Pathology & Oncology Research (2020) 26:861–865
https://doi.org/10.1007/s12253-019-00629-w

ORIGINAL ARTICLE

# TRIM72 Immunohistochemical Expression Can Predict Relapse in Colorectal Carcinoma

M. J. Fernández-Aceñero[1] · M. Cruz[2] · J. Sastre-Varela[3] · J. I. Casal[4] · M. A. Cerón Nieto[1] · L. del Puerto-Nevado[2] · J. García-Foncillas[2] · A. Cebrián[2]

Received: 8 October 2018 / Accepted: 1 March 2019 / Published online: 9 March 2019


## Abstract

Large bowel adenocarcinoma is one of the most frequent human neoplasms and despite recent insights into the pathophysiology and molecular basis of this disease, mortality remains high in advanced and metastatic cases. Most guidelines recommend adjuvant chemotherapy for tumours involving lymph nodes, but not for patients with localized stage I or II disease. However, it is well known that approximately 20% of stage II colorectal carcinoma patients eventually recur, mainly with distant or peritoneal involvement and show bad prognosis. It would be important to predict which patients are at increased risk of recurrence to guide potential adjuvant therapy use in this controversial setting. In this sense, only microsatellite stability has been proposed as a predictive tool in some guidelines. The tripartite motif family protein 72 (TRIM72) is a ubiquitin ligase, involved in the cell membrane repair machinery and known to be associated to insulin resistance. Its potential role in colon cancer has recently been proposed. The aim of this study is to determine the potential predictive value of TRIM72 immunohistochemical expression in stage II colon carcinoma. We have retrospectively reviewed a series of 95 patients with stage II colon microsatellite stable carcinomas operated with a curative intent at a single large tertiary hospital in Madrid (Spain) between 2006 and 2012. None of the patients received adjuvant chemotherapy. We reviewed the histopathological slides and constructed a tissue microarray (TMA) of three representative areas to perform immunohistochemical staining for TRIM72. In our series 30 patients (31.7%) recurred after a median follow-up of 17.5 months. Lack of immunohistochemical expression of TRIM72 in the tumor was significantly and independently associated to recurrence. A recent report by Chen et al. has shown that TRIM72 can be measured in plasma for colon carcinoma detection as an alternative to CEA or CA19.9, with lower levels in patients with carcinoma. Our report is the first one to show that lower immunohistochemical expression of TRIM72 predicts recurrence in colon stage II carcinoma. We feel this predictive influence can be related to its crucial role as a central regulator in many signaling pathways (PI3K-AKT, ERK). As an ubiquitin ligase, the lack of TRIM72 could increase the levels of several potential oncogenic molecules and therefore lead to a more aggressive phenotype. It remains to be shown whether chemotherapy could change the clinical behaviour of this bad prognosis group. We propose TRIM72 immunohistochemical analysis as a potential tool to predict recurrence risk in stage II colon carcinoma patients. Our results should be confirmed in larger series, but could open the way to management strategies refinement in this early stage group of patients.

**Keywords** Colon carcinoma · Chemotherapy · Early stage disease · Ubiquitin ligase · TRIM72 · Recurrence · Immunohistochemistry

✉ M. J. Fernández-Aceñero
mgg10167@gmail.com

[1] Department of Surgical Pathology, Hospital Clínico San Carlos, Avda Profesor Martín Lagos s, /n 28040 Madrid, Spain

[2] Translational Investigation, Hospital Fundación Jiménez Díaz, Madrid, Spain

[3] Departments of Oncology, Hospital Clínico San Carlos, Madrid, Spain

[4] Centro de Investigaciones Biológicas (CIB), Madrid, Spain

## Introduction

Large bowel carcinoma is one of the leading causes of cancer related death all over the world. [1] Recent insights into the pathophysiology of disease have led to a refinement of therapies, that has improved survival, even in advanced metastatic neoplasms. Knowledge of the molecular changes associated to this neoplasm allows selection of patients for targeted therapies with anti-EGF or anti-VEGF drugs with the subsequent increase in long term survival. [2]

Surgery remains the mainstay of therapy for early stage disease and recent reports have noted better prognosis for stage I and II diseases, with recurrence rates under 10 and 20%, respectively. [3] Overall, adjuvant therapy is not indicated in these patients. Therefore it is essential to identify potential predictive factors for early-stage disease, that can allow more aggressive therapy in selected high risk patients to improve prognosis. [4, 5]

On the other hand, the tripartite motif family protein 72 (TRIM72) or mitsugumin 53 is one molecule belonging to the tripartite motif family, traditionally involved in myocardiocyte maintenance and preconditioning. [6] Recently, some studies have suggested that the ubiquitin ligase activity of TRIM72 can relate this molecule to insulin resistance and metabolic syndrome, well known risk factors for the development of colon carcinoma. [7] Few reports have analyzed this issue so far, but a recent one by Chen et al. has proposed the potential use of TRIM72 serum levels as a sensitive marker for colon carcinoma diagnosis, as it is significantly lower in patients with disease as compared to controls. [8] Although Chen et al. have also shown a correlation between TRIM72 immunohistochemical expression and stage of colorectal carcinoma, to the best of our knowledge no study has analyzed so far the potential prognostic value of TRIM72 in colorectal carcinoma.

The aim of the present study is to analyze whether TRIM72 immunohistochemical expression can be used to predict recurrence in stage II colon carcinoma.

## Material and Methods

This is a retrospective series of patients with stage II colon carcinoma operated with a curative intent and R0 resection at a single large tertiary center in Madrid (Spain) between 2006 and 2010. Following standard guidelines, no patient received adjuvant therapy. To select patients we have retrospectively reviewed the computerized files of the Surgical Pathology Department and selected stage II intestinal type colon carcinomas. Patients were only enrolled in the study after confirming preserved expression of the proteins encoded by DNA mismatch repair genes in the archival paraffin-embedded tissue with immunohistochemistry (low risk of microsatellite instability [MSI]). We have also collected demographic data (gender, age) and also data related to the tumour (location, size, T stage, number of lymph nodes dissected in the resection specimen, focal mucinous differentiation, vascular invasion, perineural infiltration and grade). To measure outcome we chose the disease free survival (DFS) defined as the time elapsed between surgery and recurrence of disease in months.

A trained pathologist selected formalin-fixed paraffin-embedded (FFPE) tissue samples from the patients and used them for Tissue Microarray (TMA) construction. Cases in which we could not retrieve adequate material for histological analysis were excluded. The TMAs were assembled from triplicate 0.6 mm cores of FFPE biopsy tumour samples using a TMA workstation MTA-1 (Beecher Instruments). Antigen retrieval for immunohistochemistry was performed in PT-Link (Dako, Denmark) for 20 min at 95 °C in high pH buffered solution (Dako). Endogenous peroxidase was blocked, by immersing the sections in 0.03% hydrogen peroxide for 5 min. Slides were washed for 5 min with Tris buffered saline solution containing Tween 20 at pH 7.6 and incubated with the primary antibodies anti-TRIM72 (Novus) overnight at room temperature, followed by incubation with the appropriate anti-Ig horseradish peroxidase-conjugated polymer (EnVision, Dako) to detect antigen-antibody. Sections were then visualized with 3,3′-diaminobenzidine as a chromogen for 5 min and counter-stained with hematoxylin. TRIM72 was expressed in the cytoplasm of the tumour cells and expression was scored independently by two pathologists blinded to the outcome of the patients. In all the cases the intensity of the immunohistochemical expression was graded as weak, moderate or intense (1, 2 or 3, respectively) and this value was multiplied by the percentage of cells to obtain a z score with values ranging from 0 to 300. We performed a receiver-operator characteristic curve to define the best cut-off point. With this approach we chose 40 to divide cases with low TRIM72 expression ($\leq 40$) or high expression ($> 40$) for further analysis (Fig. 1).

Statistical analysis was performed with SPSS for Windows 20.0 statistical package (IBM corporation). Association between TRIM72 expression and clinicopathological and outcome variables were evaluated by chi-squared (or Fisher's exact test) or Student's t test for mean comparison, as adequate. For survival analysis we compared the Kaplan-Meier curves with the log-rank test. We also performed Cox's multivariate survival analysis. The level of statistical significance for all the tests was defined as a $p$ value less than 0.05.

Permission for this study was obtained from the Ethical Committee on Scientific Investigation of Fundación Jiménez Díaz. This study is in accordance with national regulations regarding personal data protection.

## Results

Overall 95 patients fulfilled inclusion criteria for the study. 57 patients were male and the mean age was 73.03 (51–94). Immunohistochemical expression of TRIM72 was analysed in 91 cases, for in the other 4 no representative tumor was present in the definitive TMA slides. 32 cases (35.1%) were negative according to the aforementioned cut-off values.

Table 1 summarizes the general characteristics of our series, according to the expression of TRIM72. As the Table shows, recurrence was significantly associated to

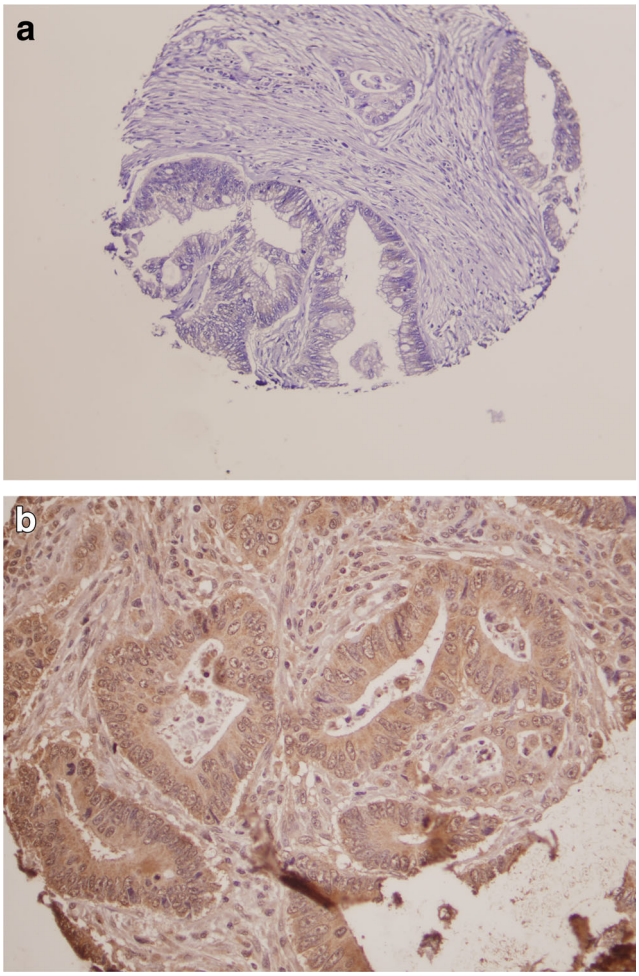


**Fig. 1** Immunohistochemical expression of TRIM72 in colon carcinoma. **a** Low expression (IHC for TRIM72, ×400); **b** High expression (IHC for TRIM72, × 400)

TRIM72 expression, for 84.3% of the patients with low TRIM72 expression in the tumour tissue recurred as opposed to 57.6% of the patients with high TRIM72 expression.

We analyzed which factors influenced recurrence and we only found that tumours with focal mucinous differentiation were significantly more aggressive with higher recurrence rates ($p = 0.03$), as also left-side tumours ($p = 0.01$). With a $p$ value = 0.1 poorly differentiated tumours showed a tendency towards more frequent recurrences. No other histopathological features (size, T stage, number of lymph nodes, vessel invasion or perineural infiltration) were shown to be associated to recurrence in this series.

We also performed a survival univariate analysis with Kaplan-Meier plots and compared them with the log rank test. Figure 2 shows the statistically significant differences between TRIM72 negative and positive lesions ($p = 0.01$).

To confirm the prognostic value of TRIM72 we performed Cox's survival multivariate analysis. TRIM72 turned out to be the only independent prognosticator of DFS with a HR 3.8 (95% CI 1.4–10.6) (p = 0.01).

## Discussion

Large bowel carcinoma is one of the most frequent malignant neoplasms with a high morbidity and mortality. Recent changes in management, mainly with more aggressive surgical therapy for advanced metastatic cases and the recent use of molecular abnormalities to guide therapy have led to a dramatic improvement in prognosis. [2] A recent population-based series from Sweden with more than 14,000 patients has shown less than 20% of recurrences for stage II disease, [3] far from the classic 30% recurrence rate described in the older literature.

Surgical resection with free margins remains the best therapeutic option for early stage carcinomas. Adjuvant therapy is not indicated in early stage lymph-node negative colon carcinoma, but it is a well-known fact that patients with disease recurrence show bad prognosis. Accordingly, there is a growing interest in defining potential prognostic factor for this selected group of patients that could guide further therapy after R0 resection. All kinds of factors have been explored. For instance, Jørgensen et al. have recently reported the results of a large randomized trial showing that more frequent

**Table 1** Characteristics of patients according to TRIM72 expression

| Characteristic | Low TRIM72 expression ($n = 32$) | High TRIM72 expression ($n = 59$) | $p$ value |
|---|---|---|---|
| Age | 71.06 (51–94) | 73.76 (54–88) | 0.2 |
| Gender | Male: 22 (68.8%) | 32 (54.2%) | 0.17 |
| | Female: 10 (31.2%) | 27 (45.8%) | |
| Mucinous | No: 28 (87.5%) | 54 (91.5%) | 0.5 |
| | Yes: 4 (12.5%) | 5 (8.5%) | |
| Location | Right colon: 17 (53.3%) | 28 (55.5%) | 0.6 |
| | Left colon: 15 (46.7%) | 31 (44.5%) | |
| Grade | Well differentiated: 4 (12.5%) | 14 (23.7%) | 0.1 |
| | Moderately differentiated: 23 (71.9%) | 42 (71.2%) | |
| | Poorly differentiated: 5 (15.6%) | 3 (5.1%) | |
| T stage | T2: 10 (31.3%) | 20 (33.9%) | 0.4 |
| | T3: 21 (65.6%) | 39 (66.1%) | |
| | T4: 1 (3.1%) | | |
| Lymph node number | 16 (9–25) | 18 (10–24) | 0.2 |
| Recurrence | No: 5 (15.6%) | 25 (42.4%) | 0.01 |
| | Yes: 27 (84.4%) | 34 (57.6%) | |

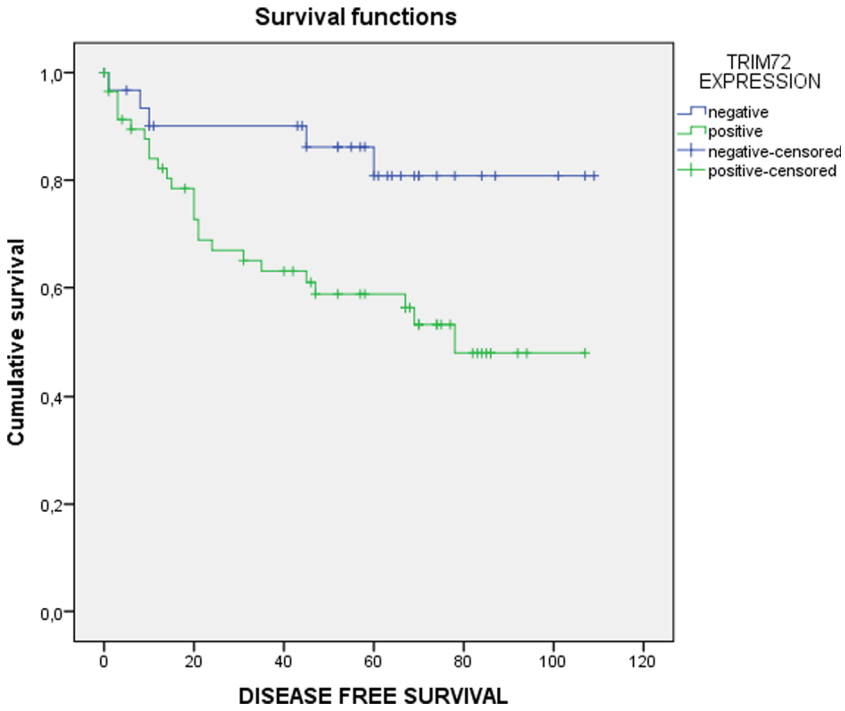


**Fig. 2** Kaplan-Meier plots comparing DFS in TRIM72 positive and negative cases

follow-up does not significantly influence prognosis. [9] As for histopathological factor, several reports, mainly retrospective series from one single hospital, have described that mucinous histology, [10] tumor budding and differentiation [11] or tumour diameter [6] can significantly influence prognosis. Other authors have centered their efforts in identifying potential molecular targets, like syalil Lewis$^x$ (high expression is associated to bad prognosis), [12] ANO9 (low level is suggestive of bad prognosis) [13] or up-regulation of transcription factor 3 (TCF3). [14] Nevertheless most of these results have not been confirmed in larger prospective series so far and at the moment the NCCN guidelines only recommend further therapy after surgery as an option for microsatellite stable cases. [15] A recent metaanalysis of 2596 studies on this topic confirmed the prognostic influence of several clinicopathological parameters, but did not support a role for adjuvant chemotherapy, as DFS was lower in the adjuvant setting than in surgically resected cases with follow-up and not further therapy (five year DFS 79.3% with adjuvant chemotherapy vs. 81.4% without adjuvant therapy). [5]

TRIM72 or mitsugumin 53 (MG53) is one of the member of the tripartite motif family and it has long been considered part of the cell membrane repair system, but it is also involved in the maintenance of myocardial cells and linked to several signalling pathways. [6] Recent reports have also suggested TRIM can induce insulin resistance and metabolic syndrome due to its E3 ligase activity. [7] It is well known that metabolic syndrome is associated to some human neoplasms and this led some authors to hypothesize that TRIM can be involved in oncogenesis through its ubiquitin ligase function. [7] Up to date very few reports have analysed the potential role of TRIM72 expression in human large bowel carcinoma. Only the recent report by Chen et al. [8] has shown that TRIM72 levels were significantly lower in colon cancer tissues than in normal tissues and also confirmed that low serum levels of this protein can serve better than CEA or CA19.9 to identify patients with colon carcinoma. Although in their series they have also shown that disease progression is associated to a reduction of TRIM72 levels, no previous report to date has analysed the potential prognostic use of this marker.

Our results clearly show that low levels of TRIM72 are associated to a worse prognosis in stage II colon carcinoma. We can attribute this finding to the ubiquitin ligase activity of TRIM72 and its relation with several signalling pathways involved in oncogenesis. The lack of TRIM72 could secondarily increase the levels of other oncogenic products (FAK, ERK, etc), that would not be normally ubiquitinated and degraded. It is still early to conclude that TRIM72 expression can be used to predict behaviour of the tumour and used as a potential criterium for adjuvant therapy, but we feel our results are promising and should be confirmed in a larger series and then used in a clinical trial to confirm whether therapy can influence this worse outcome.

In summary, we herein report the first clinical series analysing the potential prognostic influence of the ubiquitin ligase TRIM72 in colon carcinoma. Our results could open the way to further studies to better select patients with early-stage disease for further therapies.

**Acknowledgments** This report is in accordance with Ethical standards and has been approved by the Ethical Committee for Research of Hospital Fundación Jiménez Díaz.

**Author's Contribution** MJFA has selected the representative areas of the tumour, reviewed the histopathological slides, reviewed the literature and written the text.

MC has constructed the TMA and performed immunostains.

JSV has contributed to patient selection and demographic information.

JIC has contributed to the TMA construction and has read and reviewed the manuscript.

MACN has cut the slides and performed immunohistochemical staining.

LPN has contributed to immunohistochemical staining and data interpretation.

JGF has critically read and reviewed the manuscript.

AC has contributed to literature review, manuscript elaboration and manuscript writing.

All authors have read and approved the final version of the manuscript.

## Compliance with Ethical Standards

**Conflict of Interest** The authors acknowledge no conflict of interest for the present study.



## References

1. Siegel RL, Miller KD, Jemal A (2016) Cancer statistics 2016. CA Cancer J Clin 66:7–30
2. Shui L, Wu YS, Lin H, Shui P, Sun Q, Chen X (2018) Triplet chemotherapy (FOLFOXIRI) plus bevacizumab versus doublet chemotherapy (FOLFOX/FOLFIRI) plus bevacizumab in conversion therapy for metastatic colorectal Cancer: a meta-analysis. Cell Physiol Biochem 48:1870–1881
3. Osterman E, Glimelius B (2018) Recurrence risk after up-to-date Colon Cancer staging, surgery, and pathology: analysis of the entire Swedish population. Dis *Colon rectum* 61:1016–1025
4. Böckelman C, Engelmann BE, Kaprio T, Hansen TF, Glimelius B (2015) Risk of recurrence in patients with colon cancer stage II and III: a systematic review and meta-analysis of recent literature. Acta Oncol 54:5–16
5. Maeda C, Hidaka E, Mori Y, Mukai S, Miyachi H, Sawada N (2015) Tumor diameter is an easy and useful predictor of recurrence in stage II colorectal Cancer. Dig Surg 32:338–343
6. Lemekert FA, Bournazos A, Eckert DM, Kenzler M, Hawkes JM, Butler TL et al (2016) Lack of MG53 in human heart precludes utility as a biomarker of myocardial injury or endogenous cardioprotective factor. Cardiovasc Res 110:178–187
7. Liu L, Wong CC, Gong B, Yu J (2017) Functional significance and therapeutic implication of ring-type E3 ligases in colorectal cancer. Oncogene 37:148–159
8. Chen Z, Yin X, Li K, Chen S, Li H, Li Y, Zhang Q, Wang H, Qiu Y (2018) Serum levels of TRIM72 are lower among patients with colon cancer: identification of a potential diagnostic marker. Tokohu J Exp Med 245:61–68
9. Wille-Jørgensen P, Syk I, Smedh K, Laurberg S, Nielsen DT, Petersen SH, Renehan AG, Horváth-Puhó E, Påhlman L, Sørensen HT, For the COLOFOL Study Group (2018) Effect of more vs less frequent follow-up testing on overall and colorectal Cancer-specific mortality in patients with stage II or III colorectal Cancer: the COLOFOL randomized clinical trial. JAMA 319: 2095–2103
10. Hu X, Li YQ, Li QG, Ma YL, Peng JJ, Cai S (2018) Mucinous adenocarcinomas Histotype can also be a high-risk factor for stage II colorectal Cancer patients. Cell Physiol Biochem 47:630–640
11. Lee VWK, Chan KF (2018) Tumor budding and poorly-differentiated cluster in prognostication in stage II colon cancer. Pathol Res Pract 214:402–407
12. Yamadera M, Shinto E, Tsuda H, Kajiwara Y, Naito Y, Hase K, Yamamoto J, Ueno H (2018) Sialyl Lewisx expression at the invasive front as a predictive marker of liver recurrence in stage IIcolorectal cancer. Oncol Lett 15:221–228
13. Li C, Cai S, Wang X, Jiang Z (2015) Identification and characterization of ANO9 in stage II and III colorectal carcinoma. Oncotarget 6:29324–29334
14. Li C, Cai S, Wang X, Jiang Z (2014) Hypomethylation-associated up-regulation of TCF3 expression and recurrence in stage II and III colorectal cancer. PLoS One 9(11):e112005
15. NCCN Guidelines Version 3.2018 Colon Cancer. Copyright National Comprehensive Cancer Network Inc. Last accessed online 9/20/2018