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EXPRESSION AND IMPACT OF DJ-1 IN REGULATING COLON CANCER CELL INVASION AND METASTASIS

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ABSTRACT

Colon cancer is one of the most leading causes of cancer mortalities in the world. DJ-1 as an oncogene was recently stated to be involved in several types of cancer metastasis; including colon cancer. In the present study, the expression and role of DJ-1 in human colon cancer metastasis was evaluated using metastatic (SW620) and non-metastatic (SW480) colon cancer cells. The expression level of DJ-1 was markedly higher in metastatic SW620 than in non-metastatic SW480 colon cancer cells. The overexpression or knockdown of DJ-1 demonstrated no impact on cell proliferation of both colon cancer cell lines. In this respect, DJ-1 overexpression increases cell invasion and migration in both SW480 and SW620 colon cancer cells. Inversely, DJ-1 knockdown significantly represses colon cancer cell invasion and migration in both cells. Concurrently, DJ-1 overexpression increases Snail and down-regulated E-cadherin mRNA and protein expressions. Altogether, these findings suggest that DJ-1 is a good candidate for targeting colon cancer metastasis prognosis and treatment.

KEY WORDS

Colon cancer, SW480, SW620, metastasis, DJ-1

1. INTRODUCTION

Colorectal cancer is considered the third most common cancer and the third leading cause of cancer mortalities in men and women in the United States [1]. A recent study also showed that colon cancer is considered the second most leading cause of cancer mortalities worldwide [2]. Death resulted from colon cancer is not due to cancer itself but due to its ability to invade and spread to other organs and tissues. The development of metastatic cancers of the epithelial origin, which represent about 75% of all human cancers include loss of cell-cell contacts (epithelial to mesenchymal transition; EMT) and development of mesenchymal phenotype. The resulting migratory tumor cells circulate through the vasculature or the lymph system to reach different parts of the body. The main problem is how to adjust the epithelial mesenchymal transition (EMT) markers (such as Snail, N-cadherin, Vimentin, etc) and the mesenchymal epithelial transition (MET) markers (such as E-cadherin, Hakai, Occluding, etc).

Park7/DJ-1 is a multifunctional protein; acts as antioxidant against oxidative stress [3-7], involved in apoptosis regulation [8-10] and cell proliferation and cancer promotion [11, 12]. DJ-1 was recently nominated as an oncogene and involved in cancer proliferation and invasion regulation [7, 13]. It was stated that DJ-1 is positively correlated with breast [13, 14], hepatocellular [15], lung [16-18], melanoma [19], and prostate [20] cancer progression and metastasis. A protein cluster analysis study indicated that, DJ-1 is differentially expressed in SW480 and SW620 colon cancer cells; highly expressed in SW620 cells [21]. A recent research

reported that high expression of DJ-1 promotes invasion and migration of SW480 and HCT116 colorectal cancers [22]. Supporting to this, it was found that KLF17 (DJ-1downstream target) expression decreases in colorectal cancer patients [23]. The expression of DJ-1 in colon cancer cells and its role in regulating colon cancer invasion and spreading is not yet fully understood.

Therefore, through this research we aimed to assess the gene therapeutic efficiency of DJ-1 expression modulation in inhibiting colon cancer cell invasion and spreading to the surrounding and distal organs. In addition, we investigated the molecular amechanism (s) by which DJ-1 alters expression of selected genes involved in cancer invasion and EMT regulation.

2. MATERIALS AND METHODS

2.1. Cell lines and culture

Human colon cancer cell lines SW480, and SW620 were maintained in RPMI cell culture media supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were grown and maintained at 37°C in a 5% CO_2 incubator for further experiments.

2.2. Overexpression and knockdown of DJ-1

Colon cancer SW480 and SW620 cells were transiently transfected with either DJ-1 overexpression clone (DJ-1-flag) or DJ-1 siRNA (siDJ-1) as previously described [13]. Briefly, for exogenous DJ-1 overexpression, both SW480 and SW620 colon cancer cells were transfected with pc3DNA-flag-DJ-1 vector using Lipofectamine^{tm} 2000 in Opti-MEM (Invitrogen, USA). For DJ-1 silencing, both colon cells were transfected with DJ-1 siRNA (sense: 5´-CGACGAUCACUUAGAGAAATT-3´, anti-sense: 5´-UUUCUCUAAGUGAUCGUCGCA-3´) (Qiagen, USA). Cells were transfected in serum-free RPMI media, which supplanted with new serum-containing media after 12 hrs and cells were then incubated for the next experiments.

2.3. Cell proliferation assay

To study the action of DJ-1 on the proliferation of colon cancer SW480 and SW620 cells, a proper number of DJ-1-flag and si-DJ-1 transfected cells as well as control non-transfected cells were seeded into 96-well plates. Cells were then incubated for 24 hrs. Cell viability was then assessed using MTT cell viability assay (Sigma, USA) as previously shown [24].

2.4. Wound healing assay

The action of overexpression and knockdown of DJ-1 on cell migration of human colon cancer SW480 and SW620 cells was demonstrated using the wound healing assay as previously performed [13]. Briefly, both colon cancer cells were plated in 100 mM cell culture dishes. The following day, cells were transfected with either DJ-1 overexpression clone (DJ-1-flag) or DJ-1 siRNA (siDJ-1). Wounds were made immediately after transfections and then culture media were changed with new fresh media. The wounds were photographed immediately after transfection and after 24 hrs of transfection.

2.5. Cell invasion assay

The action of DJ-1 overexpression and knockdown on human colon cancer SW480 and SW620 cell invasion was investigated by cell invasion assay (Cell Biolabs, USA) as previously shown [13]. Briefly, both metastatic SW620 and non-metastatic SW480 colon cancer cell lines were transiently transfected with either DJ-1 overexpression clone (DJ-1-flag) or DJ-1 siRNA (siDJ-1). After 24 hrs of transfection, cell invasion assay was then performed according to the instructional manual using DJ-1-overexpressing and DJ-1-knockdown cells as well as controls.

2.6. Gene expression analysis

The action of DJ-1 on the metastasis and EMT regulating genes in colon cancer non-metastatic SW480 and metastatic SW620 cells has been demonstrated using real time PCR. Briefly, 1 X 10^5 cells from the two colon cancer cell lines were seeded in 100-mm cell culture plates. Both cells were then transiently transfected with either DJ-1-flag or siDJ-1 for the following 24 hrs. RNA extraction using Qiazol (Valencia, USA) was performed. Five µg of total RNA was then reverse transcribed using the first strand cDNA synthesis kit (Applied Biosystems, USA). The primers used for the real time PCR analyses were designed by the Primer Express 1.5 software. DJ-1 sense, 5`-GTCATTTGTCCTGATGCCAGC-3`, DJ-1 anti-sense, 5`-TCAGATAAATTCTGTGCGCCC-3`, E-cadherin sense, 5`-CGACCCAACCCAAGAATCTA-3`, E-cadherin anti-sense, 5`-CTCCAAGAATCCCCAGAATG-3`, Snail sense, 5`-AGCTCTCTGAGGCCAAGGATCT-3`, Snail anti-sense,5` -TGTGGCTTCGGATGTGCAT-3`, GAPDH sense, 5`-AGATCATCAGCAATGCCTCCTG-3` and GAPDH anti-sense, 5`-ATGGCATGGACTGTGGTCATG-3`. Real time PCR reactions and cycling conditions were carried out as previously shown [25] using ABI Prism 7500 (Applied Biosystems, USA). These experiments were performed three times. Data were calculated by normalizing the averages Ct values compared to its opposite GAPDH endogenous control to determine the values of Δcycle

threshold (ΔCt). Next, $2^{-\Delta\Delta Ct}$ for each treatment was calculated as previously shown [26].

2.7. Western blot analysis

The effect of DJ-1 on E-cadherin and Snail protein expression changes was investigated. Both colon cancer SW480 and SW620 cells were transiently transfected with either DJ-1 overexpressing clone (DJ-1-flag) or DJ-1 siRNA (siDJ-1) and incubated for 24 hrs. The following day, western blot analysis for observing protein expression changes was performed as previously shown [24]. Briefly, a set of forty micrograms of total proteins extracted from DJ-1-overexpressing and knockdown as well as control cells were electrophoresed using SDS-PAGE gels. Proteins were then transferred into nitrocellulose membranes, which in turn incubated in skim milk (5%) for one hr at room temperature. Membranes were then incubated with rabbit polyclonal anti-E-cadherin and anti-Snail primary antibodies (Abcam, UK) for one hr at 4℃. HRP conjugated goat anti-rabbit secondary antibody (Pierce, USA) was used for one hr at room temperature. Mouse monoclonal HRP-conjugated anti-β-actin antibody (Santa Cruz, USA) was used for normalization.

2.8. Statistical analyses

The present data were expressed as means ± standard deviation. Data were analyzed, graphed and statistically assessed. Statistical differences were investigated using Student's T-test.

3. RESULTS

3.1. DJ-1 expression in colon cancer

DJ-1 is differentially expressed in SW620 (metastatic) and SW480 (non-metastatic) colon cancer cells. We found that DJ-1 mRNA expression level is highly expressed in metastatic highly invasive SW620 cells when compared to that of non-metastatic SW480 colon cancer cells (Fig. 1A), referring to its important role in cell invasion and progression of colon metastasis.

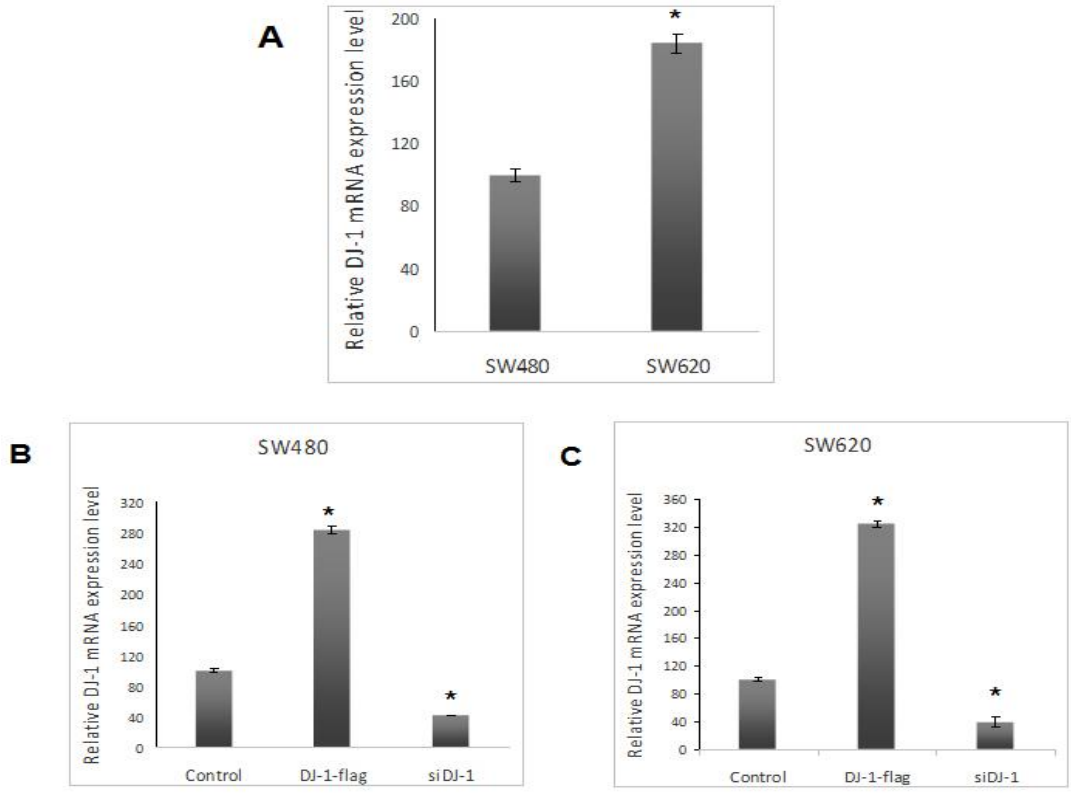

Figure 1: DJ-1 mRNA expression in non-metastatic (SW480) and metastatic (SW620) colon cancer cells (A) and the transfection efficiency of DJ-1 overexpression (DJ-1-flag) and DJ-1 knockdown (siDJ-1) in both SW480 (B) and SW620 (C). The expression of DJ-1 mRNA was assessed using real time PCR. Statistical significances were investigated using student t-test (*$p < 0.01$).

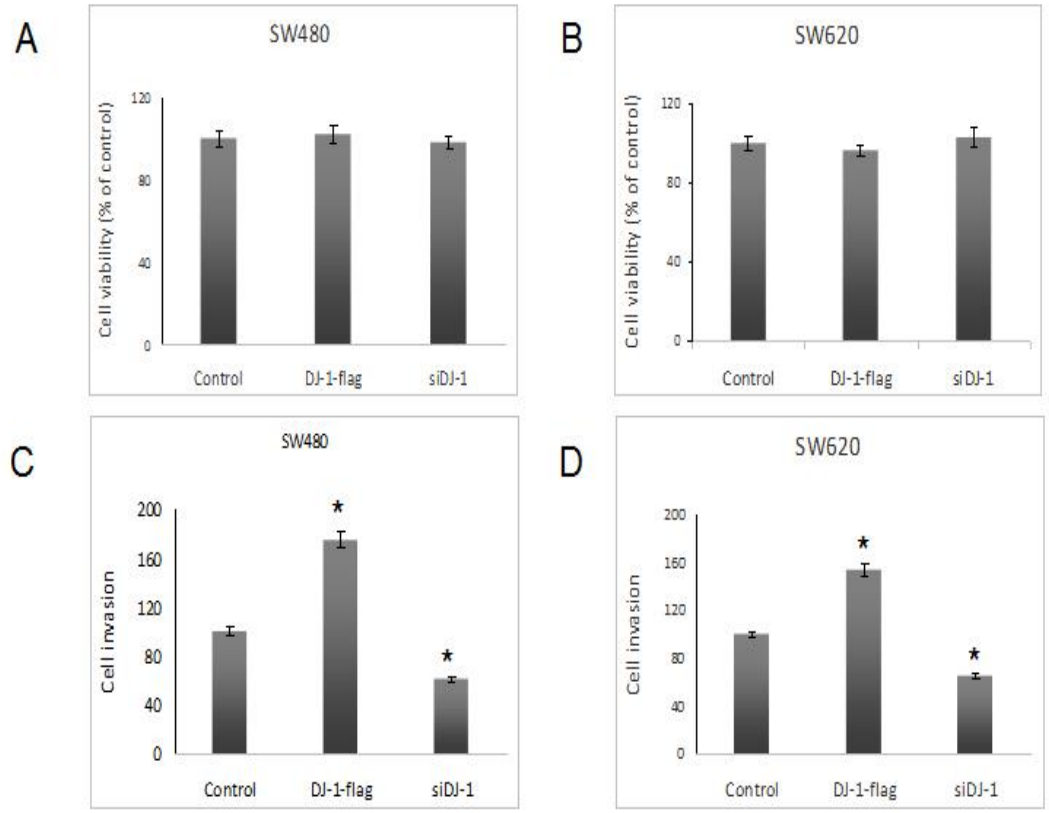

Figure 2: The impact of DJ-1 overexpression (DJ-1-flag) and DJ-1 knockdown (siDJ-1) on cell viability (A & B) and cell invasion (C & D) in both SW480 and SW620 cells. Cells were transfected and then cell viability and invasion were investigated using MTT cell viability, and cell invasion assays, respectively. Statistical differences were demonstrated using student t-test (*$p < 0.01$).

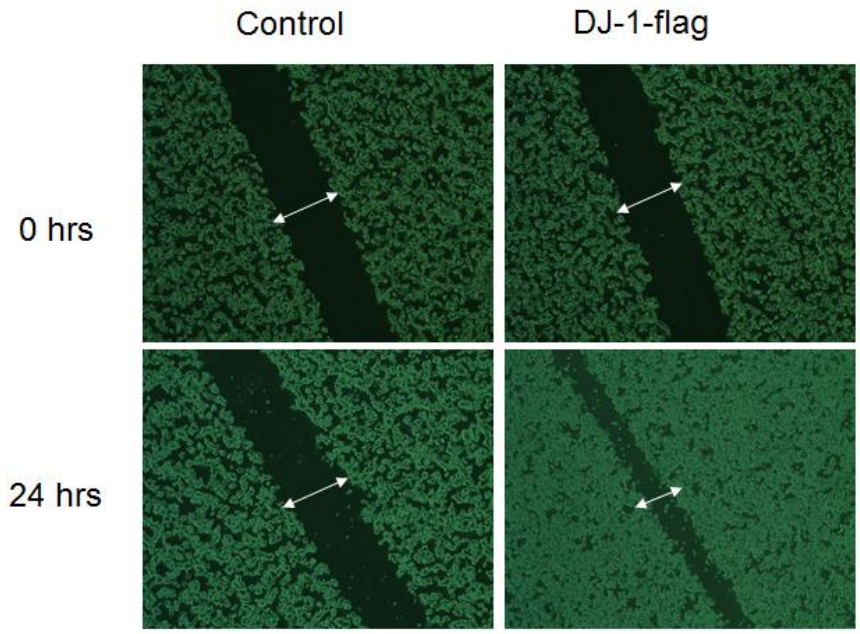

Figure 3: The effect of DJ-1 overexpression on SW480 cell migration. Cells were transfected with DJ-1 overexpression clone (DJ-1-flag) and then wounds were manually made and observed photographed immediately after transfection (0 hr) and 24 hrs after transfection using wound healing assay.

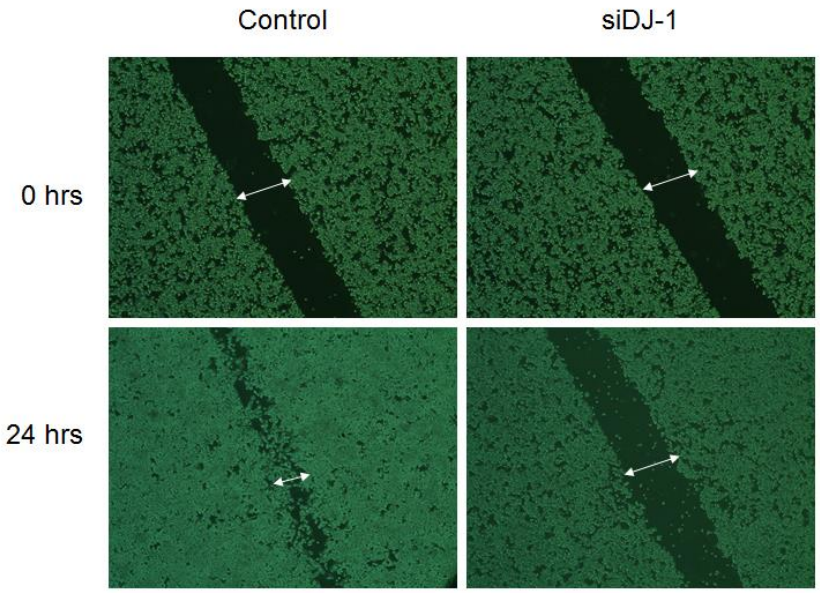

Figure 4: The effect of DJ-1 knockdown on SW620 cell migration. Wounds were made in DJ-1 overexpressing and control non-transfected cells using wound healing assay. Cells were observed and photographed immediately after transfection and after 24 hrs.

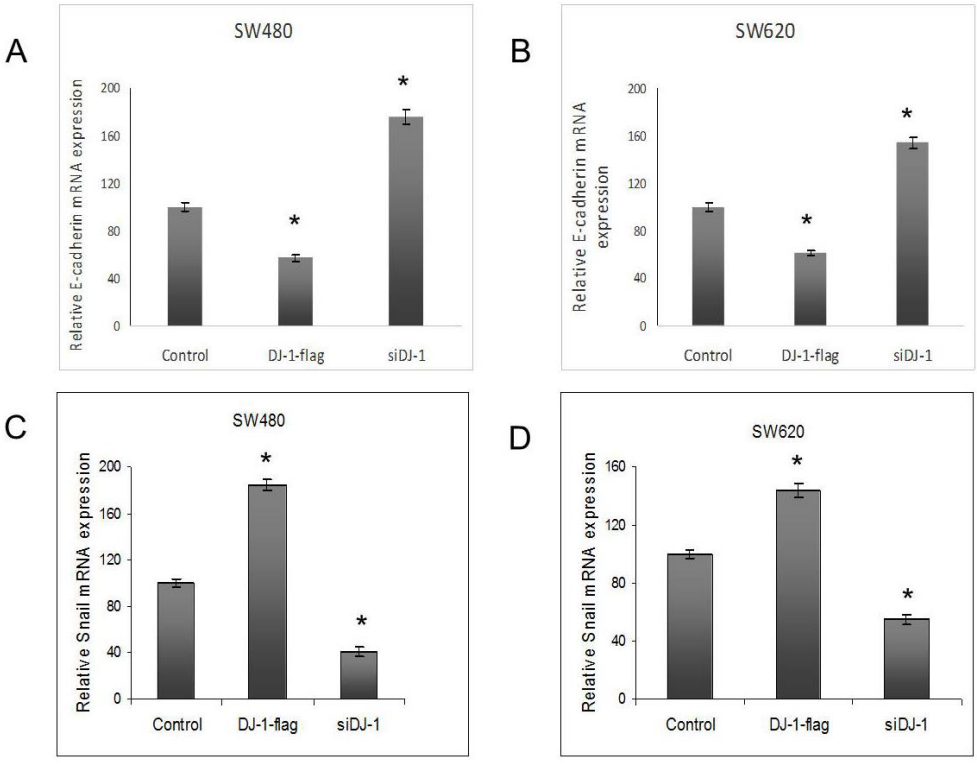

Figure 5: The impact of DJ-1 overexpression and knockdown on E-cadherin and Snail mRNA expression level in both SW480 and SW620 colon cancer cell lines. Both cells were transiently transfected for 24 hrs and then E-cadherin (A & B) and Snail (C & D) mRNA expression levels were investigated using real time PCR. Statistical analysis was performed using student t-test (*$p < 0.01$).

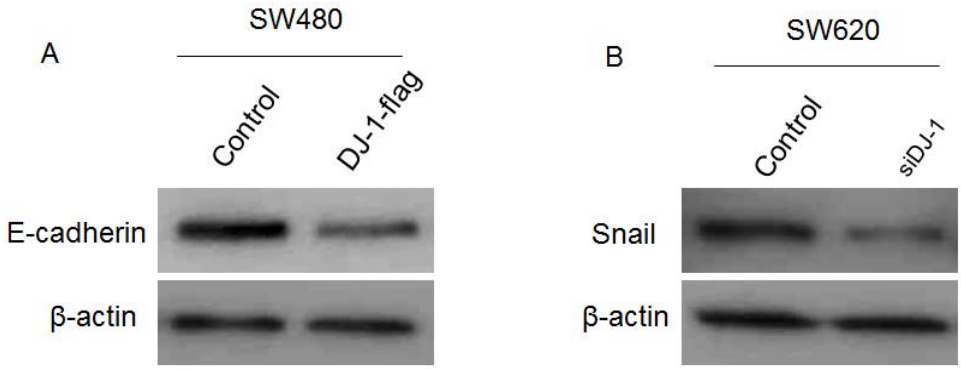

Figure 6: The action of DJ-1 overexpression on the protein level of E-cadherin in human colon cancer SW480 (A) and DJ-1 knockdown on Snail protein expression level in SW620 (B) cells. Cells were transiently transfected as shown in materials and methods and the expression of E-cadherin (A) and Snail (B) proteins were investigated using western blot analyses.

3.2. Effect of DJ-1 expression on cell proliferation

To investigate the effect of DJ-1 on colon cancer cell proliferation, both SW480 and SW620 cell lines were transiently transfected with either DJ-1 overexpression clone (DJ-1-flag) or DJ-1 siRNA (siDJ-1). The efficiency of transfection was confirmed by investigating DJ-1 mRNA expression levels in both cells using real time quantitative PCR (Figs. 1B &C). The present findings indicated that DJ-1 overexpression or knockdown showed non-significant effect on the proliferation of both SW480 and SW620 colon cancer cells (Figs. 2A & B).

3.3. Impact of DJ-1 on cell invasion and migration

The action of DJ-1 on colon cancer cell invasion and migration were investigated in non-metastatic SW480 and metastatic SW620 cell lines using cell invasion assay and wound healing assay, respectively. DJ-1 was either overexpressed or knockdown in both cells for 24 hrs, then cell invasion assay was performed for the next 24 hrs. The present findings indicated that DJ-1 overexpression markedly increased cell invasion in both SW480 (Fig. 2C) and SW620 (Fig. 2D) colon cell lines. However, DJ-1 knockdown significantly repressed cell invasion in both cells (Fig. 2C & D). Similarly, DJ-1 overexpression increased cell migration of SW480 colon cancer cells within 24 hrs (Fig. 3). On the contrary, DJ-1 silencing using DJ-1 siRNA inhibited SW620 colon cancer cell migration (Fig. 4).

3.4. Action of DJ-1 on E-cadherin and Snail expressions

The action of DJ-1 expression on the expression of epithelial marker, E-cadherin and the mesenchymal marker, Snail mRNA and protein levels were demonstrated using real time quantitative PCR and western blot analyses, respectively. Real time PCR data showed that DJ-1 overexpression repressed E-cadherin (Fig. 5A & B) with concurrent increased Snail (Fig. 5C & D) mRNA expression levels in both colon cancer cell lines. Consistently, western blot analyses indicated that E-cadherin protein expression markedly decreased by DJ-1 overexpression in SW480 (Fig. 6A). On the contrary, DJ-1 knockdown significantly enhanced the expression of E-cadherin (Fig. 5A & B) and reduced Snail (Fig. 5C & D) mRNA expression levels in both cell lines. Furthermore, DJ-1 silencing clearly inhibited Snail protein expression in SW620 cells as showed using western blot analyses (Fig. 6B).

4. DISCUSSION

According to the World Health Organization (WHO), cancer is considered the second leading cause of death worldwide. Colorectal cancer is the fourth leading cause of cancer-related deaths worldwide [27, 28]. It is stated that, in the recent decades there has been a significant increase in the incidence of colorectal cancer worldwide [27]. In this respect, colorectal cancer is considered the third most common malignancy causing death [27]. The incidence of colon cancer is the third among men and the second among women worldwide [29]. Ample data suggested DJ-1 as an oncogene, promoting cancer progression and metastasis including; breast cancer [13, 14], brain malignancies [30], metastatic uveal melanoma [19], hepatocellular carcinoma [15], pancreatic metastasis [31, 32] and lung

cancer metastasis [16-18]. Few studies were performed linking DJ-1 expression and action on colon cancer progression and metastasis. In this study, the link between DJ-1 expression and colon cancer progression, invasion and metastasis was demonstrated. Therefore, the expression of DJ-1 expression level was demonstrated in two metastatic SW620 and non-metastatic SW480 colon cancer cell lines. In the current study, DJ-1 was differentially expressed in metastatic and non-metastatic cells; DJ-1 mRNA expression level was highly expressed in metastatic SW620 cells when compared to non-metastatic SW480 cells. These data are consistent with a previously recent published study stated that DJ-1 was highly expressed in SW620 when compared to SW480 cells [21]. Next, the action of DJ-1 overexpression and knockdown on colon cancer cell proliferation, invasion and migration was evaluated. The present findings indicated that DJ-1 expression showed non-significant effect on colon cancer cell proliferation in both non-metastatic SW480 and metastatic SW620 cells. Furthermore, current data showed without doubt that, DJ-1 expression is positively correlated to the capacity of colon cancer invasion and migration. These results are consistent with recent studies found that, DJ-1 is closely correlated with the metastatic potential of colorectal cancer [21, 22], suggesting its role as a tissue marker for colorectal cancer [33]. The molecular action of DJ-1 on colon cancer metastasis regulation was demonstrated by evaluating the impact of DJ-1 expression on the expression levels of the epithelial marker; E-cadherin and mesenchymal marker; Snail at the mRNA and protein levels. Snail acts as a transcriptional inhibitor on the E-cadherin promoter [34-36]. Current data revealed that DJ-1 overexpression repressed mRNA and protein expression levels of E-cadherin in the non-metastatic colon cancer SW480 cells. However, DJ-1 knockdown reduced mRNA and protein expression levels of the metastasis-promoting marker Snail in metastatic colon cancer SW620 cells. It is reported that, DJ-1 acts through several signaling pathways to promote metastasis in several types of cancers including; activation of SRC/ERK/uPA pathway in pancreatic cancer cells [31], repressing KLF17 in breast cancer [13], regulating PTEN-AKT pathway in colorectal cancer [22], activation of AKT signaling in gastric cancer [37] and regulation of PI3K/AKT/mTOR pathway in laryngeal squamous SNU-46 cells and endometrial cancer cells [38, 39]. The action of DJ-1 and its signaling pathways involved in colon cancer metastasis regulation are poorly investigated. Therefore, further studies are yet required to uncover the full mechanism (s) of action and the signaling cascades of DJ-1 in regulating colon cancer metastasis.

The above findings suggest a crucial role of DJ-1 in colon cancer invasion and metastasis progression. Also, DJ-1 can be used as a diagnostic marker for colon cancer metastasis and DJ-1 knockdown can be useful in fighting colon cancer metastasis.

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