The Role of Aurora Kinase A (AURKA) in Regulating Translation of Oncogenes in Gastrointestinal Cancers

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KEYWORDS. Gastrointestinal cancers, inhibition of AURKA, oncogenes, translation

BRIEF. Aurora kinase A (AURKA) is frequently amplified and overexpressed in gastrointestinal cancers. In this report, we show that AURKA and EIF4E regulate the expression of the same oncogenes, suggesting that AURKA could play a role in regulating EIF4E-cap dependent translation of oncogenes. Inhibition of AURKA could be a plausible therapeutic approach in these cancers.

ABSTRACT Gastrointestinal (GI) cancers are a leading cause of cancer-related deaths and are poorly responsive to therapy. Earlier studies have shown overexpression of Aurora Kinase A (AURKA) in esophageal and gastric adenocarcinomas. AURKA regulates the cell cycle and key oncogenic signaling pathways in cancer cells. Eukaryotic translation initiation factor 4E (EIF4E) plays an important role in mRNA cap-dependent translation. Based on earlier findings showing that AURKA can phosphorylate EIF4E, we hypothesized that AURKA is involved in post-transcriptional regulation of oncogene expression through EIF4E. We investigated the expression of several oncogenes following knockdown of AURKA or EIF4E to determine the expression of oncogenes. We focused our analysis on translated mRNA by isolating polysome mRNA using innovative Translational Immunoprecipitation ChIP (TrIP-ChIP), followed by quantitative real-time PCR. The results showed that AURKA and EIF4E genetic depletion by siRNA had similar effects and caused decreased expression of several oncogenes such as BIRC3, BCL3, CSF2, CCL5, and CCND1 in FLO-1 and SNU-1 cells. The similarities in mRNA-translated gene expression following the knockdown of AURKA and EIF4E, confirmed that AURKA plays a major role in regulating EIF4E-mediated cap-dependent translation of oncogenes. Our study offers important information regarding the presence of AURKA-EIF4E axis and its role in regulating oncogenic targets. This information can rationalize the use of therapies that include inhibition of AURKA in gastrointestinal cancers.

INTRODUCTION.

Gastrointestinal (GI) cancers are the third most common cause of cancer-related deaths [1]. Upper GI cancers are poorly responsive to therapy with unfavorable outcomes [2]. Commonly used treatment modalities currently include chemotherapy, surgery and radiation, which often fail to achieve cures. As a result they lead to higher rates of recurrence, metastasis, and poor patient survival [2]. Previous studies have shown that the chromosome region 20q13 is amplified in GI cancers [3]. With the examination of this region, studies have found that there is an overexpression of the AURKA protein, which is associated with malignant GI cancers including gastric and esophageal adenocarcinoma. AURKA overexpression is a key component in the early and advanced stages of tumor development as suggested by the significant increase in AURKA expression at various stages of the disease process from gastritis to intestinal metaplasia, dysplasia and adenocarcinomas [3, 4]. AURKA is overexpressed, both at the transcriptional and translational level. It is one of the three serine/threonine kinases that regulate chromosome separation and alignment during the cell cycle [5]. In addition, AURKA also helps with the formation of the mitotic spindle fiber and activation of key signaling pathways [5]. In gastric cancer, AURKA overexpression leads to increased expression and phosphorylation of Human Doubling Minute 2 (HDM2), coupled with suppression of p53 and its downstream targets. Inhibition of AURKA with the drug alisertib (MLN8237) attenuates cancer cell survival in a dose-dependent manner [6].

EIF4E is a general translation factor that increases translation of specific messenger RNAs (mRNAs) and production of malignancy-associated pro-oncogenic proteins [7, 8]. EIF4E is a cap-dependent RNA binding protein that affects cancer cell growth and survival [7, 8]. Elevated levels of EIF4E have been found in several cancer types including cancers of the colon, breast, bladder, lung, prostate, GI tract, head and neck, Hodgkin's lymphomas and neuroblastomas, but not in typical benign lesions [7, 8] The eukaryotic translation initiation factor 4E is implicated in metastasis and may assume a role as a prognostic marker in certain cancers [9]. Our laboratory has shown that AURKA can phosphorylate and activate EIF4E in *in vitro* cell lines suggesting an active role of AURKA in regulating cap-dependent translation. In this study, we investigated the role of the AURKA-EIF4E axis in regulating translation of oncogenes. Thus, our aim is to find out the most critical translational targets of the AURKA-EIF4E complex. The genes that we examined play essential roles in carcinogenesis by regulating cancer cell survival, and proliferation.

MATERIALS AND METHODS.

Cell Culture and Transfection.

Gastric (SNU-1) and esophageal (FLO-1) cancer cell lines were cultured in Dulbecco's modified Eagle's medium (GIBCO, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA) and 1% penicillin/streptomycin (GIBCO), following standard protocols. AURKA or EIF4E was transiently silenced in FLO-1 and SNU-1 cells using standard transfection of gene specific siRNAs (Invitrogen). A negative siRNA control (Ambion) was used in each experiment. Transfections were performed using LipoJet reagent (SignaGen Laboratories, Rockville, MD). After 48 hours of transfection, cells were harvested for further analysis.

Translational Immunoprecipitation ChIP (TrIP-ChIP).

We used Translational Immunoprecipitation ChIP (TrIP-ChIP) to isolate polysomal mRNA [10]. TrIP-ChIP is based on the fact that actively translating mRNAs are associated with polysomes [10] and the newly synthesized peptide chains are closely associated with molecular chaperones such as HSP70s. In brief, 1×106 FLO-1 or SNU-1 cells were treated with 100 mg/ml cycloheximide (Sigma-Aldrich, MO) for 5 min at 37°C. 200 ml of DSP (1 mM) was introduced as a cross-linking reagent and incubation was carried out for 5 min at 37°C. The final pellets were swollen for 20 min in 500 ml of low salt buffer (LSB) (20 mM HEPES, pH 7.4, 100 mM KCl, 2 mM MgCl2) containing 1 mM dithiothreitol and lysed by the addition of 200 ml lysis buffer (1 LSB containing 1.2% Triton X-100) (Sigma-Aldrich) followed by brief vortexing. The lysate was transferred to the Ig-coated beads (the HSP70/HSP73 antibody-conjugated magnetic beads), and incubation was carried out for 2h at 4°C. After incubation with beads, the polysome complexes containing translationally active mRNA transcripts were isolated. The mRNAs were eluted from beads conjugated with HSP70/HSP73 using the ArrayPure Nano-scale RNA Purification Kit (Epicentre, Madison, WI). Quantitative real-time RT-PCR (qRT-PCR) was performed for validation of target genes in cell lines and human tissues.

Quantitative Real-Time RT-PCR.

Quantitative real-time RT-PCR was performed using de-identified human tissue samples and on polysome mRNA isolated from TrIP-ChIP after knocking down of AURKA or eIF4E. The de-identified tissue samples were obtained from the National Cancer Institute Cooperative Human Tissue Network (CHTN) and the Pathology archives at Vanderbilt University Medical Center (Nashville, TN). All tissue samples were obtained coded and de-identified in accordance with Vanderbilt University Institutional Review Board-approved

protocols. Tissue samples included five esophageal adenocarcinomas with matching adjacent normal esophageal tissue samples. RNA was isolated using the RNeasy Mini Kit (Qiagen, Germantown, MD). Total RNA (1 μg) was reverse transcribed by an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Specific Primers were designed using the online software Primer 3 (http://frodo.wi.mit.edu/primer3/). Primers were purchased from Integrated DNA Technologies (IDT, Coral-ville, IA) (Table 1).

Table 1. Sequence of qRT-PCR primers of selected genes.

EIF4E-F	CTAAGATGGCGACTGTCGAA
EIF4E-R	TCTGGGTTAGCAACCTCCTG
AURKA-F	AGTTGGAGGTCCAAAACGTG
AURKA-R	TCCAAGTGGTGCATATTCCA
HPRT1-F	ACCCTTTCCAAATCCTCAGC
HPRT1-R	GTTATGGCGACCCGCAG
CCL5-F	TGTACTCCCGAACCCATTTC
CCL5-R	TACACCAGTGGCAAGTGCTC
BCL3-F	GCACCACAGCAATATGGAGA
BCL3-R	CCTATACCCCATGATGTGCC
BIRC3-F	GTCAAATGTTGAAAAAGTGCCA
BIRC3-R	GGGAAGAGGAGAGAGAAAGAGC
CSF2-F	AAAGGGGATGACAAGCAGAA
CSF2-R	ACTACAAGCAGCACTGCCCT
18S-F	CGGACAGGATTGACAGATTGATAGC
18S-R	TGCCAGAGTCTCGTTCGTTATCG

The qRT-PCR was performed using a Bio-Rad CFX Connect Real-time System (Bio-Rad). The threshold cycle number was determined by Bio-Rad CFX manager software version 3.0. Reactions were performed in triplicate, and the threshold cycle numbers were averaged. The mRNA expression results were normalized to HPRT1 housekeeping gene as described previously [10]. For polysomal mRNA, the expression of tested genes was normalized using ribosomal RNA 18S subunit as an internal control.

Statistical Analysis.

Data are presented as means +/- standard error of mean (SEM). All *in vitro* experiments were carried out in triplicate. Statistical significance of difference between control groups and treatment groups was determined using Student's t-test. GraphPad Prism 5.0 software (GraphPad software, Inc. La Jolla, CA) was used to determine statistical analyses. P-values of less than 0.05 were considered significant.

RESULTS.

To determine if AURKA controls mRNA translation of oncogenes that regulate cancer cell survival, proliferation and invasion, we performed TrIP-ChIP and qRT-PCR following siRNA-mediated knockdown of AURKA or EIF4E in the esophageal adenocarcinoma cell line FLO-1 and gastric cancer cell line SNU-1. The knockdown of AURKA or EIF4E was verified using qRT-PCR (Figures 1A and 2A). We next examined the expression of several oncogenes (BIRC3, BCL3, CSF2, CCL5, and CCND1) in samples following knockdown of AURKA or EIF4E. Interestingly, our data showed that the knockdown of AURKA led to a significant decrease in expression of EIF4E in FLO-1 and SNU-1 cells (Figure 1B). Additionally, the knockdown of AURKA led to a significant downregulation in all five genes that we tested (Figure 1C-G)

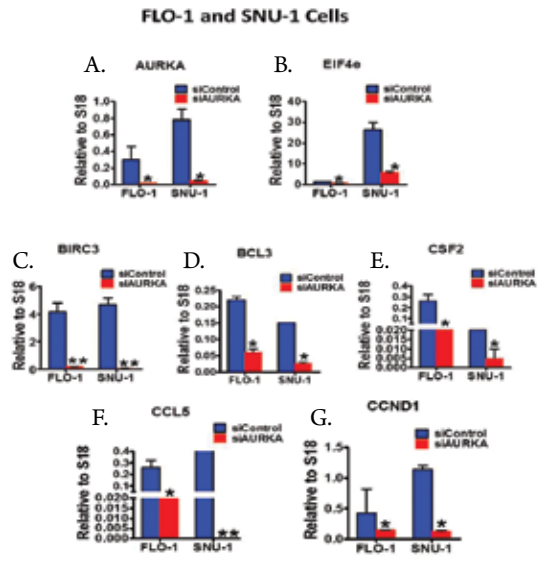

Figure 1. Quantitative real-time RT-PCR using polysomal translated mRNA following knockdown of AURKA. As shown, the knockdown of AURKA is confirmed (A). The expression of translated EIF4E was significantly down regulated (B). In addition, the expression levels of BIRC3, BCL3, CSF2, CCL5, and CCND1 were significantly downregulated (C-G). A value of $p<0.05$ was considered statistically significant; *, $P<0.05$; **, $P<0.01$

Similar results were obtained following the knockdown EIF4E alone, where the five oncogenes were also significantly downregulated (Figure 2B-F). These results indicate that AURKA regulates EIF4E and several oncogenes through the AURKA-EIF4E complex, suggesting that AURKA plays a major role in regulating EIF4E-mediated cap dependent translation of oncogenes.

To confirm if AURKA and EIF4E are important in human esophageal carcinogenesis, we tested their expression using qRT-PCR in de-identified human esophageal cancer tissue samples. Using five human esophageal adenocarcinomas and five normal esophageal tissue samples, we found that both AURKA and EIF4E were significantly overexpressed in a subset of these tumors (Figure 3A, B). Taken together, our data suggest that the AURKA-EIF4E axis is activated in a subset of tumors to regulate the expression of target oncogenesis that play important roles in carcinogenesis.

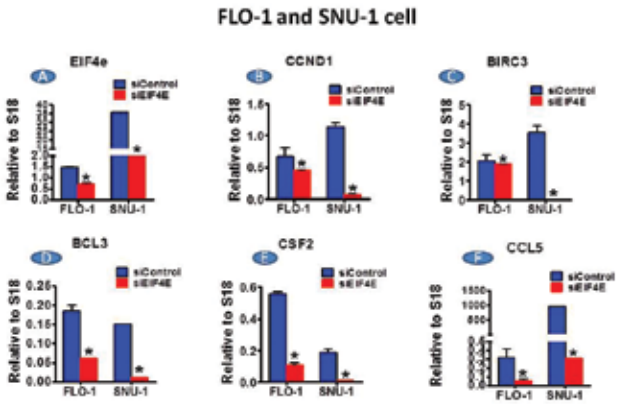

Figure 2. Quantitative real-time RT-PCR using polysomal trans-lated mRNA following knockdown of EIF4E. As shown, the knockdown of EIF4E is confirmed (A). In addition, the expression levels of CCND1, BIRC3, BCL3, CSF2, and CCL5 were significantly downregulated (B-F).

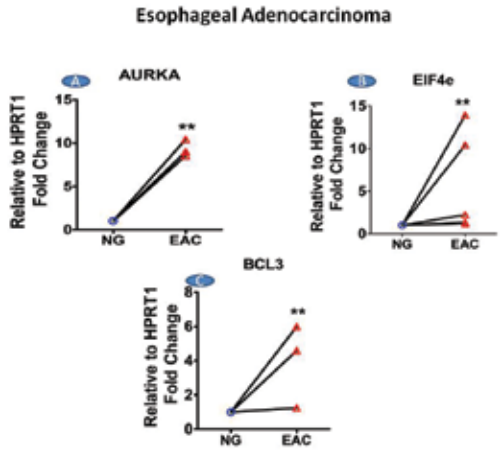

Figure 3. Overexpression of AURKA and EIF4E, in human esophageal adenocarcinoma tissue samples. qRT-PCR analyses of cancer samples, as compared to matching adjacent normal tissue samples. Each line represents data from one patient showing the expression level in the normal tissue and its adjacent cancer tissue. The results indicated significant overexpression of AURKA in all cancer samples (A) whereas EIF4E was overexpressed in 3 out of 5 (B). A value of $p<0.05$ was considered statistically significant; *, $P<0.05$; **, $P<0.01$

DISCUSSION.

Frequent overexpression of AURKA has been reported in several cancers including gastric, esophageal, breast, colon, liver, ovarian, and pancreatic cancers [7, 8]. Our laboratory has previously reported frequent overexpression of AURKA in EAC and gastric cancer [11]. In normal cells, AURKA expression is tightly regulated and plays an important role in mitosis [12]. Deregulated overexpression of AURKA causes genetic instability and oncogenic transformation. AURKA overexpression promotes tumor growth and drug resistance leading to poor clinical outcome [3, 13-15].

AURKA overexpression is associated with gastritis, intestinal metaplasia, dysplasia, and adenocarcinomas [16]. This overexpression occurs both at the transcriptional and translational level. We found that AURKA regulates the levels of EIF4E and its downstream oncogenes. This finding suggests that AURKA plays an important role in regulating cap-dependent RNA binding proteins that affect cancer cell growth and survival by promoting the translation of specific mRNAs coding for pro-oncogenic proteins. Elevated levels of EIF4E have been found in many types of tumors and cancer cell lines including cancers of the colon, breast, bladder, lung, prostate, gastrointestinal tract, head and neck, Hodgkin's lymphomas and neuroblastomas, but not in typical benign lesions [9, 17].

Our data demonstrates that the knockdown of AURKA leads to a decrease in expression of EIF4E in FLO-1 and SNU-1 cells (Figure 1B). Additionally, the knockdown of AURKA caused significant downregulation in other five oncogenes we tested - CCND1, BIRC3, BCL3, CSF2, and CCL5 (Figure 1C-G). The results indicate that AURKA and EIF4E genetic depletion by siRNA results in a major decrease in expression of oncogenes in FLO-1 and SNU-1 cells. These data indicate that AURKA regulates the expression of oncogenes that are similarly regulated by EIF4E. Our results also show a significant increase in expression of AURKA and EIF4E in EAC tissues, as compared to histologically normal tissue samples (Figure 3A, B). Taken together, these data may suggest that AURKA and EIF4E work together to regulate the expression of the same cellular oncogenes.

CONCLUSION.

In conclusion, our study offers important information regarding the role of AURKA-EIF4E in regulating oncogenes in gastrointestinal cancers. Targeting AURKA in these cancers can be a promising therapeutic approach for future investigations.

ABBREVIATIONS.

BIRC3 - Baculovirus Inhibitor of Apoptosis Repeat

BCL3 - B-Cell Lymphoma3

CSF2 - Colony Stimulating Factor

CCL5 C - C chemokine Ligand 5

CCND1 - Cyclin D1

ACKNOWLEDGMENTS.

I would like to thank my mentors Dr. Wang and Dr. El-Rifai for their guidance and support throughout the project, completing the research and revising the manuscript.

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