Arch Biol Sci. 2025;77(2):147-157 https://doi.org/10.2298/ABS250114012I

The role of ADAM10 in the prediction of neoadjuvant chemoradiotherapy response in patients with locally advanced rectal cancer

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Received: January 14, 2025; **Revised**: March 4, 2025; **Accepted**: March 18, 2025; **Published online**: May 16, 2025

Abstract: Protease ADAM10, a member of the A disintegrin and metalloproteinase protein family, plays a role in cytokine/growth factor release, the shedding of receptor molecules from the membrane, and intracellular signaling. ADAM10 is implicated in colorectal cancer development and progression and has been identified as a potential predictive biomarker in this disease. This study evaluates *ADAM10* expression in tumor and non-tumor tissue and ADAM10 serum concentrations in patients with locally advanced rectal cancer (LARC) treated with neoadjuvant chemoradiotherapy (nCRT). The study included samples taken before nCRT from 23 histopathologically confirmed LARC patients. Expression of *ADAM10* was assessed using RT-qPCR, while ELISA measured ADAM10 concentrations in the serum, and the correlation of the results with nCRT response was evaluated. We found statistically significantly higher expression of *ADAM10* in tumor tissue compared to healthy tissue in 83% of LARC patients. Serum concentrations of ADAM10 varied widely (66.6-1119.1 pg/mL) and did not correlate with tissue expression levels. Neither the tissue expression level nor ADAM10 serum concentrations predicted the response to nCRT. Our results confirm the involvement of ADAM10 in rectal cancer initiation and warrant further research on ADAM10 substrates, signaling pathways involved in its activity, and its potential as a therapeutic target.

Keywords: ADAM10, locally advanced rectal cancer, LARC, nCRT, RT-qPCR, ELISA.

Abbreviations: ACTB – β-actin; ADAM – A disintegrin and metalloproteinase; cCR – complete clinical response; CRC – colorectal cancer; LARC – locally advanced rectal cancer; nCRT – neoadjuvant chemoradiotherapy; pCR – pathologic complete response; RCRG – rectal cancer regression grade; RTK – receptor tyrosine kinase; W&W – watch-and-wait approach

INTRODUCTION

The A disintegrin metalloproteinase (ADAM) protein family is represented by 21 transmembrane multidomain proteins, with 13 having proteolytic activity and belonging to zinc-proteases [1]. They irreversibly cleave the extracellular domains of transmembrane proteins, converting them into soluble forms through a process known as "ectodomain shedding." ADAM10 is the first disintegrin-metalloproteinase with confirmed proteolytic activity [2,3], and, together with ADAM17, it is the most studied enzyme from the family. In recent years, research has elucidated the roles of proteolytically active ADAM9, ADAM12, ADAM17, ADAM22, and ADAM28 [4-8].

The proteolytical activity of ADAMs can be constitutive or stimulated by different extracellular signals, including growth factors, cytokines, reactive oxygen species, and inflammatory signals. As such ADAMs play a key role in the cellular response to changes in the extracellular compartment [9]. The substrates of ADAM proteases are diverse, and ADAMs are involved in cytokine/growth factor release, the shedding of

receptor molecules from the membrane, and intracellular signal transduction via limited proteolysis followed by regulated intramembrane proteolysis [10]. The important roles of ADAMs in the context of human disease development are currently being investigated, including their involvement in the triggering of systemic inflammation and sepsis due to the release of TNF-α and soluble IL-6 receptor [11], their role in kidney inflammation and disease [12], and their contribution to the pathophysiology of autoimmune diseases such as pemphigus [13]. On the other hand, several studies have investigated the potential protective role of ADAMs in neurodegenerative diseases, primarily Alzheimer's disease [14]. Given the importance of ADAMs in cell proliferation, adhesion, and migration, as well as their regulation of the bioavailability of growth factors (such as IGF, EGFR, and TGF-α), their role in carcinogenesis is being actively researched. This includes investigating their involvement in cancer treatment, monitoring, and resistance. In addition, the downstream effects of ectodomain shedding can either downregulate receptor activity or have a dysregulated activation effect. Known receptor substrates of the ADAMs are the Notch receptors, TGF-β receptors, receptor tyrosine kinases (RTK) such as HER2, HER4 and VEGFR2, MET, and the TAM-family RTKs [15].

Different members of the ADAM family are expressed in the gastrointestinal system and have a significant role in intestinal development and homeostasis, especially in signaling between intestinal epithelial cells and cells in the lamina propria, such as endothelial cells, myofibroblasts, and immune cells. The dysregulation of the crosstalk between these cells through aberrant or inadequate ADAM function can be associated with chronic inflammation, inflammation-associated cancer, and tumorigenesis [16]. *In vitro* studies suggest that ADAM9 and ADAM10 overexpression can promote tumor metastasis [17,18], while genetic deficiency of *ADAM10* reduces colon adenoma in mice [9]. There are reports that ADAM overexpression in colorectal cancer (CRC) correlates with disease progression and poor prognosis [19,20]; the expression levels of *ADAM17* in CRC tissue samples may be used as a prognostic marker in advanced stages of the disease [21]. Another study found that ADAM28 was overexpressed in both tumor tissue and histopathologically confirmed normal tissue surrounding the tumor in overweight and obese CRC patients [22]. The levels of ADAMs in the sera of patients with CRC were described in one preliminary study, suggesting that the concentrations of ADAM10 and ADAM28 correlate with the histopathological grade of cancer and the presence of distant metastases [23].

There is growing evidence of the inadequacy of the term colorectal cancer (CRC) due to accumulating knowledge confirming that colon and rectal cancer are two distinct entities [24]. Colon and rectal cancer rank third among the leading cancer types for estimated new cancer cases and deaths in both sexes in the USA in 2024 [25]. Consequently, they have attracted significant attention from the perspective of therapeutic strategy. The standard of care for patients with locally advanced rectal cancer (LARC), defined as stage II (T3-4, node-negative) or stage III (node-positive) of the disease, currently consists of neoadjuvant chemoradiotherapy (nCRT) or total neoadjuvant treatment (TNT), followed by total mesorectal surgery. This approach raises significant concerns about the quality of patients' lives [26]. The degrees of response to nCRT vary, from complete regression to further growth, and can be evaluated clinically (clinical complete response – cCR) or by pathohistological assessment (pathologic complete response – pCR). pCR is defined as the absence of cancer in tissue samples post-treatment and was reported in 10.3% of LARC patients following nCRT [27]. It can only be achieved after rectal resection. To assess cCR without surgery, the response to nCRT is evaluated by digital rectal examination, rectoscopy (with or without biopsy), and radiological methods (including CT, MRI, and/or PET/CT). cCR refers to the disappearance of the rectal tumor with a virtual absence of viable malignant cells and is used as a parameter when the watch-and-wait (W&W) approach is considered as a therapeutic strategy. The W&W approach offers a noninvasive alternative to radical surgery, aiming for organ preservation and reduced morbidity in LARC patients with cCR after nCRT [28]. Several studies of the W&W approach have been conducted, each with unique criteria and follow-up protocols; however, no international expert consensus exists on the definition of cCR that is essential for the proper selection of LARC patients for W&W, and thus the search for new biomarkers continues. A recent pilot proteomic study has identified ADAM10 and CAD (involved in pyrimidine nucleotide biosynthesis) as

potential biomarkers of resistance to nCRT in LARC patients who had undergone surgical treatment [29].

Here, we report on ADAM10 expression levels in tumor and surrounding tissue, as well as serum ADAM10 levels in LARC patients who underwent nCRT, aiming to better understand its role in cancer tumorigenesis and its potential as a biomarker.

MATERIALS AND METHODS

Ethics statement

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine, University of Belgrade, Approval No.1550/V-2, which follows the Declaration of Helsinki Ethical Guidelines. Informed consent was obtained from all participants.

Study participants

This study included 23 LARC patients diagnosed and treated at the Clinic for Digestive Surgery, University Clinical Center of Serbia, between April 2019 and October 2020. Demographic data (age and sex) and tumor marker CEA and CA19-9 levels were obtained. All patients underwent a diagnostic biopsy, during which samples of primary tumor tissue and adjacent healthy mucosa were collected. The diagnosis of adenocarcinoma was confirmed by histopathology in all patients, and tumor staging was determined according to the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging criteria. The absence of distant metastases was confirmed using computed tomography (CT) of the thorax and CT or magnetic resonance imaging (MRI) of the abdomen and pelvis. Sera samples were collected from patients at the time of diagnosis, and appropriately processed and stored at -80°C.

Upon confirmation of diagnosis, all patients underwent nCRT, which consisted of a total radiation dose of 50.4 Gy delivered in 28 fractions, combined with two or three cycles of chemotherapy (5-fluorouracil 425 mg/m² and leucovorin 20 mg/m²). Eight to 12 weeks after nCRT, patients underwent surgical resection of the primary tumor. The pathological response to the nCRT was estimated according to the Rectal Cancer Regression Grade (RCRG).

Analysis of *ADAM10* expression in tumor and non-tumor tissue samples

TRIzol reagent (Thermo Fisher Scientific, USA) was used to extract total RNA from collected tissue samples according to the manufacturer's instructions. The Ultrospec 3300 Pro spectrophotometer (Amersham Biosciences, UK) was used to estimate the total RNA concentration and purity by measuring the absorbance at 260 and 280 nm. The High-Capacity cDNA Kit (Applied Biosystems, USA) was used to synthesize the cDNA from 1 μg of total RNA in a 20 μL reaction according to the manufacturer's instructions. The reaction conditions were 10 min at 25°C, 120 min at 37°C, and 5 min at 85°C.

The expression of the *ADAM10* gene was analyzed by quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR). The reactions were carried out using SYBR Green in a 7900 HT Fast Real-Time PCR system (Applied Biosystems, USA). The specific primers used for RT-qPCR amplification of *ADAM10* were as follows: forward 5'-CTCTGATCATGCTAATGGCTGGA-3'; reverse 5'-GCTGCAGTTAGCGTCTCATGTGT-3'. The amplification of the β-actin (*ACTB*) gene used the following primers: forward 5'-GGACTTCGAGCAAGAGATGG-3'; reverse 5'-AGGAAGGAAGGCTGGAAGAG-3', serving as an internal control.

The reaction mixtures contained 25 ng cDNK, 1 × Power SYBR® Green PCR Master Mix (Applied Biosystems, USA), and 4 pmol of each gene-specific primer in a final reaction volume of 10 μl. The cycling conditions were as follows: denaturation of the template at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and at 60°C for 1 min.

Each RT-qPCR reaction was performed in triplicate, and melting curve analysis validated the specificity of the products. Relative expression of *ADAM10* mRNA was normalized to the expression of the *ACTB* gene and calculated by applying the 2^{-dCt} method.

Analysis of ADAM10 levels in serum

The serum ADAM10 levels in patients were measured using a sandwich ELISA with the Human ADAM10 (A Disintegrin and Metalloprotease 10) ELISA Kit (Elabscience, USA). The ELISA plates are pre-coated with an antibody specific to human ADAM10; a biotinylated antibody serves as the detection antibody, while avidin-horseradish peroxidase catalyzes the reaction with substrate in which a blue color is produced. Upon addition of the stop solution, the color turns yellow, and the intensity of the color was measured at 450 nm. All samples were analyzed in duplicate, and the concentrations were determined by comparing the values with standards using a four-parameter logistic curve in GraphPad Prism according to the manufacturer's instructions.

ADAM10 expression analysis in rectal cancer using The Cancer Genomic Atlas

ADAM10 expression was evaluated using The University of California Santa Cruz (UCSC) Xena platform (http://xena.ucsc.edu/), in rectal adenocarcinoma (READ) compared to normal tissue, utilizing data from the Genomic Data Commons – The Cancer Genome Atlas (GDC TCGA) database [30]. This database has data for 187 samples. The samples were filtered for the available *ADAM10* expression data and stage of the disease, with 166 samples left for the analyses. Of these, 156 samples were primary rectal tumors and 10 were non-tumor solid tissue. There were 9 matched samples of primary tumors and normal tissues. When the data was filtered for LARC (stages II and III), there were 106 samples of which 102 were tumor tissue and only 4 were non-tumor tissue. There were 4 matched samples of primary tumors and normal tissue for LARC cohort of patients. The *ADAM10* expression data was downloaded from the UCSC Xena platform for further statistical analysis in GraphPad Prism software.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous data are presented as the means (standard deviation, SD) or median (minimum and maximum values), while categorical data are reported as percentages (%). The Shapiro-Wilk test was used to assess the normality of continuous data. The Kruskal-Wallis test was used to analyze the differences between independent data. The related samples Wilcoxon signed-rank test was used to analyze the differences between matched data. The non-parametric Spearman rank correlation coefficient was used to examine the degree of association between the data.

RESULTS

Patients

This study included a group of 23 LARC patients treated with nCRT. The patients' demographic and clinical characteristics are given in Table 1.

ADAM10 tissue expression

In tissue samples, the relative expression of *ADAM10* gene was measured by RT-qPCR. We observed a statistically significant difference in *ADAM10* expression between tumor and corresponding non-tumor tissue samples (P=0.04). The expression of *ADAM10* was increased in tumor tissues compared to non-tumor tissues in 83.3% of cases (Fig. 1).

Table 1. Characteristics of the study group

	LARC patients (n=23)
Age (years), median (range)	67 (34-83)
Males, n (%)	15 (65.2)
CEA (IU/mL), median (range)	3.3 (0.6-218.3)
CA 19-9 (IU/mL), median (range)	10.0 (2.0-30.0)
Stage at diagnosis	
T stadium, n (%)	
T2	1 (4.3)
T3	15 (65.3)
T4	7 (30.4)
N stadium, n (%)	
N1	4 (17.4)
N2	19 (82.6)
Response to nCRT, n (%)	
RCRG1	4 (17.4)
RCRG2	10 (43.5)
RCRG3	9 (39.1)

LARC – locally advanced rectal cancer; SD – standard deviation; CEA – carcinoembryonic antigen; CA 19-9 – carbohydrate antigen 19-9; nCRT – neoadjuvant chemoradiotherapy; RCRG – rectal cancer regression grade

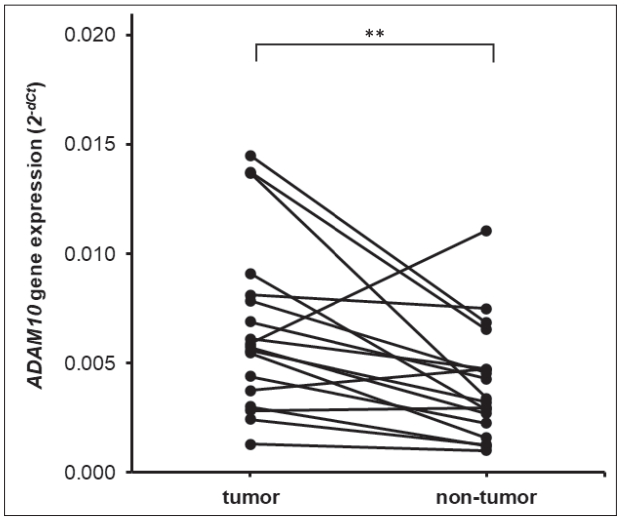

Fig. 1. *ADAM10* gene expression in tumor and surrounding non-tumor tissue in LARC patients. Relative expression of *ADAM10* mRNA was normalized to the expression of the β-actin (*ACTB)* gene and calculated using the 2^{-dCt} method. Statistical difference between tumor and non-tumor tissue was assessed using the related samples Wilcoxon signed-rank test. n=18, **P<0.01.

ADAM10 serum concentration and correlation with tissue expression

Serum samples were obtained from all patients to measure ADAM10 levels using the ELISA method. The obtained concentrations of ADAM10 in serum samples ranged from 66.6 pg/mL to 1119.1 pg/mL. We examined the correlation between ADAM10 serum levels and *ADAM10* tumor tissue expression (Fig. 2) but found no association (R=0.19; P=0.46).

Predictive value of ADAM10

To evaluate the predictive significance of ADAM10 serum levels and *ADAM10* tissue expression, patients were divided into three subgroups according to the pathological response as follows: RCRG1 (complete or good response), RCRG2 (moderate response), and RCRG3 (little or no response). Protein serum levels, tumor tissue gene expression, and the differences in gene expressions between tumor and non-tumor tissue samples were compared among patients with different RCRG stages (Table 2). However, an association with the pathological response was not found (P>0.05) (Fig. 3).

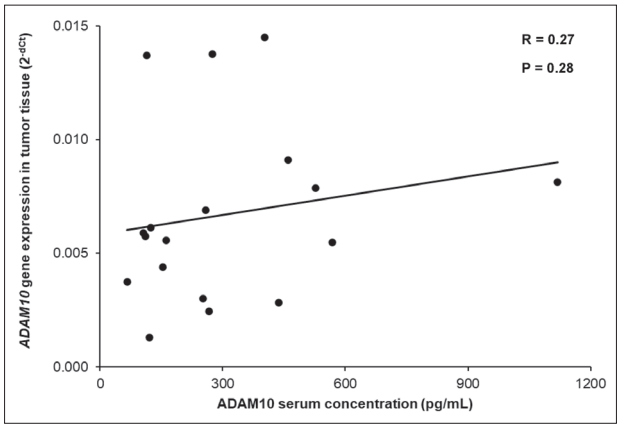

Fig. 2. Correlation between *ADAM10* gene expression in tumor tissue and ADAM10 serum concentration in LARC patients. The degree of association between data was examined using a non-parametric Spearman rank correlation coefficient. R – correlation coefficient, n=18.

GDC TCGA cohort *ADAM10* tissue expression in rectal cancer

Analysis of the publicly available data in GDC TCGA READ database using UCSC Xena showed higher *ADAM10* expression in non-tumor tissue compared to tumor tissue (P=0.002) in the cohort of all rectal cancer patients. In accordance with this was the result of higher expression in non-tumor tissue compared with tumor tissue in matched samples from 9 rectal cancer patients (P=0.02). In the case of samples from patients with LARC, there was no difference in *ADAM10* expression in tumor and non-tumor tissue (P=0.09). Similarly, no difference in expression was observed in tumor and non-tumor tissue in 4 matched samples in LARC patients (P=0.25) (Fig. 4).

DISCUSSION

This study aimed to assess ADAM10's role in LARC and its potential as a predictive biomarker for nCRT. To the best of our knowledge, this is the first study to investigate both *ADAM10* expression in tumors and surrounding healthy tissue, and ADAM10 serum levels in treatment-naive LARC patients. We suggest a potential role of ADAM10 in local tumor progression, explore the relationship between gene tissue expression levels and serum levels, and evaluate the possible predictive value of both parameters on patients' responses to standard neoadjuvant chemoradiotherapy.

Table 2. Serum ADAM10 concentration, *ADAM10* expression, tumor marker levels, tumor stadium and RCRG score for patients participating in the study

Patient No	ADAM10 concentration (pg/mL)	*ADAM10* tissue expression			CEA (IU/mL)	CA 19-9 (IU/mL)	stage		RCRG
		tumor (2^{-dCt})	non-tumor (2^{-dCt})	tumor/non-tumor ratio					
1	121.0	0.001295	0.001022	1.267513	3.5	#	T3	N1	1
2	568.0	0.005479	0.001583	3.460547		#	T3	N2	1
3	124.6	0.006130	0.004740	1.293249	*	2	T3	N2	3
4	527.6	0.007867	0.004569	1.721898	3	13	T3	N2	3
5	403.0	0.014498	0.006848	2.116969	2.6	#	T3	N2	2
6	161.3	0.005574	0.003226	1.727876	5.9	23	T4	N2	3
7	430.1				19.7	20	T4	N2	3
8	309.8				2.7	9	T3	N2	3
9	274.9	0.013754	0.006551	2.099433	5.7	#	T3	N2	2
10	153.2	0.004398	0.002270	1.937236	4.7	4	T3	N2	1
11	258.7	0.006901	0.004281	1.612166	0.6	3	T3	N2	1
12	105.4	0.005896	0.011064	0.532923	4.6	16	T4	N2	2
13	146.1				2	#	T3	N2	2
14	252.2	0.003014	0.001216	2.479415	2.2	21	T4	N2	2
15	437.1	0.002838	0.002964	0.957271	*	4	T3	N2	2
16	215.8				4.5	#	T2	N2	3
17	114.5	0.013706	0.000339	40.42007	3.5	11	T3	N2	3
18	459.4	0.009112	0.002889	3.153601	2.7	10	T3	N1	3
19	316.2				1.73	19	T4	N1	2
20	111.7	0.005747	0.002715	2.116969	218.3	30	T4	N1	2
21	1119.1	0.008133	0.007505	1.083726	3.6	6	T3	N2	2
22	266.3	0.002448	0.001289	1.898684	2.1	5	T4	N2	2
23	66.6	0.003755	0.004746	0.791137	2.6	3	T3	N2	3

dCt – delta cycle of threshold; CEA – carcinoembryonic antigen; CA 19-9 – carbohydrate antigen 19-9; RCRG – rectal cancer regression grade; * – below the lower limit of detection (1.72 IU/ml); # – below the lower limit of detection (2.06 IU/mL)

ADAM10 is likely involved in breast cancer progression [31]. It promotes cell growth, migration, and invasion in osteosarcoma [32] and is identified as a key player in the diagnosis, prognosis, and metastasis of non-small cell lung cancer [33]. It is also recognized as significant in cancer immunology [34] as it impairs the recognition of cancer cells by T or NK cells in many types of malignances, including melanoma, various carcinomas, chronic lymphocytic leukemia, acute myeloid leukemia, non-Hodgkin and Hodgkin's lymphomas [35,36]. The silencing of *ADAM10*, both *in vitro* and *in vivo*, has been shown to inhibit the proliferation of hepatocellular carcinoma cells [37]. In CRC cell culture, ADAM10 was found to be overactivated and its expression induced metastasis [18,38].

The role of overexpression of *ADAM10* in colorectal cancer development is probably related to Notch signaling [39]. In general, the activation of the Notch signaling cascade primarily regulates stem cell maintenance and differentiation but can also be responsible for tumor development [40]. ADAM10 is the major extracellular protease for Notch [41], and upon cleavage, regulated intramembrane proteolysis occurs, followed by intracellular Notch domain translocation to the nucleus and expression of genes involved in proliferation and differentiation. We found that *ADAM10* expression was significantly higher in tumor tissue than in non-tumor tissue in over 80% of cases. This is in line with a study that investigated ADAM10 in CRC patients by tissue-array immunohistochemistry that postulated an association of ADAM10 with higher tumor stage [42]. Conversely, a recently published study that measured ADAM10 concentrations found lower levels in tumor tissue than in tissue of surgical margins for all clinical stages but stage IV in CRC patients [43]. The authors hypothesize that higher ADAM10 protein concentration in surgical margins than tumor tissue suggests its significant role in early cancer stages linked to Notch signaling. In line with this finding is our analysis of GDC TGCA, which revealed higher expression of *ADAM10* in non-tumor tissue than in tumor samples of rectal cancer. No difference was observed in LARC patients. However, it is noteworthy that this database consisted of only 4 matched tumor and non-tumor samples, whereas our study group included 18 paired samples for expression analysis. Expression of *ADAM10* has been studied in various gastrointestinal cancers. Increased levels of *ADAM10* mRNA have been observed in gastric cancer [44]. High ADAM10 content, determined by immunohistochemical analysis, has been linked to lymph node involvement, the presence of metastases, and unfavorable prognosis [45]. One of the limitations of our study is that we were not able to perform subgroup analysis due to the low number of enrolled patients belonging to each TNM group, and

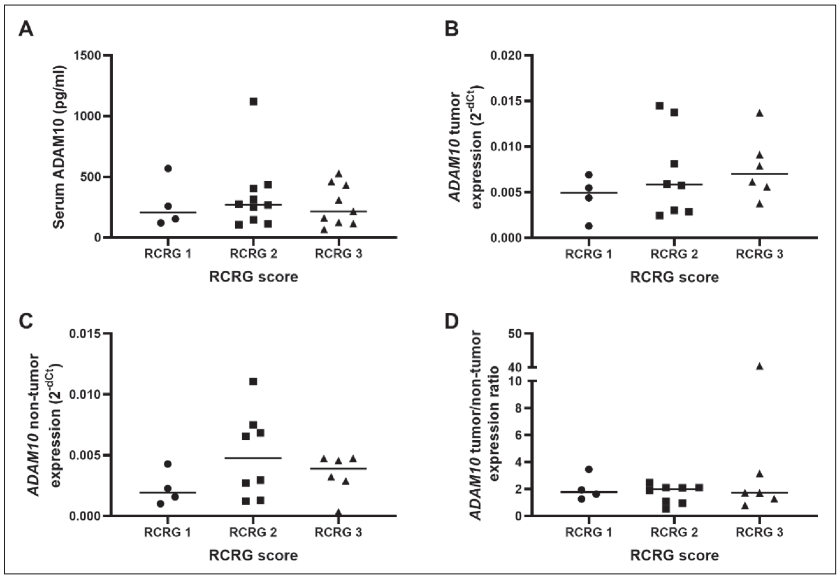

Fig. 3. ADAM10 serum concentration and expression levels do not differ in LARC patients with different RCRG scores. Patients with complete or good response (RCRG 1), moderate response (RCRG 2), little or no response (RCRG 3) to nCRT did not have statistically different (P>0.05) levels of serum ADAM10 concentration (**A**), expression of *ADAM10* in tumor tissue (**B**), expression of *ADAM10* in the surrounding healthy tissue (**C**) and the ratio of expression between these tissues was also not different (**D**). The data given in the graphs are medians (line) for each group, with each patient represented by the symbol. The differences between the groups were analyzed using the Kruskal-Wallis test. RCRG – rectal cancer regression grade; nCRT – neoadjuvant chemoradiotherapy.

our group included only patients with locally advanced disease. To clarify ADAM10's role in rectal cancer, future studies should enroll more patients and measure both gene expression and protein concentration of ADAM10 in tumor tissue. Also, the majority of research data on ADAM10 is related to CRC, although it is now clear that colon cancer and rectal cancer are two distinct entities, and this issue needs to be addressed in future research [24].

The measured concentration of ADAM10 in the serum of LARC patients in our study varied widely, in the range of~50 pg/mL to 1 ng/mL. These concentrations are significantly lower than previously reported in a preliminary report for the same protein (2-300 ng/mL) [46]. The authors also reported the levels of ADAM10 in the extended CRC patient group of 85 subjects and controls [23], with a mean value in the patient group of ~100 ng/mL. They postulated that the concentrations of ADAM10 and ADAM28 are significantly higher in CRC patients than in controls (~1 ng/mL) and that the patient levels correlate with the clinical and histopathological stage of the disease. The significant discrepancies in ADAM10 concentrations between this study and ours may be due to the different ELISA kits used, as the manufacturer did not provide detailed information on antibody specificity. There are not many studies that measured ADAM10 concentration in the serum in general, and the available results for healthy people are also conflicting. An article indicates that the concentrations of ADAM10 in healthy subjects fall within the low ng/mL range [47], similar to the findings of Walkiewicz et al. Nevertheless, other studies have reported lower concentrations of approximately 150 pg/mL in control groups [48,49]. Further studies should use and compare different assays, establish reference intervals for ADAM10 in a sufficient number of healthy subjects, to properly interpret results in various pathological conditions.

Finally, we analyzed the association of ADAM10 serum concentration and *ADAM10* tissue expression pre-treatment with the pathological response after nCRT in LARC patients. The rationale behind this analysis is that the changes in ectodomain shedding catalyzed by ADAMs are an adaptive response to kinase inhibition promoting therapeutic resistance in tumors. In melanoma, BRAF and MEK inhibitors reduce the shedding of RTKs, resulting in drug resistance through alternative signaling pathways such as Jnk/cJUN and PI3K/Akt [15]. A similar mechanism of resistance was determined in EGFR dysregulated lung cancer, triple-negative breast cancer, glioblastoma, and *KRAS* mutated colon cancer [50]. Preclinical data

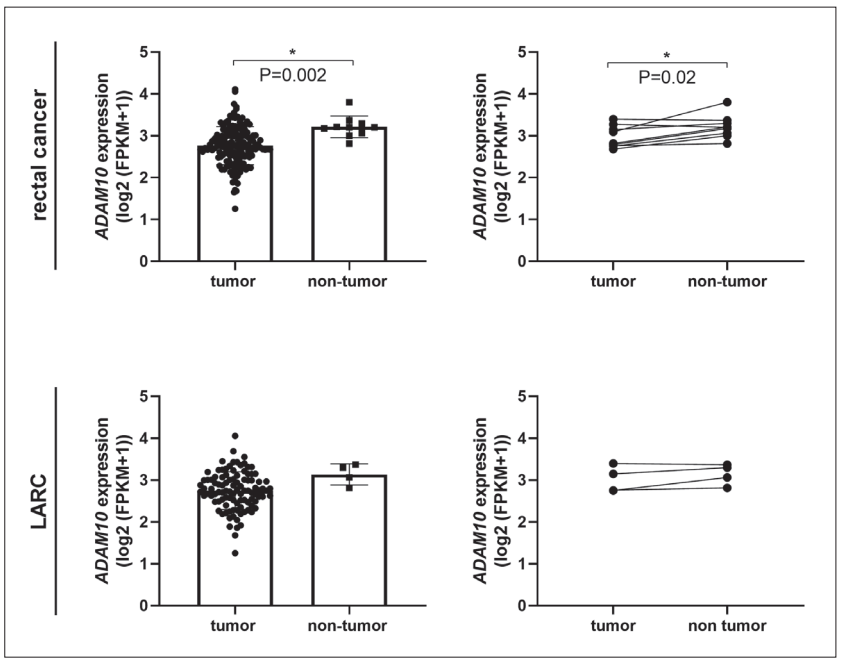

Fig. 4. Expression of *ADAM10* in tumor and non-tumor tissue from the GDC TCGA READ database. Upper panel – the data obtained from the GDC TCGA database for all rectal cancer (left, n=166) and matched samples (right, n=9). Lower panel – the data only for LARC patients (left, n=106) and matched samples for this group of patients (right, n=4).

and animal models also suggest that inhibitors of ADAM10 and ADAM17 overcome the resistance to EGFR inhibition by preventing EGFR ligand shedding from tumor cells [51]. In addition, the role of ADAM10 in promoting cell fusion in the metastatic model of colon carcinoma has been described, which induces resistance to 5-fluorouracil and oxaliplatin [52]. In our study group of LARC patients, neither *ADAM10* expression in tumor and surrounding non-tumor tissue nor ADAM10 serum concentrations correlated with the pathological response to nCRT. It should be noted that the result might have been different if the study included a larger number of patients, and if the mutation status of the analyzed tissue/patient was known. A recent pilot proteomic comparative analysis of pre-treatment tumor samples from responders and non-responders has identified ADAM10 as a leading biomarker candidate for predicting resistance to nCRT in LARC patients, as it was detected exclusively in non-responders. The authors of the study suggested the use of ADAM10 as a potential therapeutic target for overcoming the resistance to standard nCRT protocols [29]. Finding a new reliable biomarker for the prediction of the response to nCRT is of great importance to improve the criteria for the proper selection of LARC patients for the W&W treatment approach. No tissue or blood protein biomarkers have been identified for predicting or tracking response to nCRT in W&W studies [28]. However, Renehan et al.'s W&W study [53] included at least two CEA measurements during the first two years post-nCRT as part of the follow-up protocol. This highlights the need to identify cCR and explore new biomarkers for the W&W approach.

This study is the first to measure both tissue (tumor and non-tumor) expression and serum levels of ADAM10 in LARC patients before nCRT, aiming to validate ADAM10 as a predictive biomarker in rectal cancer. However, although the tumor tissue exhibited increased *ADAM10* expression compared to non-tumor tissue, we found no correlation between tissue expression and blood levels in LARC patients. *ADAM10* tissue expression and blood levels showed no correlation with nCRT response, as evaluated by pathohistology and graded as RCRG1, 2, 3 (complete-good/moderate/little-no response). It appears that despite the previously demonstrated predictive potential of tissue overexpression of ADAM10 for resistance to treatment [29], the serum levels of this protein could not be used as predictive biomarker of nCRT response in LARC patients. This result suggests the relationship between ADAM10 transcription, tissue, and serum levels is complex and requires further study.

CONCLUSIONS

Colon and rectal cancer were leading causes of new cancer cases and deaths last year. Research continues on better therapies and strategies to evaluate treatment response. A growing body of literature suggests the importance of ADAMs in gastrointestinal tumor initiation, progression, and resistance to treatment, especially of ADAM10 in rectal cancer. We found that *ADAM10* expression is significantly higher in tumor

tissue than in healthy tissue in LARC patients before therapy, with no correlation to serum concentration or response to nCRT. Additional research is necessary to confirm these findings, identify ADAM substrates and the ADAM-protease-activated signaling pathways involved in tumor initiation and progression, explore potential novel targets for cancer treatment, and clarify the role of ADAM10 in therapy resistance.

Funding: This work was funded by the Strategic Project of the Serbian Academy of Sciences and Arts "Molecular basis of response to chemoradiotherapy in rectal cancer – MOHERATEKA", F-69.

Acknowledgments: The authors want to thank Prof. Katarina Zeljić, Faculty of Biology University of Belgrade, for assistance in the analysis of the GDC TCGA database.

Author contributions: Conceptualization, AN and SMD; methodology AN and SMD; software, AMI, MU; validation, AMI, MU; formal analysis, AMI, SD, MU, and SMD; investigation AMI, SD, MU, MM, GB, SMD; resources, GB, AN; SMD; data curation AMI, SMD; writing and original draft preparation, AMI; writing, review and editing, AMI, SD, MU, MM, GB, AN, and SMD; visualization, AMI; supervision AN and SMD; project administration AN, SMD; funding acquisition, AN. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors have no financial or non-financial interests to disclose.

Data availability: The data referring to the clinical and pathological characteristics of the patients are not publicly available due to privacy. The raw data underlying this article is available as an online supplementary research dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Isakovic%20et%20al_Research%20Dataset.pdf

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SUPPLEMENTARY MATERIAL

RESEARCH DATASET

The raw data underlying this article is available as an online supplementary research dataset:
https://www.serbiosoc.org.rs/NewUploads/Uploads/Isakovic%20et%20al_Research%20Dataset.pdf