The RNA binding protein neuro-oncological ventral antigen 1 (NOVA1) regulates IL-6 mRNA stability to enhance JAK2-STAT3 signaling in CRC

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ABSTRACT

The molecular mechanisms governing the metastasis of colorectal cancer (CRC) are incompletely understood. In the present study, we found NOVA1 to be expressed at higher levels in CRC cell lines and tissue samples, and this upregulation was positively correlated with TNM stage (p = 0.034), poor differentiation (p = 0.001), and lymph node metastasis (p = 0.008). Both overall survival (OS) and relapse-free survival (RFS) were both significantly decreased in patients with high NOVA1 expression relative to those with low expression. Through a multivariate analysis, we determined that NOVA1 independently predicted poor outcomes in those with CRC. In further functional studies, we found that NOVA1 expression controlled the proliferation and invasive characteristics of CRC cells via a mechanism wherein NOVA1 bound and stabilized the IL6 mRNA, enhancing IL-6/JAK2/STAT3 signaling to in turn upregulate matrix metalloproteinases (MMPs) 2, 7, and 9. NOVA1 therefore plays key functional roles in regulating CRC progression, and our results further indicate that it serve as a valuable prognostic biomarker and potentially a target for therapeutic treatment in individuals with CRC.

1. Introduction

Colorectal cancer (CRC) is among the most common forms of cancer [1], with its metastasis being the leading cause of death among those affected [2]. In individuals in which primary tumors are detected early, surgery or other interventions may prove effective, but when the disease is more advanced there are few effective treatment avenues. The metastasis of CRC is the result of a well-studied series of molecular events common to many forms of cancer, such as the epithelial-to-mesenchymal transition (EMT) and constitutive signaling by certain kinase [3]. While research into these mechanisms has aided in the development of novel treatments, metastasis remains a complex and multifaceted process which is not fully explored at present.

RNA-binding proteins (RBPs) have recently been shown to be key post-transcriptional regulators of translational activity [4–7], with several having been studied in the context of influencing melanoma progression and prognosis [8–10]. One such RBP linked with cancer is neuro-oncological ventral antigen 1 (NOVA1) [11,12]. Normally

expressed in central nervous system tissues, NOVA1 is able to facilitate specific alternative splicing events in cells via binding to conserved motifs [13–15]. In certain tumor types such as lymphomas, gastric cancer (GC), hepatocellular carcinomas (HCC), astrocytomas, and oligodendrogliomas, NOVA1 expression has been found to be dysregulated [12,16–18]. Zhang et al. [19] found that NOVA1 overexpression was both linked to GABA regulation and to the enhancement of HCC cell growth. Consistent with this finding, Shen et al. [20] found that miR-339, which suppresses NOVA1 expression, was able to disrupt the growth and metastasis of tumor cells. The specific role of NOVA1 in CRC, however, has not been studied.

Herein, we investigated how NOVA1 influences CRC, identifying a positive correlation between its expression and poor outcomes in those with CRC. In patients who underwent surgery, we further found NOVA1 expression to independently predict poorer survival and higher rates of tumor recurrence. In functional studies, we also found that NOVA1 expression enhanced the migratory abilities of CRC cells, and that this enhancement was at least in part due to its ability to regulate IL-6/
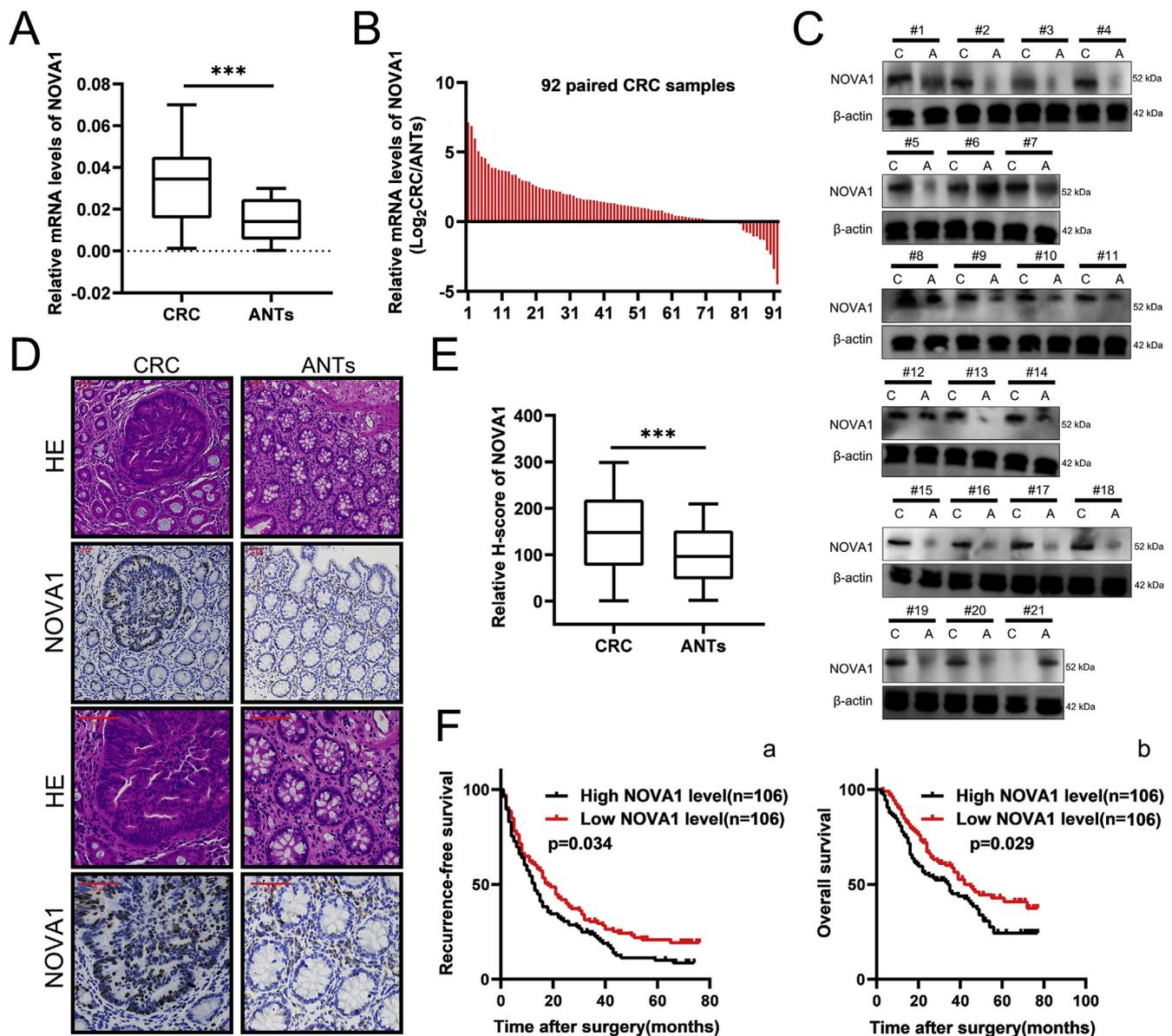
Fig. 1. NOVA1 expression is associated with CRC progression

(A and B) NOVA1 mRNA levels were assessed in 92 pairs of CRC and adjacent normal tissues (ANT) via qRT-PCR, with Student's t-tests used to gauge significance. (C) NOVA1 protein levels in CRC and ANT tissues were assessed via Western blotting.(D) NOVA1 IHC staining results in CRC samples. Student's t-tests were used to gauge significance. (E) Representative IHC images of NOVA1 protein levels in CRC and ANT samples. Scale bar, 100 mm. (F) RFS (a) and OS (b) in NOVA1-high and -low patients, with significance assessed via a 2-sided log-rank test.

JAK2/STAT3 signaling. This study for the first time demonstrates that NOVA1 is able to stabilize IL6 mRNA via bindings its 3′UTR, thereby enhancing the JAK2/STAT3 signaling associated with the progression of CRC.

2. Materials and methods

2.1. Patient samples

We performed immunohistochemistry (IHC) using stored tissue samples from 212 patients who had stage I–III CRC and who had undergone curative surgery in the Changhai Hospital from January 2010 to December 2012. Pathological analyses confirmed that all of these patients had adenocarcinoma histology. For details regarding clinicopathological findings in these patients, see Table S1. Patient follow-up was conducted from date of operation until the end of the study period (December 2017) or death. In patients, we measured both

overall survival (OS), which was the period of time from surgery until CRC-related death, as well as relapse-free survival (RFS), which was the period of time from surgery until the disease recurred. For RFS analyses, patients who had died or did not exhibit recurrence were censored at the time of last follow-up or death. Study exclusion criteria included: disruptions in the function of the heart, lungs, kidneys, or liver; previous receipt of any anti-cancer treatments; death from any other cause. The 7th Edition tumor-node-metastasis (TNM) classification system was used to stage tumors based on the guidelines of the American Joint Committee on Cancer Staging. Any patients with stage III disease, as well as those with stage II disease who also exhibited pT4 or poor tumor differentiation, were treated via 5-fluoruracil-based adjuvant chemotherapy. In addition to this subset of FFPE tissue sections, we obtained a further 92 freshly frozen CRC tissue samples from patients with stage I–III primary CRC treated via surgery at Changhai Hospital from January 2015 to December 2016 (Table S2). These samples were utilized for qRT-PCR and western blotting experiments. In

Table 1
Correlation of Clinico-pathologic characteristics with NOVA1 protein level.

Characteristics	No.of patients (N = 212)	NOVA1 protein level		p-vaule[a]
		High	Low	
Gender				0.391
Female	93	48	45	
Male	119	58	61	
Age(years)				0.325
< 60	62	29	33	
≥60	150	77	73	
Tumor location				0.339
Rectum	94	45	49	
Colon	118	61	57	
Differentiation grade				**0.001**
Well + Moderate	161	70	91	
Poor	51	36	15	
Tumor size(cm)				0.061
< 5	84	36	48	
≥5	128	70	58	
Local invasion				0.426
pT1-T2	34	18	16	
pT3-T4	178	88	90	
Lymph node metastasis				**0.008**
N0–1	132	57	75	
N2–3	80	49	31	
TNM stage				**0.034**
I + II	128	57	71	
III	84	49	35	
Adjuvant chemotherapy				0.445
No	100	49	51	
Yes	112	57	55	
CA19–9,U/mL				0.115
< 37	149	79	70	
≥37	63	27	36	
CEA,ng/mL				0.110
< 5	153	81	72	
≥5	59	25	34	

[a] Pearson chi-sequare test was used for comparison between subgroups. Bold type indicates statistical significance. Abbreviations: TNM tumor-node-metastasis, CA19–9 carbohydrate antigen 19–9, CEA carcinoembryonic antigen.

all cases, patients had provided written informed consent. This study was performed in a manner consistent with the Declaration of Helsinki, and the institutional Ethics Committee of Changhai Hospital approved this study. The ethical clearance number was 2017-148-01.

2.2. qRT-PCR

qRT-PCR was conducted based on previous protocols [21]. Relative gene expression was determined based upon relative Ct values normalized to β-actin expression. All primers and probes used are listed in Table S3.

2.3. Western blotting

Western blotting was conducted based on previous protocols [21]. Cell Signaling Technology was the source of all primary antibodies against NOVA1, E-cadherin, N-cadherin, Vimentin, MMP2,MMP7, MMP9, Phospho-Akt(p-Akt), total Akt (T-Akt), Phospho-ERK(p-ERK), total ERK(T-ERK), Phospho-JNK(p-JNK), total JNK (T-JNK), Phospho-p38(p-p38), total p38(T-p38),Phospho-STAT3(p-STAT3), total STAT3(T-STAT3), JAK2, Phospho-JAK2(at Y1007 and Y1008). β-actin served as a loading control, and anti-β-actin was from Santa Cruz Biotechnology.

2.4. RNA binding assay

The RiboTrap Kit (MBL International) was used for RNA binding

assays based on the provided instructions. A pBluescript plasmid encoding the 3′UTR of IL6 was used in order to randomly incorporate 5-bromo-UTP (BrU) into this mRNA sequence. This sequence was then bound by Gbead-conjugated anti-BrU. Lysates from the indicated cell lines stably expressing shNOVA1 or shNT were then mixed for 2 h with these beads, after which samples were washed, and protein was eluted and assessed via western blotting.

2.5. Statistical analysis

GraphPad Prism (GraphPad Software) was used for all analyses. Data are means ± s e m unless otherwise indicated. The following statistical tests were used as appropriate: Student's two-tailed t tests, Wilcoxon signed-rank tests, Pearson chi-square tests, Pearson correlation analyses, Log-rank tests, Fisher's exact tests, nonparametric Mann-Whitney U tests, and Cox proportional hazards regression models. $p < 0.05$ was the threshold of significance; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

3. Results

3.1. CRC tissues exhibit increased NOVA1 levels

To explore the expression of NOVA1 in human CRC, we collected 92 pairs of primary CRC samples and adjacent normal tissues (ANTs). When we analyzed NOVA1 expression in these samples via qRT-PCR, expression was significantly higher in the CRC samples (Fig. 1A), of which 82.6% (76/92) exhibited higher NOVA1 expression than the matched control tissue (Fig. 1B). To confirm and expand upon this result, we further conducted Western blotting to assess NOVA1 protein levels in 21 CRC and ANT tissues, revealing increased NOVA1 protein in CRC samples (Fig. 1C). IHC was additionally conducted in 212 FFPE primary CRC samples, revealing higher NOVA1 expression in CRC tissue samples relative to ANT controls (Fig. 1D and E), consistent with a role for NOVA1 in tumorigenesis. Together, these findings confirm that NOVA1 expression is increased in CRC.

3.2. Increased NOVA1 expression corresponds to poorer outcomes and disease characteristics

In the 212 CRC patient samples processed via IHC, we additionally explored the association between NOVA1 expression and patient outcomes by grouping patients into NOVA1-high and -low groups based on median staining density values in this cohort. Based on the available clinicopathological data from these patients, we found a strong positive correlation between the expression of NOVA1 and poor differentiation (p = 0.001), TNM stage (p = 0.034), and lymph node metastasis (p = 0.008) (Table 1). There were also clear reductions in OS and RFS in NOVA1-high patients relative to their NOVA1-low counterparts (Fig. 1F). In a multivariate regression model of the data available for these patient samples, we found NOVA1 levels to independently predict the aggressiveness of CRC disease and patient outcomes (Table 2). As such, NOVA1 has clear value as a prognostic indicator for CRC in patients.

3.3. Impairing NOVA1 expression disrupts CRC cell metastatic potential

As our patient sample findings clearly underscore an association between NOVA1 expression and the aggressive nature of CRC tumors, we next sought to assess whether NOVA1 directly facilitates metastatic potential in these cells. We first assessed NOVA1 expression in human CRC cell lines (HT29, HCT116, SW480, Lovo and SW620), revealing that the more invasive and metastatic cell lines (Lovo and SW620) exhibited higher NOVA1 expression than did the other cell lines assessed(Fig. 2A). To further explore the functional role of NOVA1 in these cells, we produced 4 NOVA1-specific shRNAs to silence the

Table 2
Univariate and multivariate analyses of clinicopathologic parameters associated with recurrence-free survival and overall survival.

Variable	Categories	Univariate analysis			Multivariate analysis		
		HR	95% CI	p-vaule	HR	95% CI	p-vaule
Disease-free survival							
Gender	Male/Female	1.065	0.739–1.472	0.845	–	–	NA
Age(years)	≥60/ < 60	1.271	0.820–1.806	0.308	–	–	NA
Tumor location	Colon/rectum	1.162	0.809–1.618	0.471	–	–	NA
Tumor size(cm)	≥5/ < 5	1.264	1.1890–1.813	0.035	–	–	NS
Differentiation grade	Poor/well + moderate	1.444	1.017–2.206	0.037	–	–	NS
Local invasion	pT3-4/pT1-2	2.048	1.169–3.543	0.011	1.402	1.178–2.534	0.039
Lymph mode metastasis	N2–3/N0–1	1.961	1.358–2.711	0.013	1.197	1.132–2.362	0.021
TNM stage	III/I + II	1.912	1.403–2.800	0.019	1.894	1.068–3.520	0.041
Adjuvant chemotherapy	Yes/no	1.182	0.244–2.503	0.087	–	–	NA
CA19–9,U/Ml	≥37/ < 37	1.507	1.2981–2.318	0.018	1.247	1.084–1.955	0.043
CEA, ng/mL	≥5/ < 5	1.935	1.834–2.819	0.035	–	–	NS
NOVA1 expression	High/low	1.945	1.310–2.865	0.029	1.859	1.238–2.767	0.037
Over-all survival							
Gender	Male/Female	1.088	0.744–1.635	0.813	–	–	NA
Age(years)	≥60/ < 60	1.255	0.791–1.954	0.372	–	–	NA
Tumor location	Colon/rectum	1.094	0.752–1.656	0.659	–	–	NA
Tumor size(cm)	≥5/ < 5	1.334	0.906–2.045	0.167	–	–	NA
Differentiation grade	Poor/well + moderate	1.604	1.066–2.527	0.033	–	–	NS
Local invasion	pT3-4/pT1-2	2.213	1.120–4.136	0.025	1.469	1.172–2.838	0.035
Lymph mode metastasis	N2–3/N0–1	2.415	1.685–3.685	0.022	2.129	1.159–3.830	0.018
TNM stage	III/I + II	2.484	1.665–3.642	0.027	2.717	1.380–5.455	0.005
Adjuvant chemotherapy	Yes/no	2.094	1.380–3.105	0.071	–	–	NA
CA19–9,U/Ml	≥37/ < 37	1.613	1.4967–2.611	0.036	–	–	NS
CEA, ng/mL	≥5/ < 5	1.241	0.785–1.896	0.406	–	–	NA
NOVA1 expression	High/low	2.37	1.536–4.021	0.027	2.25522	1.424–3.818	0.035

Note. NA: not adopted; NS: not significant.

endogenous NOVA1 expression (shNOVA1) in the Lovo and SW620 cells, using the construct which achieved the best knockdown efficiency in subsequent assays (Fig. 2B). Cells in which NOVA1 had been knocked down exhibited substantially impaired proliferation relative to those cells expressing a non-targeting control shRNA (shNT) (Fig.2C, a–b). Transwell migration and invasion assays further confirmed a decrease in the migratory capacity of these NOVA1-KD cells (Fig.2D, a–b). To extend this work into an animal model, we performed murine tail vein injections of Lovo-PGL cells transfected with shNT or shNOVA1 in order to generate a model of lung metastasis, and we monitored metastatic disease progression every 2–3 weeks using a luciferase-based in vivo imaging apparatus. We found that shNOVA1 significantly disrupted the metastasis of these CRC cells into the lungs (Fig. 2E and F). When lung samples were isolated following an 8 week study period and stained using hematoxylin and eosin (H&E), we observed higher numbers of micrometastases in shNT samples relative to shNOVA1 samples (Fig. 2G and H). These findings therefore confirm that knocking down NOVA1 can impair the metastatic potential of CRC cells in vitro and in vivo.

3.4. NOVA1 enhances STAT3 signaling to induce MMP-2/7/9 expression

We next sought to determine how NOVA1 facilitates this increased metastatic potential in CRC cells, first via assessing the role of this RBP in regulating the epithelial-mesenchymal transition (EMT) given its central importance in metastasis. However, we did not find any significant shifts in EMT-related gene expression (vimentin, E-cadherin, or N-cadherin), suggesting that NOVA1 exerts its effects in an EMT-independent fashion (Fig. 3A). The production of proteolytic MMPs is also known to be a central process in tumor invasion, as these enzymes are able to degrade stromal tissue in order to facilitate better migratory ability in tumors. We found that the expression levels of MMP-2/7/9 were markedly reduced in shNOVA1 CRC cell lines (Fig. 3B). We also found that levels of p-STAT3 were reduced in these shNOVA1 cell lines relative to shNT controls, whereas phosphorylation levels of ERK, JNK, AKT, and p38 were unchanged (Fig. 3C). When we transiently

transfected shNOVA1, shNT, or pmyr-STAT3 into Lovo/SW620 cells, we found that ectopic STAT3 expression in cells in which NOVA1 had been knocked down led to a significant increase in both cell migratory ability (Fig. 3D) and expression of MMP-2/7/9 (Fig. 3E). There was also a clear positive correlation between the p-STAT3 and NOVA1 levels in CRC patient samples (Fig. 3F). Together, these results further support a direct role for NOVA1 in the positive regulation of CRC metastasis at least in part via regulating STAT3 signaling.

3.5. NOVA1 stabilizes the IL6 mRNA via binding its 3′UTR

STAT3 activation is governed by the activation of specific receptor tyrosine kinases by STAT3-activating ligands including IL-6, IL-10, IL-17A, IL-21, and IL-23A. We therefore assessed the expression of these cytokines in CRC cell lines, revealing significantly decreased IL-6 mRNA levels in shNOVA1 cell lines relative to shNT controls (Fig. 4A). We also found that JAK2 phosphorylation was markedly decreased by NOVA1 knockdown (Fig. 4B), suggesting that an IL-6/JAK2/STAT3 signaling pathway may regulate the survival and metastasis of CRC cells. As NOVA1 is an RBP, and the binding of factors to mRNA 3′UTR regions can modulate mRNA stability [22], we next explored the possibility that NOVA1 may bind the IL6 3′UTR to slow its degradation in CRC cells. We therefore conducted a RiboTrap RNA–protein binding assay using this IL6 3′UTR region. Lysates from shNOVA1 or shNT cells were mixed with protein G bead-conjugated BrdU-labeled IL6 3′UTR, and eluted proteins were assessed by Western blotting. We found that NOVA1 was indeed able to bind this IL6 3′UTR region (Fig. 4C). To further confirm that this was associated with increased stability of the IL6 mRNA, we treated cells with α-amanitin to disrupt RNA synthesis and then assessed decreases IL-6, β-actin, and 18S rRNA over 24 h. We observed that NOVA1 knockdown significantly reduced the half-life or the IL6 mRNA transcript (Fig. 4D). We further assessed how NOVA1 expression influenced IL-6 secretion, revealing that NOVA1-KD cells secreted significantly less IL-6 than did shNT controls (Fig. 4E). We then assessed IL-6 mRNA expression in the 92 CRC tissue samples previous assessed in Fig. 1A, identifying a correlation between NOVA1 and IL-6 expression
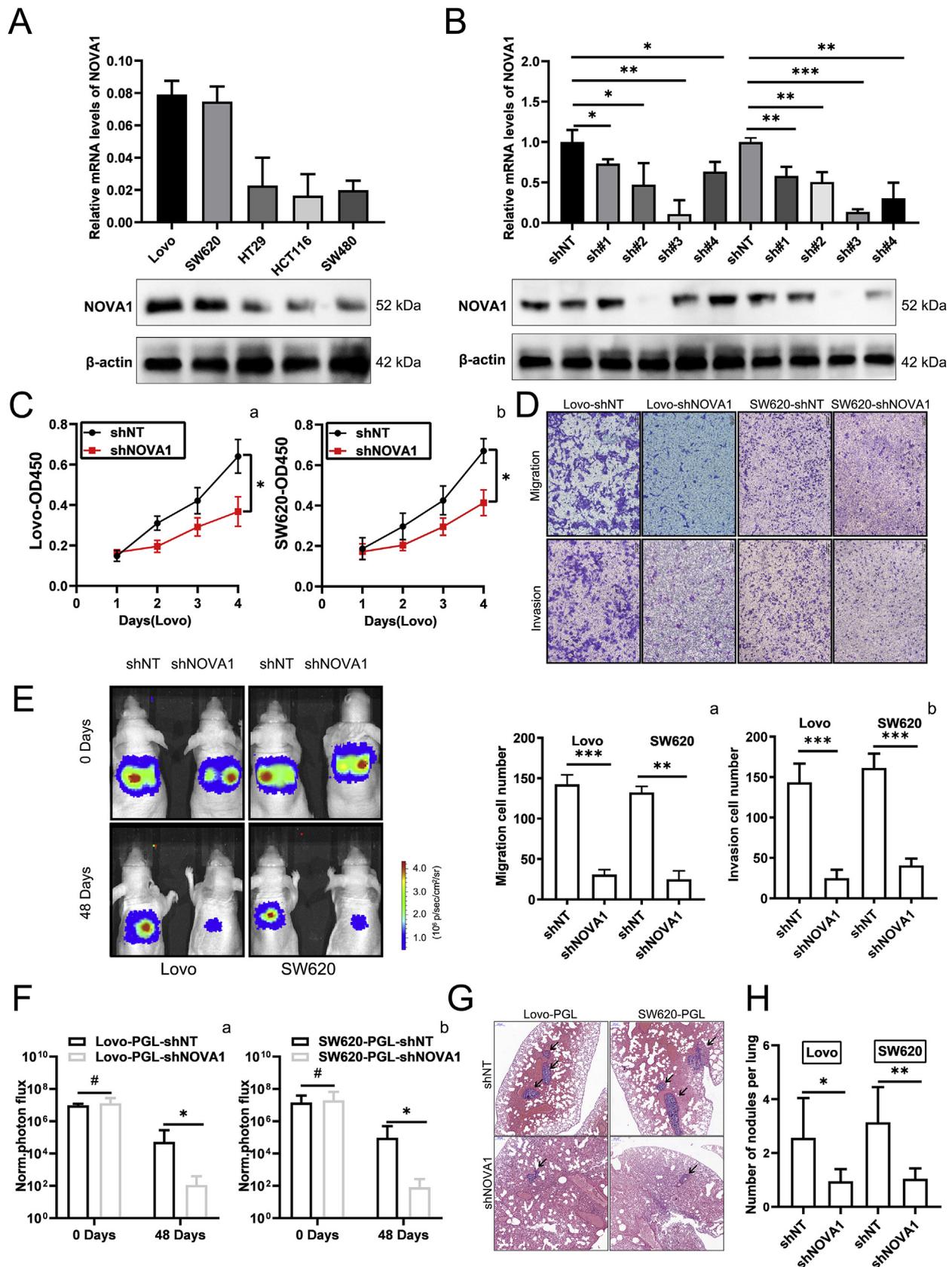
Fig. 2. NOVA1 knockdown impaired CRC cell metastasis in vivo and in vitro

(A) NOVA1 expression in a range of CRC cell lines. (B) NOVA1 shRNA transduction (shRNA 1–
4) (LV-sh-NOVA1) in Lovo and SW620
cells led to a significant decrease in NOVA1 expression as measured via qRT-PCR and Western blotting.(C and D) NOVA1 knockdown influence on CRC cell in vitro proliferation (C), migration, and invasion (D). Scale bar, 100 μm. (E) Lung metastasis images from mice injected via the tail vein with Lovo-PGL cells measured using the IVIS Imaging System. Images of luciferase activity are shown both upon initial injection and after 48 days, with a statistical analysis in (F). (G) Representative H&E stained images of lung metastases from G. Scale bar, 50 mm, with a statistical analysis in (H). Data are means ± s e m, and were analyzed via Student's t-tests.
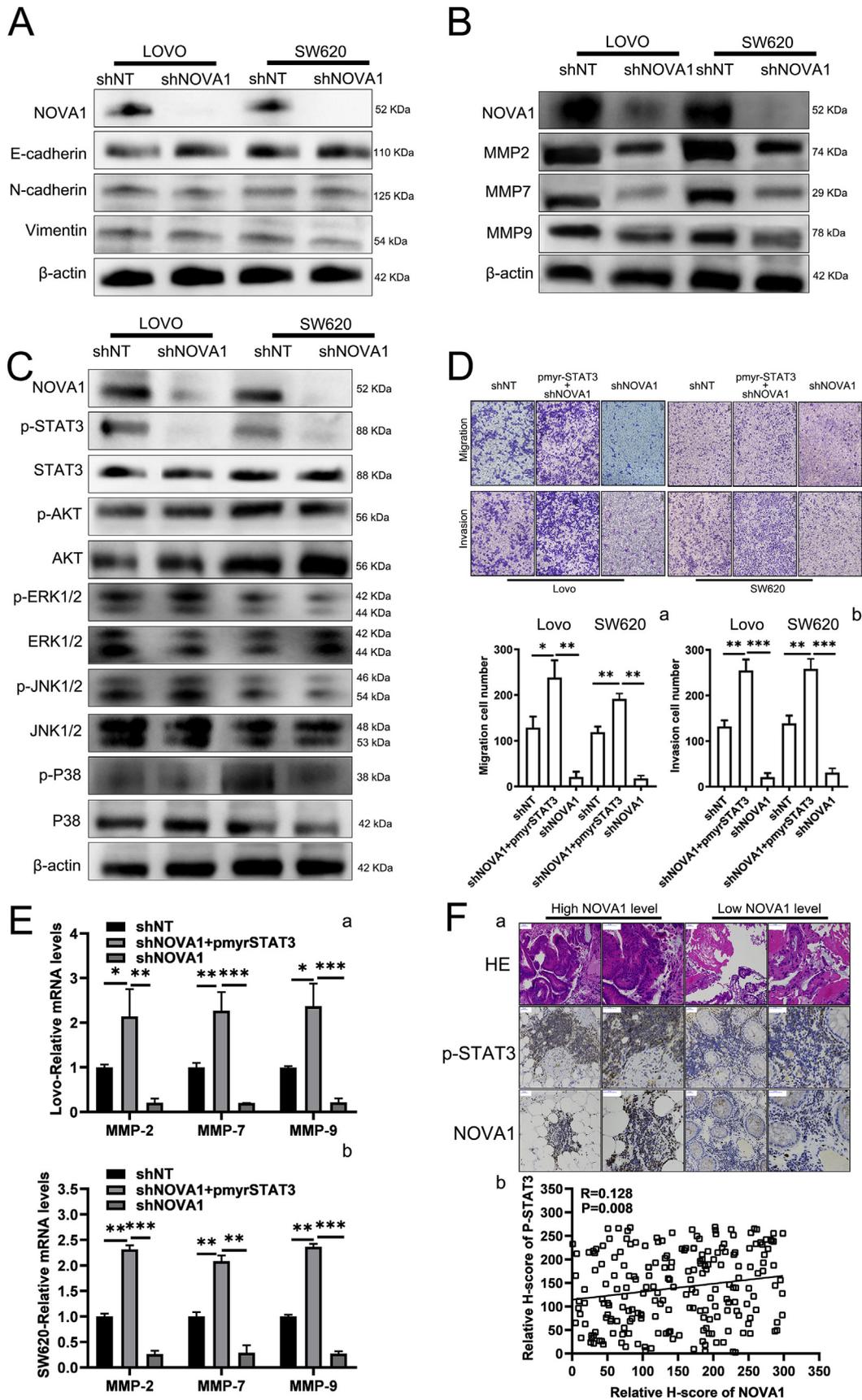
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Fig. 3. NOVA1 regulates STAT3 signaling to promote MMP-2/7/9 expression

(A) Western blotting was used to assess expression of vimentin, as well as E − and N-cadherinl. (B) MMP-2/7/9 expression was measured via Western blotting. (C) Western blotting was used to assessed levels of total and phosphorylated STAT3 (p-STAT3), AKT (p-AKT), ERK1/2 (p-ERK1/2), c-Jun-N-terminal kinase (p-JNK), and p38 (p-p38). (D) 24 h after transfecting Lovo and SW620 cells using shNT, shNOVA1, or pmyr-STAT3, cells were assessed via migration and invasion assays with representative images shown. Scale bar, 50 mm. Significance was calculated via Student's t-tests. (E) Relative MMP-2/7/9 expression was assessed via qRT-PCR after cell treatment. Significance was calculated via Student's t-tests. (F)(a) Levels of NOVA1 and p-STAT3 were assessed by IHC in tissue sections, with a positive correlation between NOVA1 and p-STAT3 expression levels. Scale bar, 50 μm. (b) The correlation between H-score, NOVA1, and p-STAT3 was assessed in 212 patients, with P values assessed via Pearson's correlation test.
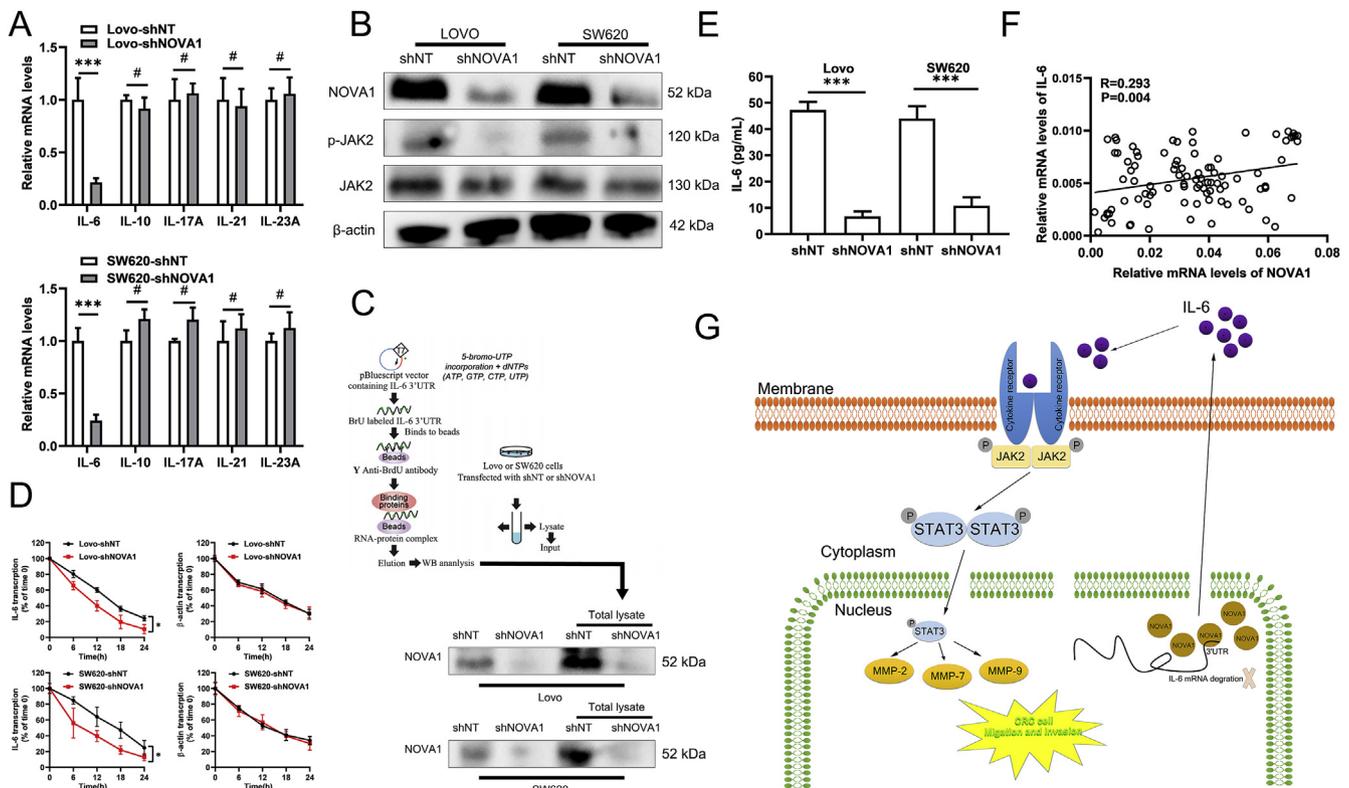
Fig. 4. NOVA1 stabilizes IL6 mRNA via 3′UTR binding

(A) qRT-PCR was used to assess IL-6, IL-10, IL-17A, IL-21, IL-23A, and IL-6 expression in treated cells, with Student's t-tests used to gauge significance. (B) Western blotting was used to assess levels of NOVA1, JAK2, and p-JAK2 in cell lysates.(C) Assessment of NOVA1 binding to a BrdU-labeled IL6 3′UTR mRNA, using shRNA-transduced Lovo and SW620 lysates mixed with binding protein G beads and the labeled mRNA constructs. Eluted protein was analyzed for NOVA1 expression via Western blotting. (D) qRT-PCR was used to assess IL-6 and β-actin mRNA stability following the addition of α-amanitin (50 μM) to disrupt RNA synthesis, with normalization to 18S rRNA (produced by RNA polymerase I and unaffected byα-amanitin). Significance was determined via Student's t-tests.(E) IL-6 levels in cell culture supernatants as assessed by ELISA, with significance assessed via Student's t-tests.(F) NOVA1 and iL-6 mRNA correlations were assessed in CRC tissue samples, with Pearson's correlation test used to assess the significance of this correlation. (G) An overview of the proposed NOVA1-regulated IL-6/JAK2/STAT3 signaling mechanism as a regulator of the metastasis of CRC.

in these samples (Fig. 4F). Together these findings shown that NOVA1 can enhance IL6 transcript stability via 3′UTR binding.

4. Discussion

CRC remains among the deadliest forms of cancer, with high rates of metastasis and recurrence following surgical tumor removal. The specific mechanisms governing this metastatic behavior are incompletely understood, and there are still many markers of tumor recurrence which have yet to be identified. As such, efforts to identify novel biomarkers of these disease processes will prove invaluable to improving disease monitoring efforts and patient outcomes. These biomarkers may further shine light on new potential therapeutic targets that can be leveraged in order to more effectively treat CRC.

There is abundant evidence that RBPs play central roles in regulating processes essential to cell survival, including apoptotic cell death, proliferation, migration, and invasion [23–25]. Consistent with their central roles, many specific RBPs are linked to the development

and progression of certain tumor types [25,26]. In previous studies have suggested that the RBP play important role in a variety of malignant tumor progression [17,27–29]. What role NOVA1 plays in CRC has not previously been addressed, leading us to address it in the present study. We found that both CRC cell lines and tissue samples exhibited increased NOVA1 expression, and this expression was positively correlated with poor differentiation, advantaged TNM stage, and lymph node metastasis. Elevated NOVA1 expression within a tumor was an independent predictor of decreased OS and RFS in CRC patients, suggesting that NOVA1 levels directly correlate with poor outcomes. When NOVA1 was knocked down in vitro and in vivo, tumor cell growth and metastasis was significantly suppressed. Together our results indicate that NOVA1 both serves a key functional role in mediating CRC metastasis and that it can serve as a novel prognostic biomarker of CRC patient outcomes.

As an important inflammatory factor, interleukin-6 (IL-6) activates several downstream pathways, such as the JAK2/STAT3 and P13K/AKT pathways. The JAK2/STAT3 pathway is a signal transduction pathway

from the membrane to the nucleus. The activation of JAK2 protein kinase can catalyze the phosphorylation of STAT3 protein into the nucleus, which can regulate the expression of oncogene-related genes [30–32]. With respect to the regulatory mechanisms whereby NOVA1 governs the metastasis of CRC cells, we identified a mechanism whereby it is able to bind the IL6 mRNA 3′UTR in order to stabilize this transcript. This stabilization was linked to JAK2/STAT3 signaling, and the upregulation of MMP-2/7/9, thereby promoting tumor growth and metastasis (Fig. 4G). Several pieces of experimental evidence were consistent with this model: (i) the substantially decreased expression of MMP-2/7/9 following shRNA-mediated NOVA1 knockdown in CRC cells; (ii) decreased p-JAK2/p-STAT3 levels following NOVA1 knockdown in CRC cells, and improved cell migration/invasion following ectopic STAT3 expression in these NOVA1-KD cells; (iii) RiboTrap RNA–protein binding assays providing direct evidence for NOVA1 binding to the IL6 mRNA 3′UTR, as well as reduced IL6 half-life and IL-6 secretion following NOVA1 knockdown; and (iv) a positive correlation between NOVA1, IL6, and p-STAT3 levels in human CRC tissue samples. It is essential that the metabolism of RNA transcripts is carefully regulated in order to maintain normal cellular homeostasis and dysregulation of such stability can mediate disease pathology. RBPs can play a number of different roles in regulating RNA expression, from mediating alternative splicing to altering transcript polyadenylation and stability [33]. Many previous studies have found that miRNAs are able to modulate the stability of mRNA transcripts, and there is also evidence for the importance of RBPs to mRNA stability in the context of cancer, mediating both oncogenesis and disease progression. We have identified a novel mechanism wherein NOVA1 stabilizes IL6 transcripts, thereby enhancing JAK2/STAT3 signaling to promote CRC pathogenesis and progression, although future studies will be needed to fully explore and validate this mechanism.

In conclusion, we have experimentally identified NOVA1 as a novel regulator of the metastasis of CRC cells, owing at least in part to its ability to regulate the IL-6/JAK2/STAT3 signaling pathway via binding to and stabilization of the IL6 mRNA 3′UTR. These findings offer novel insights into the biology of CRC, and suggest potential future therapeutic avenues wherein NOVA1 may be targeted in order to better control or disrupt CRC metastasis.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.suronc.2019.09.009.

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