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AURKA regulates JAK2–STAT3 activity in human gastric and esophageal cancers

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ABSTRACT

Aurora kinase A is a frequently amplified and overexpressed gene in upper gastrointestinal adenocarcinomas (UGCs). Using *in vitro* cell models of UGCs, we investigated whether AURKA can regulate Signal Transducer and Activator of Transcription 3 (STAT3). Our data indicate that overexpression of AURKA in FLO-1 and AGS cells increase STAT3 phosphorylation at the Tyr705 site, whereas AURKA genetic depletion by siRNA results in decreased phosphorylation levels of STAT3 in FLO-1 and MKN45 cells. Immunofluorescence analysis showed that AURKA overexpression enhanced STAT3 nuclear translocation while AURKA genetic knockdown reduced the nuclear translocation of STAT3 in AGS and FLO-1 cells, respectively. Using a luciferase reporter assay, we demonstrated that AURKA expression induces transcriptional activity of STAT3. Pharmacological inhibition of AURKA by MLN8237 reduced STAT3 phosphorylation along with down-regulation of STAT3 pro-survival targets, BCL2 and MCL1. Moreover, by using clonogenic cells survival assay, we showed that MLN8237 single dose treatment reduced the ability of FLO-1 and AGS cells to form colonies. Additional experiments utilizing cell models of overexpression and knockdown of AURKA indicated that STAT3 upstream non-receptor tyrosine kinase Janus kinase 2 (JAK2) is mediating the effect of AURKA on STAT3. The inhibition of JAK2 using JAK2-specific inhibitor AZD1480 or siRNA knockdown, in presence of AURKA overexpression, abrogated the AURKA-mediated STAT3 activation. These results confirm that the AURKA-JAK2 axis is the main mechanism by which AURKA regulates STAT3 activity. In conclusion, we report, for the first time, that AURKA promotes STAT3 activity through regulating the expression and phosphorylation levels of JAK2. This highlights the importance of targeting AURKA as a therapeutic approach to treat gastric and esophageal cancers.

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1. Introduction

Upper gastrointestinal adenocarcinomas (UGCs), adenocarcinomas of the stomach and esophagus, are characterized by poor response to current chemotherapeutics (Hohenberger and Gretschel, 2003; Kelsen et al., 1998). Gastric cancer is the fourth most common malignancy and the second leading cause of cancer-related deaths in the world (Ferlay et al., 2010; Inoue and Tsugane, 2005), whereas esophageal cancer is the eighth most common cancer worldwide (Ferlay et al., 2010) and its incidence is continuously increasing (Devesa et al., 1998). Despite the recent advancement in understanding the biology of UGC, full elucidation of mechanisms and signaling molecules that promote tumorigenesis and survival remains elusive.

Aurora kinase A (AURKA) amplification and overexpression are frequent findings in UGCs as well as several other malignancies (Chung et al., 2005; Dar et al., 2008b; Marumoto et al., 2005; Sehdev et al., 2012). AURKA is a serine/threonine kinase that localizes to spindle poles and ensures its correct assembly in normal cells (Lens et al., 2010). Several studies have shown that AURKA overexpression promotes drug resistance and tumor recurrence (Cammareri et al., 2010; Otto et al., 2009; Yang et al., 2006). We and others reported that AURKA overexpression counteracts the functions of tumor suppressor genes p53 and p73 (Dar et al., 2008a; Katayama et al., 2012; Sehdev et al., 2014). In cancer cells, AURKA overexpression results in activating several oncogenic pathways including PI3K/AKT, β-catenin, and NF-κB (Dar et al., 2009; Katsha et al., 2013). These reports suggest that AURKA could serve as a signaling hub that connects and regulates several oncogenic signaling networks. The pharmacological inhibition of AURKA using small molecule inhibitor MLN8237, also known as alisertib, has shown significant inhibition of tumor growth in pre-clinical xenograft tumor models (Katsha et al., 2013; Sehdev et al., 2013) with promising results in phase II clinical trials (Friedberg et al., 2014; Matulonis et al., 2012).

Signal Transducer and Activator of Transcription 3 (STAT3) is an important transcription factor that regulates the expression of several cytokines, growth factors, and pro-survival genes that modulate various cellular events; including survival, cell cycle, invasion, and angiogenesis (Aggarwal et al., 2009; Bromberg, 2002; Kortylewski and Yu, 2007; Yu et al., 2007). STAT3 is activated through receptor tyrosine kinases and cytokines; and upon its activation, it dimerizes and translocates into the nucleus where it regulates its target genes (Jarnicki et al., 2010). In addition, non-receptor tyrosine kinases such as Janus kinase 2 (JAK2), can phosphorylate and activate STAT3 (Jarnicki et al., 2010; Kortylewski and Yu, 2007). Of note, STAT3 activation has been linked to chemoresistance in a number of malignancies including lung cancer (Kulesza et al., 2013), head and neck cancer (Bourguignon et al., 2012), and breast cancer (Lieblein et al., 2008). In gastric cancer, STAT3 activation has been associated with *Helicobacter pylori* infection and pre-neoplastic progression (Jackson et al., 2007). Furthermore, STAT3 activation is a common criterion of inflammatory and non-inflammatory gastric cancer mouse models (Giraud et al., 2012), which reflects the overall important role of STAT3 in gastric carcinogenesis.

In this report, we show that AURKA promotes STAT3 activity, nuclear translocation, and expression of pro-survival proteins (BCL2 and MCL1), possibly through regulating the JAK2–STAT3 axis. AURKA knockdown or pharmacological inhibition reversed these pro-survival effects. Collectively, our novel data demonstrate the importance of developing and moving AURKA specific inhibitors in clinical studies of upper gastrointestinal carcinomas.

2. Materials and methods

2.1. Cell culture and reagents

Human gastric (AGS and MKN45) and esophageal (FLO-1) adenocarcinoma cell lines were maintained 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). AURKA investigational inhibitor MLN8237 (Millennium Pharmaceuticals, Inc., Cambridge, MA) was prepared and stored according to the manufacturer's instructions. Specific antibodies against p-AURKA (Thr288), AURKA, p-STAT3 (Tyr705), STAT3, p-JAK2 (Tyr221), JAK2, BCL2, MCL1, and β-actin were purchased from Cell Signaling Technology (Beverly, MA). STAT3 inhibitor AZD1480 was purchased from Sigma–Aldrich (Milwaukee, WI). MLN8237 (alisertib) was purchased from Selleck Chemicals (Houston, TX). Transfection reagent Fugene 6 was purchased from Promega (Madison, WI).

2.2. AURKA expression and plasmids

Flag-tagged coding sequence of AURKA was sub-cloned into Xba I and BamH I sites of the adenoviral shuttle vector (pACCMV), and the recombinant adenovirus was generated by co-transfecting HEK-293AD cells with the shuttle and adenoviral backbone (pJM17) plasmids using the Calcium Phosphate Transfection Kit (Applied Biological Materials, Inc., Richmond, BC). The expression plasmid for AURKA was generated by PCR amplification of the full-length coding sequence of AURKA and cloned in frame into pcDNA3.1 (Invitrogen Life Technologies). A synthetic Flag-tag sequence was added at the N-terminus of AURKA. Cloning of AURKA was confirmed by sequencing and restriction enzyme digestion.

2.3. Western blotting

Cells were scraped, centrifuged, and pellets were resuspended in lysis buffer (PBS and 1% Triton X100) containing Halt Protease Inhibitor Cocktail and Halt Phosphatase Inhibitor Cocktail (Pierce Biotechnology, Inc., Rockford, IL) on ice. Protein concentration was measured using the Bradford Protein Assay (Bio-Rad Laboratories, Hercules, CA). Proteins (25 μg) from each sample were subjected to SDS/PAGE and transferred onto nitrocellulose membranes. Membranes were probed with specific primary antibodies and HRP-coupled secondary antibodies (Cell Signaling). Protein bands were visualized using a commercial Immobilon Western Chemiluminescent HRP Substrate kit (Millipore, Billerica, MA).

2.4. *Immunofluorescence*

AGS and FLO-1 cells were seeded in 8-well chambers (BD Falcon, Bedford, MA) and incubated overnight. Next day, AGS cells were infected with control or AURKA adenoviruses particles (5 MOI), whereas FLO-1 cells were transfected with scramble or AURKA siRNA (Cell Signaling) and cultured for 48 h. Cells were washed with PBS and fixed with fresh 4% paraformaldehyde solution for 45 min at room temperature. After washing with ice-cold PBS, cells were incubated with permeabilization solution (0.1% Triton X-100 in 0.1% Sodium Citrate) for 2 min on ice. Then, cells were washed again with PBS twice for 1 min each followed by incubation with 10% non-immune goat serum blocking solution (Invitrogen) for 20 min at room temperature. Next, cells were incubated in a specific primary antibody against STAT3 or AURKA diluted in PBS (1:400) for 2 h at room temperature. After washing 3 times with ice-cold PBS, cells were incubated in fluorescein isothiocyanate (FITC)-tagged secondary antibody (1:1000; Invitrogen) for 45 min at room temperature. Cells were then washed in PBS, mounted with Vectashield/DAPI (Vector Laboratories, Burlingame, CA), visualized, and randomly selected images were taken using an Olympus BX51 fluorescence microscope (Olympus Co., Japan). The percentage of nuclear STAT3-positive cells was calculated from at least 200 cells from each experiment using ImageJ software (http://www.uhnresearch.ca/facilities/wcif/imagej/).

2.5. *Luciferase reporter assay*

STAT-Luc reporter vector (Clontech, Mountain View, CA) was used to measure the activity of STAT3. Cells were seeded in 24-well plates and transiently co-transfected with 0.5 μg of STAT3-Luc reporter in combination with 0.5, 1.0 or 1.5 μg of AURKA vector or 1.0 μg of pcDNA3.1 vector using Fugene 6 according to the manufacturer's instructions. In a separate experiment, MKN45 cells were transfected with STAT3-Luc reporter (0.5 μg) in combination with shAURKA (0.1, 0.25 or 0.5 μg) or shScramble (0.5 μg). Luciferase activity was measured using the Dual-Luciferase Reporter Assay kit (Promega) according to the manufacturer's instructions. For the rescue experiment, we transfected the FLO-1 cells with siScramble or siAURKA along with 0.5 μg STAT3-Luc reporter and cells allowed to settle for 24 h. Then, we transfected cells with 2.0 μg of pcDNA3.1 or AURKA-pcDNA3.1 vectors using Fugene 6. The STAT3 luciferase reporter activity was measured after an additional 24 h.

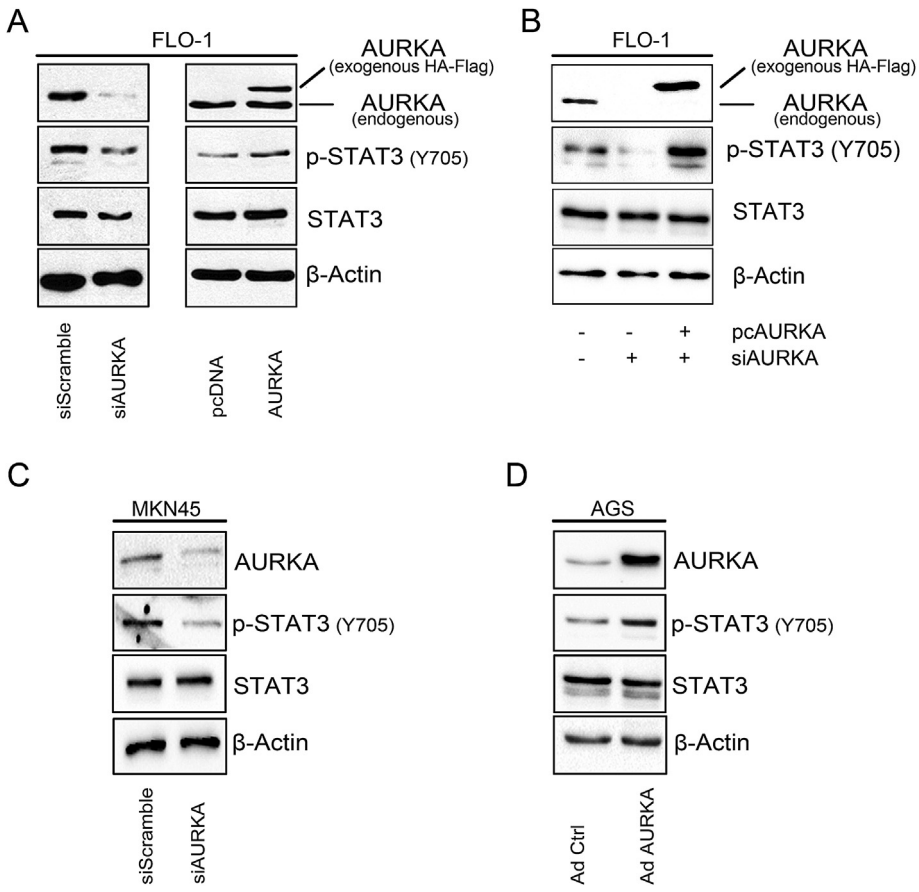

Figure 1 – AURKA promotes phosphorylation of STAT3 in upper gastrointestinal cancer cells. (A) AURKA knockdown (left panel) or stable overexpression (right panel) in FLO-1 cells led to a decrease or increase in p-STAT3 (Tyr705), respectively. (B) Knockdown of endogenous AURKA led to a decrease in the p-STAT3 (Tyr705). Reconstitution of AURKA, after knockdown of endogenous levels, restored the STAT3 phosphorylation levels. (C) AURKA knockdown in MKN45 cells resulted in decreased phosphorylation of STAT3 (Tyr705). (D) AGS cells showed increased p-STAT3 (Tyr705) protein level in response to transient AURKA overexpression.

2.6. AURKA silencing by small interfering RNA (siRNA)

Cells were transfected with siScramble, siAURKA or siJAK2 (Cell Signaling) for 24 h in 0% FBS. Next day, 20% FBS-DMEM medium was added, and cells were allowed to recover for another 24 h before harvesting. In a separate experiment, and in order to check whether AURKA reconstitution, after its knockdown, would restore STAT3 phosphorylation, we transfected the FLO-1 cells with siScramble or siAURKA for 24 h. Then, we transfected cells with 2.0 μg of pcDNA3.1 or AURKA-pcDNA3.1 vectors using Fugene 6. Cells were harvested after an additional 24 h and subjected to Western blot analysis.

2.7. Clonogenic cell survival assay

FLO-1 and AGS cells were seeded at 5000 cells/well in 6-well plates. Next day, cells were treated with 0.5 μM of MLN8237 for 24 h. After washing with PBS, the cells were incubated in drug-free complete DMEM medium for 10 days. Subsequently, cell colonies were fixed with 2% Paraformaldehyde solution for 10 min, and stained overnight with crystal violet (0.05% Crystal Violet in 50% Methanol). Next, colonies were gently washed with PBS and photographed. Cell survival was determined by quantifying the dye signal in each well with ImageJ analysis software.

2.8. RNA extraction and real-time RT-PCR

Total RNA from cells was extracted using a miRNeasy extraction kit (Qiagen, Valencia, CA). RNA (2 μg) was converted to cDNA using an iScript cDNA synthesis kit (Bio-Rad). Quantitative RT-PCR (qRT-PCR) was performed using an iCycler (Bio-Rad) with the threshold cycle number determined by use of iCycler software version 2.1. Reactions were performed in triplicate, and the threshold cycle numbers were averaged. The results of the genes were normalized to *HPRT1* housekeeping gene for both cells as described previously (El-Rifai et al., 2002). The primers used in qRT-PCR analysis are shown in Supplemental Table 1.

2.9. Statistical analysis

Data are presented as means ± standard error of mean. All *in vitro* experiments were carried out in triplicate. The statistical significance of the studies was determined by the

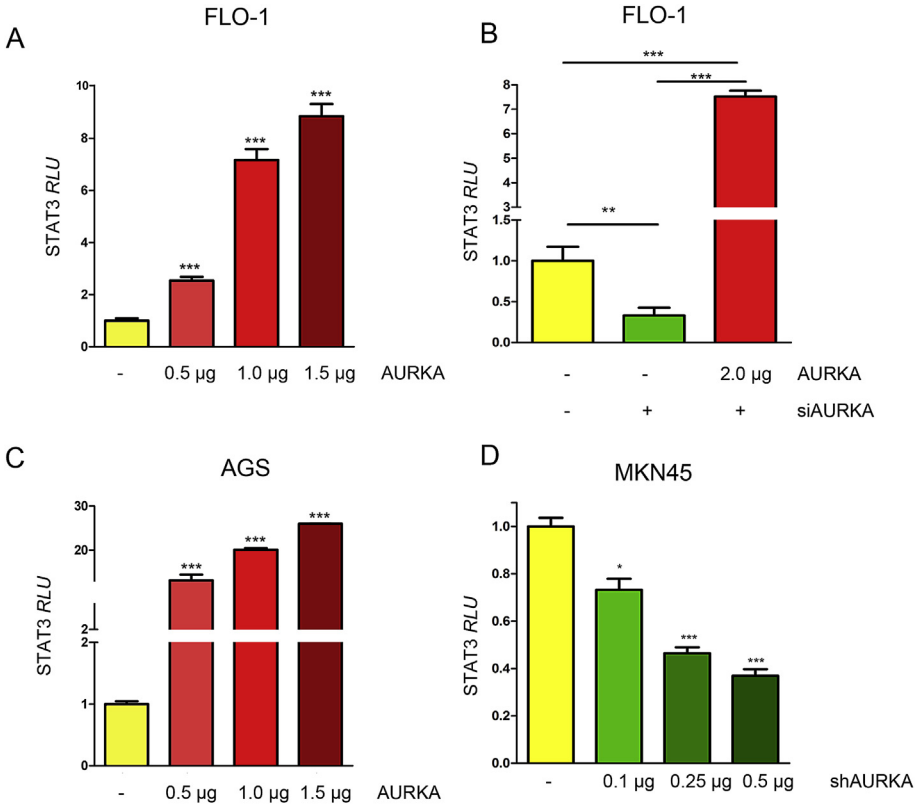

Figure 2 – AURKA overexpression induces STAT3 transcriptional activity. (A) FLO-1 cells were transfected with pcDNA or AURKA in combination with STAT3 luciferase reporter plasmids, and STAT3 luciferase reporter activity was measured. The data indicate that AURKA can enhance STAT3 transcriptional activity. (B) Knockdown of endogenous AURKA decreases STAT3 luciferase reporter activity, whereas re-introducing AURKA rescued the STAT3 activity levels. (C) AGS cells were transfected with pcDNA or AURKA in combination with STAT3 luciferase reporter plasmids, and STAT3 luciferase reporter activity was measured. The data indicate that AURKA can enhance STAT3 transcriptional activity. (D) MKN45 cells were transfected with shScramble or shAURKA in combination with STAT3 luciferase reporter plasmids, and STAT3 luciferase reporter activity was assessed. The data show that knockdown of endogenous AURKA decreases STAT3 transcriptional activity in a dose-dependent fashion.

Student's *t* test using GraphPad Prism 5 software (GraphPad software, Inc. La Jolla, CA). Differences with p values ≤ 0.05 are considered significant.

3. Results

3.1. *AURKA induces STAT3 phosphorylation and activity*

In order to investigate whether changes in AURKA levels would affect phosphorylation of STAT3, we modulated AURKA levels in FLO-1, AGS, and MKN45 cell lines and assessed the changes in STAT3 phosphorylation by Western blotting. Our data indicated that in FLO-1 cells, and after knocking down AURKA, p-STAT3 (Tyr705) levels were decreased (Figure 1A, left panel). On the other hand, FLO-1 cells stably overexpressing AURKA showed higher STAT3 phosphorylation compared to control cells (Figure 1A, right panel). In a rescue experiment, we confirmed that knockdown of endogenous AURKA by siRNA followed by overexpression of exogenous AURKA, restored AURKA-induced phosphorylation of STAT3 in FLO-1 cells (Figure 1B). To confirm these observations, we also knocked down endogenous AURKA in MKN45 cells and examined STAT3 phosphorylation. Indeed, Western blot analysis data showed a decrease in p-STAT3 levels (Tyr705), as compared to scramble control transfected cells (Figure 1C). Additionally, by using an adenovirus system we transiently overexpressed AURKA in AGS cells and evaluated p-STAT3 (Tyr705) levels. Our data indicated an increase in STAT3 phosphorylation in Adeno-AURKA infected cells as compared to control adenovirus infected cells (Figure 1D). Next, to investigate if the increase of STAT3 phosphorylation is accompanied with increased STAT3 activity, we transfected FLO-1 cells with 0.5, 1.0 or 1.5 μg of AURKA vector in combination with STAT3 luciferase reporter plasmid (0.5 μg), and STAT3 transcriptional activity was measured. Our data showed a dose-dependent significant induction of STAT3 activity (Figure 2A). For a rescue experiment, we knocked down the endogenous AURKA for 24 h followed by its reconstitution using exogenous AURKA transfection. The STAT3 transcriptional activity was measured and demonstrated that knockdown of endogenous AURKA reduced the STAT3 reporter activity whereas overexpression of AURKA restored the activity of the STAT3 reporter (Figure 2B). In AGS cells, STAT3 activity showed dose-dependent increase in response to AURKA transfection (Figure 2C). Conversely, knockdown of endogenous AURKA in MKN45 cells reduced STAT3 transcriptional activity in a dose-dependent manner (Figure 2D). Together, these data suggest that AURKA promotes phosphorylation and activation of STAT3.

3.2. *STAT3 nuclear translocation is affiliated with AURKA levels*

Upon phosphorylation, STAT3 translocates to the nucleus where it binds to and activates expression of its transcription target genes. To elucidate whether AURKA can alter STAT3 nuclear translocation, we performed immunofluorescence analysis for STAT3 in FLO-1 and AGS cells after AURKA knockdown or overexpression, respectively. Our data clearly indicated that AURKA knockdown in FLO-1 cells led to a significant ($p < 0.01$) decrease of cells showing positive nuclear staining of STAT3 relative to control cells (Figure 3A). In contrast, AURKA overexpression in AGS cells resulted in a dramatic increase ($p < 0.01$) of cells depicting positive nuclear

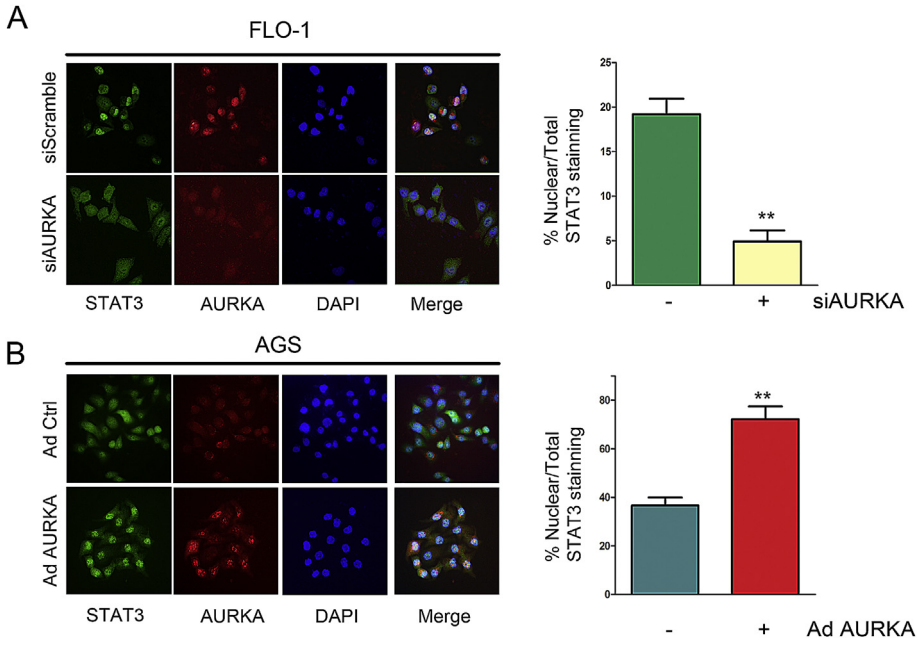

Figure 3 – AURKA expression modulates STAT3 nuclear translocation. Representative images of STAT3 immunofluorescence analysis in FLO-1 cells (A) after AURKA knockdown, or in AGS cells (B) after AURKA overexpression by adenovirus are shown. Original magnification is shown at ×400. AURKA knockdown or overexpression significantly decreased ($p < 0.01$) or increased ($p < 0.01$) nuclear STAT3-positive staining relative to vehicle-treated control, respectively.

staining of STAT3, as compared to control cells (Figure 3B). Collectively, these data clearly show that AURKA regulates phosphorylation and nuclear localization of STAT3.

3.3. *Pharmacological inhibition of AURKA attenuates STAT3 phosphorylation and suppresses cell survival*

Based on the aforementioned data, we decided to examine whether AURKA kinase activity is involved in regulating STAT3. Therefore, we treated FLO-1, AGS and MKN45 cells with AURKA specific inhibitor MLN8237 (0.5 μM) for 30 min or 1 h, and subjected the cell lysates to Western blot analysis. Our data showed that AURKA inhibition decreased STAT3 phosphorylation at Tyr705 in all cell lines (Supplemental Figure 1A–C). Next, we treated FLO-1 and AGS cells for 72 h with MLN8237 (0.5 μM) and examined the expression levels of pro-survival proteins, BCL2 and MCL1, known protein targets of STAT3 with anti-apoptotic functions (Bhattacharya et al., 2005; Liu et al., 2003). Indeed, our data indicated that MLN8237 treatment led to a decrease in STAT3 phosphorylation and BCL2 and MCL1 expression levels (Figure 4A and B). The detected transient increase of total unphosphorylated AURKA protein level following MLN8237 treatment could be a cellular response to inhibition of its activity and function,

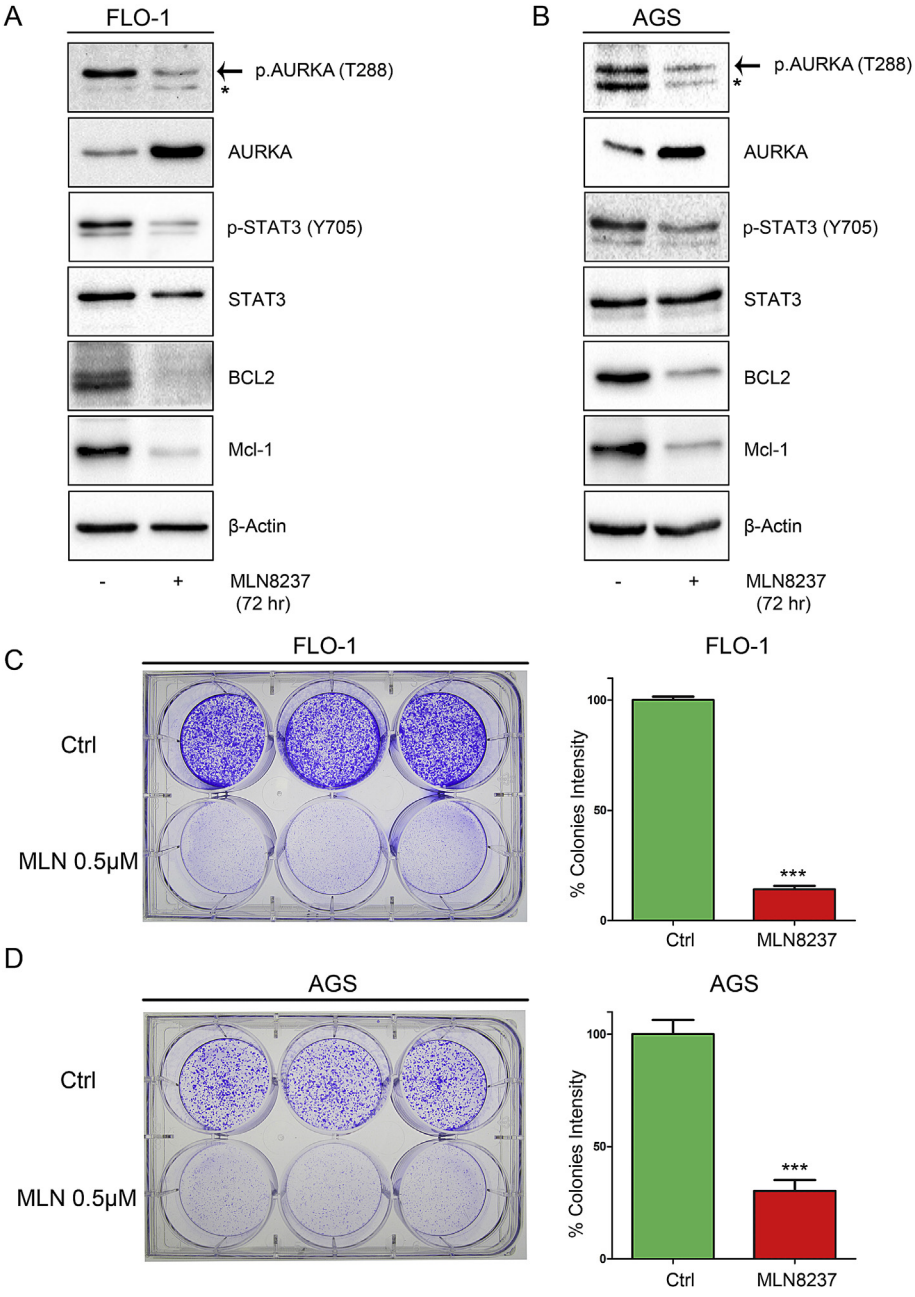

Figure 4 – AURKA pharmacological inhibition decreases STAT3 phosphorylation and reduces cell survival. FLO-1 (A) and AGS (B) cells treated with MLN8237 (0.5 μM) for 72 h and subjected to Western blot analysis. Data indicated down-regulation of p-STAT3 (Tyr705) and STAT3 targets, BCL2 and MCL1, protein expression. FLO-1 (C) and AGS (D) cells treated with vehicle or MLN8237 (0.5 μM) for 24 h and then cultured in fresh media for 10 days. Graphs show colonies quantitative data (right panels).

which has been reported previously (Do et al., 2014; Li and Rana, 2012). To demonstrate the biological effect of AURKA inhibition on cell growth, we performed long-term clonogenic cell survival assay. AGS and FLO-1 cells were treated with MLN8237 (0.5 μM) for only 24 h and cultured for 10 days. Our data showed that MLN8237 significantly impaired the cells ability to form colonies as indicated by the quantitative analysis at the end of the experiment (Figure 4C and D). Collectively, these data suggest that AURKA inhibition suppresses the STAT3 pathway leading to decreased survival of cancer cells.

3.4. *AURKA regulates phosphorylation and expression of JAK2*

To elucidate the exact mechanism by which AURKA regulates STAT3, we examined STAT3 upstream molecules, specifically Janus kinase 2 (JAK2). Our results indicated for the first time that knockdown of AURKA by siRNA resulted in a significant decrease in phosphorylation and expression of JAK2 in FLO-1 (Figure 5A, left panel) and MKN45 (Supplemental Figure 2). To determine whether the decrease in JAK2 protein levels is

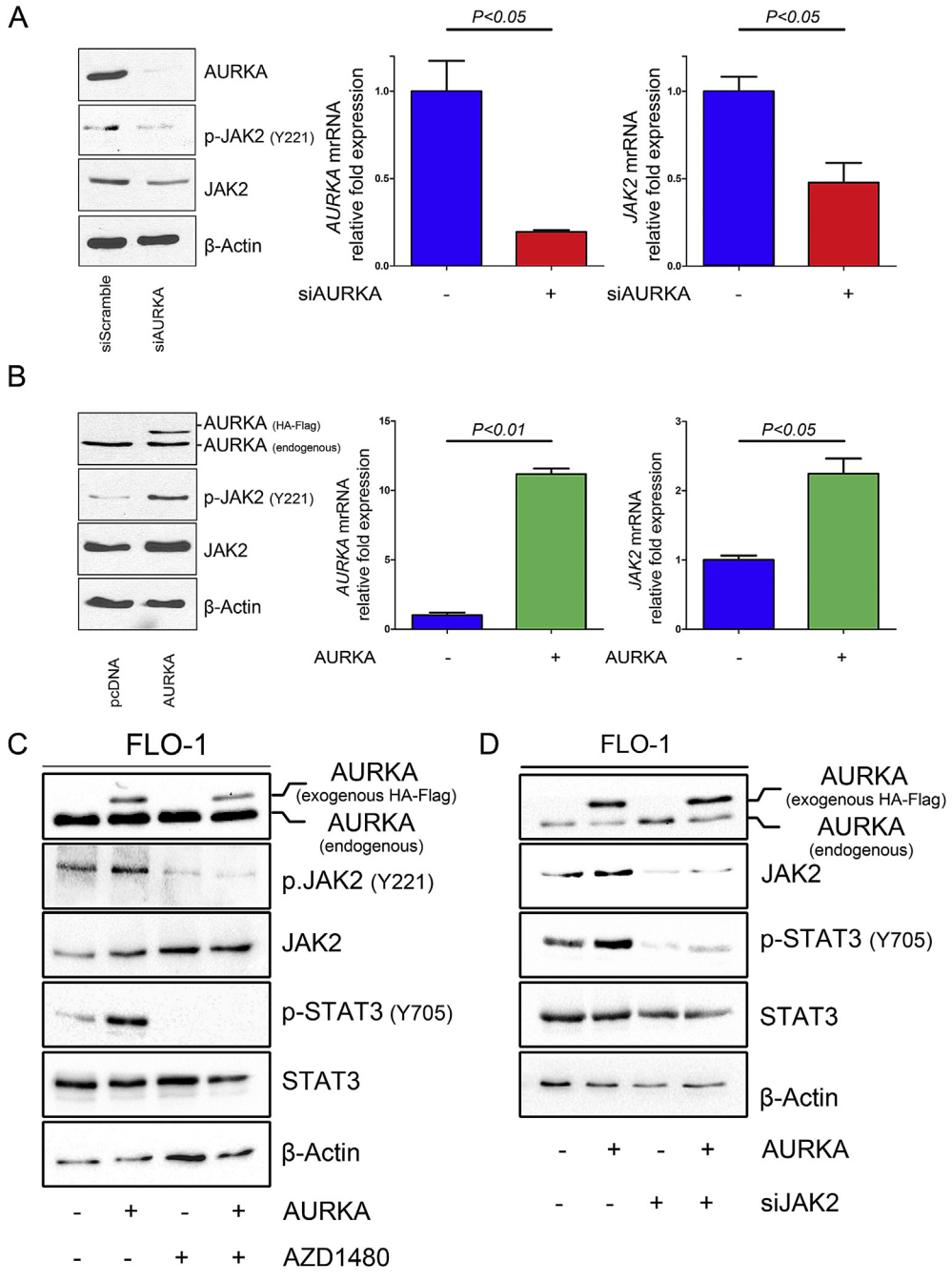

Figure 5 – AURKA regulates JAK2 expression. (A) Silencing AURKA (siAURKA) in FLO-1 cells led to decreased JAK2 at both protein and mRNA levels. (B) FLO-1 cells stably overexpressing AURKA showed an increase in JAK2 at both protein and mRNA levels. (C, D) Inhibition of JAK2 using pharmacologic inhibitor (AZD1480) (C) or genetic knockdown JAK2 using siRNA (siJAK2) (D) abrogated AURKA-induced phosphorylation of STAT3 in FLO-1 cells.

initiated transcriptionally, we assessed JAK2 mRNA expression after knockdown or overexpression of AURKA by qRT-PCR in FLO-1 cells. Our data showed that AURKA knockdown resulted in decreased JAK2 mRNA levels (Figure 5A, right panel). Conversely, AURKA overexpression led to an increase in JAK2 phosphorylation at Y221 and JAK2 protein and mRNA levels (Figure 5B). Previously, it was reported that JAK2-specific inhibitor AZD1480 could block STAT3 phosphorylation and signaling (Hedvat et al., 2009; Scuto et al., 2011). Therefore, to determine if JAK2 is necessary for AURKA-mediated phosphorylation of STAT3, we treated FLO-1 cells overexpressing AURKA with AZD1480. Western blot analysis indicated that inhibition of JAK2 abrogated AURKA-induced phosphorylation of STAT3 (Figure 5C). Consistent with these results, knockdown of JAK2 in FLO-1 cells stably overexpressing AURKA significantly reduced the AURKA-induced STAT3 phosphorylation (Figure 5D). Taken together, these results clearly indicate that JAK2 mediates the effect of AURKA on STAT3.

4. Discussion

In this study, we demonstrate for the first time that AURKA regulates the phosphorylation, nuclear localization, and transcription activity of STAT3. Our results indicate that AURKA regulation of STAT3 is mediated by JAK2. Importantly, our data suggest that pharmacological inhibition of AURKA could be a plausible approach to suppress the JAK2-STAT3 oncogenic axis.

STAT3 plays a major role in tumorigenesis by mediating activation of several pro-oncogenic functions such as proliferation, survival, invasion, and angiogenesis (Yu et al., 2007, 2009). Several studies have shown that *H. pylori* infection leads to STAT3 activation through regulation of IL-6 and IL-11 (Bronte-Tinkew et al., 2009; Lee et al., 2012). In esophageal cancer, it has been shown that STAT3 contributes to survival and proliferation of cancer cells (Wang et al., 2013; Zhang et al., 2012). These reports show the important role of STAT3 in gastric and esophageal cancer progression and thus, it is of high importance to target STAT3 as a therapeutic approach for both malignancies. To date, there are no active clinical trials to test the efficacy of STAT3 inhibitors in UGC (clinicaltrials.gov). In our current report, we show that AURKA knockdown or pharmacological inhibition can significantly decrease STAT3 phosphorylation/activity and expression of STAT3 downstream targets, BCL2 and MCL1. We also demonstrated that MLN8237 can suppress survival of AGS and FLO-1 cells, and it is possible that other physiological functions could be affected as well. Constitutive activation of STAT3 or activating mutations in its upstream molecule, JAK2, trigger tumorigenesis, thereby promoting invasive growth and metastasis (Yu et al., 2007). In our study, we showed that the levels and phosphorylation JAK2 were affected by AURKA expression, suggesting that AURKA regulates the JAK2–STAT3 signaling. On the other hand, a previous report showed that activating mutations in JAK2, up-regulate AURKA expression through c-Myc, and this promotes chemoresistance to cisplatin (Sumi et al., 2011). Taken together with our data, this may suggest the presence of a positive feedback loop through which the cancer cells maintain constant activation of STAT3 and overexpression of AURKA, collectively promoting cancer cell survival. In addition, AURKA can regulate cell migration and adhesion in ovarian cancer through regulation of SRC (Do et al., 2014), which is an upstream regulator of STAT3. This highlights the role of AURKA in modulating oncogenic signaling pathways related to STAT3 in cancer.

Our recent study has indicated that AURKA can also regulate the pro-inflammatory NF-κB signaling in gastric cancer (Katsha et al., 2013). STAT3 is known to play important roles in promoting the pro-inflammatory signaling (Yu et al., 2007, 2009). Therefore, it is possible that AURKA overexpression could be a major molecular event that promotes inflammation and carcinogenesis by activating NF-κB and STAT3 signaling pathways. Our current data clearly indicate that pharmacologic or genetic inhibition of AURKA abrogates the JAK2–-STAT3 signaling axis. This finding is a significant addition to our knowledge about the role of AURKA in carcinogenesis that has been shown to implicate the regulation of other key signaling pathways such as β-catenin (Dar et al., 2009), NF-κB (Katsha et al., 2013), and p53 family proteins (Katayama et al., 2012). Collectively, these data suggest that AURKA is placed at a major signaling hub in carcinogenesis. Therefore, targeted inhibition of AURKA, which is capable of regulating several important pathways in cancer cells, could be a novel therapeutic approach to target multiple oncogenic signaling pathways. Of note, phase II clinical trials using MLN8237, also known as alisertib, demonstrated promising results (Friedberg et al., 2014; Matulonis et al., 2012). The implementation of this strategy could be successful in overcoming drug resistance and eliminating cancer cells in UGCs. However, a single agent therapy may not be successful and a combination with existing chemotherapy regimens may be more beneficial and need to be examined in clinical trials.

In summary, our results demonstrate the JAK2–STAT3 axis as a novel signaling pathway regulated by AURKA in upper gastrointestinal cancers. Ongoing early phase clinical trials and future studies are likely to provide important information for moving AURKA inhibitors into the clinical practice.

Authors' contribution

Ahmed Katsha: Design of experiments and acquisition of data; analysis and interpretation of data; drafting of the manuscript; technical and material support.

Janet Arras: Experimental support and acquisition of data.

Mohammed Soutto: Analysis and interpretation of data.

Abbes Belkhiri: Analysis and interpretation of data; experimental troubleshooting; drafting of the manuscript; critical revision of the manuscript.

Wael El-Rifai: Study concept and design; obtained funding; study supervision; experimental troubleshooting; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

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Disclosure of potential conflicts of interest

All authors indicated "no conflict of interest".

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molonc.2014.05.012.

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