RESEARCH ARTICLE

Editorial Process: Submission:10/04/2024 Acceptance:03/11/2025

Role of Lyn Immunohistochemical Staining in Progression of Colorectal Carcinoma

Hany Y. Sayed*, Reda F Abdelmeguid, Dalia M. Abd El-Rehim, Nisreen A.A. Osman, Nisreen DM Toni

Abstract

Objective: To investigate the immunohistochemical expression of Lyn in colorectal adenocarcinoma with its corresponding lymph node metastasis and correlation with clinicopathological characteristics. **Methods:** Immunohistochemical analysis of Lyn expression was performed on 70 colorectal cancer (CRC) tissue specimens of hemicolectomy and their corresponding lymph node metastases. Clinicopathological data, including age, gender, tumor size, location, TNM stage, modified Dukes stage, tumor grade, tumor-infiltrating lymphocytes (TILs), poorly differentiated clusters (PDCs), vascular invasion, and perineural invasion (PNI), were collected and analyzed to assess correlations with Lyn expression. **Results:** High Lyn expression was observed in 34.3% of CRC cases. Significant associations were found between high Lyn expression and positive nodal metastasis ($p < 0.001$), higher TNM stage ($p = 0.003$), and advanced modified Dukes stage ($p = 0.001$). No significant associations were found between Lyn expression and age, gender, tumor size, primary tumor location, tumor grade, TILs, PDCs, vascular invasion, or PNI ($p > 0.05$ for all). A significant correlation was observed between Lyn expression in primary tumors and their corresponding lymph node metastases ($p = 0.033$). **Conclusion:** Lyn expression is significantly associated with unfavorable clinicopathological parameters, which are lymph node metastasis and advanced tumor stage, suggesting its potential role as a prognostic marker in colorectal cancer.

Keywords: Colorectal cancer (CRC)- Lyn, Immunohistochemical- Prognosis

Asian Pac J Cancer Prev, **26 (3)**, 943-948

Introduction

Colorectal cancer (CRC) ranks third globally in terms of cancer diagnoses, following lung and prostate cancer in men and breast and lung cancer in women. It accounts for 1.93 million new cases annually, representing 10% of all cancer diagnoses out of an estimated 19.3 million cases globally. CRC is the second leading cause of cancer-related deaths worldwide, contributing to 0.94 million deaths annually (9.4% of all cancer-related deaths) out of an estimated 10 million cancer deaths [1].

In Egypt, CRC is the seventh most common cancer after liver, breast, bladder, non-Hodgkin lymphoma (NHL), lung, and leukemia, accounting for approximately 6,194 newly diagnosed cases (3.9% of all cancers) in 2022. The incidence rate is higher in men than in women, with a male-to-female ratio of 1.4:1. CRC ranks as the ninth leading cause of cancer mortality in Egypt, with 3,096 deaths in 2022 out of 95,275 total cancer deaths [2].

Lck-related novel protein tyrosine kinase (Lyn), also referred to as LCK/YES novel protein tyrosine kinase or JTK8, belongs to the Src family of tyrosine kinases (SFKs), which includes Src, Fyn, Yes, Lck, Hck, Blk, Fgr, Lyn, and Yrk [3]. The Lyn gene, located on chromosome 8q3, encodes this protein and regulates various cellular signaling pathways. Lyn is activated through the phosphorylation of tyrosine residues within the catalytic domain [4–6].

Within cells, Lyn modulates key signaling pathways and plays dual roles in promoting or inhibiting cellular responses [7]. It is critical for B-cell receptor (BCR) signaling, promoting B-cell proliferation, differentiation, and antibody production. Lyn also regulates the activity of macrophages and neutrophils, controlling migration, phagocytosis, and reactive oxygen species production [8]. Emerging evidence suggests that Lyn plays a significant role in tumor development and progression by suppressing apoptosis and promoting cellular proliferation [9]. Aberrant expression of Lyn has been reported in several cancers, including lung, prostate, and breast cancers [10–12]. In CRC, Lyn activates the Akt (anti-apoptotic) pathway, promoting chemoresistance through PI3-K/PKB-mediated cell survival mechanisms [13,14]. Furthermore, Lyn is a molecular target of minocycline, a

*Department of Pathology, Faculty of Medicine, Minia University 61511, El-Minia, Egypt. *For Correspondence: hany.yousri@mu.edu.eg*

chemotherapeutic agent that binds and inactivates Lyn, leading to STAT3 inactivation, suppression of epithelial-mesenchymal transition (EMT), and inhibition of CRC metastasis [15,16].

While previous studies have highlighted Lyn's involvement in CRC progression, its role in metastatic dissemination and its correlation with clinicopathological parameters remain underexplored. This study aims to address this gap by investigating the immunohistochemical expression of Lyn in colorectal adenocarcinoma and its corresponding lymph node metastases, correlating its expression with clinicopathological features to further understand its prognostic significance.

Materials and Methods

The present study comprised 70 randomly selected paraffin blocks of primary colorectal adenocarcinoma cases, including 41 cases with their available corresponding blocks of metastatic lymph nodes. They were obtained from the archives of histopathological laboratories of Minia University Hospital and Minia Oncology Center in the period between 2020 and 2023.

To confirm the diagnosis, all pathology reports containing slides stained with hematoxylin and eosin (H&E) were examined. The clinicopathological data included in this study are patients' age, gender, tumor location, tumor size, tumor grade according to the 5th edition of WHO, poorly differentiated clusters (PDCs) (cancer clusters in the stroma that are made up of five or more cancer cells and not forming glands) grade, regional lymph node involvement, TNM stage, modified Dukes stage, lymphovascular invasion (LVI), tumor-infiltrating lymphocytes (TILs), and perineural invasion (PNI).

Immunohistochemical (IHC) Procedure

The manual IHC staining method has been used in our study. Five µm tissue sections on positively charged slides were treated with 3% hydrogen peroxide for 30 minutes to inhibit the endogenous peroxidase activity after being deparaffinized and rehydrated using xylene and graded ethanol solutions. To retrieve the antigen, sections were treated for 20 minutes in a 700-W microwave oven with 0.1 mol/L citrate, pH 6.0. A polyclonal rabbit Lyn antibody (0.1 ml, concentrated, Bioss, USA) was the primary antibody used. It was incubated at 4°C for the entire night. Using diaminobenzidine (DAB) to serve as a chromogen, the reaction was identified using the avidin-biotin detection kit. Lastly, Mayer's hematoxylin was used as a counterstain on the sections. An internal positive control for Lyn was assessed using the lymphocytes within the stained sections. PBS was used to incubate the slides after processing negative control tissue sections, which did not include the primary antibody.

Scoring of Immunohistochemical Staining

The slides were independently reviewed by two pathologists. Regarding Lyn, estimation of expression was made using a score obtained by multiplying the values of the intensity and percentage of immunoreactive cells. The intensity was graded as 0 negative, 1 weak, 2 moderate, and 3 intense staining. The percentage of positive cells was graded from 0 to 4 (0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (76–100%)). High Lyn expression was defined if the score was ≥4. Cases with scores less than 4 were considered low Lyn expression [14].

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 25 was used for the statistical analysis. Using the Chi-squared test and Fisher's exact test, the association between Lyn expression and any of the patient clinicopathological features was confirmed. The association between Lyn expression in the original tumors and the corresponding lymph node metastasis was determined using the Wilcoxon test. It was found that a P value of 0.05 or less was significant.

Results

The mean age of the patients was 54.3±15.6 years (range 17-89 years) with a median of 55 years. Forty cases (57.1%) were males, and 30 (42.9%) were females. The mean tumor size was 6.7±2.5 cm (range 2-14.5 cm) with a median of 6 cm. 60 (85.7%) tumors were in the colon while 10 (14.3%) tumors were located on the rectum. Thirty-eight (54.3%) tumors were low grade, while 32 (45.7%) tumors were high grade. Regarding PDC grade, 55 (78.6%) tumors were PDC grade 1, 11 (15.7%) tumors were PDC grade 2, and 4 (5.7%) tumors were PDC grade 3. Forty-one (58.6%) cases had regional lymph node metastasis at the time of diagnosis. Concerning modified Dukes staging, 29 (41.4%) cases were stage B, 36 (51.4%) cases were stage C, and 5 (7.1%) cases were stage D. Lymphovascular invasion and perineural invasion were present in 39 (55.7%) cases and 6 (8.6%) cases, respectively. Sixty-eight (97.1%) of the tumors' stroma showed lymphocytic infiltration (Table 1).

Lyn was detected in the cytoplasm of malignant cells (Figure 1). 34.3% of cases showed high cytoplasmic Lyn expression, whereas negative/low expression was found in 46 (65.7%) cases. There was no statistically significant association discovered between Lyn expression and the following patient characteristics: age, gender, primary tumor location, size of the tumor, grade of the tumor (Figure 2), TILs, PDCs, lymphovascular invasion, and PNI ($p = 0.439, 0.346, 0.532, 0.138, 0.593, 0.178, 0.916, 0.475, 0.322$, respectively). However, there is a statistically significant association between high Lyn expression and positive nodal metastasis, high TNM stage, and advanced modified Dukes stage (Figure 3) ($p = < 0.001, 0.003$, and 0.001, respectively) (Table 1).

Lyn expression in the primary tumors and its expression in the corresponding lymph node metastasis were found to be significantly correlated ($p=0.033$) (Figure 4) (Table 2).

Discussion

Colorectal cancer is regarded as one of the most prevalent cancers in humans and one of the main causes of death from cancer [16]. Lyn is utilized by colon cancer cells to activate the Akt (anti-apoptotic) pathway, and these

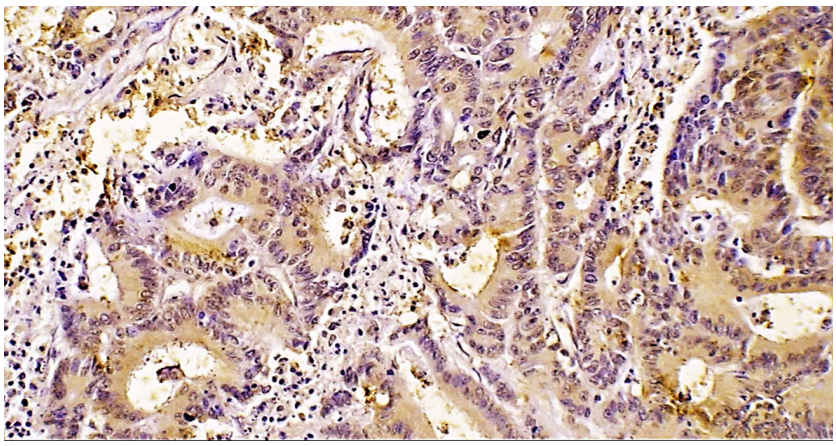

Figure 1. High Cytoplasmic Lyn Expression in Low Grade Colorectal Adenocarcinoma (DAP as chromogen and hematoxylin as counterstain X200).

Table 1. Association between Cytoplasmic Lyn Expression and Clinicopathological Features for Patients with Colorectal Adenocarcinoma (n=70)

Clinicopathological Features		Total 70 (100%)	Cytoplasmic Lyn Expression		P value
			Negative/low expression n=46	High expression n=24	
Age, (years)	<50	24 (34.3%)	15 (62.5)	9 (37.5)	0.439
	≥50	46 (65.7%)	31 (67.4)	15 (32.6)	
Gender	Male	40 (57.1%)	25 (62.5)	15 (37.5)	0.346
	Female	30 (42.9%)	21 (70)	9 (30)	
Location	Colon	60 (85.7%)	38 (63.3)	21 (36.7)	0.532
	Rectum	10 (14.3%)	7 (70)	3 (30)	
Tumor size, (cm)	<6	25 (35.3%)	11 (44)	14 (56)	0.138
	≥6	45 (64.7%)	35 (77.8)	10 (22.2)	
Tumor grade	Low grade	38(54.3%)	25 (65.8)	13 (34.2)	0.593
	High grade	32 (45.7%)	21 (65.6)	11 (34.4)	
Nodal status	Negative	29 (41.2%)	26 (89.7)	3 (10.3)	< 0.001*
	Positive	41 (58.6%)	20 (48.8)	21 (51.2)	
TNM stage	I	10 (14.3%)	10 (100)	0 (0)	0.003*
	II	19 (27.1%)	16 (84.2)	3 (15.8)	
	III	31 (44.3%)	16 (51.6)	15 (48.4)	
	IV	10 (14.3%)	4 (40)	6 (60)	
Modified Dukes staging	B1	7 (10%)	6 (85.7)	1 (14.3)	0.001*
	B2	22 (31.4%)	21 (95.5)	1 (4.5)	
	C1	12 (17.1%)	6 (50)	6 (50)	
	C2	24 (34.3%)	12 (50)	12 (50)	
	D	5 (7.1%)	1 (20)	4 (80)	
TILs	Absent	2 (2.9%)	1 (50)	1 (50)	0.178
	Mild	25 (35.7%)	19 (76)	6 (24)	
	Moderate	32 (45.7%)	17 (53.1)	15 (46.9)	
	Marked	11 (15.7%)	9 (81.8)	2 (18.9)	
Poorly differentiated clusters	G1	55 (78.6%)	36 (65.5)	9 (34.5)	0.916
	G2	11 (15.7%)	7 (63.6)	4 (36.4)	
	G3	4 (5.7 %)	3 (75)	1 (25)	
Lymphovascular invasion	Absent	31 (44.3%)	21 (67.7)	10 (32.3)	0.475
	Present	39 (55.7%)	25 (64.1)	14 (35.9)	
Perineural invasion	Absent	64 (91.4%)	41 (64.1)	23 (35.9)	0.322
	Present	6 (8.6%)	5 (83.3)	1 (16.7)	

*Tests of significance: chi-square test and Fisher's exact test; P - value < 0.05 is considered statistically significant

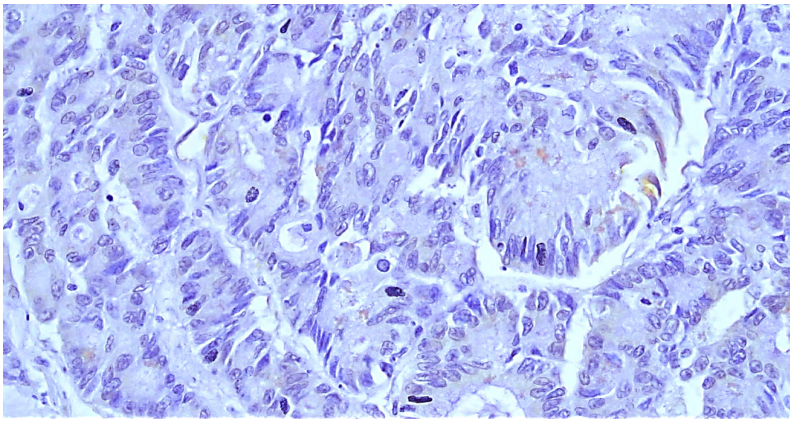

Figure 2. Negative/Low Cytoplasmic Lyn Expression in High Grade Colorectal Adenocarcinoma (DAP as chromogen and hematoxylin as counterstain X200).

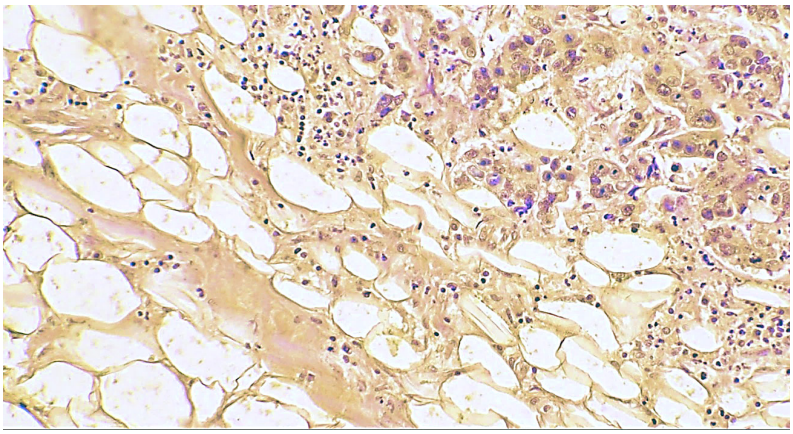

Figure 3. High Cytoplasmic Lyn Expression in Colorectal Adenocarcinoma Infiltrating the Pericolic Fat (DAP as chromogen and hematoxylin as counterstain X200).

Table 2. The Change in Lyn Expression Scores in 41 Pairs of Primary Tumor and Their Corresponding LN Metastases.

Lyn expression	Change in expression pattern			P.= 0.033
	P = M[a]	M > P[b]	P > M[c]	
	19 (46.3%)	16 (39%)	8 (14.6%)	

P, Primary colorectal adenocarcinoma; M, metastatic malignant LN; a, Equal expression in primary tumor and LN metastasis; b, Higher expression in LN metastasis compared to primary tumor; c, Higher expression in primary tumor compared to LN metastasis; *Test of significance: Wilcoxon test; * P - value < 0.05 is considered significant.

cells showed increased Lyn kinase activity when exposed to chemotherapy [13]. In our current study, Lyn high expression was observed in 34.3% of CRC cases. This is in concordance with Hao et al., who reported Lyn expression positivity in 40% of CRC cases [14]. This finding is in contrast with Su et al., who reported Lyn expression positivity in 93% of cases [15]. This finding is due to the different scoring system used, considering positive Lyn expression when 10% or more of cells are stained. In the present study, positive Lyn expression shows no

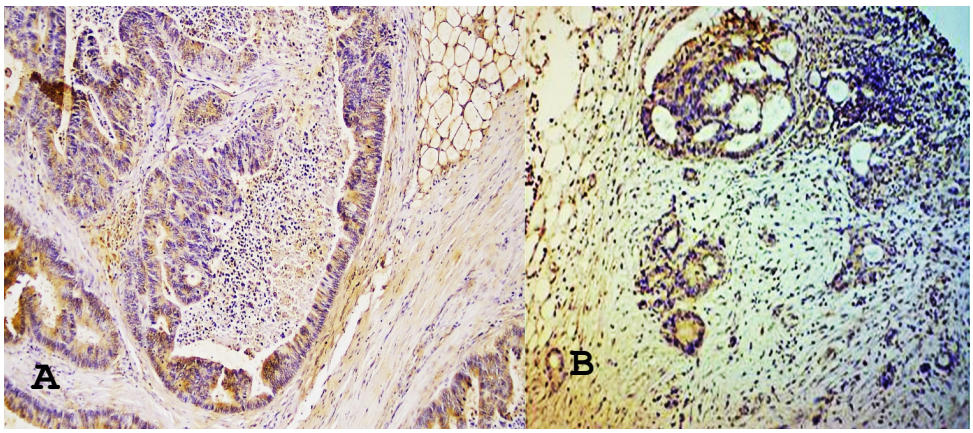

Figure 4. Immunohistochemical Expression of Lyn in Primary Colorectal Adenocarcinoma and Its Corresponding lymph Node Metastasis. A, High expression of Lyn in primary colorectal adenocarcinoma (Streptavidin-biotin-immunoperoxidase, original magnification x200). B, High expression of Lyn in corresponding lymph node metastasis (Streptavidin-biotin-immunoperoxidase, original magnification x200).

significant associations with patients' age, gender, tumor size, or primary tumor location. This is in line with Hao et al. and Su et al. [14, 15]. Our study shows no significant association between Lyn expression and tumor grade. This is in contrast with Hao et al. and Su et al., who found a significant association with tumor grade [14, 15]; this may be due to the difference in scoring system and the higher number of high-grade tumors included in the study. In the present study, Lyn expression is significantly associated with advanced tumor stage. Lyn was expressed more in higher TNM stages ($p = 0.003$). It was expressed in stage C and D more than in stage A and B of modified Dukes staging ($p = 0.001$). This result is in line with previous studies of Hao et al., Su et al., and Yang et al. [14-16]. High Lyn is significantly associated with positive nodal metastasis ($p < 0.001$). This is in line with Hao et al. and Su et al., who found a significant association between Lyn expression and lymph node metastasis in CRC [14, 15]. Our study also found no significant association between Lyn expression and TILs, PDCs, vascular invasion, and PNI. As far as we are aware, this research is the first to examine the expression of Lyn in primary tumors and the expression of Lyn in corresponding lymph node metastases. We found a significant correlation between the expression of Lyn in the primary tumors and the lymph node metastases that corresponded to them ($p = 0.033$). This supports the significant role of Lyn in colorectal cancer progression and metastasis.

This study has one limitation, which is the fewer number of included cases. However, the study of Lyn expression by immunohistochemistry in the primary tumor and corresponding lymph nodes offers new and potentially useful information for CRC patients.

In conclusion, the expression of Lyn was observed in the cytoplasm of malignant cells. High expression of Lyn was significantly associated with poor prognostic clinicopathological parameters, including advanced tumor stage and lymph node metastasis. This highlighted the significant role of Lyn in CRC progression and metastasis. This study affords a pivotal step for further research on the use of Lyn in the management of CRC.

Author Contribution Statement

All authors shared in the manuscript writing and approval of the final version.

Acknowledgements

Approval

It is not a part of an approved student thesis.

Ethical Declaration

Specimens' collection and patients' medical data privacy were in accordance with the Institutional Ethical Committee's (Faculty of Medicine Minia University, Egypt) guidelines (Approval number: 727/4/2023).

Data Availability

Data is available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin. 2023;73(3):233-54. https://doi.org/10.3322/caac.21772
2. Global Cancer Observatory: Cancer Today [Internet]. Lyon (France): International Agency for Research on Cancer; 2024. Available from: https://gco.iarc.who.int/today.
3. Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol. 1997;13(1):513-9. https://doi.org/10.1146/annurev.cellbio.13.1.513.
4. Yamanashi Y, Fukushige SL, Semba K, Sukegawa J, Miyajima N, Matsubara K, et al. The yes-related cellular gene lyn encodes a possible tyrosine kinase similar to p56lck. Mol Cell Biol. 1987;7(7):2370-6. https://doi.org/10.1128/mcb.7.1.237-243.1987
5. Lowell CA. Src-family kinases: rheostats of immune cell signaling. Mol Immunol. 2004;41(6-7):631-43. https://doi.org/10.1016/j.molimm.2004.04.010
6. Xu Y, Harder KW, Huntington ND, Hibbs ML, Tarlinton DM. Lyn tyrosine kinase: accentuating the positive and the negative. Immunity. 2005;22(1):9-18. https://doi.org/10.1016/j.immuni.2004.12.004
7. Ingley E. Functions of the Lyn tyrosine kinase in health and disease. Cell Commun Signal. 2012;10(1):21. https://doi.org/10.1186/1478-811X-10-21
8. Brodie EJ, Infantino S, Low MS, Tarlinton DM. Lyn, lupus, and (B) lymphocytes: a lesson on the critical balance of kinase signaling in immunity. Front Immunol. 2018;9:401. https://doi.org/10.3389/fimmu.2018.00401
9. Kumar A, Jaggi AS, Singh N. Pharmacology of Src family kinases and therapeutic implications of their modulators. Fundam Clin Pharmacol. 2015;29(2):115-30. https://doi.org/10.1111/fcp.12097
10. Huang TH, Huo L, Wang YN, Xia W, Wei Y, Chang SS, et al. Epidermal growth factor receptor potentiates MCM7-mediated DNA replication through tyrosine phosphorylation of Lyn kinase in human cancers. Cancer Cell. 2013;23(6):796-810. https://doi.org/10.1016/j.ccr.2013.04.027.
11. Tabariès S, Annis MG, Hsu BE, Tam CE, Savage P, Park M, et al. Lyn modulates Claudin-2 expression and is a therapeutic target for breast cancer liver metastasis. Oncotarget. 2015;6(11):9476-87. https://doi.org/10.18632/oncotarget.3269.
12. Wang X, Wen J, Li R, Qiu G, Zhou L, Wen X. Gene expression profiling analysis of castration-resistant prostate cancer. Med Sci Monit. 2015;21:205-11. https://doi.org/10.12659/MSM.891193
13. Bates RC, Edwards NS, Burns GF, Fisher DE. A CD44 survival pathway triggers chemoresistance via lyn kinase and phosphoinositide 3-kinase/Akt in colon carcinoma cells. Cancer Res. 2001;61(13):5275-83.
14. Hao JM, Chen JZ, Sui HM, Si-Ma XQ, Li GQ, Liu C, et al. A five-gene signature as a potential predictor of metastasis and survival in colorectal cancer. J Pathol. 2010;220(4):475-89. https://doi.org/10.1002/path.2668
15. Su N, Peng L, Xia B, Zhao Y, Xu A, Wang J, et al. Lyn is involved in CD24-induced ERK1/2 activation in colorectal cancer. Mol Cancer. 2012;11:65. https://doi.org/10.1186/1476-4598-11-43
16. Yang L, Yang J, Liu H, Lan J, Xu Y, Wu X, et al. Minocycline binds and inhibits LYN activity to prevent STAT3-

mediated metastasis of colorectal cancer. Int J Biol Sci. 2022;18(6):2540-51. https://doi.org/10.7150/ijbs.70708.