The EGF/hnRNP Q1 axis is involved in tumorigenesis via the regulation of cell cycle-related genes

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Abstract

Heterogeneous nuclear ribonucleoprotein (hnRNP) Q1, an RNA-binding protein, has been implicated in many post-transcriptional processes, including RNA metabolism and mRNA splicing and translation. However, the role of hnRNP Q1 in tumorigenesis remains unclear. We previously performed RNA immunoprecipitation (RIP)-seq analysis to identify hnRNP Q1-interacting mRNAs and found that hnRNP Q1 targets a group of genes that are involved in mitotic regulation, including Aurora-A. Here, we demonstrate that altering the hnRNP Q1 level influences the expression of the Aurora-A protein, but not its mRNA. Stimulation with epidermal growth factor (EGF) enhances both binding between hnRNP Q1 and Aurora-A mRNA as well as the efficacy of the hnRNP Q1-induced translation of Aurora-A mRNA. The EGF/hnRNP Q1-induced translation of Aurora-A mRNA is mediated by the mTOR and ERK pathways. In addition, we show that hnRNP Q1 up-regulates the translation of a group of spindle assembly checkpoint (SAC) genes. hnRNP Q1 overexpression is positively correlated with the levels of Aurora-A and the SAC genes in human colorectal cancer tissues. In summary, our data suggest that hnRNP Q1 plays an important role in regulating the expression of a group of cell cycle-related genes. Therefore, it may contribute to tumorigenesis by up-regulating the translation of these genes in colorectal cancer.

Introduction

Genetic instability is a major event in the tumorigenesis of various cancers, including colorectal cancer. To maintain genome integrity and fidelity, it is important that cells regulate the expression of proteins involved in the cell cycle checkpoint mechanism, as well as control chromosome replication and separation during cell division1. Aurora-A is a serine/threonine kinase involved in regulating cell cycle progression, cell survival and malignant transformation2. Overexpression of Aurora-A has been found in many cancer cells, including breast, colon, pancreas, liver, and stomach cancer3. The deregulation of Aurora-A kinase may result in chromosomal instability, indicating a link with tumorigenesis4. Aurora-A is regarded as an oncoprotein. The oncogenic role of Aurora-A has been frequently mentioned, and this protein has attracted substantial interest as a therapeutic target in various cancers5. Previous studies have suggested that the overexpression of Aurora-A in cancers may result from gene amplification6,7, transcriptional up-regulation8,9 or enhanced protein stability10,11. In our previous reports, we found that Aurora-A is translationally up-regulated by heterogeneous nuclear ribonucleoprotein Q1 (hnRNP Q1)12, and epidermal growth factor (EGF) increases the expression of the Aurora-A protein via the ERK and Akt pathways13. However, the mechanism by which EGF mediates the translational up-regulation of Aurora-A expression and the translational regulatory factors that

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contribute to the increased expression of Aurora-A remains unclear.

hnRNPs are a family of RNA-binding proteins in eukaryotic cells. The hnRNP family comprises at least one or more RNA recognition motifs (RRMs) and combines with an RGG box or acidic domain for protein–protein interaction or RNA binding. hnRNPs are trans-acting elements that play an important role in multiple aspects of nucleic acid metabolism, including the packaging of nascent transcripts, alternative splicing and translational regulation. Recently, two studies showed that aberrant hnRNP expression can cause a wide variety of diseases, including neurodegeneration and cancer. Seven isoforms of hnRNP Q (also known as SYNCRIP or NSAP1) have been identified in human cells. These hnRNP Q isoforms (hnRNP Q1-Q7) result from the alternative splicing of a single gene, SYNCRIP. They prefer to bind to mRNAs with AU-rich RNA sequences. hnRNP Q contains three RRM domains, one RGG-rich region (RGG box) and one acidic domain (AcD). Recent studies have shown that hnRNP Q plays a role in mRNA processing events, including pre-mRNA splicing, mRNA transport, and translational regulation. hnRNP Q1 may participate in mRNA localization, translation, and turnover. Consistent with this assumption, we previously demonstrated that hnRNP Q1 translationally up-regulates Aurora-A mRNA and increases tumorigenesis in colorectal cancer. However, the role of hnRNP Q1 in tumorigenesis is not well characterized.

In the present study, we demonstrate that hnRNP Q1 acts as a trans-acting factor to up-regulate Aurora-A expression, and EGF enhances the hnRNP Q1-induced translational efficacy of Aurora-A mRNA. hnRNP Q1-induced Aurora-A mRNA translation might be mediated by the mTOR and ERK pathways. In addition to Aurora-A, hnRNP Q1 translationally up-regulates a group of genes known to be involved in the spindle assembly checkpoint (SAC). The expression of hnRNP Q1 is positively associated with the levels of Aurora-A and these SAC genes in colorectal cancer. In conclusion, we propose that hnRNP Q1 plays a novel role in tumorigenesis by translationally up-regulating cell cycle-related mRNAs in colorectal cancer.

### Materials and methods

#### Cell culture, siRNA, and transfection

SW480 and HCT116 human colorectal cancer cells were grown in Leibovitz’s L-15 medium (L-15; Invitrogen, Carlsbad, CA) and RPMI 1640 (Invitrogen), respectively. All media were supplemented with 10% fetal bovine serum (Invitrogen), 100 µg/ml streptomycin and 100 U/ml penicillin. The cells were maintained at 37 °C in 5% CO2. hnRNP Q (SYNCRIP) siRNA was purchased from Dharmacon™ (L-016218) and was transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

#### Antibodies and reagents

Anti-EGFR (sc-03), anti-hnRNP Q (sc-56703), anti-phospho-ERK (sc-7383), anti-Lamin A/C (sc-7292), anti-ribosomal protein S6 (sc-74459), anti-Bub1 (sc-47743), Mps1 (sc-540), and anti-GAPDH (sc-25778) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-ERK1/2 (06-182) antibody was purchased from Upstate (Charlottesville, VA, USA), and anti-Aurora-A (35C1) antibody was purchased from GeneTex (Irvine, CA, USA). Anti-BubR1 (sc#612502) antibody was purchased from Becton Dickinson (Franklin Lakes, New Jersey, USA), and anti-phospho-mTOR/ Ser2448 (#2971), anti-p-4E-BP1/Thr70 (#9455) and anti-4E-BP1 (#9452) antibodies were purchased from Cell Signaling (Beverly, MA, USA). Anti-Aurora-B (A5102) antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-GFP (JL-8) antibody was purchased form Clontech (Mountain View, CA, USA). Human recombinant EGF was purchased from Promega (Madison, WI, USA). U0126 was purchased from Sigma-Aldrich.

#### Quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen) according to the manufacturer’s instructions. Reverse transcription was performed using 1 µg of total RNA and a high-capacity cDNA Archive Kit (Applied BioSystems, Foster City, CA). Diluted cDNA (10 ng) was used as a template for RT-qPCR using the SYBR Advantage qPCR Premix (Bio-Rad, Hercules, CA). The relative expression of each mRNA was evaluated using the CFX Connect™ Real-Time PCR Detection System (Bio-Rad). Actin mRNA was used as the internal control. At least two independent analyses were performed for each sample. The following primer sequences were used: for Aurora-A, forward, 5′-ATGCTCTGTCTTCTGTTCTC-3′ and reverse, 5′-TCAGAGATCCACCTTCCTC-3′; for Bub1, forward, 5′-GGAGGAGGCTTCTGTCAGCA-3′ and reverse, 5′-TCACAAAAACTCTTTCACTG-3′; for Mad2, forward, 5′-GGTCAAGAGCTTGAGG-3′ and reverse, 5′-CTCCTCTTGTTCTG-3′; for Mps1, forward, 5′-CCAGGGCAACTTGAGG-3′ and reverse, 5′-ATTCATCGGCTA-3′; and for Actin, forward, 5′-CTGGACTTCGAGCAAGAGATG-3′ and reverse, 5′-TGATGGAGTGGTATTTCCG-3′.
Preparation of cell extracts and western blot analysis

The cells were harvested in RIPA lysis buffer (50 mM Tris-HCl/pH 8.0, 150 mM NaCl, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, 1 mM DTT, 10 mM β-glycerol phosphate, and 1 mM EGTA) containing a protease inhibitor cocktail. The protein lysates were cleared using centrifugation at 13,000 rpm for 10 min, and protein concentrations were determined using the Bradford method and a Protein Assay Kit (Bio-Rad). Equivalent protein lysates were used to perform western blot analysis using antibodies as indicated in the text. The levels of proteins were quantitated and normalized to the level of GAPDH using ImageJ software.

Ribosomal protein S6 immunoprecipitation assay (S6-IP)

The cells were harvested and lysed using S6-IP buffer (10 mM HEPES/pH 8.0, 100 mM KCl, 5 mM MgCl2, 1% Triton-X100, 0.5% sodium deoxycholate, 100 units/ml RNase inhibitor, and 100 μg/ml cycloheximide). The cell lysates were pre-cleaned with protein A beads for 1 h at 4°C and then immunoprecipitated using anti-ribosomal protein S6 antibodies for 2 h. Protein A beads were then added and incubated for additional 1 h at 4°C. The beads were washed three times with S6-IP buffer, and the RNA immunoprecipitated by the beads was extracted with Trizol reagent (Invitrogen). The S6-bound mRNAs were evaluated using RT-qPCR.

RNA-immunoprecipitation assay (RIP)

The cell lysates for RIP were collected in RIP buffer (10 mM HEPES/pH 8.0, 40 mM KCl, 3 mM MgCl2, 5% glycerol, 2 mM DTT, 0.5% sodium deoxycholate, 100 units/ml RNase inhibitor and protease inhibitor). The cytoplasmic extracts were incubated with anti-GFP antibody in RIP buffer at 4°C for 2 h. The protein A/G beads were pre-cleaned with 1 mg/ml tRNA for 2 h and then added to the immunoprecipitated lysates. The mixture was then incubated at 4°C for 2 h. The beads were washed three times with RIP buffer, and the RNA was isolated using Trizol reagent (Invitrogen) and processed for RT-qPCR.

In vivo translation assay

The in vivo translation assay was performed as previously described13. Firefly and renilla luciferase activities were detected using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer’s instructions.

Biotin pull-down assay

A biotinylated RNA probe was synthesized using an in vitro transcription system (Promega) according to the manufacturer’s instructions. The cells were lysed using RIPA lysis buffer, and total cell lysates were pre-cleaned using streptavidin beads (Sigma-Aldrich) at 4°C for 1 h. The lysates were then diluted with 5 × EMSA buffer (50 mM Hepes/pH 8.0, 250 mM KCl, 10 mM MgCl2, 5 mM DTT, 25% glycerol, 0.5 mg/ml tRNA, 0.7 mg/ml heparin, 100 unit/ml RNase inhibitor, and protease inhibitors) and incubated with biotin-labeled RNA probes at 4°C for 2 h. The mixtures were subjected to UV-crosslinking (120 ml/cm2) three times for 5 min each and then incubated with streptavidin beads while rotating at 4°C for 1 h. The beads were washed three times with wash solution (10 mM Hepes/pH 8.0, 40 mM KCl, 3 mM MgCl2, 2 mM DTT, 5%, glycerol, 0.5% SDS and 2% NP-40) and then subjected to western blot analysis.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed as previously described13 using formalin-fixed and paraffin-embedded tissue sections. The sections were incubated with anti-EGFR (sc-03, Santa Cruz), anti-hnRNP Q1 (AP7852c, Abgent) or anti-Aurora-A (35C1, GeneTex) antibodies. The sections were then counterstained with hematoxylin.

Patient specimens

Clinical colorectal cancer specimens were used according to the tenets of the Declaration of Helsinki, and the laboratory protocol used in this study was approved by the Institutional Review Board (IRB) of National Cheng Kung University Hospital, Tainan, Taiwan (A-ER-100-403).

ONCOMINE microarray datasets

Microarray datasets for colorectal cancer (Kaiser Colon Dataset, 2007, GSE5206; Skrzypczak Colorectal 2 Dataset, 2010, GSE20916; Sabates-Bellver Colon Dataset, 2007, GSE8671; and Ki Colon Dataset, 2007, GSE6988) were accessed from the ONCOMINE Cancer Profiling Database (www.oncomine.org) and used to investigate the expression of hnRNP Q1 and Aurora-A mRNA.

The Cancer Genome Atlas (TCGA) dataset

We downloaded TCGA colon adenocarcinoma and rectum adenocarcinoma datasets (version: 2015-02-24) via the UCSC Xena (http://xena.ucsc.edu). The gene expression profile was measured experimentally using the Illumina HiSeq 2000 RNA Sequencing platform of the University of North Carolina TCGA genome characterization center. These datasets show the gene-level transcription estimates, expressed as log2-transformed RSEM normalized counts. The median age of the patients was 68 years (range, 31 to 90 years). Other clinicopathological information is shown in Supplementary Table 1. The mean level of Aurora-A expression was 10.02. The mean level of hnRNP Q1 expression was 12.24. Values above the mean were considered high levels of Aurora-A and...
hnRNP Q1 and those below the mean were considered low levels of Aurora-A and hnRNP Q1.

Statistical analysis
All statistical analyses were performed using GraphPad Prism (La Jolla, CA, USA) and SPSS 17.0 software (SPSS, Chicago, IL, USA). All experimental data were expressed as the means ± S.D. Two-tailed t-test was used to compare means between two groups. One-way ANOVA was used to compare means among three groups. The Chi-square test and Spearman’s correlation coefficient were used to analyze the clinical specimen data. Significance was accepted at p < 0.05.

Results
EGF and hnRNP Q1 cooperatively enhance the translation of Aurora-A mRNA
Our previous report indicated that, in colorectal cancer, EGF increases Aurora-A protein expression by enhancing the translational efficiency of Aurora-A mRNA via ERK and Akt pathways\textsuperscript{13}. However, the regulatory factors involved in the EGF-induced translational
up-regulation of Aurora-A mRNA remain unknown. Recently, we found that hnRNP Q1, an RNA-binding protein, can bind to and translationally up-regulate Aurora-A mRNA\(^{12}\). Therefore, we sought to further examine whether the EGF signaling pathway also contributes to the hnRNP Q1-mediated translational up-regulation of Aurora-A mRNA. To address this question, we transiently transfected cells with GFP or GFP-hnRNP Q1, and the total cell lysates were then collected to determine the expression level of Aurora-A. The results showed that hnRNP Q1 and EGF each increases the protein level of Aurora-A and that treating cells with both EGF and hnRNP Q1 result in a synergistic effect on Aurora-A expression. The

**Fig. 2 EGF enhances the ability of hnRNP Q1 to bind to the Aurora-A mRNA 5′-UTR.** a Total cell lysate, the cytosolic and nuclear fractions were obtained from SW480 cells after treatment with 10 nM EGF and were used to perform western blot analysis with the indicated antibodies.

b Cytoplasmic extracts obtained from GFP- or GFP-hnRNP Q1-expressing cells treated with EGF were used to perform RIP using anti-GFP antibodies. The bound Aurora-A mRNA was measured using RT-qPCR. \(\gamma\)-Actin was used as the negative control for RIP. The mean ± S.D. was obtained from three independent experiments (*p < 0.05; **p < 0.01).

c SW480 cells were treated with (+) or without (−) EGF for 2 h, and then total cell lysates were harvested to perform biotin-pull down assays using the biotin-labeled Aurora-A mRNA 5′-UTR 257 nt probe (Biotin-257). Beads were used as the negative control.
Fig. 3 The mTOR and ERK pathways regulate the hnRNP Q1-induced translation of Aurora-A mRNA following stimulation with EGF.

**a** SW480 cells were transfected with GFP-hnRNP Q1 for 24 h. Serum-starved cells were pre-treated with rapamycin (100 nM) for 2 h and then were treated with EGF (10 nM) for an additional 2 h. The level of Aurora-A protein was evaluated using western blot analysis and was quantified as a ratio.

**b** Quantification of the expression level of Aurora-A mRNA in GFP-hnRNP Q1-expressing cells treated with rapamycin and EGF, as described in (a).

**c** The translational efficiency of the Aurora-A mRNA was determined using S6-IP and was quantitated using RT-qPCR.

**d, e** GFP-hnRNP Q1-expressing SW480 cells were serum-starved and then pre-treated with U0126 (10 μM) for 30 min followed by treatment with EGF for another 2 h. Cell lysates were collected to determine the protein expression of Aurora-A by western blot analysis (d) and the mRNA expression of Aurora-A using RT-qPCR (e). GFP-hnRNP Q1-expressing cells were treated with U0126 and then EGF, as described above. The cell lysates were then collected for S6-IP (f) or RIP assays using anti-GFP antibodies (g). The bound Aurora-A mRNA was measured using RT-qPCR. The mean ± S.D. was obtained from three independent experiments. (*p < 0.05; **p < 0.01)
Fig. 4 (See legend on next page.)
level of the homologous protein Aurora-B was not affected in hnRNP Q1-expressing or EGF-treated cells (Fig. 1a and Supplementary Figure S1A). These results indicate that hnRNP Q1 may be involved in the EGF-mediated translational up-regulation of Aurora-A mRNA, and this effect is specific to Aurora-A. Quantitative real-time PCR (RT-qPCR) was performed to determine that neither hnRNP Q1 nor EGF treatment affected Aurora-A mRNA expression (Fig. 1b and Supplementary Figure S1B).

To further evaluate the effect of hnRNP Q1 and EGF on the up-regulation of the translational efficiency of Aurora-A mRNA, we performed a ribosomal protein S6-immunoprecipitation assay (S6-IP) using hnRNP Q1-expressing cells. The results showed that the S6 subunit-associated Aurora-A mRNA was increased in hnRNP Q1-overexpressing or EGF-treated cells (Fig. 1c), and hnRNP Q1 enhanced the translational efficacy of Aurora-A mRNA in response to EGF treatment (Fig. 1c and Supplementary Figure S1C). To determine the contribution of hnRNP Q1 to the EGF-mediated translational up-regulation of Aurora-A mRNA, an hnRNP Q siRNA was used to knock down endogenous hnRNP Q1 expression. We then examined the expression of Aurora-A using western blot analysis. The data showed that knocking down hnRNP Q1 abolished the EGF-enhanced protein expression of Aurora-A (Fig. 1d and Supplementary Figure S1D). This result implies that the EGF-enhanced expression of Aurora-A is hnRNP Q1 dependent. These results demonstrate that hnRNP Q1 regulates the translation of Aurora-A, and the EGF signaling pathway may play an important role in controlling the hnRNP Q1-mediated translational regulatory effect on Aurora-A expression in colorectal cancer cells.

**EGF increases the ability of hnRNP Q1 to bind to the Aurora-A mRNA 5′-UTR**

According to a previous report, hnRNP Q1 is distributed throughout the cell but is localized primarily in the cytoplasm19. Here, we investigated whether EGF alters the subcellular localization of hnRNP Q1. The results revealed that EGF did not affect the expression or subcellular localization of hnRNP Q1 (Fig. 2a). Our previous report indicated that hnRNP Q1 directly binds to the Aurora-A mRNA 5′-UTR at its RBD2 and RBD3 domains13. Therefore, we used a RIP to evaluate the capacity of GFP-hnRNP Q1 to bind to the Aurora-A mRNA following stimulation with EGF. As expected, GFP-hnRNP Q1 was associated with Aurora-A mRNA, and EGF treatment enhanced its ability (Fig. 2b). γ-Actin, a gene that is not targeted by hnRNP Q122, was used as the negative control for RIP. A biotin pull-down assay was then performed using the biotin-labeled Aurora-A mRNA 5′-UTR 257 nt RNA probe to demonstrate the direct interaction between hnRNP Q1 and Aurora-A mRNA13. The results showed that endogenous hnRNP Q1 interacted with the Aurora-A mRNA 5′-UTR 257 nt RNA probe, and EGF enhanced this interaction (Fig. 2c). These results indicate that hnRNP Q1 acts as a trans-acting factor to translationally up-regulate the Aurora-A mRNA, and EGF enhances the binding ability and translational efficacy of hnRNP Q1.

**EGF enhances the hnRNP Q1-mediated translational up-regulation of Aurora-A mRNA through the mTOR and ERK pathways**

Our previous data indicated that EGF translationally regulates Aurora-A mRNA through the ERK and Akt pathways in colorectal cancer13. Here, we sought to determine whether the ERK and Akt pathways contribute to the hnRNP Q1-mediated translational up-regulation of Aurora-A mRNA. We first analyzed the cap-dependent/mTOR signaling pathway. Similar to the results of a previous report15, we found that EGF enhanced Aurora-A expression and mTOR activation in GFP-hnRNP Q1-overexpressing cells (Fig. 3a, compare lanes 1 and 2). The addition of rapamycin, a mTOR inhibitor, blocked the EGF-mediated GFP-hnRNP Q1-enhanced expression of Aurora-A (Fig. 3a, compare lanes 1, 2, and 4). Neither EGF nor rapamycin treatment altered Aurora-A mRNA levels in GFP-hnRNP Q1-expressing cells (Fig. 3b). This result suggested that the mTOR pathway might
contribute to the hnRNP Q1-mediated regulation of Aurora-A mRNA translation, and EGF enhanced this effect by modulating the mTOR pathway. In addition, the EGF-enhanced recruitment of ribosomal protein S6 to Aurora-A mRNA was abolished by rapamycin treatment in GFP-hnRNP Q1-expressing cells (Fig. 3c). These results indicate that hnRNP Q1 translationally increases Aurora-A expression via the mTOR pathway.

Next, the ERK pathway was examined to evaluate its involvement in the hnRNP Q1-mediated translation of Aurora-A mRNA. As shown in Fig. 3d, adding U0126, an ERK inhibitor, eliminated the effect of EGF on the hnRNP Q1-enhanced expression of the Aurora-A protein (Fig. 3d, compare lanes 1, 2, and 4) but did not affect the level of Aurora-A mRNA (Fig. 3e). The EGF-enhanced association of ribosomal protein S6 with Aurora-A mRNA (Fig. 3f)

Table 1  The correlation between clinicopathological variables and the expression of hnRNP Q1 in data obtained from the TGCA dataset

| Clinical variables | HnRNP Q high | HnRNP Q low | p Value* |
|--------------------|--------------|-------------|----------|
| Age                |              |             | 0.996    |
| <65                | 80 (52.6%)   | 72 (47.4%)  |          |
| ≥65                | 101 (52.6%)  | 91 (47.4%)  |          |
| Gender             |              |             | 0.313    |
| Male               | 102 (55.1%)  | 83 (44.9%)  |          |
| Female             | 79 (49.7%)   | 80 (50.3%)  |          |
| Aurora-A           |              |             | <0.001   |
| High               | 118 (65.6%)  | 63 (38.4%)  |          |
| Low                | 62 (34.4%)   | 101 (61.6%) |          |
| MMR                |              |             | 0.832    |
| dMMR               | 27 (54.0%)   | 23 (46.0%)  |          |
| pMMR               | 154 (52.4%)  | 140 (47.6%) |          |
| TNM stage          |              |             | 0.235    |
| T0                 | 0 (0.0%)     | 1 (100.0%)  |          |
| T1                 | 3 (33.3%)    | 6 (66.7%)   |          |
| T2                 | 28 (59.0%)   | 27 (41.1%)  |          |
| T3                 | 132 (55.9%)  | 104 (44.1%) |          |
| T4                 | 18 (52.6%)   | 25 (47.4%)  |          |
| N0                 | 103 (53.4%)  | 90 (46.6%)  | 0.310    |
| N1                 | 51 (56.7%)   | 39 (43.3%)  |          |
| N2                 | 27 (44.3%)   | 34 (55.7%)  |          |
| M0                 | 158 (52.8%)  | 141 (47.2%) | 0.828    |
| M1                 | 23 (51.1%)   | 22 (48.9%)  |          |
| Stage              |              |             | 0.941    |
| 0–2                | 97 (52.4%)   | 88 (47.6%)  |          |
| 3–4                | 84 (52.8%)   | 75 (47.2%)  |          |
| Site               |              |             | 0.014    |
| Colon              | 127 (48.8%)  | 133 (51.2%) |          |
| Rectum             | 54 (64.3%)   | 30 (35.7%)  |          |

*The data were analyzed using Pearson’s chi-square test

Table 2 The correlation between clinicopathological variables and the expression of Aurora-A in data obtained from the TGCA dataset

| Clinical variables | Aurora-A high | Aurora-A low | p Value* |
|--------------------|---------------|--------------|----------|
| Age                |               |              | 0.919    |
| <65                | 80 (52.6%)    | 72 (47.4%)   |          |
| ≥65                | 100 (52.1%)   | 92 (47.9%)   |          |
| Gender             |               |              | 0.634    |
| Male               | 99 (53.5%)    | 86 (46.5%)   |          |
| Female             | 81 (50.9%)    | 78 (49.1%)   |          |
| Aurora-A           | <0.001        |              |          |
| High               | 118 (65.6%)   | 62 (34.4%)   |          |
| Low                | 63 (38.4%)    | 101 (61.6%)  |          |
| MMR                | 0.832         |              |          |
| dMMR               | 15 (30.0%)    | 35 (70.0%)   |          |
| pMMR               | 165 (56.1%)   | 129 (43.9%)  |          |
| TNM stage          |               |              | 0.018    |
| T0                 | 0 (0.0%)      | 1 (100.0%)   |          |
| T1                 | 6 (66.7%)     | 3 (33.3%)    |          |
| T2                 | 33 (60.0%)    | 22 (40.0%)   |          |
| T3                 | 128 (54.2%)   | 108 (45.8%)  |          |
| T4                 | 30 (69.8%)    | 13 (30.2%)   |          |
| N0                 | 112 (58.0%)   | 81 (42.0%)   | 0.019    |
| N1                 | 45 (50.0%)    | 45 (50.0%)   |          |
| N2                 | 23 (37.7%)    | 38 (62.3%)   |          |
| M0                 | 162 (54.2%)   | 137 (45.8%)  | 0.076    |
| M1                 | 18 (40.0%)    | 27 (60.0%)   |          |
| Stage              |               |              | 0.046    |
| 0–2                | 106 (57.3%)   | 79 (42.7%)   |          |
| 3–4                | 74 (46.5%)    | 85 (53.5%)   |          |
| Site               |               |              | 0.129    |
| Colon              | 130 (50.0%)   | 130 (50.0%)  |          |
| Rectum             | 50 (59.5%)    | 34 (40.5%)   |          |

*The data were analyzed using Pearson’s chi-square test
and the binding of hnRNP Q1 to Aurora-A mRNA (Fig. 3g) were also abolished in cells treated with U0126.

**hnRNP Q1 expression is positively correlated with Aurora-A levels in human colorectal cancer**

Previous reports have indicated that hnRNP Q1 may be aberrantly overexpressed in cancers, and our results showed that hnRNP Q1 translationally up-regulates Aurora-A protein expression. These findings collectively suggest that the level of hnRNP Q1 may be increased in cancer cells in parallel with the Aurora-A expression, which is regulated by hnRNP Q1. To address this issue, we analyzed the expression of *hnRNP Q1* mRNA in colorectal cancer. We first tested cancer microarray datasets that are publicly available from Oncomine (https://www.oncomine.org) to survey many colorectal cancer specimens. We found that the relative levels of both the hnRNP Q1 and Aurora-A mRNAs were significantly higher in colorectal cancer tissues than in normal tissues across four different cohorts (Fig. 4a, b). We also analyzed the correlation between the *hnRNP Q1* and *Aurora-A* mRNA levels in colorectal cancer using The Cancer Genome...
Fig. 6 Parallel changes in the overexpression of EGFR, hnRNP Q1, Aurora-A and spindle assembly checkpoint genes in colorectal cancer tissues. a Western blot analysis was used to determine the expression levels of the EGFR, hnRNP Q1, Aurora-A, Mad2, Bub1, BubR1, and Mps1 proteins in human colorectal tumors (T) and adjacent normal colon mucosal tissues (N). Six representative specimens (P1–P6) are shown. b The expression levels of the Mad2, Bub1, BubR1, and Mps1 mRNAs in human colorectal cancer tissues (T) and their normal colon mucosal tissues (N) were analyzed using RT-qPCR. (N = 40; *p < 0.05; **p < 0.01; ***p < 0.001)
hsRNPs (TCGA) dataset. As shown in Fig. 4c, there was a significant positive correlation between the levels of the 
hsRNPs Q1 and Aurora-A mRNA in colorectal cancer tissues (Spearman’s correlation: $r = 0.3563; p < 0.0001$). A clinicopathological analysis indicated that 
hsRNPs Q1 is overexpressed in colorectal cancer and is significantly correlated with Aurora-A expression ($p < 0.001$). 
hsRNPs Q1 was detected more often in rectal carcinomas than in the carcinomas of the proximal colon ($64.3 \%$ vs $48.8\%$, 
$p = 0.014$) (Table 1). Aurora-A is significantly correlated with MMR ($p < 0.001$) and TNM stage (T stage: $p = 0.018$; 
N stage: $p = 0.019$) (Table 2). The expression of Aurora-A is closely associated with the tumor stage, and it is more 
frequently found in the early stage of colorectal cancer ($57.3 \%$ vs $46.5\%$, $p = 0.046$). hsrNPs Q1 and Aurora-A expression is not significantly correlated with age and gender.

To further evaluate the correlation between the EGFR signaling pathway and hsrNPs Q1-regulated expression of 
Aurora-A, 54 paired colorectal cancer tissues were collected to analyze the expression patterns of Aurora-A, 
hsRNPs Q1, and EGFR. Consistent with our previous findings, the results of RT-qPCR indicated that the 
expression levels of hsrNPs Q1 and Aurora-A are positively correlated (Pearson’s Chi-square analysis, $p < 0.001$) 
(Fig. 4d). Our previous study indicated that hsrNPs Q1 and Aurora-A are co-expressed in $69.6\%$ (78/129) of 
colorectal cancer tissues (ref. 12 and Supplementary Figure S2). Here, we observed that the high expression level of both 
hsRNPs Q1 and Aurora-A was positively correlated with the high level of EGFR (Pearson’s Chi-square analysis, $p = 0.046$) (Fig. 4e). Overall, these results indicate that the expression of hsrNPs Q1 is associated with 
Aurora-A protein expression in colorectal cancer; an increased level of Aurora-A mRNA may augment the 
effect of hsrNPs Q1 on its translational regulation, and the EGFR pathway may be involved in the hsrNPs Q1-mediated translational up-regulation of Aurora-A mRNA.

hsRNPs Q1 plays an important role in regulating the 
expression of a group of SAC genes

We previously used RIP analysis followed by next-generation sequencing (NGS) to show that a group of 
SAC genes are potential targets of hsrNPs Q1 (GEO accession number GSE76457 12). However, the NGS 
results were preformed using GFP-hsrNPs Q1-overexpressing cells that are not treated with EGF. Here, we 
evaluated the effect of hsrNPs Q1 and EGF on the translational regulation of the expression of these SAC 
genes. Four genes, including Mad2, Bub1, BubR1, and Mps1, were chosen for further analysis. The results of RIP 
indicated that hsrNPs Q1 binds to the Mad2, Bub1, BubR1, and Mps1 mRNAs, and EGF only slightly 
enhances the binding ability of GFP-hsrNPs Q1 to these 
four genes (Fig. 5a). There were no obvious changes in the expression of these four genes at the mRNA level in either 
EGF-hsrNPs Q1-overexpressing or EGF-treated cells (Fig. 5b). An S6-IP assay further demonstrated that while 
hsrNPs Q1 translationally up-regulated the expression of these four genes, EGF slightly increased the hsrNPs Q1-
mediated translation of these genes (Fig. 5c). These data suggest that hsrNPs Q1 is an important regulator in 
translationally up-regulating SAC genes and Aurora-A in colorectal cancer; EGF is involved in hsrNPs Q1-
modulated Aurora-A mRNA translation and may participate in hsrNPs Q1-mediated SAC mRNA translation. A more detailed mechanism regarding the effect of EGF 
treatment in hsrNPs Q1-mediated SAC mRNAs translational regulation needs to be further investigated.

Co-expression of SAC proteins, EGFR, and Aurora-A in 
human colorectal tissues

The expression of SAC genes in human colorectal cancer tissues was further evaluated using western blot 
analysis and RT-qPCR. The results showed that the expression levels of the Mad2, Bub1, BubR1, and Mps1 
proteins and mRNAs were significantly higher in colorectal cancer tissues than in paired adjacent normal tissues 
(Fig. 6a, b). Next, we investigated the correlation between overexpressed Mad2, Bub1, BubR1, and Mps1 with 
hsrNPs Q1 in human colorectal tissues. However, due to the difficulty in obtaining the protein expression profile 
from the public database, and because overexpressed mRNA can also contribute to increased protein expres-
sion levels through up-regulating translational efficiency, we analyzed the mRNA expression pattern of these genes 
using the TCGA dataset. Data from the TCGA dataset support the positive correlation between hsrNPs Q1 and 
Mad2, Bub1, BubR1, and Mps1 mRNAs in colorectal cancer (Fig. 6b and Supplementary Figure S3). These 
results suggest that EGF signaling may modulate hsrNPs Q1 to increase the expression of Aurora-A and SAC 
proteins to promote the development of colorectal cancer.

Discussion

The dysregulation of Aurora-A plays a vital role in 
tumorigenesis. It has been shown that the overexpression 
of Aurora-A can be achieved through gene amplification, 
RNA transcriptional activation, or increased protein sta-
bility. No previous reports have indicated that Aurora-A is 
naturally deficient in human tumors 13. In our previous 
report, we found that EGF increases the expression of the 
Aurora-A protein via the translational regulatory 
machinery. These data suggested a potential explanation 
for the abnormal expression of Aurora-A in cancers 13. 
Recently, we found that a novel trans-acting factor, 
hsrNPs Q1, regulates the translation of Aurora-A mRNA 
by binding to its 5’UTR 12. In this report, we used IHC,
western blot analysis, and RT-qPCR to show that hnRNP Q1 translationally up-regulates Aurora-A mRNA and the overexpression of Aurora-A is positively correlated with the overexpression of hnRNP Q1 in colorectal cancer tissues.

The results of RIP showed that EGF enhances the binding ability of hnRNP Q1 to Aurora-A mRNA. We speculate that EGF may play a role in post-translational modifying hnRNP Q1 to influence its ability to bind to its target mRNAs, thereby enhancing its translational efficiency. Indeed, previous reports have suggested that EGF controls the functions of many hnRNPs through post-translational modifications. For example, EGF treatment led to the ubiquitylation of hnRNP A1 and was associated with cell migration;24 EGF activates SR proteins, which are RNA-binding proteins, via Akt-SR protein kinase (SRPK) to regulate Rac1 mRNA splicing;25 the phosphorylation of the ARE-binding protein DAZAP1 [DAZ (deleted in azoospermia)-associated protein 1] by EGF/ERK2 may contribute to the induction of translation;26 and EGF induces the acetylation of hnRNP A1 and hnRNP L in colorectal cancer cells.27 However, more evidence is needed to determine whether EGF controls the post-translational modification of hnRNP Q1.

The activation of EGFR may also play an important role in chromosome instability.28 It was previously reported that EGF induces centrosome separation and promotes mitotic progression by activating Akt,29 and the EGFR/MEK/ERK/CDK5 pathway induces p-FAK/Ser732, which promotes mitosis and cell proliferation.30 These findings indicate that EGF regulates mitotic progression by initiating signaling transduction but does not reveal its specific molecular mechanism. In this study, we provide the first evidence to demonstrate the correlation between the expression of EGFR and SAC proteins in colorectal cancer tissues (Fig. 6b) and that EGF signaling might be an important regulator of the augmented expression of SAC genes. In conclusion, these data indicate that hnRNP Q1 up-regulates the expression levels of Aurora-A (this study and12) and a group of SAC genes, and the expression of hnRNP Q1 is positively associated with the levels of Aurora-A and SAC proteins in colorectal cancer. Our study shows that hnRNP Q1 plays a potentially oncogenic role during tumorigenesis.

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YCW, BWL, JCL, YY, and LYH designed the experiments; YCW and LYH wrote the manuscript; YCW, KCC, CHL, LYL, YY, CSL, SJY, and CTL generated the data; KCC, BWL, JCL, YY, PCL, CTL, and LYH analyzed the clinical results; YCW, KCC, CHL, YY, CTL, and LYH analyzed the data.

Conflict of interest
The authors declare that they have no conflict of interest.

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