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Official Title: The Effect of ADAR1 Expression Level on Total Neoadjuvant Treatment Response in Locally Advanced Rectal Cancer

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Title

The effect of ADAR1 expression level on total neoadjuvant treatment response in locally advanced rectal cancer

Summary

Total neoadjuvant therapy (TNT) is currently the standard treatment for locally/locally advanced rectal cancer due to better response and fewer distant metastases. In TNT, sequential chemotherapy (CT) and chemoradiotherapy (CRT) are planned and depending on the treatment response, either surgery is performed or a watch and wait approach is applied. Depending on tumor localization and patient performance status, CT is planned as induction or consolidation. Most of the time, mFOLFOX6 and CAPOX are preferred as CT regimen. In proximal rectal cancer, surgery can be performed without CRT and only after CT. In locally/locally advanced rectal cancer, the aim is to avoid surgery as much as possible or to perform sphincter-sparing surgery if possible. Colonoscopy and pelvic MR at the time of diagnosis are the most important steps in staging, treatment selection and decision making. These two diagnostic methods should be repeated especially for watch and wait decision and for response evaluation after TNT.
ADAR 1 (Adenosine deaminase acting on RNA1) is an RNA editing enzyme that catalyzes the deamination of adenosine to inosine (A-to-I), a dynamic modification that can lead to a diverse transcriptome in a combinatorial manner. A defect in ADAR1-mediated RNA modification results in abnormal regulation of substrates that can affect phenotypic changes in cancer. This phenomenon of over-regulation is seen in many cancers such as colon, liver, lung, breast and esophageal cancers and in many cases promotes tumor progression. In studies, increased ADAR1 expression has been associated with lower survival and worse prognosis, especially in metastatic colon and gastric cancer. ADAR1 is also predicted to increase proliferation through both the AKT pathway and the mTOR pathway and therefore may be targeted in the near future.
ADAR1 expression is monitored by RNA-based real time PCR. In order to demonstrate increased expression, biopsies should be taken from the malignant tissue and the intact tissue of the patient and

the biopsy should be stored under -80 C conditions immediately after biopsy to prevent RNA degradation. The tissue will not come into contact with nitrogen or formaldehyde.
In our study, sufficient biopsies from cancerous and intact tissue will be taken from patients with suspected rectal cancer, confirmed by pelvic MRI and consent for participation in the study, and fresh tissue will be stored at -80 C in the genetics laboratory. After the TNT plan is made by us and the treatment is completed, both pelvic MRI and control rectoscopy will be performed for preoperative evaluation. Again, biopsies will be taken from diseased and healthy tissue and ADAR1 expression will be evaluated. The study is planned to include 50 patients and a period of one year is foreseen for tissue procurement/storage.
Our aim in this study will be to determine whether ADAR1 expression level changes after TNT, whether this predicts clinical and pathological response, whether responses change according to the selected CT, whether there is a difference between CT induction-consolidation/RT short or long course, and the relationship between tumor DNA mismatch repair enzyme status and ADAR1 level. Our primary endpoint will be the effect of the change in ADAR1 expression level on the response after TNT (ORR). Our secondary endpoints will be recurrence-free survival (RFS) and overall survival (OS).

Original Value and Hypothesis of the Study

Colorectal cancer is the third most common cancer in both sexes, but it ranks second in mortality after lung cancer, with one in ten cancer deaths attributed to colorectal cancer[1]. Total neoadjuvant therapy (TNT) is currently considered the standard of care for the treatment of locally advanced rectal cancer[2]. In this approach, all non-surgical interventions, including multi-agent chemotherapy and concurrent chemoradiotherapy (CRT), are administered prior to surgical resection or the decision to opt for non-operative treatment. Accumulating evidence suggests that TNT improves survival in patients with locally advanced rectal cancer and is expected to reduce distant metastasis through systemic chemotherapy, thereby preventing the onset of micrometastases[3]. TNT has also been associated with improved compliance, reduced toxicity, decreased need for and duration of ileostomy, and improved anal sphincter preservation rates following a watch-and-wait strategy, along with increased complete clinical response[4, 5]. Studies have shown that the rate of pCR in patients receiving TNT therapy is approximately 20–40%[6, 7]. Although numerous randomized controlled trials have evaluated different TNT strategies compared to standard KRT treatment in terms of KRT sequencing, systematic chemotherapy, radiotherapy modality, and TNT intensity, it is known that the TNT approach demonstrates superior survival benefit compared to adjuvant chemotherapy following neoadjuvant KRT. However, TNT theoretically offers several surgical advantages, including an increased likelihood of sphincter-preserving surgery and a reduced need for ileostomy; however, results across studies are inconsistent[8].

ADAR 1 (Adenosine deaminase acting on RNA1) catalyzes the C6 deamination of adenosine (A) to produce inosine (I) in RNA regions characterized by a double-stranded structure, a process known as adenosine (A) to inosine (I) RNA editing (ATIRE)[9]. This process is crucial for altering RNA structures and sequences in both coding and non-coding RNA, influencing tumor characteristics, tumor stage, drug responses, and patient survival, thereby significantly contributing to cancer progression[10]. The role of ADAR1 in promoting tumorigenesis via the ATIRE pathway is becoming increasingly evident in various cancers, including stomach cancer, esophageal squamous cell carcinoma, breast cancer, hepatocellular carcinoma, and CRC[11-14].

ADAR1, ADAR2, and ADAR3 are members of the ADAR family; while ADAR1 and ADAR2 are widely expressed, ADAR3 is primarily expressed in the brain and lacks catalytic activity. ADAR1 contains RNA-binding domains (RBDs) and Zα domains, whereas ADAR2 contains only RBDs. Zα domains enable ADAR1 to bind to newly synthesized RNA and inhibit the activation of pathogenic

interferons (IFNs)[15]. ADAR1 has two isoforms: an IFN-inducible form (p150) and a constitutively expressed form (p110). It has been found that ADAR1-p110 reduces the chemotactic potential of melanoma cells and promotes immune exclusion [16]. Loss of ADAR1 function results in unregulated RNA accumulation, which can be mistakenly recognized as foreign by RIG-I (Retinoic acid-inducible gene I), thereby triggering its activation and subsequent IFN response. Additionally, DNA released from damaged cells can activate the cGAS (GMP–AMP synthase)-STING (stimulator of interferon genes) pathway, leading to the production of type I IFNs through both pathways. The interaction between ADAR1 and the cGAS-STING and RIG-I pathways highlights a crucial balance in immune regulation[17, 18]. ADAR1's ability to interact with IFNs, regulate IFN production, and its complex roles in cancer therapy, coupled with its higher expression in MSI-H (microsatellite instability-high) patients, its positive correlation with high TMB (tumor mutation burden), PD-1/PD-L1 levels, naive B cells, active memory CD4 T lymphocytes, and M1 macrophage cells suggests its potential for modulating responses to cancer therapies [19, 20]. A potential mechanism has been proposed whereby increased ADAR1 expression in gastric cancer leads to proliferation and migration of various diseases through the mTOR/p70S6 kinase/S6 ribosomal protein pathway. To investigate the functional relationship between ADAR1 and mTOR signaling, the mTOR kinase inhibitor rapamycin was used to treat gastric cancer cells and observe its effects on ADAR1-overexpressed cell proliferation and migration. The results showed that ADAR1 overexpression significantly promoted cell proliferation and migration as expected, but these effects were significantly attenuated in cells treated with rapamycin. This suggests that rapamycin may block the effects of ADAR1 overexpression on gastric cancer cell growth and migration. These results indicate that the mTOR signaling pathway plays an important role in ADAR1-mediated gastric cancer progression[21].

Recently, the effect of increased ADAR1 expression on survival in metastatic colon cancer was investigated. Both overall survival (OS) and relapse-free survival (RFS) were significantly reduced in the group with increased ADAR1 expression; however, the study did not include patients with early-stage rectal cancer[20]. For all these reasons, the prognostic value of ADAR1 expression levels and/or changes in locally advanced rectal cancer patients for TNT response will be investigated.

Purpose and Objectives

1. To investigate the relationship between ADAR1 expression levels and pathological and clinical response to TNT

2. To evaluate the relationship between potential differences in KT and RT administered in TNT and ADAR1 expression levels

3. To determine the relationship between changes in ADAR1 expression and RFS and OS in patients undergoing surgery or watch and wait after TNT

1. Study Design

The study will include 50 patients diagnosed with locally advanced rectal cancer who applied to the General Surgery Department of Necmettin Erbakan University Faculty of Medicine. The control group will be formed with healthy rectal tissue samples taken from the same patients. Within the scope of the study, tissue samples will be collected from each patient at two different time points: before and after TNT. A total of 200 tissue samples will be examined, including 50 cancerous rectal tissue samples and 50 healthy rectal tissue samples before TNT, and 50 cancerous rectal tissue samples and 50 healthy rectal tissue samples after TNT.

Inclusion criteria

Histological and stage diagnosis of local/locally advanced rectal cancer

Age range between 18 and 90

ECOG performance score between 0 and 2

No contraindications for CT and/or RT

Exclusion criteria

Patients with metastatic rectal cancer

Those with suspected rectal cancer

Those who did not approve the informed consent form

Those with contraindications for CT and/or RT

2. Dermographic data collection

Obtaining and recording dermographic data from patients and controls included in the study

3. Storage and evaluation of biopsy materials

Tissue samples will be stored at -80°C until the day of analysis in the Medical Genetics Department laboratory of Necmettin Erbakan University Faculty of Medicine and total RNA will be isolated from the tissue samples. During this process, 5-30 mg of tissue will be homogenized in a 1.5 mL homogenization tube, and extraction will be performed according to the kit manufacturer's instructions to obtain 30-50 µl of total RNA. cDNA will be obtained from the RNA samples whose quantities have been calculated. In the PCR protocol, primer binding will be performed at 25°C for 10 minutes, reverse transcription will be performed at 42°C for 15 minutes, and finally, enzyme inactivation will be performed at 85°C for 5 minutes. Gene expression levels will be determined by the real-time PCR method. The p150 isoform, which is larger in size and has been used in previous studies, will be used. The reaction will be performed in a real-time PCR device with an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 5 seconds, 55°C/59°C for 30 seconds (reading). The relative mRNA expression levels of the ADAR1 gene obtained via the device will be determined using the R package and the 2-ΔΔCt method. A total of 200 gene expression data obtained from 50 patients before and after treatment will be statistically compared.

Data Collection and Statistical Analysis

Clinical, pathological, and demographic characteristics, along with laboratory results, will be obtained from patient files and the hospital database.

For descriptive statistics, the Chi-square test and Fisher's exact tests will be used. For measurable evaluations, if the distribution is normal, the Student's t-test will be performed using the mean and standard deviation; if the distribution is not normal, the Mann-Whitney U test will be performed using the median value.

Non-parametric related tests will be used to compare two or more variables between non-parametric dependent variables. Kaplan-Meier survival curves will be used to calculate survival, and the log-rank test will be used to compare differences in survival times. All variables affecting prognosis and survival will first be evaluated using univariate Cox regression analysis, followed by multivariate Cox regression analysis. To determine the correlation between categorical variables, the chi-square test or Fisher's exact test will be used. All statistical analyses will be performed using IBM SPSS Statistics 25.0. Two-sided p-values <0.05 will be considered statistically significant.