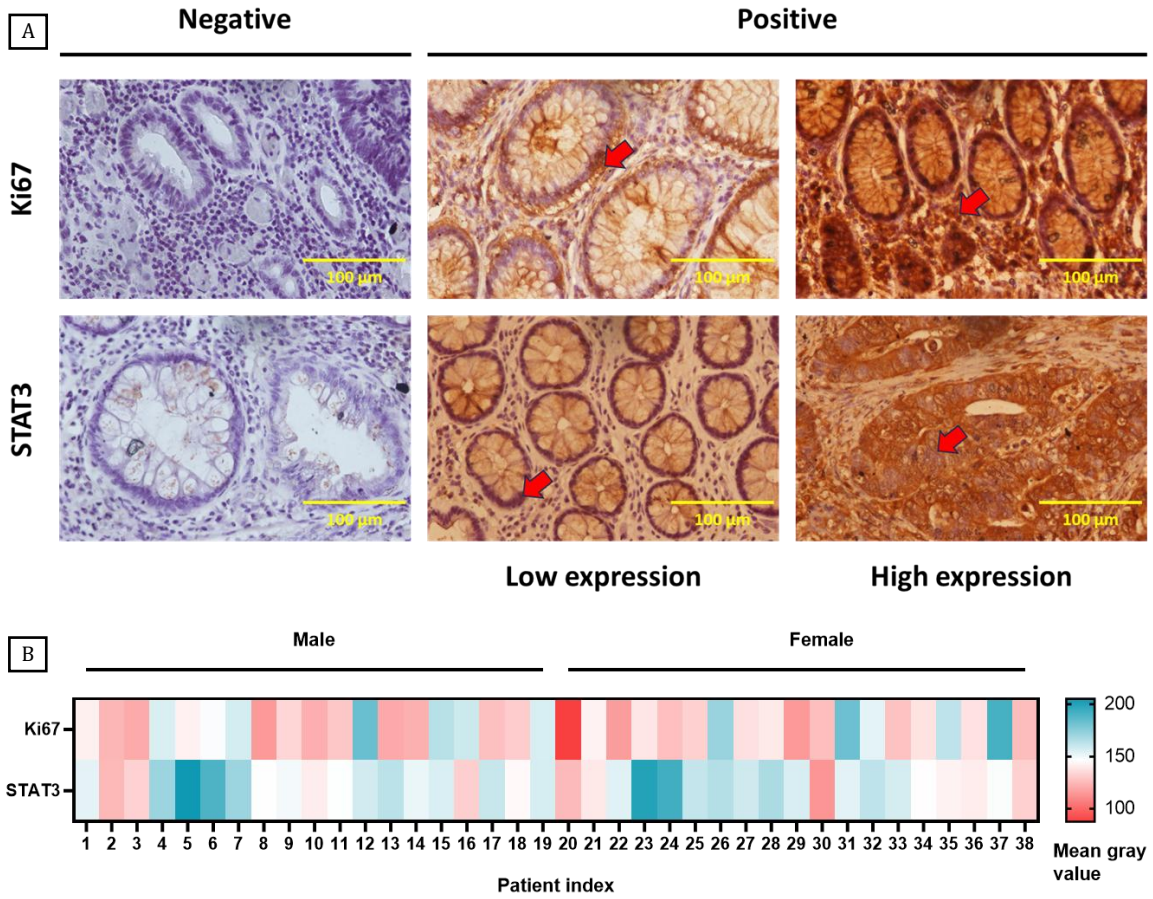

Figure 1. Ki-67 and STAT3 IHC expression. (A) Representative images of immunohistology Ki-67 and STAT3 expression (400× magnification) and (B) gender-based expression pattern heatmap of Ki-67 and STAT3 in CRC. The mean gray value is based on the average of two observers. The red arrow represents positively stained cells.

Table 2. Inter-observer reproducibility of Ki-67 and STAT3 protein expression.

Marker	ICC	95% CI	*p*-value
Ki-67	0.929	0.868–0.962	<0.001*
STAT3	0.802	0.636–0.895	<0.001*

*Statistically significant ($p < 0.05$). ICC, intraclass correlation coefficient (two-way random effects model, with absolute agreement); 95% CI, 95% confidence interval; p-value, level of significance.

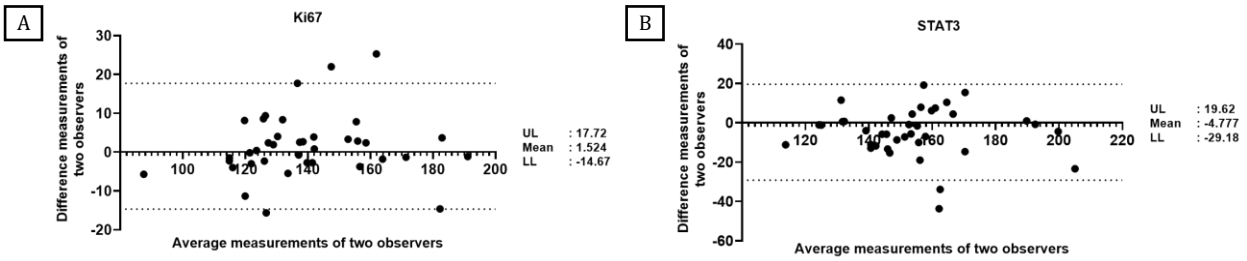

Figure 2. Bland-Altman plot analysis comparing for measurements of immunohistology expression between two observers. (A) Ki-67 expression analysis and (B) STAT3 expression analysis. UL, upper 95% limits of agreement; LL, lower 95% limits of agreement

3.3. Expression of Ki-67 and STAT3 in Relation to Tumor Infiltration and Tumor Sizes

The expression pattern of Ki-67, a protein associated with cell proliferation, and STAT3, a transcription factor involved in various cellular processes, was evaluated and compared among the dichotomized clinicopathological groups. The graphical heatmap of Ki-67 and STAT3 expression shows relative intensity in the mean gray value of each CRC sample (Figure 1B). Both markers were significantly higher in advanced tumor infiltration (pT3–T4 vs. pT1–T2; Ki-67: p = 0.021, STAT3: p = 0.008) and in larger tumor size (>5 cm vs. ≤5 cm; Ki-67: p = 0.017, STAT3: p = 0.006), but no significant differences were observed with respect to age, sex, tumor differentiation, morphology, nodal status, or angioinvasion (all p > 0.05, Figure 3). Correlation analysis revealed that Ki-67 and STAT3 were individually associated with tumor infiltration and size. However, they were not correlated with each other (r = 0.224, p = 0.175), suggesting distinct biological pathways (Figure 4). In multivariable logistic regression, the associations of Ki-67 with tumor infiltration (OR 1.84, 95% CI (0.71–4.78, p = 0.270, EPV = 14.5) and tumor size (OR 2.66, 95% CI 0.93–7.31, p = 0.068, EPV = 12) were no longer significant after adjustment for STAT3 expression, whereas STAT3 remained independently associated with both tumor infiltration (OR 4.11 , 95% CI 1.28–13.29, p = 0.043, EPV 14.5) and tumor size (OR 3.65, 95% CI 1.06–12.52, p = 0.040, EPV = 12) (Table 3). Taken together, these findings indicate that only STAT3 retains independent significance, underscoring its role as a more robust biomarker of tumor aggressiveness in colorectal carcinoma.

Our study demonstrates that both Ki-67 and STAT3 are upregulated in larger colorectal tumors and in those with deeper invasion (Figure 3D), supporting their role as biomarkers of tumor aggressiveness (9). Ki-67 expression was significantly higher in tumors with deep infiltration compared to superficial infiltration, indicating greater proliferative activity in more invasive tumors (10). Similarly, STAT3 was markedly expressed in deep invasion, consistent with its role in tumor progression through inflammatory and pro-survival signaling pathways (8). Larger tumor size (>5 cm) also showed enhanced expression of both Ki-67 and STAT3 (Figure 3H), underscoring their association with the biological aggressiveness of CRC (17,20). These findings suggest that Ki-67 and STAT3 are closely linked to tumor progression parameters than the other clinicopathological parameters, highlighting the potential of these biomarkers in the diagnosis and treatment of colorectal cancer.

Although Ki-67 and STAT3 are both implicated in tumor progression, no significant correlation was found between their levels of expression in our study (Figure 4A), suggesting that both markers may represent distinct biological pathways in CRC. There is minimal study directly assessing the correlation between Ki-67 and STAT3 correlation in CRC. However, Ki-67 and STAT3 have been positively correlated with each other in gastric cancer and cervical intraepithelial neoplasia (CIN) (26–28). Ki-67 and STAT3 exhibited an identical pattern of correlation with clinicopathological indices of tumor progression, namely, tumor invasion and size (Figure 4B). Such parallelism indicates a potential convergence in downstream effects with divergent upstream regulation (29). Importantly, multivariate logistic regression analysis revealed that STAT3 still preserved its independent association with tumor infiltration and size after adjustment for Ki-67 expression. In contrast, the association of Ki-67 with these indices became nonsignificant after inclusion of STAT3 in the model. This finding suggests that STAT3 makes a stronger and more stable contribution to tumor progression prediction compared to Ki-67, supporting its potential as a better biomarker in CRC. The lack of collinearity between the two markers also supports the hypothesis that STAT3 can mediate invasive and proliferative activity through mechanisms beyond mere cell proliferation, including possibly inflammatory signaling and microenvironmental regulation, potentially through the IL-6/JAK2/STAT3 pathway (16,30,31).

Despite its association with tumor size and invasion, Ki-67 did not emerge as an independent predictor of progression in our multivariable analysis. This result is increasingly consistent with recent insights that Ki-67 is not indispensable for cell proliferation. Instead, it plays a role in nuclear organization, particularly in maintaining the peri chromosomal layer and interphase heterochromatin (32). Rather than acting as a driver of proliferation-linked malignant behavior, Ki-67 appears to function as a structural scaffold whose intrinsically disordered domains and charge distribution facilitate extensive protein–protein interactions and potentially phase-separated nuclear compartments (33). Within this framework, Ki-67 serves more as a cellular marker of cycling activity than as an effector of invasion, angiogenesis, or immune escape. This paradigm shift provides a compelling explanation for why elevated Ki-67 expression, although reflecting high proliferative activity, does not independently capture the complexity of colorectal cancer progression. Methodologically, it is also possible that our use of whole-tumor mean gray values in IHC quantification may have underestimated focal proliferative activity at invasive fronts. However, this appears secondary to broader biological considerations.

In contrast, STAT3 retained its significance as an independent factor, underscoring its multifaceted role in colorectal cancer progression that extends beyond proliferation. STAT3 activation primarily occurs through cytokine-mediated JAK signaling, particularly via IL-6, which induces phosphorylation at Tyr705, dimerization, and nuclear translocation of the active p-STAT3 form. In the nucleus, p-STAT3 regulates transcriptional programs that promote epithelial–mesenchymal transition (EMT), tumor cell migration, invasion, angiogenesis, and immune evasion. Typically constrained by negative regulators such as SOCS3, PIAS3, and protein tyrosine phosphatases, this pathway is frequently hyperactivated in tumors, sustaining malignant behavior even in the absence of increased proliferative activity (18,27,34). In addition, STAT3

modulates the tumor microenvironment by recruiting immunosuppressive myeloid cells and inducing angiogenic factors such as VEGF (35). The independence of STAT3 from Ki-67 aligns with reports that STAT3 activation can enhance the metastatic potential of even in cancers with relatively modest proliferative indices (18). Thus, linking the multivariate finding to its biological implications clarifies that STAT3's oncogenic influence extends to microenvironmental remodeling, immune evasion, and metastatic dissemination—distinct from its role in pure proliferative control. Importantly, this dissociation emphasizes that tumor aggressiveness is not solely determined by growth kinetics but also by the capacity to remodel the surrounding stroma and evade immune surveillance. From a translational standpoint, these results reinforce the rationale for therapeutic strategies targeting STAT3 signaling, which could suppress invasion and metastatic spread even in tumors that remain highly proliferative.

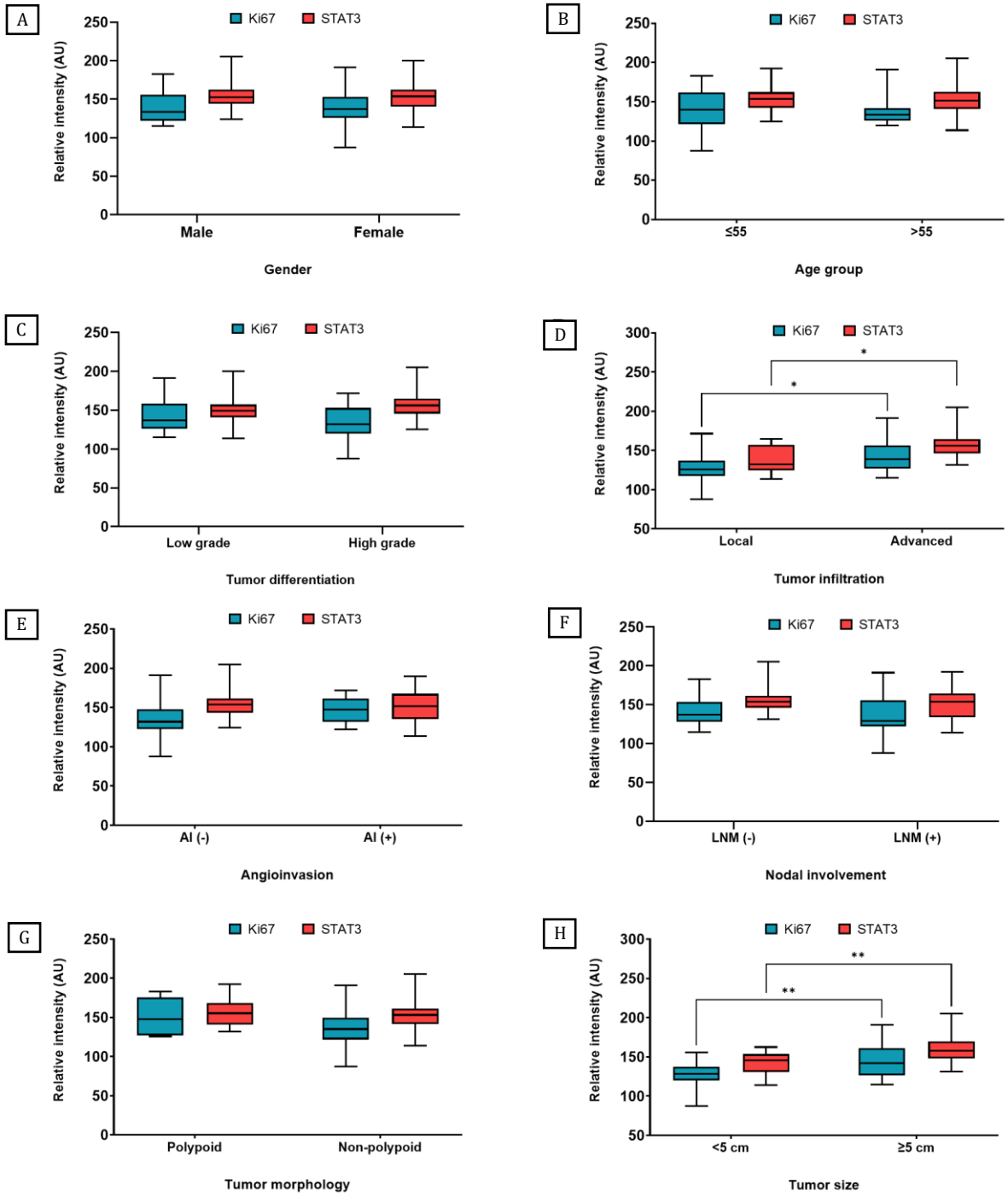

Figure 3. Characterization of Ki-67 and STAT3 expression in colorectal carcinoma according to clinicopathological characteristics. (A) gender, (B) age group, (C) tumor differentiation, (D) tumor infiltration, (E) angioinvasion, (F) nodal involvement, (G) tumor morphology, and (H) tumor size-based expression pattern. Data are presented as minimum to maximum and median values in each group. *p < 0.05, **p < 0.01. LNM, lymph node metastasis; AI, angioinvasion.

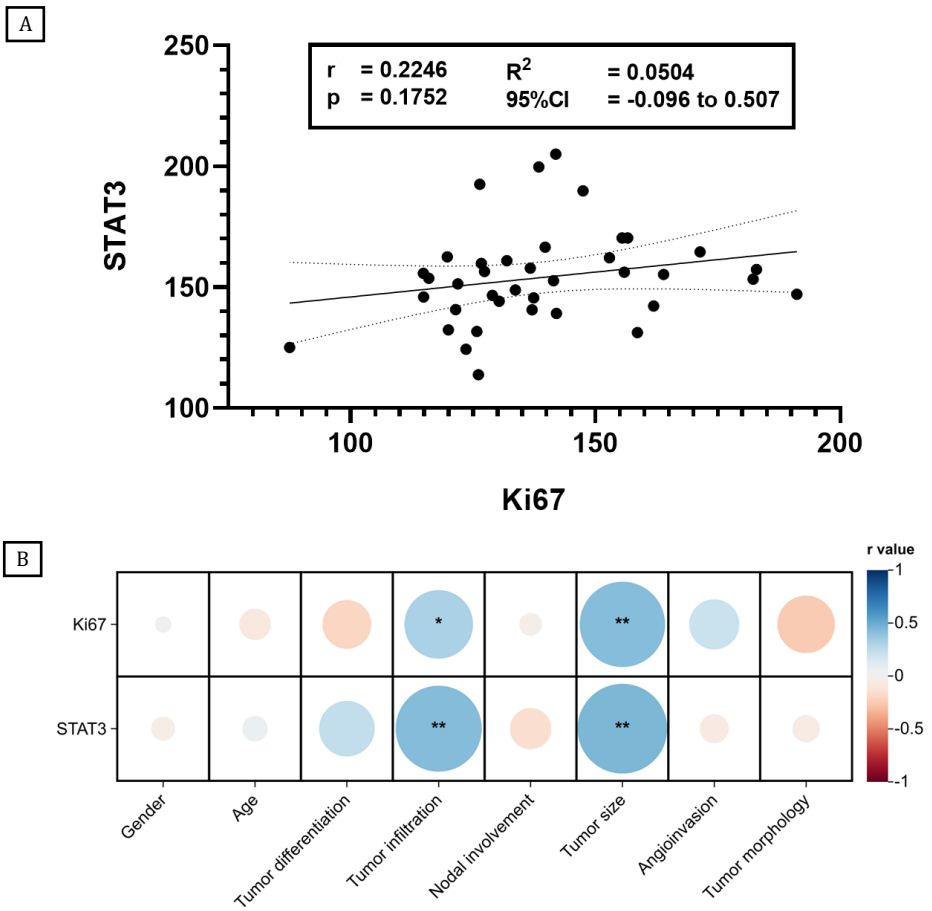

Figure 4. Correlation analysis of Ki-67 and STAT3. (A) Correlation of Ki-67 vs. STAT3 and (B) Ki-67 and STAT3 vs. colorectal carcinoma clinicopathological features. Positive R-value favors female, older age, high-grade tumor, advanced infiltration, LNM, large tumor, positive angioinvasion, and non-polypoid type. *p < 0.05, **p < 0.01. M, male; F, female.

Table 3. Logistic regression analysis of Ki-67 and STAT3 associated with tumor infiltration and size.

Dependent variable	Biomarker	Univariate analysis		Multivariate analysis	
		OR 1-SD (95% CI)	*p*-value	OR 1-SD (95% CI)	*p*-value
Tumor infiltration	Ki-67	2.69 (1.07–7.72)	0.067	1.84 (0.71–4.78)	0.270
	STAT3	5.40 (1.67–19.38)	0.015*	4.11 (1.28–13.29)	0.043*
Tumor size	Ki-67	3.12 (1.33–9.04)	0.021*	2.66 (0.93–7.31)	0.068
	STAT3	4.66 (1.63–15.41)	0.014*	3.65 (1.06–12.52)	0.040*

*Statistically significant (p < 0.05). Association of STAT3 with tumor size and infiltration in multivariate analysis was adjusted for Ki-67 expression, and vice versa. OR and 95% CI expressed as per 1-SD increment. OR, odds ratio; 95% CI, 95% confidence interval.

3.4. Limitations and Future Directions

This study has several limitations that must be considered in future research. The cross-sectional design limits causal inference, and the relatively small sample size at a single center may decrease generalizability and statistical power. The use of FFPE samples may introduce variability in antigen preservation and staining intensity. To minimize these limitations, future studies could utilize fresh-frozen or prospectively collected tissue specimens, employ standardized pre-analytical handling procedures, or employ multiplex digital immunohistochemistry or quantitative image analysis to enhance biomarker accuracy. Although inter-observer agreement was excellent, IHC quantitation remains semiquantitative. Future research should include larger, multi-institutional cohorts, collect survival data, and control for molecular features such as microsatellite instability (MSI) status, gene mutations (TP53, APC, BRAF, KRAS, PIK3CA, and SMAD4), and tumor site to improve the validity and prognostic value of biomarker correlation (36).

4. CONCLUSIONS

In conclusion, this study highlights the differential roles of STAT3 and Ki-67 in colorectal cancer progression. While both markers are associated with deeper tumor infiltration and larger tumor size, only STAT3 retained an independent association, underscoring its stronger prognostic relevance. The lack of correlation between STAT3 and Ki-67 suggests distinct biological pathways, with STAT3 potentially driving tumor aggressiveness via inflammation-related signaling beyond proliferation. Clinically, STAT3 expression could complement existing prognostic systems by identifying tumors at higher risk of invasion and metastasis, aiding in patient stratification. Translationally, these findings support ongoing efforts to target STAT3 signaling in colorectal cancer, including emerging therapeutic strategies such as small-molecule inhibitors, antisense oligonucleotides, and pathway-specific combination therapies, highlighting its potential as both a biomarker and therapeutic target.

Author contributions: IT, BN, AW: Conceptualization. AW, IT, IP: Methodology. AW; Software. IT, IP, BN: Validation, Investigation. AW, BN, IT: Formal analysis. IT, BN: Resources. AW, VW, BN: Writing – Original draft. AW, VW: Writing – Review & editing. AW: Visualization. All authors read and approved the final manuscript.

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REFERENCES

1. Sharma R, Abbasi-Kangevari M, Abd-Rabu R, Abidi H, Abu-Gharbieh E, Acuna JM, et al. Global, regional, and national burden of colorectal cancer and its risk factors, 1990–2019: A systematic analysis for the global burden of disease study 2019. Lancet Gastroenterol Hepatol. 2022;7(7):627-647. https://doi.org/10.1016/S2468-1253(22)00044-9
2. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174. https://doi.org/10.1016/j.tranon.2021.101174
3. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi DJ, John A, et al. Colorectal cancer: A review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. Cancers (Basel). 2022;14(7):1732. https://doi.org/10.3390/cancers14071732
4. Theodora I, Sudiana IK, Budipramana VS, Erwin F, Dewi S, Novita BD. Tumor differentiation is correlated with Estrogen Receptor Beta (ERβ) expression but not with Interleukin-6 (IL-6) expression in colorectal carcinoma. Indones Biomed J. 2024;16(5):481–6. https://doi.org/10.18585/inabj.v16i5.3324
5. Roshandel G, Ghasemi-Kebria F, Malekzadeh R. Colorectal cancer: Epidemiology, risk factors, and prevention. Cancers (Basel). 2024;16(8):1530. https://doi.org/10.3390/cancers16081530
6. Parmar S, Easwaran H. Genetic and epigenetic dependencies in colorectal cancer development. Gastroenterol Rep (Oxf). 2022:10:goac035.. https://doi.org/10.1093/gastro/goac035
7. Cortés-Ballinas L, López-Pérez TV, Rocha-Zavaleta L. STAT3 and the STAT3-regulated inhibitor of apoptosis protein survivin as potential therapeutic targets in colorectal cancer (Review). Biomed Rep. 2024;21(6):175. https://doi.org/10.3892/br.2024.1863
8. Lin H, Ho A, Huang H, Yang B, Shih B, Lin H, et al. STAT3-mediated gene expression in colorectal cancer cells-derived cancer stem-like tumorspheres. Adv Dig Med. 2021;8(4):224–33. https://doi.org/10.1002/aid2.13223
9. Lişcu HD, Verga N, Atasiei DI, Badiu DC, Dumitru AV, Ultimescu F, et al. Biomarkers in colorectal cancer: Actual and future perspectives. Int J Mol Sci. 2024;25(21):11535. https://doi.org/10.3390/ijms252111535
10. Liu Q, Ran D, Wang L, Feng J, Deng W, Mei D, et al. Association between Ki67 expression and therapeutic outcome in colon cancer. Oncol Lett. 2023;25(6):272. https://doi.org/10.3892/ol.2023.13858
11. Uxa S, Castillo-Binder P, Kohler R, Stangner K, Müller GA, Engeland K. Ki-67 gene expression. Cell Death Differ. 2021;28(12):3357-3370. https://doi.org/10.1038/s41418-021-00823-x
12. Lei HT, Yan S, He YH, Xu N, Zhao M, Yu CJ, et al. Ki67 testing in the clinical management of patients with non-metastatic colorectal cancer: Detecting the optimal cut-off value based on the Restricted Cubic Spline model. Oncol Lett. 2022;24(6):420. https://doi.org/10.3892/ol.2022.13540
13. Spitzner M, Roesler B, Bielfeld C, Emons G, Gaedcke J, Wolff HA, et al. STAT3 inhibition sensitizes colorectal cancer to chemoradiotherapy in vitro and in vivo. Int J Cancer. 2014;134(4):997–1007. https://doi.org/10.1002/ijc.28429
14. Calu V, Ionescu A, Stanca L, Geicu OI, Iordache F, Pisoschi AM, et al. Key biomarkers within the colorectal cancer related inflammatory microenvironment. Sci Rep. 2021;11(1):7940. https://doi.org/10.1038/s41598-021-86941-5
15. Tjahjono Y, Caroline, Foe K, Wijaya H, Dewi BDN, Karnati S, et al. 2-(3-(Chloromethyl)benzoyloxy) benzoic Acid reduces prostaglandin E-2 concentration, NOX2 and NFKB expression, ROS production, and COX-2 expression in lipopolysaccharide-induced mice.

Prostaglandins Other Lipid Mediat. 2024:174:106866. https://doi.org/10.1016/j.prostaglandins.2024.106866

16. Zhuang M, Ding X, Song W, Chen H, Guan H, Yu Y, et al. Correlation of IL-6 and JAK2-STAT3 signaling pathway with prognosis of nasopharyngeal carcinoma patients. Aging (Albany NY). 2021;13(12):16667-16683. https://doi.org/10.18632/aging.203186
17. Tong G, Zhang G, Liu J, Zheng Z, Chen Y, Niu P, et al. Cutoff of 25% for Ki67 expression is a good classification tool for prognosis in colorectal cancer in the AJCC-8 stratification. Oncol Rep. 2020;43(4):1187–98. https://doi.org/10.3892/or.2020.7511
18. Han C, Sun B, Zhao X, Zhang Y, Gu Q, Liu F, et al. Phosphorylation of STAT3 promotes vasculogenic mimicry by inducing epithelial-to-mesenchymal transition in colorectal cancer. Technol Cancer Res Treat. 2017;16(6):1209–1219. https://doi.org/10.1177/1533034617742312
19. Grund B, Sabin C. Analysis of biomarker data: Logs, odds ratios, and receiver operating characteristic curves. Curr Opin HIV AIDS. 2010;5(6):473–9. https://doi.org/10.1097/COH.0b013e32833ed742
20. Morikawa T, Baba Y, Yamauchi M, Kuchiba A, Nosho K, Shima K, et al. STAT3 expression, molecular features, inflammation patterns, and prognosis in a database of 724 colorectal cancers. Clin Cancer Res. 2011;17(6):1452-62. https://doi.org/10.1158/1078-0432.CCR-10-2694
21. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: A leading role for STAT3. Nat Rev Cancer. 2009;9(11):798-809. https://doi.org/10.1038/nrc2734
22. Rahadiani N, Habiburrahman M, Abdullah M, Jeo WS, Stephanie M, Handjari DR, et al. Analysing 11 years of incidence trends, clinicopathological characteristics, and forecasts of colorectal cancer in young and old patients: a retrospective cross-sectional study in an Indonesian national referral hospital. BMJ Open. 2022;12(9):e060839. https://doi.org/10.1136/bmjopen-2022-060839
23. Schmuck R, Gerken M, Teegen EM, Krebs I, Klinkhammer-Schalke M, Aigner F, et al. Gender comparison of clinical, histopathological, therapeutic and outcome factors in 185,967 colon cancer patients. Langenbecks Arch Surg. 2020;405(1):71-80. https://doi.org/10.1007/s00423-019-01850-6
24. Shaukat A, Kahi CJ, Burke CA, Rabeneck L, Sauer BG, Rex DK. ACG clinical guidelines: Colorectal cancer screening 2021. Am J Gastroenterol. 2021;116(3):458-479. https://doi.org/10.14309/ajg.0000000000001122
25. Crowe A, Yue W. Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated Protocol. Bio Protoc. 2019;9(24):e3465. https://doi.org/10.21769/bioprotoc.3465
26. Yang SF, Yuan SSF, Yeh YT. Positive association between STAT3 and Ki-67 in cervical intraepithelial neoplasia. Kaohsiung J Med Sci. 2006;22(11):539-46. https://doi.org/10.1016/S1607-551X(09)70350-X
27. Yang SF, Yuan SSF, Yeh YT, Wu MT, Su JH, Hung SC, et al. The role of p-STAT3 (ser727) revealed by its association with Ki-67 in cervical intraepithelial neoplasia. Gynecol Oncol. 2005;98(3):446–52. https://doi.org/10.1016/j.ygyno.2005.05.032
28. Wu L-J, Li H-X, Luo X-T, Lu R-Z, Ma Y-F, Wang R, et al. STAT3 activation in tumor cell-free lymph nodes predicts a poor prognosis for gastric cancer. Int J Clin Exp Pathol. 2014;7(3):1140–6. https://pmc.ncbi.nlm.nih.gov/articles/PMC3971319/
29. Koveitypour Z, Panahi F, Vakilian M, Peymani M, Seyed Forootan F, Nasr Esfahani MH, et al. Signaling pathways involved in colorectal cancer progression. Cell Biosci. 2019;97(9):1-14. https://doi.org/10.1186/s13578-019-0361-4
30. Zhang X, Hu F, Li G, Li G, Yang X, Liu L, et al. Human colorectal cancer-derived mesenchymal stem cells promote colorectal cancer progression through IL-6/JAK2/STAT3 signaling. Cell Death Dis. 2018;9(2):25. https://doi.org/10.1038/s41419-017-0176-3
31. Xiao L, Li X, Cao P, Fei W, Zhou H, Tang N, et al. Interleukin-6 mediated inflammasome activation promotes oral squamous cell carcinoma progression via JAK2/STAT3/Sox4/NLRP3 signaling pathway. J Exp Clin Cancer Res. 2022;41(1):166. https://doi.org/10.1186/s13046-022-02376-4
32. Andrés-Sánchez N, Fisher D, Krasinska L. Physiological functions and roles in cancer of the proliferation marker Ki-67. J Cell Sci. 2022;135(11): jcs258932. https://doi.org/10.1242/jcs.258932
33. Mrouj K, Andrés-Sánchez N, Dubra G, Singh P, Sobecki M, Chahar D, et al. Ki-67 regulates global gene expression and promotes sequential stages of carcinogenesis. Proc Natl Acad Sci U S A. 2021;118(10):e2026507118. https://doi.org/https://doi.org/10.1073/pnas.2026507118
34. Zhang G, Hou S, Li S, Wang Y, Cui W. Role of STAT3 in cancer cell epithelial-mesenchymal transition (Review). Int J Oncol. 2024;64(5):48. https://doi.org/10.3892/ijo.2024.5636
35. Rébé C, Ghiringhelli F. STAT3, a master regulator of anti-tumor immune response. Cancers (Basel). 2019;11(9):1280. https://doi.org/10.3390/cancers11091280
36. Su Z, El Hage M, Linnebacher M. Mutation patterns in colorectal cancer and their relationship with prognosis. Heliyon. 2024;10(17):e36550. https://doi.org/10.1016/j.heliyon.2024.e36550