Expression of PD-L1 and PD-1 in stage T4 rectal cancer tissues and surrounding metastatic lymph nodes and correlation with prognosis

Yu Wang[1], Haofeng Lu[2], Mi Li[1*]

[1] Department of Hepatobiliary Surgery, The First Affiliated Hospital of Yangtze University, The First Clinical MedicalCollege of Yangtze University, Jingzhou, Hubei434000, China

[2] Department of Hepatobiliary Surgery, The First Affiliated Hospital of Yangtze University, Jingzhou, Hubei434000, China

ARTICLE INFO

ABSTRACT

The expressions of programmed death ligand 1 (PD-L1) and programmed death receptor 1 (PD-1) in T4 rectal cancer tissues and surrounding metastatic lymph nodes were analyzed and correlated with prognosis. For this purpose, ninety-eight patients with T4 rectal cancer treated in our hospital from July 2021 to July 2022 were selected, and surgically resected rectal cancer tissues as well as para carcinoma tissue samples, and surrounding metastatic lymph node tissues were obtained from all patients. Analysis of PD-L1 and PD-1 expression in rectal cancer tissues as well as in adjacent tissue specimens and surrounding metastatic lymph node tissues were performed using immunohistochemical staining. PD-L1 and PD-1 expression were analyzed in relation to lymph node metastasis, maximum tumor diameter, as well as histological analysis, and the relationship between the two and prognosis was analyzed. Immunohistochemistry for PD-L1, PD-1 revealed that both proteins were expressed in association with the target cytoplasm as well as within the cell membrane; The number of cases with positive expression of PD-L1 and PD-1 in cancer tissues was significantly higher than the number of cases with expression in adjacent tissues, which was statistically significant (P < 0.05); The expression rates of PD-L1, PD-1 in poor expression in progression-free survival as well as in progression survival were significantly higher than those in medium and high expression with statistical significance (P < 0.05); Compared with patients without lymph node metastasis, patients with T4 rectal cancer with lymph node metastasis had a higher number of cases with high expression levels of PD-L1 and PD-1 proteins, and the difference was statistically significant (P < 0.05); PD-L1 and PD-1 in T4 stage rectal cancer prognosis is closely related, distant metastasis as well as lymph node metastasis has a greater effect on PD-L1 and PD-1. PD-L1 and PD-1 showed abnormal expression in T4 rectal cancer tissues as well as in surrounding metastatic lymph nodes, and PD-L1 and PD-1 were closely related to prognosis in T4 rectal cancer, distant metastasis, as well as lymph node metastasis had a greater effect on PD-L1 and PD-1. Its detection is able to provide a certain data reference for the prognosis of T4 rectal cancer.

Introduction

Rectal cancer is the third most common cancer worldwide, with approximately 1.4 million new cases each year, ranking the third most common cancer in men and second in women. Approximately 30-40% of patients are found to have local progression or combined distant metastases at the time of initial diagnosis, which cannot be cured by surgery (1). Due to the poor prognosis of advanced rectal cancer, the average survival time for advanced disease is close to 30 months, despite significant advances in surgical treatment, chemotherapy and biological therapy. Metastatic rectal cancer remains the fourth most common cause of cancer death. The 5-year survival rates for patients with combined liver and lung metastases are 50% and 43.7%, respectively (2,3). In patients with peritoneal metastases, the 3-year survival rate is only 18%, and the life expectancy of peritoneal metastases detected during the same period is less than 9 months (4). Clinical studies have shown that programmed death receptor-1 (PD-1) plays an important role in the tumor microenvironment as well

as in the immune system after binding to the ligand (PD-L1). However, there are fewer studies on the expression of PD-L1 and PD-1 in stage T4 rectal cancer tissues and surrounding metastatic lymph nodes and their correlation with prognosis (5,6).

In this study, we selected patients with stage T4 rectal cancer, surgically extracted stage T4 rectal cancer tissues, paraneoplastic tissues and surrounding lymph nodes, analyzed the expression of PD-L1 and PD-1 in stage T4 rectal cancer tissues and surrounding metastatic lymph nodes, and analyzed their prognostic correlation with stage T4 rectal cancer.

Materials and Methods

Ninety-eight patients with stage T4 rectal cancer admitted to our hospital from July 2021 to July 2022 were selected, including 56 males and 42 females, aged 41-69 years, with a mean of (55.1±11.0) years. The degree of histological differentiation included 47 cases of low differentiation, 33 cases of medium differentiation, and 18 cases

* Corresponding author. Email: 2622258339@qq.com

of high differentiation. There were 43 cases of lymph node metastasis and 55 cases without lymph node metastasis. The study was approved by our hospital ethics committee and all subjects gave informed consent.

Inclusion criteria: all enrolled patients with stage T4 rectal cancer met the diagnostic criteria for stage T4 rectal cancer proposed by the National Health and Wellness Commission of the People's Republic of China (7), had complete medical records, and all underwent surgical extraction of the cancerous tissue as well as paraneoplastic tissue.

Exclusion criteria: those with incomplete medical records; those with other stages of rectal cancer; those who have received related treatment; those with cardiovascular disease; those with communication disorders.

Specimen collection

The specimens were prepared by surgical resection of cancerous tissues, paraneoplastic tissues 5 cm away from the cancerous tissues and tissues in the surrounding metastatic lymph nodes of patients with lymph node metastasis, fixed in 4% neutral formalin and embedded in paraffin with a thickness of 3μm, and serially sectioned for immunohistochemical labeling.

Immunohistochemical staining

The specimens to be tested were dewaxed, treated with hydration and immersed in 0.01 mol/L, 95°C citrate buffer, incubated for 10 min in 3% H2O2 environment, dropped into goat serum, incubated for 30 min in 26°C environments, withdrawn from the blocking solution, added primary antibody, incubated overnight in 5°C environments, added secondary antibody the next day, incubated for 0.5 h in 37°C thermostat, washed and DAB color development and blocked.

PD-L1 and PD-1 immunohistochemical determination

(I) The results of all sections were observed, evaluated, and determined by two experienced pathologists using a double-blind method. Positive protein expression was noted when PD-L1 and PD-1 proteins appeared as yellow or brown particles in the cytoplasm/plasm. The semi-quantitative integral method was used to determine the positivity in combination with the proportion of positive cells. Scoring of target cells: no staining was scored as 0, pale yellow as 1, brown as 2 and tan as 3. Percentage of target cells: 25% was scored as 1, 26%~50% as 2, 51%~75% as 3, and ≥76% as 4.

(II) Judgment criteria for PD-L1 and PD-1: the staining intensity and the percentage of target cells were multiplied by two points, of which 0~2 points were recorded as negative and ≥3 points were recorded as positive.

(III) High expression, moderate expression, and low expression were determined based on the number of positive cells detected. Where low expression was recorded as positive cells >20%, medium expression as >40%, and high expression as >65%.

Statistical processing

SPSS 21.0 software was used for data statistics. The measurement data were described by ($\bar{x} \pm s$). The f-value test was performed for multiple group comparisons, and an independent sample t-test was conducted for comparison between groups. The correlations were analyzed by Pearson correlation analysis. $P < 0.05$ was statistically significant.

Results

PD-L1, PD-1 immunohistochemical maps

As shown in Figures 1 and 2, immunohistochemistry for PD-L1 and PD-1 revealed that both proteins were expressed in the target cytoplasm as well as in the cell membrane

Expression of PD-L1 and PD-1 in cancerous and paraneoplastic tissues

As shown in Table 1, the number of positive expression cases of PD-L1 and PD-1 in cancer tissues was significantly higher than the number of expression cases in paraneoplastic tissues, which was statistically different ($P < 0.05$).

PD-L1 and PD-1 expression in survival

As shown in Table 2, the expression rates of PD-L1 and PD-1 in progression-free survival as well as in progressive survival were significantly higher in low expression than in medium and high expression, with statistical differences ($P < 0.05$).

Relationship between PD-L1 and PD-1 protein expression and lymph node metastasis in patients with stage T4 rectal cancer

As shown in Table 3, the number of cases with high PD-L1 and PD-1 protein expression in patients with stage T4 rectal cancer with lymph node metastasis was higher compared with patients without lymph node metastasis, and the difference was statistically significant ($P < 0.05$).

Prognostic relationship between PD-L1 and PD-1 in stage T4 rectal cancer

As shown in Table 4, PD-L1 and PD-1 were closely related to the prognosis of stage T4 rectal cancer, and distant metastasis as well as lymph node metastasis had a greater
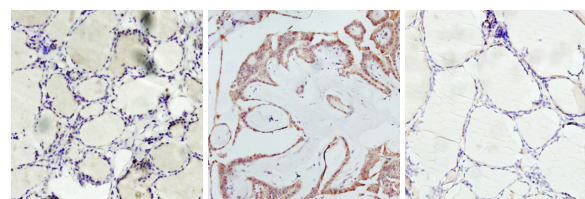
Paraneoplastic tissues   Cancerous tissues   Peripheral metastatic lymph nodes

Figure 1. Immunohistochemical staining of PD-L1 protein expression in cancer tissues, paraneoplastic tissues and surrounding metastatic lymph nodes.
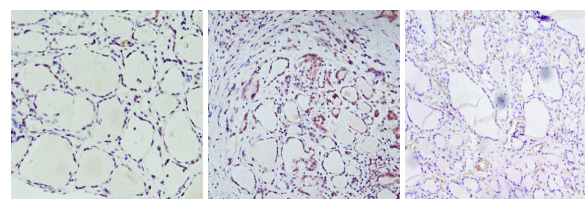
Paraneoplastic tissues   Cancerous tissues   Peripheral metastatic lymph nodes

Figure 2. Immunohistochemical staining of PD-1 protein expression in cancer tissues, paraneoplastic tissues and surrounding metastatic lymph nodes.

Table 1. Expression of PD-L1 and PD-1 in cancer tissues as well as in paraneoplastic tissue [n, %].

Tissue	Number of cases (n)	PD-L1 expression		PD-1 expression	
		Positive	Negative	Positive	Negative
Cancerous tissue	98	82	16	83	15
Paraneoplastic tissue	98	14	84	17	81
X^2		94.406		88.935	
P		0.001		0.001	

Table 2. Comparison of PD-L1 and PD-1 expression rates in survival.

Survival period	PD-L1			PD-1		
	Low	Medium	High	Low	Medium	High
No progression	49 (50.00)	34 (34.69)	15 (15.31)	45 (45.92)	30 (30.61)	23 (23.47)
Progression	50 (51.02)	33 (33.67)	15 (15.31)	48 (48.98)	34 (34.69)	16 (16.33)

Table 3. Relationship between PD-L1 and PD-1 protein expression and lymph node metastasis in patients with stage T4 rectal cancer [n, %].

Lymph node metastasis	Number of cases (n)	PD-L1			PD-1		
		Low expression	Medium expression	High expression	Low expression	Medium expression	High expression
No	41	19	13	9	23	10	8
Yes	57	6	18	33	12	16	29
X^2		2.619			3.491		
P		0.031			0.019		

Table 4. Prognostic relationship between PD-L1 and PD-1 in stage T4 rectal cancer.

Projects	Risk coefficient	95%CI	P
Distant metastasis	1.035	1.002~2.035	0.001
Lymph node metastasis	1.216	1.035~2.103	0.001

impact on PD-L1 and PD-1.

Discussion

As a common type of malignant tumor in the digestive system, rectal cancer ranks fourth among malignant tumors worldwide. The mortality rate is the second highest, with serious implications for both social security and families. The pathogenesis of rectal cancer is complex, and it is of great social value to find effective means of prevention and treatment by exploring its pathogenesis (8).

With the continuous progress and development of medical technology in recent years, new chemotherapeutic drugs as well as molecularly targeted drugs have been used and have achieved better results. Surgery is one of the main options previously used to treat rectal cancer, but the prognosis for patients after surgery is less favorable (9,10). As an important strategy for treating tumors, immunotherapy has a good clinical effect, but the existence of immune tolerance can affect the therapeutic effect of immunotherapy. Clinical investigations have shown that cancer cells protect themselves at the site of cancer by various mechanisms, which are carried out by adjusting the expression of immunosuppressive molecules to evade the body's immune response (11,12). PD-1, an immuno-suppressive molecule that has received more attention in recent years, is expressed in most tumor cells as well as in immune cells (13). It has been pointed out in the study that PD-1/PD-L1 are key negative regulatory molecules

in immune checkpoints and both play important roles in regulating the immune system as well as in the tumor microenvironment. The combination of the two can induce apoptosis or failure of activated T cells, which in turn negatively regulates the immune response, thereby causing tumor cells to evade host immune surveillance, leading to the development of immune escape and ultimately promoting the growth and development of clinical tumors (14,15). In this study, we found that the positive expression rate of PD-1 and PD-L1 in T4 rectal cancer tissues was higher than that in the paraneoplastic tissues, which was basically consistent with the above results, indicating that PD-1 and PD-L1 were closely related to rectal cancer and showed abnormal expression in rectal cancer tissues.

PD-L1 can also be referred to as (B7-H1 or CD274), which is mainly expressed by cells of bone marrow origin. Clinical investigations have shown that PD-L1 can also be expressed when the organism is subjected to IFN-γ and TNF-α after the development of inflammation (16,17). It plays a crucial role in the immune response as an apoptosis-associated protein (18). Clinical investigations have shown that PD-L1 acts as an inducer of the binding of its ligand PD-1 to lymphocytes, which in turn transmits its suppressive signal to T lymphocytes, ultimately serving to regulate immune tolerance in peripheral T cells (19,20). Recent research investigations have revealed that PD-1 is able to be highly expressed in a variety of tumors including non-small cell lung cancer, melanoma, breast cancer, and kidney cancer (21). It can be used to determine the

biological behavior of tumor invasion and metastasis, and it can also be regarded as an important indicator of prognosis (22). In this study, we found that the expression rates of PD-L1 and PD-1 in progression-free survival as well as in progressive survival were significantly higher in low expression than in medium and high expression, and the number of cases of high PD-L1 and PD-1 protein expression was higher in patients with lymph node metastasis T4 stage rectal cancer compared with patients without lymph node metastasis. This result is approximately the same as the above results. Thus, it can be seen that the detection of PD-L1 and PD-1 in rectal cancer can play a better role and can be used as one of the important indicators to judge the prognosis.

In summary, PD-L1 and PD-1 showed aberrant expression in stage T4 rectal cancer tissues and surrounding metastatic lymph nodes. Moreover, PD-L1 and PD-1 were closely related to the prognosis of stage T4 rectal cancer, and distant metastasis and lymph node metastasis had a greater impact on PD-L1 and PD-1.

Acknowledgments

Interest conflict

The authors declare that they have no conflict of interest.

References

1. Nicolas AM, Pesic M, Engel E, Ziegler PK, Diefenhardt M, Kennel KB, Buettner F, Conche C, Petrocelli V, Elwakeel E, Weigert A, Zinoveva A, Fleischmann M, Häupl B, Karakütük C, Bohnenberger H, Mosa MH, Kaderali L, Gaedcke J, Ghadimi M, Rödel F, Arkan MC, Oellerich T, Rödel C, Fokas E, Greten FR. Inflammatory fibroblasts mediate resistance to neoadjuvant therapy in rectal cancer. Cancer Cell. 2022 Feb 14;40(2):168-184.e13. doi: 10.1016/j.ccell.2022.01.004. Epub 2022 Feb 3. PMID: 35120600.

2. Otegbeye EE, Mitchem JB, Park H, Chaudhuri AA, Kim H, Mutch MG, Ciorba MA. Immunity, immunotherapy, and rectal cancer: A clinical and translational science review. Transl Res. 2021 May;231:124-138. doi: 10.1016/j.trsl.2020.12.002. Epub 2020 Dec 8. PMID: 33307273; PMCID: PMC8016725.

3. Guan X, Wei R, Yang R, Lu Z, Liu E, Zhao Z, Chen H, Yang M, Liu Z, Jiang Z, Wang X. Association of Radiotherapy for Rectal Cancer and Second Gynecological Malignant Neoplasms. JAMA Netw Open. 2021 Jan 4;4(1):e2031661. doi: 10.1001/jamanetworkopen.2020.31661. PMID: 33416884; PMCID: PMC7794669.

4. Childs DD, Rocha Lima CMSP, Zhou Y. Mucin-Containing Rectal Cancer: A Review of Unique Imaging, Pathology, and Therapeutic Response Features. Semin Roentgenol. 2021 Apr;56(2):186-200. doi: 10.1053/j.ro.2020.07.010. Epub 2020 Jul 30. PMID: 33858645.

5. Filippone A, Lanza M, Mannino D, Raciti G, Colarossi C, Sciacca D, Cuzzocrea S, Paterniti I. PD1/PD-L1 immune checkpoint as a potential target for preventing brain tumor progression. Cancer Immunol Immunother. 2022 Sep;71(9):2067-2075. doi: 10.1007/s00262-021-03130-z. Epub 2022 Jan 29. PMID: 35092481; PMCID: PMC9374620.

6. Gao Y, Li S, Xu D, Chen S, Cai Y, Jiang W, Zhang X, Sun J, Wang K, Chang B, Wang F, Hong M. Prognostic value of programmed death-1, programmed death-ligand 1, programmed death-ligand 2 expression, and CD8(+) T cell density in primary tumors and metastatic lymph nodes from patients with stage T1-4N+M0 gastric adenocarcinoma. Chin J Cancer. 2017 Jul 29;36(1):61.

7. National Health and Wellness Commission of the People's Republic of China. Chinese treatment norms for colorectal cancer (2020 version). Chin J Surg 2020;58(8):561-585.

8. Glynne-Jones R, Glynne-Jones S. The concept and use of the neoadjuvant rectal score as a composite endpoint in rectal cancer. Lancet Oncol. 2021 Jul;22(7):e314-e326. doi: 10.1016/S1470-2045(21)00053-X. Epub 2021 May 25. PMID: 34048686.

9. Brar MS, de Buck van Overstraeten A, Baxter NN. Rectal Cancer Incidence is Low Following Rectal Diversion or Subtotal Colectomy for IBD: Results of a Population-based Study. J Crohns Colitis. 2021 Nov 8;15(11):1787-1798. doi: 10.1093/ecco-jcc/jjab090. PMID: 34165532.

10. Lam J, Tam MS, Retting RL, McLemore EC. Robotic Versus Laparoscopic Surgery for Rectal Cancer: A Comprehensive Review of Oncological Outcomes. Perm J. 2021 Dec 14;25:21.050. doi: 10.7812/TPP/21.050. Erratum in: Perm J. 2022 Jun 29;26(2):181. PMID: 35348098; PMCID: PMC8782436.

11. Zhang Y, Wu Y, Gong ZY, Ye HD, Zhao XK, Li JY, Zhang XM, Li S, Zhu W, Wang M, Liang GY, Liu Y, Guan X, Zhang DY, Shen B. Distinguishing Rectal Cancer from Colon Cancer Based on the Support Vector Machine Method and RNA-sequencing Data. Curr Med Sci. 2021 Apr;41(2):368-374. doi: 10.1007/s11596-021-2356-8. Epub 2021 Apr 20. PMID: 33877555.

12. Manzo-Merino J, Del-Toro-Arreola S, Rocha-Zavaleta L, Peralta-Zaragoza Ó, Jiménez-Lima R, Madrid-Marina V. IMMUNOLOGY OF CERVICAL CANCER. Rev Invest Clin. 2020;72(4):188-197. doi: 10.24875/RIC.20000057. PMID: 33064686.

13. Dong Y, Sun Q, Zhang X. PD-1 and its ligands are important immune checkpoints in cancer. Oncotarget. 2017 Jan 10;8(2):2171-2186. doi: 10.18632/oncotarget.13895. PMID: 27974689; PMCID: PMC5356790.

14. Peng Q, Qiu X, Zhang Z, Zhang S, Zhang Y, Liang Y, Guo J, Peng H, Chen M, Fu YX, Tang H. PD-L1 on dendritic cells attenuates T cell activation and regulates response to immune checkpoint blockade. Nat Commun. 2020 Sep 24;11(1):4835. doi: 10.1038/s41467-020-18570-x. PMID: 32973173; PMCID: PMC7518441.

15. Gao T, Mao Z, Li W, Pei R. Anti-PD-L1 DNA aptamer antagonizes the interaction of PD-1/PD-L1 with antitumor effect. J Mater Chem B. 2021 Jan 28;9(3):746-756. doi: 10.1039/d0tb01668c. PMID: 33319876.

16. Scandiuzzi L, Ghosh K, Hofmeyer KA, Abadi YM, Lázár-Molnár E, Lin EY, Liu Q, Jeon H, Almo SC, Chen L, Nathenson SG, Zang X. Tissue-expressed B7-H1 critically controls intestinal inflammation. Cell Rep. 2014 Feb 27;6(4):625-32. doi: 10.1016/j.celrep.2014.01.020. Epub 2014 Feb 13. PMID: 24529703; PMCID: PMC3962725.

17. Jin P, Zhao Y, Liu H, Chen J, Ren J, Jin J, Bedognetti D, Liu S, Wang E, Marincola F, Stroncek D. Interferon-γ and Tumor Necrosis Factor-α Polarize Bone Marrow Stromal Cells Uniformly to a Th1 Phenotype. Sci Rep. 2016 May 23;6:26345. doi: 10.1038/srep26345. PMID: 27211104; PMCID: PMC4876328.

18 Mukherjee S, Biswas T. Activation of TOLLIP by porin prevents TLR2-associated IFN-γ and TNF-α-induced apoptosis of intestinal epithelial cells. Cell Signal. 2014 Dec;26(12):2674-82. doi: 10.1016/j.cellsig.2014.08.009. Epub 2014 Aug 22. PMID: 25152369.

19. Kuol N, Stojanovska L, Nurgali K, Apostolopoulos V. PD-1/PD-L1 in disease. Immunotherapy. 2018 Feb;10(2):149-160. doi: 10.2217/imt-2017-0120. PMID: 29260623.

20. Arrieta O, Montes-Servín E, Hernandez-Martinez JM, Cardona AF, Casas-Ruiz E, Crispín JC, Motola D, Flores-Estrada D, Barrera L. Expression of PD-1/PD-L1 and PD-L2 in peripheral T-

cells from non-small cell lung cancer patients. Oncotarget. 2017 Oct 24;8(60):101994-102005. doi: 10.18632/oncotarget.22025. PMID: 29254220; PMCID: PMC5731930.

21. Maekawa N, Konnai S, Okagawa T, Nishimori A, Ikebuchi R, Izumi Y, Takagi S, Kagawa Y, Nakajima C, Suzuki Y, Kato Y, Murata S, Ohashi K. Immunohistochemical Analysis of PD-L1 Expression in Canine Malignant Cancers and PD-1 Expression on Lymphocytes in Canine Oral Melanoma. PLoS One. 2016 Jun 8;11(6):e0157176. doi: 10.1371/journal.pone.0157176. PMID: 27276060; PMCID: PMC4898770.

22. Saigusa S, Toiyama Y, Tanaka K, Inoue Y, Mori K, Ide S, Imaoka H, Kawamura M, Mohri Y, Kusunoki M. Implication of programmed cell death ligand 1 expression in tumor recurrence and prognosis in rectal cancer with neoadjuvant chemoradiotherapy. Int J Clin Oncol. 2016 Oct;21(5):946-952. doi: 10.1007/s10147-016-0962-4. Epub 2016 Feb 26. PMID: 26919982.