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# ADAR1 as a prognostic marker for patients with colorectal cancer and synchronous liver metastasis and a predictor of chemotherapy efficacy

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**RNA editing by adenosine deaminase acting on RNA (ADAR) enzymes plays a role in cancer progression. However, its clinical significance in metastatic colorectal cancer (CRC) remains unclear. This study aimed to evaluate whether ADAR1 expression predicts prognosis and treatment response in colorectal cancer (CRC) with synchronous liver metastasis. This study included 40 patients with stage IV CRC and synchronous liver metastases. ADAR1 expression in tumor tissues was evaluated using immunohistochemistry. Expression levels were quantified using the immunoreactive score, and associations with clinicopathological features, overall survival (OS), and chemotherapy response were examined. High ADAR1 expression was significantly associated with multiple liver metastases ($P = 0.0206$), lymph node metastasis ($P = 0.0241$), and reduced response to chemotherapy ($P = 0.0224$). Significantly shorter OS was observed in patients with high ADAR1 expression in the nucleus ($P = 0.0458$). ADAR1 expression was an independent prognostic factor comparable to the presence of extrahepatic metastases. Low ADAR1 expression was correlated with a higher likelihood of achieving a response to chemotherapy. ADAR1 expression can reflect tumor aggressiveness and chemotherapy resistance in patients with CRC and synchronous liver metastasis. ADAR1 has considerable potential as a dual-purpose biomarker for stratifying patients based on prognosis and optimizing treatment intensity.**

**Keywords** RNA editing, Liver metastasis, Chemotherapy, Biomarker, Colorectal cancer

Colorectal cancer (CRC) is a common malignant tumor with a high incidence and mortality rate worldwide. Controlling distant metastasis is crucial for improving treatment outcomes. Distant metastases to the liver, lungs, peritoneum, and other organs are frequently observed in advanced CRC, and the presence of these metastases can impede radical treatment and significantly worsen prognosis. Hepatic metastasis is the most common form of distant metastasis in patients with CRC and is estimated to be present at initial diagnosis in approximately 20%–25% of cases[1]. Additionally, the possibility of surgical resection, which is a key factor in determining prognosis, is influenced by this condition. Therefore, understanding the mechanism of distant metastasis development in CRC and identifying new predictive factors and treatment targets are crucial to controlling distant metastasis.

With advances in anticancer drugs and the introduction of molecularly targeted therapies, it has become possible for even patients with advanced recurrent disease to achieve long-term survival. Some patients with synchronous liver metastases can be successfully treated with surgical resection. However, surgery alone is not sufficient to control distant metastases, and surgery should be combined with chemotherapy. Particularly,

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predicting the efficacy of chemotherapy is crucial. Although potent chemotherapy is desirable for stage IV CRC that is resistant to treatment, biomarkers for predicting chemotherapy efficacy are required.

Genetic information is transmitted from DNA to RNA and then to proteins. RNA editing is a mechanism that creates diversity in gene expression by directly modifying RNA sequences. The adenosine deaminase acting on RNA (ADAR) and APOBEC families are RNA-editing enzymes that play essential physiological roles, including controlling development and the immune system[1,2]. Conversely, abnormalities in RNA editing are involved in cancer development and progression. These enzymes have attracted attention as new targets for cancer control[2]. ADAR1 is a representative RNA-editing enzyme that converts adenosine to inosine. The expression of this enzyme is upregulated in CRC tumor tissues[3]. High ADAR1 expression has been reported to be associated with the metastatic tendency of tumors, especially metastasis to lymph nodes and distant organs, and its expression level has been reported to affect the biological malignancy and prognosis of tumors[3]. Additionally, RNA editing is involved in the interaction between tumor cells and surrounding stromal cells. Particularly, cancer-associated fibroblasts have been reported to promote invasion and metastasis by increasing editing activity in response to tumor signals[4].

Therefore, this study aimed to investigate whether the RNA-editing enzyme ADAR1 is clinically useful as a prognostic and predictive marker of chemotherapy response in patients with CRC and synchronous liver metastasis.

## Methods

### Patients

A total of 40 patients with stage IV CRC who were diagnosed and treated at Okayama University Hospital were included in this study. All patients were confirmed to have CRC based on histopathological findings. Staging was performed according to the American Joint Committee on Cancer TNM classification.

### Immunohistochemistry

Formalin-fixed paraffin-embedded specimens were sectioned, and the sections were deparaffinized with xylene and ethanol. Endogenous peroxidase activity was inhibited by hydrogen peroxide. Antigen activation was performed by high-pressure steam treatment at 121 °C for 15 min. ADAR1 antibody (Abcam, 1:100 dilution) was used as the primary antibody. The reaction was allowed to proceed overnight at 4 °C. The secondary antibody and color development were performed using the EnVision + Dual Link Kit. Hematoxylin was used for nucleus staining. As a negative control, slides without the primary antibody were processed simultaneously.

### Evaluation of staining score

The ADAR1 expression level was quantitatively evaluated using the immunoreactive score (IRS)[5]. The IRS was calculated as the product of the percentage of stained cells and the staining intensity. The percentage of stained cells was graded on a 5-point scale ranging from 0 to 4: 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%). The staining intensity was graded on a 4-point scale ranging from 0 to 3: 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). Each sample was independently assessed by three blinded evaluators. The average value was taken as the representative score.

### Ethical considerations

This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences and Okayama University Hospital (1903–037) and conducted in accordance with the relevant ethical guidelines and regulations. Written informed consent was obtained from all patients.

### Statistical analysis

Statistical analyses were performed using JMP Pro software (Version 17.0, SAS Institute Inc., Cary, NC, USA). Intergroup comparisons were performed using Wilcoxon's rank-sum, chi-square, and Steel's tests. Survival analysis was performed using the Kaplan–Meier method. Differences between groups were assessed using the log-rank test. A two-sided $P$ value $< 0.05$ indicated statistical significance.

## Results

### The presence of extrahepatic metastasis and chemotherapy resistance are poor prognostic factors for patients with stage IV CRC

First, the impact of the presence or absence of extrahepatic metastasis and the response to preoperative chemotherapy on overall survival (OS) was preliminarily examined in patients with stage IV CRC and synchronous liver metastasis at Okayama University Hospital. Figure 1A shows the Kaplan–Meier survival curves for patients with liver metastasis, with or without distant metastasis to extrahepatic organs. A significantly better prognosis was observed in patients without extrahepatic metastasis ($n = 32$) than in those with extrahepatic metastasis ($n = 8$) ($P = 0.0333$). This indicates that metastasis to extrahepatic organs reflects the degree of CRC progression and may be a poor prognostic factor. Figure 1B shows the survival curve stratified by the response to preoperative chemotherapy. Patients who achieved a partial response (PR) ($n = 17$) tended to have longer survival than those who achieved stable disease (SD) or progressive disease (PD) ($n = 22$). However, this difference was not statistically significant ($P = 0.0707$). These findings indicate that the presence or absence of extrahepatic metastasis and sensitivity to chemotherapy are significantly associated with the prognosis of patients with stage IV CRC and synchronous liver metastasis.

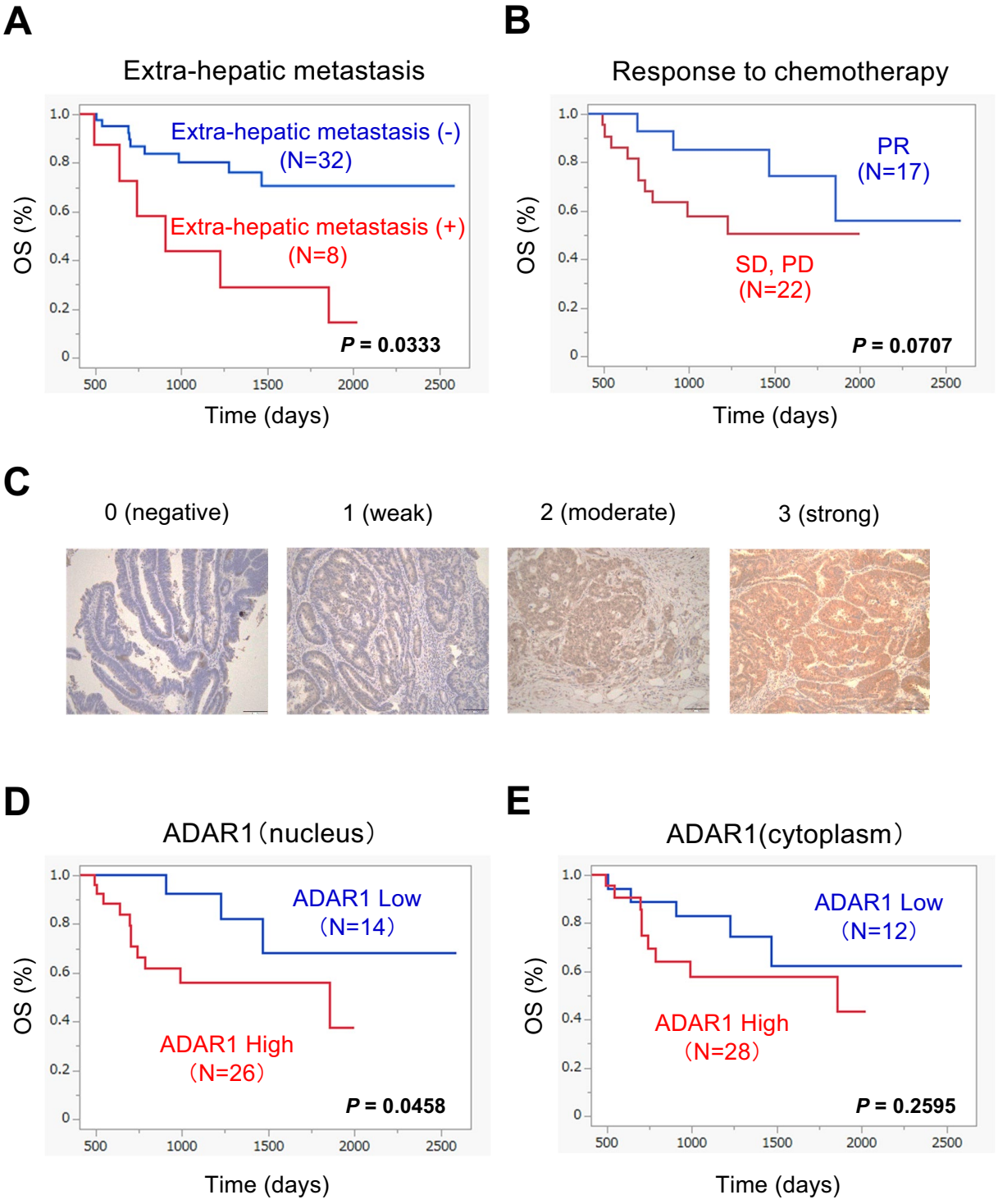


**Fig. 1**. Prognostic impact of ADAR1 expression and clinical factors in colorectal cancer with liver metastasis. (**A**) Kaplan–Meier survival curves comparing patients with or without extrahepatic metastases. The presence of extrahepatic metastasis was associated with significantly shorter overall survival (OS) ($P = 0.0333$). (**B**) Kaplan–Meier survival curves comparing patients with partial response (PR) with those with stable disease or progressive disease (SD/PD) after chemotherapy. Despite being not statistically significant ($P = 0.0707$), a trend toward improved survival was observed in patients with PR. (**C**) Representative immunohistochemical staining images of ADAR1 in tumor tissues. The staining intensity was classified into four levels: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). (**D**) Kaplan–Meier survival curves stratified by ADAR1 expression in the nucleus. An association was observed between high ADAR1 expression in the nucleus and significantly shorter OS ($P = 0.0458$). (**E**) Kaplan–Meier survival curves based on cytoplasmic ADAR1 expression levels. No significant difference in survival was observed ($P = 0.2595$).

## Evaluation of ADAR1 immunostaining intensity

The RNA-editing enzyme ADAR1 is associated with increased malignancy in CRC[3]. Therefore, we hypothesized that ADAR1 could serve as both a prognostic and predictive marker for chemotherapy efficacy in stage IV CRC with synchronous liver metastasis. The expression of ADAR1 in CRC tissues was evaluated by immunohistochemistry. The intensity of expression was classified into four levels: negative, weakly positive, moderately positive, and strongly positive (Fig. 1C). In the negative cases, no obvious staining was observed in the tumor epithelium, and the staining level remained at the background level. In the weakly positive cases, mild cytoplasmic staining was observed in some tumor cells. In the moderately positive cases, clear cytoplasmic staining was observed across a wide tumor area. In the strongly positive cases, strong and uniform cytoplasmic staining was observed throughout the tumor. These evaluations were conducted following the method previously described by Remmele et al. and quantified using the IRS[5]. Based on the evaluations, cases were classified into low-expression and high-expression groups, and the relationship with clinicopathological factors and prognosis was analyzed. The scoring was performed independently by three specialists. In cases where differences in evaluation were observed, the final judgment was made after consultation.

## Patient characteristics

A total of 40 patients with stage IV CRC and synchronous liver metastasis were included in this study (Table 1). Based on ADAR1 expression levels, 26 patients were included in the high-expression group, and 14 were included in the low-expression group. Of the 26 patients in the high-expression group, 15 were males, and 11 were females. Of the 14 patients in the low-expression group, 8 were males, and 6 were females. No significant difference was observed in gender ($P = 0.9733$). The tumor depth was T1 or T2 in 2 patients (5.0%) and T3 or T4 in 38 patients (95.0%). No significant association was observed between tumor depth and ADAR1 expression ($P = 0.6482$). Regarding the distribution of distant metastases, 32 cases (80.0%) were limited to liver metastases only, whereas 8 cases (20.0%) had metastases involving other organs, without significant difference observed between the groups ($P = 0.8684$). Regarding the number of liver metastases, 28 cases (70.0%) had < 5 metastases, 12 cases (30.0%) had ≥ 5 metastases, and the proportion of patients with multiple liver metastases of ≥ 5 was significantly higher in the ADAR1 high-expression group ($P = 0.0206$). The pretreatment carcinoembryonic antigen levels were low in 16 cases (40.0%) and high in 24 cases (60.0%). No significant correlation was observed between pretreatment carcinoembryonic antigen levels and ADAR1 expression ($P = 0.3435$). Of the 40 patients, 11 (27.5%) received preoperative chemotherapy with a regimen that included 5-FU, L-OHP (oxaliplatin), and

| Characteristics | | Total | High ADAR1 | Low ADAR1 | *P* value |
|---|---|---|---|---|---|
| No. of patients | | 40 | 26 | 14 | |
| Age (years) | < 64 | 21 | 15 | 6 | 0.3702 |
| | ≥ 64 | 19 | 11 | 8 | |
| Sex | Male | 23 | 15 | 8 | 0.9733 |
| | Female | 17 | 11 | 6 | |
| Location | Right | 11 | 9 | 2 | 0.1696 |
| | Left | 29 | 17 | 12 | |
| Tumor depth | T1 or T2 | 2 | 1 | 1 | 0.6482 |
| | T3 or T4 | 38 | 25 | 13 | |
| **Lymph node metastasis** | **Negative** | **5** | **1** | **4** | **0.0241*** |
| | **Positive** | **35** | **25** | **10** | |
| Distant metastasis | Liver-limited | 32 | 21 | 11 | 0.8684 |
| | Other organs | 8 | 5 | 3 | |
| H score | 1 | 21 | 15 | 6 | 0.3702 |
| | 2 or 3 | 19 | 11 | 8 | |
| **Number of liver metastases** | **< 5** | **28** | **15** | **13** | **0.0206*** |
| | **≥ 5** | **12** | **11** | **1** | |
| RAS | Wild-type | 27 | 16 | 11 | 0.2726 |
| | Mutant | 13 | 10 | 3 | |
| Pretreatment CEA | Low | 16 | 9 | 7 | 0.3435 |
| | High | 24 | 17 | 7 | |
| Pretreatment CA19-9 | Low | 32 | 20 | 12 | 0.6555 |
| | High | 7 | 5 | 2 | |
| **5-FU and L-OHP ± CPT-11** | **Yes** | **11** | **10** | **1** | **0.0344*** |
| | **No** | **29** | **16** | **13** | |
| **Chemotherapy response** | **PR** | **17** | **8** | **9** | **0.0224*** |
| | **SD and PD** | **22** | **18** | **4** | |

**Table 1**. Clinicopathological features. CA19-9 = carbohydrate antigen 19–9, CEA = carcinoembryonic antigen, L-OHP = oxaliplatin, PR = partial response, SD = stable disease, PD = progressive disease. *$P < 0.05$.

CPT-11 (irinotecan). The induction rate was significantly higher in the ADAR1 high-expression group than in the low-expression group ($P=0.0344$). This may be due to the fact that the ADAR1 high-expression group had a high proportion of cancers with a high degree of malignancy from a clinical perspective. Additionally, the presence of lymph node metastases tended to be higher in the ADAR1 high-expression group than in the ADAR1 low-expression group, indicating that it may be a useful indicator of pathological progression and metastatic potential. Additionally, the proportion of patients who achieved a PR to chemotherapy was significantly lower in the ADAR1 high-expression group ($P=0.0224$). These findings indicate a significant association between high ADAR1 expression and multiple liver metastases, lymph node metastasis, and chemotherapy resistance.

### Association between ADAR1 expression in the nucleus and cytoplasm and prognosis in patients with stage IV CRC

We focused on the subcellular localization of ADAR1 (nucleus or cytoplasmic). The impact of the expression level in each localization on the survival of patients with CRC was examined. Figure 1D shows the results of the analysis of OS in which patients were stratified into high-expression ($n=26$) and low-expression groups ($n=14$) based on ADAR1 expression in tumor cell nuclei. The ADAR1 high-expression group had significantly lower survival rates than the low-expression group ($P=0.0458$), indicating that high expression of ADAR1 in nuclei may be a poor prognostic factor. Figure 1E shows a similar analysis based on ADAR1 expression in the cytoplasm of tumor cells. A difference in the survival curves was observed between the high-expression ($n=28$) and low-expression groups ($n=12$), but this difference was not statistically significant ($P=0.2595$). These findings indicate that ADAR1 activity is involved in the malignancy and prognosis of stage IV CRC with liver metastasis.

### Relationship between ADAR1 expression intensity and lymph node metastasis, number of liver metastases, and response to chemotherapy

We used quantitative scores based on the IRS for analysis to evaluate ADAR1 expression levels as a continuous variable because the chi-square test alone cannot rule out the possibility of bias in the cutoff setting. The relationship between ADAR1 expression and tumor malignancy and treatment response was then examined, focusing on the presence or absence of lymph node metastasis, the number of liver metastases, and response to chemotherapy (Fig. 2). Regarding the relationship with the presence or absence of lymph node metastasis, the IRS for ADAR1 was significantly higher in the group with lymph node metastasis (nucleus: $P=0.0007$; cytoplasm: $P=0.0045$), and the intensity of ADAR1 expression was positively correlated with lymph node metastasis (Fig. 2A). Regarding the relationship with the number of liver metastases, the IRS for ADAR1 was significantly higher in the group with $\geq 5$ metastatic foci (cytoplasm: $P=0.0369$) (Fig. 2B). Regarding the relationship with the response to chemotherapy (PR vs. SD/PD), the IRS for ADAR1 was significantly higher in the poor response group (SD/PD) (nucleus: $P=0.03$; cytoplasm: $P=0.06$) (Fig. 2C). These findings indicate that the intensity of ADAR1 expression is closely related to the metastatic and invasive properties of tumors and their response to chemotherapy.

### The expression intensity of ADAR1 is a prognostic factor comparable to the presence of extrahepatic metastasis

Univariate and multivariate analyses were performed using the Cox proportional hazards model to identify factors affecting OS in patients with stage IV CRC and synchronous liver metastasis (Table 2). The multivariate analysis identified the presence of extrahepatic metastasis (hazard ratio: 3.76, $P=0.0266$) and high ADAR1 (hazard ratio: 3.75, $P=0.0487$) as independent poor prognostic factors.

### Association between ADAR1 expression and response to chemotherapy: significance as a predictive factor

A logistic regression analysis was performed to identify clinical factors that could predict response to chemotherapy (Table 3). The analysis examined whether each factor could predict response to chemotherapy. The multivariate analysis revealed that ADAR1 expression was independently associated with response to chemotherapy, with a higher response rate observed in patients with low ADAR1 expression (odds ratio: 5.76, $P=0.0258$). These findings indicate that high ADAR1 expression may indicate chemotherapy resistance. Therefore, ADAR1 may be a useful biomarker for determining treatment strategies.

## Discussion

This study investigated the relationship between intratumoral expression of the RNA-editing enzyme ADAR1 and clinical characteristics, prognosis, and response to chemotherapy in patients with stage IV CRC and synchronous liver metastasis. The results showed that the expression intensity of ADAR1 was significantly associated with the extent of tumor progression (multiple liver metastasis and lymph node metastasis) and chemotherapy resistance and could be a poor prognostic factor for OS.

ADAR1 is an enzyme responsible for A-to-I RNA editing and creates functional diversity in proteins that are translated by modifying the RNA sequence after transcription[6]. Recently, ADAR1 has been reported to affect the malignant phenotype of cancer and the immune environment[7] and has been suggested to be involved in tumor invasion and metastasis in CRC[3]. Recent studies have demonstrated that ADAR1 promotes tumor aggressiveness by facilitating epithelial–mesenchymal transition and suppressing innate immune responses through RNA editing[8]. ADAR1-mediated A-to-I editing can alter the function of oncogenic or tumor suppressor RNAs, thereby contributing to enhanced proliferation, invasion, and metastasis. Furthermore, ADAR1 has been implicated in editing double-stranded RNAs to evade immune recognition, creating an immunosuppressive tumor microenvironment[7]. In this study, the analysis showed that the number of liver metastases and the

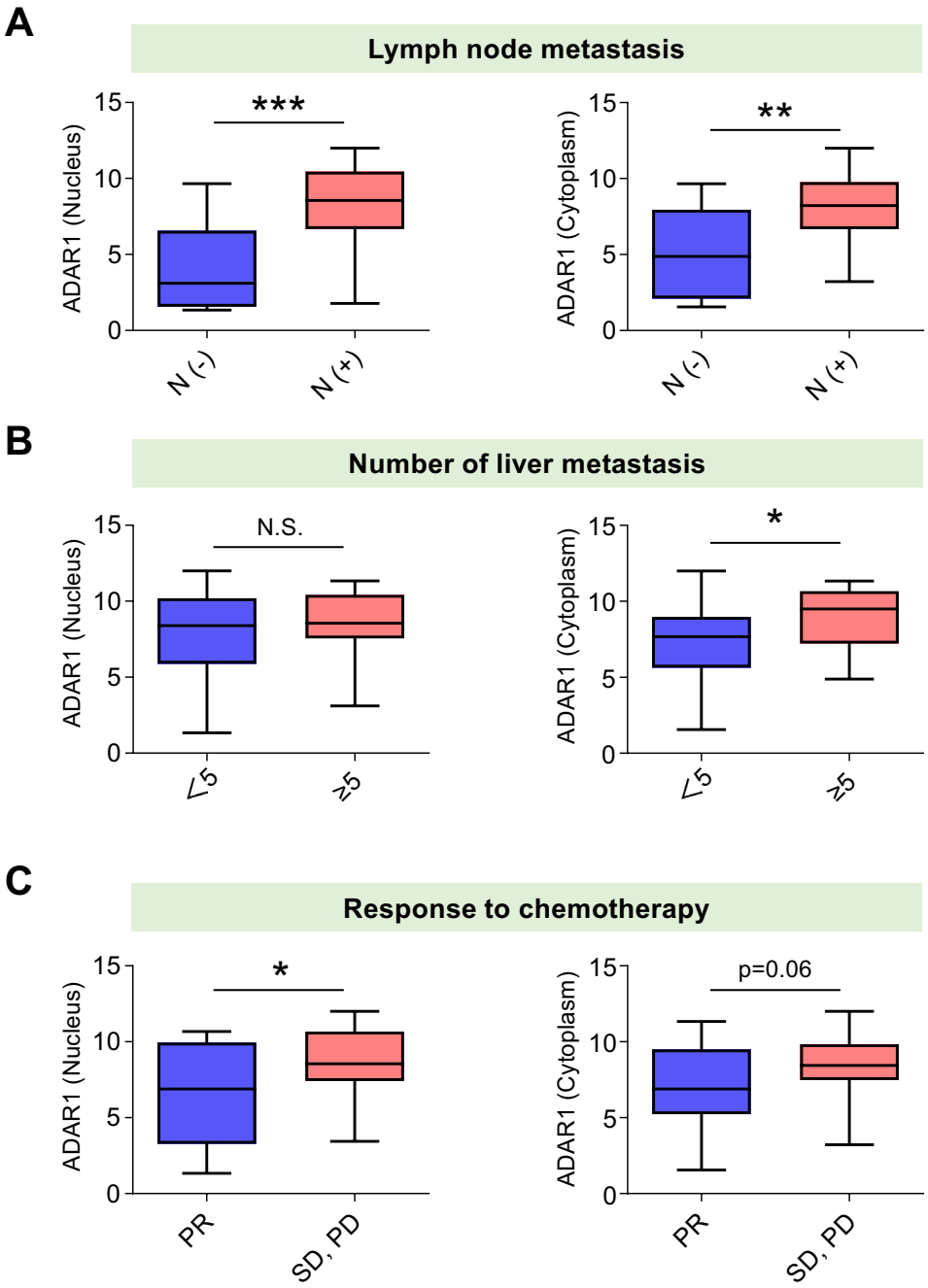


**Fig. 2**. Correlation between ADAR1 expression and metastatic potential and chemotherapy response. (**A**) Comparison of the ADAR1 immunoreactive score (IRS) in the tumor nuclei and cytoplasm between patients with and without lymph node metastasis. High ADAR1 expression was significantly associated with lymph node involvement (nucleus: $P = 0.0007$; cytoplasm: $P = 0.0045$). (**B**) Comparison of ADAR1 expression according to the number of liver metastases ($< 5$ vs. $\geq 5$). ADAR1 expression was significantly higher in cases with multiple liver metastases (cytoplasm: $P = 0.0369$). (**C**) Comparison of ADAR1 expression in tumors from patients with PR and those with SD/PD after chemotherapy. High ADAR1 expression was correlated with chemotherapy resistance (nucleus: $P = 0.03$; cytoplasm: $P = 0.06$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

frequency of lymph node metastasis were significantly higher in the ADAR1 high-expression group than in the ADAR1 low-expression group, indicating a close association with disease progression.

ADAR1 expression in the nucleus was significantly associated with a poor prognosis. ADAR1 is an enzyme that primarily functions in the cytoplasm. However, it influences splicing control and RNA stability through RNA editing in the nucleus[9]. Consequently, its localization in the nucleus may play a specific role in tumor progression. Therefore, further studies are needed to elucidate the molecular mechanisms that focus on the functions of ADAR1 based on localization.

| Variables | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR | *P* value | HR | *P* value |
| Age < 64 years | 1.06 | 0.9129 | | |
| Female | 1.45 | 0.4914 | | |
| Left-sided | 1.53 | 0.5127 | 1.46 | 0.6019 |
| **Extrahepatic metastasis** | **3.59** | **0.0189** | **3.76** | **0.0266*** |
| H score of 2 or 3 | 1.34 | 0.5907 | | |
| RAS mutant | 1.00 | 0.9977 | | |
| High pretreatment CA19-9 | 1.39 | 0.6139 | | |
| **High ADAR1** | **2.91** | **0.1026** | **3.75** | **0.0487*** |

**Table 2**. ADAR1 as a prognostic marker for patients with stage IV colorectal cancer and liver metastasis. CA19-9 = carbohydrate antigen 19–9, HR = hazard ratio. *$P < 0.05$.

| Variables | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR | *P* value | OR | *P* value |
| Age < 64 years | 1.71 | 0.4066 | | |
| **Male** | **2.88** | **0.1132** | **4.67** | **0.0469*** |
| Left-sided | 2.67 | 0.1903 | | |
| Extrahepatic metastasis | 1.38 | 0.6826 | | |
| H score of 1 | 3.21 | 0.0771 | 3.54 | 0.0944 |
| RAS wild | 2.25 | 0.2487 | | |
| High pretreatment CA19-9 | 1.84 | 0.4660 | | |
| **Low ADAR1** | **5.06** | **0.0216** | **5.76** | **0.0258*** |

**Table 3**. ADAR1 as a predictive marker of response to chemotherapy. CA19-9 = carbohydrate antigen 19–9, OR = odds ratio. *$P < 0.05$.

Furthermore, ADAR1 has been reported to affect the efficacy of chemotherapy. In this study, patients with low ADAR1 expression showed a higher response rate, indicating that ADAR1 is involved in resistance to treatment. Recent studies have reported a relationship between ADAR1 and drug resistance in various cancer types. For example, in CRC, ADAR1 has been reported to activate the AKT pathway and be involved in chemoresistance[9]. Additionally, ADAR1-mediated RNA editing of SCD1 has been reported to drive drug resistance and self-renewal in gastric cancer[10]. Furthermore, ADAR1 has been suggested to promote cisplatin resistance in intrahepatic cholangiocarcinoma by regulating BRCA2 expression through an A-to-I editing mechanism[11]. ADAR promotes chemoresistance in CRC by regulating PARP1 expression[12]. These findings are consistent with those of our study, which showed that high ADAR1 expression was associated with chemotherapy resistance in CRC, indicating that ADAR1 plays a role in a novel molecular mechanism that regulates response to drugs.

In this study, the Cox proportional hazards model analysis revealed that high ADAR1 expression was significantly associated with shorter OS and was a powerful prognostic factor comparable to the presence or absence of extrahepatic metastasis. These findings indicate that ADAR1 comprehensively reflects cancer progression and treatment resistance rather than merely indicating tumor presence. Patients with high ADAR1 expression are less sensitive to chemotherapy and may show resistance to treatment. Therefore, more powerful initial treatment strategies are needed for these cases. FOLFOXIRI (5-FU + oxaliplatin + irinotecan) combined with bevacizumab has been reported to have a high response rate and a significant tumor reduction effect[13], making it a potentially powerful initial treatment option for advanced CRC with high ADAR1 expression. Additionally, if the cancer is RAS wild-type and presents on the left side of the colon, FOLFOX combined with an anti-EGFR antibody (cetuximab or panitumumab) should be considered[13,14]. According to the DEEPER trial, FOLFOXIRI combined with cetuximab may also be used[15]. However, because ADAR1 has been reported to be involved in the immune environment and interferon signaling[7], combining it with immune checkpoint inhibitors or introducing new treatments that target RNA editing should be considered. By considering the expression level of ADAR1, it is possible to construct a personalized treatment strategy that optimizes the treatment intensity for each patient. Further prospective studies are needed to establish a treatment algorithm based on ADAR1.

This study has some limitations. First, the number of cases was relatively small, and the statistical power of the subgroup analysis was limited. We had intended to compare ADAR1 expression with prognosis and chemotherapy response by regimen, but due to the insufficient number of cases, we have decided to postpone this analysis. Second, the direct relationship between high ADAR1 expression and increased RNA-editing activity was not evaluated. Further studies are needed to perform functional analyses and investigate the interaction with other factors that affect treatment efficacy and prognosis, such as RAS/BRAF mutation, MSI status, and immune cell infiltration.

In conclusion, ADAR1 plays an important role in both tumor progression and treatment response in stage IV advanced CRC with liver metastasis. ADAR1 is a molecule that is expected to be clinically applied as a new biomarker for prognosis and response to chemotherapy.

## Data availability

All data generated or analysed during this study are included in this published article.

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## Author contributions

K.N., K.S., K. Yoshida, S.T., H.K., H.M., H.Y., Yuhei Kondo, E.M., H.T., S.K., and T. Fujiwara conceived and designed experiments. K.N., H.U., T.T., M.K., and S.N. performed experiments. K.N., Yoshitaka Kondo, Y.M., T. Fuji, K. Yasui, Y.Y., R.S., K.T., N.K., and Yoshihiko Kakiuchi contributed to reagents, materials, and other analytical tools. K.N. and K.S. wrote the manuscript.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Ethics approval

Written informed consent was obtained from all patients. This study was approved by the Institutional Review Board, the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences and Okayama University Hospital (No. 1903–037).

## Additional information

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