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Co-expression of epithelial mesenchymal transition markers NEDD9 and Twist1 in carcinoma of the uterine cervix and their clinical significances

Ola A. Megahed, Ola A. Harb, Rham Z. Ahmed, Safa A. Balata, Amr AbdAlmohsen Alnemr

ABSTRACT

Aims: Aims of our study were to detect NEDD9 and Twist1 co-expressions in carcinoma of the uterine cervix, to correlate such expressions with clinicopathological criteria of the patients and to explore their significances in response to cancer therapy, progression, recurrence and patients' survival. Methods: This is a prospective cohort study where we assessed NEDD9 and Twist1 co-expressions by immunohistochemical-technique in fifty paraffin blocks of carcinoma of the uterine cervix, we have followed our patients for three years then we detect relations between markers expressions, pathological criteria, and cancer response to therapy, progression, recurrence and patients' survival rates. Results: We detected high expression of NEDD9 in 29 out of 50 (58%) cases and high expression of Twist1 in 23 out of 50 (46%) cases of carcinoma of the uterine cervix. High-expression of both NEDD9 and Twist1 was positively correlated with carcinoma higher grade, advanced FIGO stage, presence of lupus nephritis & distant metastases, higher incidence of carcinoma progression, poor response to therapy, higher rate of recurrence, poor progression free and three year overall survival rates (p < 0.001). Conclusion: NEDD9 and Twist1 are markers of poor prognosis and can be used as therapeutic targets in carcinoma of the uterine cervix patients.

Keywords: Carcinoma of the uterine cervix, Immunohistochemistry, NEDD9 and Twist1, Prognosis

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INTRODUCTION

Carcinoma of the uterine cervix is the fourth most common cancer in females all over the world [1]. The current therapeutic modalities as radical hysterectomy and chemo-radiotherapy had promising results but this type of cancer have shown to metastasized early to lymph nodes which is believed to be a poor prognostic factor. Hence, the need for profound exploration of

the underlying mechanisms of invasion and spread of such cancer type which will aid in the revelation of effective novel therapies. The epithelial-mesenchymal-transition (EMT) is the route by which malignant cells lose their epithelial features and transform to cells with mesenchymal criteria that are able to invade the extracellular environment and metastasize [2]. The EMT plays a fundamental role in cancer progression, invasion and spread [3]. So, studying EMT detailed molecular pathogenesis that could allow its inhibition became a recent point of research for metastases and invasion prevention. NEDD9 was first detected in precursor of neuronal cells in year 1992, which resulted in down-regulation in mice central nervous system development [4]. Formerly, aberrant expression of NEDD9 protein has contributed to invasion and metastasis in plethora of cancers [5, 6]. NEDD9 was found to have many important roles in cells adhesion, motility, invasion, and EMT induced cancer spread [7, 8]. Twist1, is a basic helix-loop–helix proteins family member, it is a DNA-basic-binding domain which targets E-box sequences and a helix-loop-helix domains consensus [9]. Twist1 is very important for neural crest migration and mesoderm formation [10]; stimulation of EMT process in cancer invasion and spread [11]; and regulation of many cancer-related functions like angiogenesis and extracellular matrix degradation [12]. Twist plays a crucial role in promoting EMT. Recent findings have demonstrated that Twist overexpression plays a key role in solid cancers [13].While NEDD9 and Twist were found to have a role in metastasis in various cancers, but few studies up-to-date determined their detailed role in carcinoma of the uterine cervix and while no previous research had demonstrated the correlation of both together such type of cancer.

Aim of our research was to detect NEDD9 and Twist1 co-expressions in carcinoma of the uterine cervix, to correlate such expressions with clinicopathological criteria of the patients and to explore their significances in response to cancer therapy, progression, recurrence and patients' survival.

MATERIALS AND METHODS

This is a prospective cohort-study that was done at pathology, medical oncology and clinical oncology and nuclear medicine and gynecology departments, Zagazig University, faculty of medicine, the study design was accepting by the IRB ethical committee in faculty of medicine, Zagazig university hospitals in the period from January 2014 to January 2017.

We included 50 cases of carcinoma of the uterine cervix, in this study. Patient's demographic data as patient's age, tumor size, grade, stage and follow-up data from the archives of the shared departments, used the staging system of international-federation-of-gynecology-and-obstetrics' (FIGO) for staging of carcinoma of the uterine cervix [14]. All patients were managed according to their stage by surgery in oncology unit in department of gynecology and obstetrics, chemotherapy (platinum based chemotherapy), and radiotherapy or combined modalities.

Expressions of NEDD9 and Twist1 were evaluated in sections from all fifty paraffin blocks of carcinoma of the uterine cervix.

Immunohistochemical technique of staining

We used Streptavidin-biotin method [15], incubated slides overnight with mouse mono-clonal anti-NEDD9 (NEDD9 clone ab18056, dilution 1: 100Abcam, UK, Cambridge) and rabbit poly-clonal anti-Twist (1:50, ab50581; Abcam, UK, Cambridge).

NEDD9 positive control was pancreatic carcinoma tissue [16] and Twist1 positive control was thyroid cancer tissue [17], while negative controls was done by omission of the primary antibodies replacing them with usual saline. The slides were assessed, evaluated and scored by two pathologists.

Evaluation of NEDD9 immunohistochemical expression

Only cytoplasmic expression was considered positive for NEDD9. We evaluated both extent and intensity of stain in all slides. We graded stain-intensity as: zero (negative stain); one (weak stain); two (moderate stain); and three (strong stain). We graded stain-extent as: zero, positive cells less than 1%; (one) 2–25%; (two) 26–50%; (three) 51–75%; (four) more than 75%, then we multiplied both the intensity and extent of stain in each other to reach final staining score from 0–12 final grades we used the value of 4 as final cut-off above which is considered high expression and below which is considered low expression [17].

Evaluation of immunostaining intensity of Twist1

Only nuclear expression was considered positive for Twist1. We evaluated both extent and intensity of stain in all slides by the same way as NEDD9, but we summated the stain intensity and extent scores to reach the final staining score (zero to seven). We used the value of 3 as final cut-off above which is considered high expression and below which is considered low expression [18, 19].

Statistical analysis

SPSS 22.0 for windows (SPSS Inc., USA Chicago, IL) and MedCalc-windows (MedCalc-Software 13, Ostend, bvba Belgium) in is the program used for performing the statistics. We used Mann-Whitney-U test for comparison between two non-normally distributed variables s, used Pearson's chi-square or Fisher's-exact tests for comparison between categorical variables percent,

calculated overall-survival (OS) and progression-free-Survival (PFS) rates-stratifications according to NEDD9 and Twist1 expressions, by the method of Kaplan-Meier curve. We considered the p-value <0.05 as significant.

RESULTS

Patient characteristics

We illustrated the clinico-pathological criteria of our patients in Table 1.We included 50 patients in our study with carcinoma of the uterine cervix in our study, which were divided into 32 (64%) cases with squamous cell carcinoma and 18 (36%) cases with adenocarcinoma with age ranged from (39–72) years (mean: 55.92±8.25 years).

Interpretation of immunohistochemical results

Regarding NEDD9 expression, we detected high expression of NEDD9 in 29 out of 50 (58%) cases of carcinoma of the uterine cervix and its expression was significantly positively correlated with increased tumor size (p = 0.003), higher grade, advanced FIGO stage, and distant metastases and presence of lymphovascular invasion (p < 0.001), but we found no significant correlations between NEDD9 expression, age of the patient or histopathological subtypes of the carcinoma (Tables 2 and 3, Figures 1 and 2).

Regarding progression and survival analysis we found that cases with high NEDD9 expression were significantly positively correlated with higher incidence of carcinoma progression, poor response to therapy, higher rate of recurrence (p<0.001). Patients with high NEDD9 expression had poor progression free and three year overall survival rates (p < 0.001) (Tables 4 and 5, Figure 5).

Regarding Twist1 expression, we detected high expression of Twist1 in 23 out of 50 (46%) cases of carcinoma of the uterine cervix and its expression was significantly positively correlated with increased tumor size (p = 0.019), higher grade, advanced FIGO stage, and distant metastases and presence of lymphovascular invasion (p<0.001). We found no significant correlations between Twist1 expression, age of the patient or histopathological subtypes of the carcinoma (Tables 2 and 3, Figures 3 and 4).

Regarding progression and survival analysis we found that cases with high Twist1 expression were significantly positively correlated with higher incidence of carcinoma

Table 1: Demographic and follow-up data of our patients

Characteristics	All (N = 50)		Characteristics	All (N = 50)	
	No.	(%)		No.	(%)
Age (years)			FIGO stage		
Mean±SD	55.92	±8.15	Stage I	6	(12%)
Median (Range)	56	(39–72)	Stage II	21	(42%)
<55 years	23	(46%)	Stage III	12	(24%)
>55 years	27	(54%)	Stage IV	11	(22%)
Histopathological			Treatment		
SCC	32	(64%)	Surgery	6	(12%)
Adenocarcinoma	18	(36%)	Concurrent CRT	35	(70%)
Size			Chemotherapy alone	9	(18%)
<4 cm	6	(12%)	Response to treatment		
>4 cm	44	(88%)	CR	25	(50%)
Grade			PR	3	(6%)
Grade I	10	(20%)	SD	7	(14%)
Grade II	28	(56%)	PD	9	(18%)
Grade III	12	(24%)	N/A	6	(12%)
LVI			OAR	28	(56%)
Absent	37	(74%)	NR	16	(32%)
Present	13	(26%)	N/A	6	(12%)
Lupus nephritis			Follow-up duration (months)		
Negative	27	(54%)	Mean ± SD	28.32	±8.97
Positive	23	(46%)	Median (Range)	30	(10–36)
Distant metastasis			Events		
Negative	39	(78%)	Recurrence	15	(30%)
Positive	11	(22%)	Died	17	(34%)

Table 2: Correlation between clinicopathological criteria, NEDD9 and Twist1 expression in our patients

Characteristics	All (N = 50)		NEDD9				p-value	Twist1				p-value
			Low (N = 21)		High (N = 29)			Low (N = 27)		High (N = 23)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Age (years)												
Mean±SD	55.92	±8.15	54.80	±8.36	56.72	±8.04	0.418*	56.55	±8.44	55.17	±7.92	0.566*
Median (Range)	56	(39–72)	55	(39–72)	56	(39–72)		57	(39–72)	56	(39–70)	
<55 years	23	(46%)	11	(47.8%)	12	(52.2%)	0.441‡	12	(52.2%)	11	(47.8%)	0.811‡
>55 years	27	(54%)	10	(37%)	17	(63%)		15	(55.6%)	12	(44.4%)	
Histopathological												
SCC	32	(64%)	14	(43.8%)	18	(56.3%)	0.738‡	17	(53.1%)	15	(46.9%)	0.869‡
Adenocarcinoma	18	(36%)	7	(38.9%)	11	(61.1%)		10	(55.6%)	8	(44.4%)	
Size												
<4 cm	6	(12%)	6	(100%)	0	(0%)	0.003‡	6	(100%)	0	(0%)	0.025‡
>4 cm	44	(88%)	15	(34.1%)	29	(65.9%)		21	(47.7%)	23	(52.3%)	
Grade												
Grade I	10	(20%)	10	(100%)	0	(0%)	<0.001§	10	(100%)	0	(0%)	<0.001§
Grade II	28	(56%)	11	(39.3%)	17	(60.7%)		15	(53.6%)	13	(46.4%)	
Grade III	12	(24%)	0	(0%)	12	(100%)		2	(16.7%)	10	(83.3%)	
LVI												
Absent	37	(74%)	21	(56.8%)	16	(43.2%)	<0.001‡	26	(70.3%)	11	(29.7%)	<0.001‡
Present	13	(26%)	0	(0%)	13	(100%)		1	(7.7%)	12	(92.3%)	
Lupus nephritis												
Negative	27	(54%)	20	(74.1%)	7	(25.9%)	<0.001‡	24	(88.9%)	3	(11.1%)	<0.001‡
Positive	23	(46%)	1	(4.3%)	22	(95.7%)		3	(13%)	20	(87%)	
Distant metastasis												
Negative	39	(78%)	21	(53.8%)	18	(46.2%)	0.001‡	27	(69.2%)	12	(30.8%)	<0.001‡
Positive	11	(22%)	0	(0%)	11	(100%)		0	(0%)	11	(100%)	
FIGO stage												
Stage I	6	(12%)	6	(100%)	0	(0%)	<0.001§	6	(100%)	0	(0%)	<0.001§
Stage II	21	(42%)	14	(66.7%)	7	(33.3%)		18	(85.7%)	3	(14.3%)	
Stage III	12	(24%)	1	(8.3%)	11	(91.7%)		3	(25%)	9	(75%)	
Stage IV	11	(22%)	0	(0%)	11	(100%)		0	(0%)	11	(100%)	
NEDD9												
Low	21	(42%)						20	(95.2%)	1	(4.8%)	<0.001‡
High	29	(58%)						7	(24.1%)	22	(75.9%)	
Twist												
Low	27	(54%)	20	(74.1%)	7	(25.9%)	<0.001‡					
High	23	(46%)	1	(4.3%)	22	(95.7%)						

* Independent samples Student's t-test
‡ Chi-square test
§ Chi-square test for trend
p<0.05 is significant

Table 3: Correlation between clinicopathological features and expression of both NEDD9 and Twist1 together in our patients

Characteristics	All (N = 50)		NEDD9/Twist1						p-value
			Both High (N = 21)		One of them High (N = 9)		Both Low (N = 20)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Age (years)									
Mean ± SD	55.92	±8.15	55.04	±8.22	61	±5.56	54.55	±8.49	0.116*
Median (Range)	56	(39–72)	56	(39–70)	60	(53–72)	55	(72–33)	
<55 years	23	(46%)	10	(43.5%)	2	(8.7%)	11	(47.8%)	0.256‡
>55 years	27	(54%)	11	(40.7%)	7	(25.9%)	9	(33.3%)	
Histopathological									
SCC	32	(64%)	13	(40.6%)	6	(18.8%)	13	(40.6%)	0.962‡
Adenocarcinoma	18	(36%)	8	(44.4%)	3	(16.7%)	7	(38.9%)	
Size									
<4 cm	6	(12%)	0	(0%)	0	(0%)	6	(100%)	0.006‡
>4 cm	44	(88%)	21	(47.7%)	9	(20.5%)	14	(31.8%)	
Grade									
Grade I	10	(20%)	0	(0%)	0	(0%)	10	(100%)	<0.001§
Grade II	28	(56%)	12	(42.9%)	6	(21.4%)	10	(35.7%)	
Grade III	12	(24%)	9	(75%)	3	(25%)	0	(0%)	
LVI									
Absent	37	(74%)	10	(27%)	7	(18.9%)	20	(54.1%)	0.001‡
Present	13	(26%)	11	(84.6%)	2	(15.4%)	0	(0%)	
Lupus nephritis									
Negative	27	(54%)	3	(11.1%)	4	(14.8%)	20	(74.1%)	<0.001‡
Positive	23	(46%)	18	(78.3%)	5	(21.7%)	0	(0%)	
Distant metastasis									
Negative	39	(78%)	11	(28.2%)	8	(20.5%)	20	(51.3%)	0.001‡
Positive	11	(22%)	10	(90.9%)	1	(9.1%)	0	(0%)	
FIGO stage									
Stage I	6	(12%)	0	(0%)	0	(0%)	6	(100%)	<0.001§
Stage II	21	(42%)	3	(14.3%)	4	(19%)	14	(66.7%)	
Stage III	12	(24%)	8	(66.7%)	4	(33.3%)	0	(0%)	
Stage IV	11	(22%)	10	(90.9%)	1	(9.1%)	0	(0%)	

*One Way ANOVA test; ‡Chi-square test; §Chi-square test for trend; p<0.05 is significant.

progression, poor response to therapy, higher rate of recurrence ($p < 0.001$). Patients with high Twist1 expression had poor progression free and three year overall survival rates ($p < 0.001$). We found a significant correlations between NEDD9 and Twist1 expressions in carcinoma of the uterine cervix ($p < 0.001$) (Tables 4 and 5, Figure 5).

DISCUSSION

Although there is marked development in screening, diagnostic and therapeutic modalities carcinoma of the uterine cervix remains the fourth cause of malignancy-related fatality in females globally [1]. We detected and compared the NEDD9 expression

Table 4: Correlation between NEDD9 & Twist1 expression and outcome of our patients

Outcome	All (N = 50)		NEDD9/Twist1						p-value
			Both High (N = 21)		One of them High (N = 9)		Both Low (N = 20)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Treatment									
Surgery	6	(12%)	0	(0%)	0	(0%)	6	(30%)	<0.001‡
Concurrent CRT	35	(70%)	12	(57.1%)	9	(100%)	14	(70%)	
Chemotherapy alone	9	(18%)	9	(42.9%)	0	(0%)	0	(0%)	
Response to treatment									
CR	25	(50%)	4	(19%)	7	(77.8%)	14	(70%)	<0.001‡
PR	3	(6%)	2	(9.5%)	1	(11.1%)	0	(0%)	
SD	7	(14%)	6	(28.6%)	1	(11.1%)	0	(0%)	
PD	9	(18%)	9	(42.9%)	0	(0%)	0	(0%)	
N/A	6	(12%)	0	(0%)	0	(0%)	6	(30%)	
OAR	28	(56%)	6	(28.6%)	8	(88.9%)	14	(70%)	<0.001‡
NR	16	(32%)	15	(71.4%)	1	(11.1%)	0	(0%)	
N/A	6	(12%)	0	(0%)	0	(0%)	6	(30%)	
Recurrence									
Absent	16	(32%)	0	(0%)	0	(0%)	16	(80%)	<0.001‡
Present	15	(30%)	4	(19%)	7	(77.8%)	4	(20%)	
N/A	19	(38%)	17	(81%)	2	(22.2%)	0	(0%)	
Disease free survival									
Mean (month) (95%CI)	30.42 month (28.02–32.81)		22.25 month (17.04 – 27.46)		22.57 month (18.82–26.32)		34.80 month (33.75–35.85)		<0.001†
12 month DFS (%)	100%		100%		100%		100%		
24 month DFS (%)	77.4%		50%		57.1%		100%		
36 month DFS (%)	51.6%		----		----		80%		
Mortality									
Absent	33	(66%)	7	(33.3%)	6	(66.7%)	20	(100%)	<0.001‡
Present	17	(34%)	14	(66.7%)	3	(33.3%)	0	(0%)	
Overall survival									
Mean (month) (95%CI)	30.06 month (27.43–32.68)		23.47 month (18.83–28.11)		31.11 month (27.30–34.92)		36 month		<0.001†
12 month OS (%)	88%		71.4%		100%		100%		
24 month OS (%)	76%		52.4%		77.8%		100%		
36 month OS (%)	64.2%		23.9%		64.8%		100%		

‡Chi-square test; †Log rank test; p< 0.05 is significant.

by immunohistochemical analysis in 50 cases of carcinoma of the uterine cervix, our results revealed that NEDD9 overexpression was correlated with bad clinicopathological parameters like higher incidence of lymph node and distant metastasis, higher grade and advanced FIGO stage of cervical carcinoma patients. We also demonstrated that cases with high NEDD9 expression would have higher incidence of carcinoma progression, poor response to therapy, higher rate of recurrence. Our results were similar to those reported by Sima et al., in cervical cancer [20], Li et al., in colon cancer [21], Zhang et al., in bladder cancer [22], Kim et al. [23] and Lucas et al. [24] in HNSCC and melanoma respectively, which were in line with our results that NEDD9 overexpression may have a role in carcinoma of the uterine cervix progression, invasion and spread. We also found that the carcinoma of the uterine cervix patients with a NEDD9 overexpression was associated with a

Table 5: correlation between expression of both NEDD9 and Twist1 together and outcome of our patients

Outcome	All (N = 50)		NEDD9				p-value	Twist1				p-value
			Low (N = 21)		High (N = 29)			Low (N = 27)		High (N = 23)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Treatment												
Surgery	6	(12%)	6	(28.6%)	0	(0%)	0.001‡	6	(22.2%)	0	(0%)	<0.001‡
Concurrent CRT	35	(70%)	15	(71.4%)	20	(69%)		21	(77.8%)	14	(60.9%)	
Chemotherapy alone	9	(18%)	0	(0%)	9	(31%)		0	(0%)	9	(39.1%)	
Response to treatment												
CR	25	(50%)	15	(71.4%)	10	(34.5%)	<0.001‡	20	(74.1%)	5	(21.7%)	<0.001‡
PR	3	(6%)	0	(0%)	3	(10.3%)		0	(0%)	3	(13%)	
SD	7	(14%)	0	(0%)	7	(24.1%)		1	(3.7%)	6	(26.1%)	
PD	9	(18%)	0	(0%)	9	(31%)		0	(0%)	9	(39.1%)	
N/A	6	(12%)	6	(28.6%)	0	(0%)		6	(22.2%)	0	(0%)	
OAR	28	(56%)	15	(71.4%)	13	(44.8%)	<0.001‡	20	(74.1%)	8	(34.8%)	<0.001‡
NR	16	(32%)	0	(0%)	16	(55.2%)		1	(3.7%)	15	(65.2%)	
N/A	6	(12%)	6	(28.6%)	0	(0%)		6	(22.2%)	0	(0%)	
Recurrence												
Absent	16	(32%)	16	(76.2%)	0	(0%)	<0.001‡	16	(59.3%)	0	(0%)	<0.001‡
Present	15	(30%)	5	(23.8%)	10	(34.5%)		10	(37%)	5	(21.7%)	
N/A	19	(38%)	0	(0%)	19	(65.5%)		1	(3.7%)	18	(78.3%)	
Disease free survival												
Mean (month) (95%CI)	30.42 month (28.02–32.81)		34.48 month (30.30–35.65)		21.90 month (18.94–24.86)		<0.001†	31.77 month (29.35–34.19)		23.40 month (18.78–28.02)		<0.001†
12 month DFS (%)	100%		100%		100%			100%		100%		
24 month DFS (%)	77.4%		100%		30%			80.8%		60%		
36 month DFS (%)	51.6%		76.2%		----			61.5%		----		
Mortality												
Absent	33	(66%)	21	(100%)	12	(41.4%)	<0.001‡	24	(88.9%)	9	(39.1%)	<0.001‡
Present	17	(34%)	0	(0%)	17	(58.6%)		3	(11.1%)	14	(60.9%)	
Overall survival												
Mean (month) (95%CI)	30.06 month (27.43–32.68)		36 month		25.54 month (21.76–29.33)		<0.001†	34.62 month (33.07–36.16)		24.59 month (20.11–29.08)		<0.001†
12 month OS (%)	88%		100%		79.3%			100%		73.9%		
24 month OS (%)	76%		100%		58.6%			92.6%		56.5%		
36 month OS (%)	64.2%		100%		31.8%			88.7%		31.4%		

‡Chi-square test; †Log rank test; p< 0.05 is significant.

shorter overall survival rate, and our results were in line with Peng Li et al. [21] in colon cancer patient, Our results were explained by that NEDD9 could regulate TGF-β and and Wnt-signaling pathways which played many roles in invasion and spread of cancers of several organs [18, 25]. The invasion and metastasis is of tumors a complex continuous process and NEDD9 may act through focal adhesion kinase (FAK) phosphorylation and that their interaction is essential for cancer cell movement, invasion and spread [26]. NEDD9 is a down-stream molecule of FAK which stimulated cancer movement and promoted cancer cells invasion and spread [23]. Sima et al. found

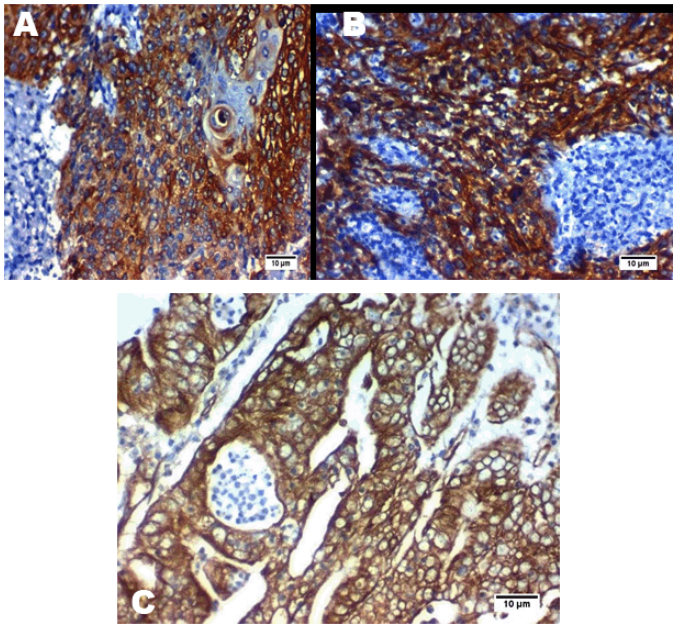

Figure 1: Immunohistochemical expression of NEDD9 in carcinoma of the uterine cervix (A) High expression in the cytoplasm of poorly differentiated squamous cell carcinoma (magnification: x400), (B) High expression in the cytoplasm of poorly differentiated adenocarcinoma (magnification: x400), and (C) High expression in the cytoplasm of moderately differentiated adenocarcinoma (magnification: x400).

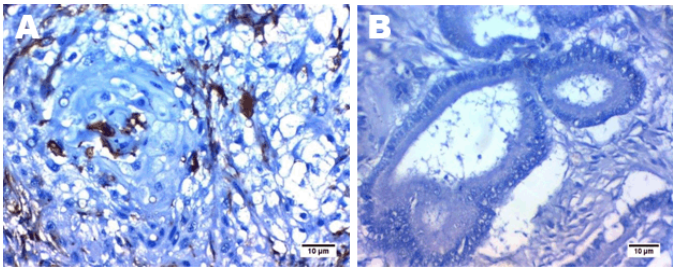

Figure 2: Immunohistochemical expression of NEDD9 in carcinoma of the uterine cervix (A) Low expression in the cytoplasm of well differentiated squamous cell carcinoma stage II (magnification: x400), (B) Negative expression in the cytoplasm of well differentiated adenocarcinoma stage II (magnification: x400).

overexpressed NEDD9 stimulates cervical cancer cells motility and invasion by a positive feedback mechanism of tyrosine phosphorylation between FAK and NEDD9 that was in agree with our results [20].

In addition to previous explanation of the role of NEDD9 in cervical cancer progression it also has a fundamental role in the EMT [7], as it increased the removal of E-cadherin from cell junctions leading to its lysosomal degradation [27].

Peng Li et al. also proved in colorectal cancer that NEDD9 has a role in phosphorylation FAK- tyrosine and also in EMT regulation [21]. The prognostic role of NEDD9 had been assessed and its over expression has shown to be promising for lung cancer prognosis [6].

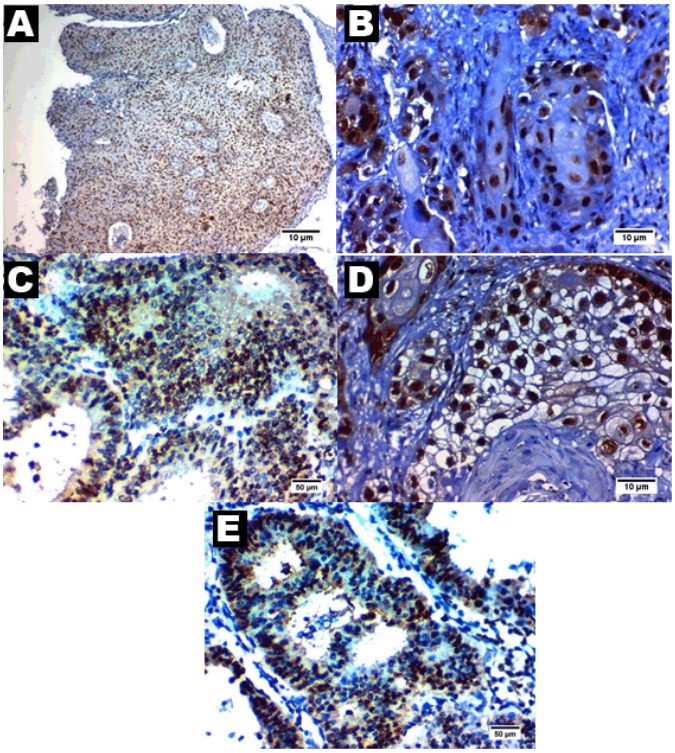

Figure 3: Immunohistochemical expression of Twist1 in carcinoma of the uterine cervix (A) High expression in nucleus of poorly differentiated squamous cell carcinoma x100, (B) High expression in nucleus of poorly differentiated squamous cell carcinoma (magnification: x400), (C) High expression in nucleus of poorly differentiated adenocarcinoma (magnification: x400), (D) High expression in nucleus of moderately differentiated squamous cell carcinoma (magnification: x400), and (E) High expression in nucleus of moderately differentiated adenocarcinoma (magnification: x400).

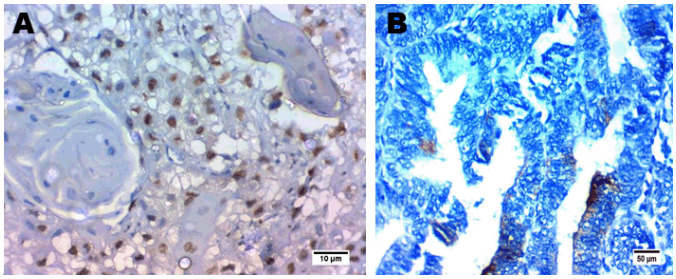

Figure 4: Immunohistochemical expression of Twist1 in carcinoma of the uterine cervix (A) Low expression in nucleus of well differentiated squamous cell carcinoma (magnification: x400), and (B) Negative expression in nucleus of well differentiated adenocarcinoma (magnification: x400).

As vimentin and E-cadherin are essential EMT markers Tikhmyanova et al., proved that down regulation of NEDD9 could reduce vimentin and increased E-cadherin expressions and also NEDD9 overexpression could down-regulate E-cadherin and upregulat vimentin expressions [27] which were in line with our results that NEDD9 might have a molecular role in cancer cervix progression and metastasis.

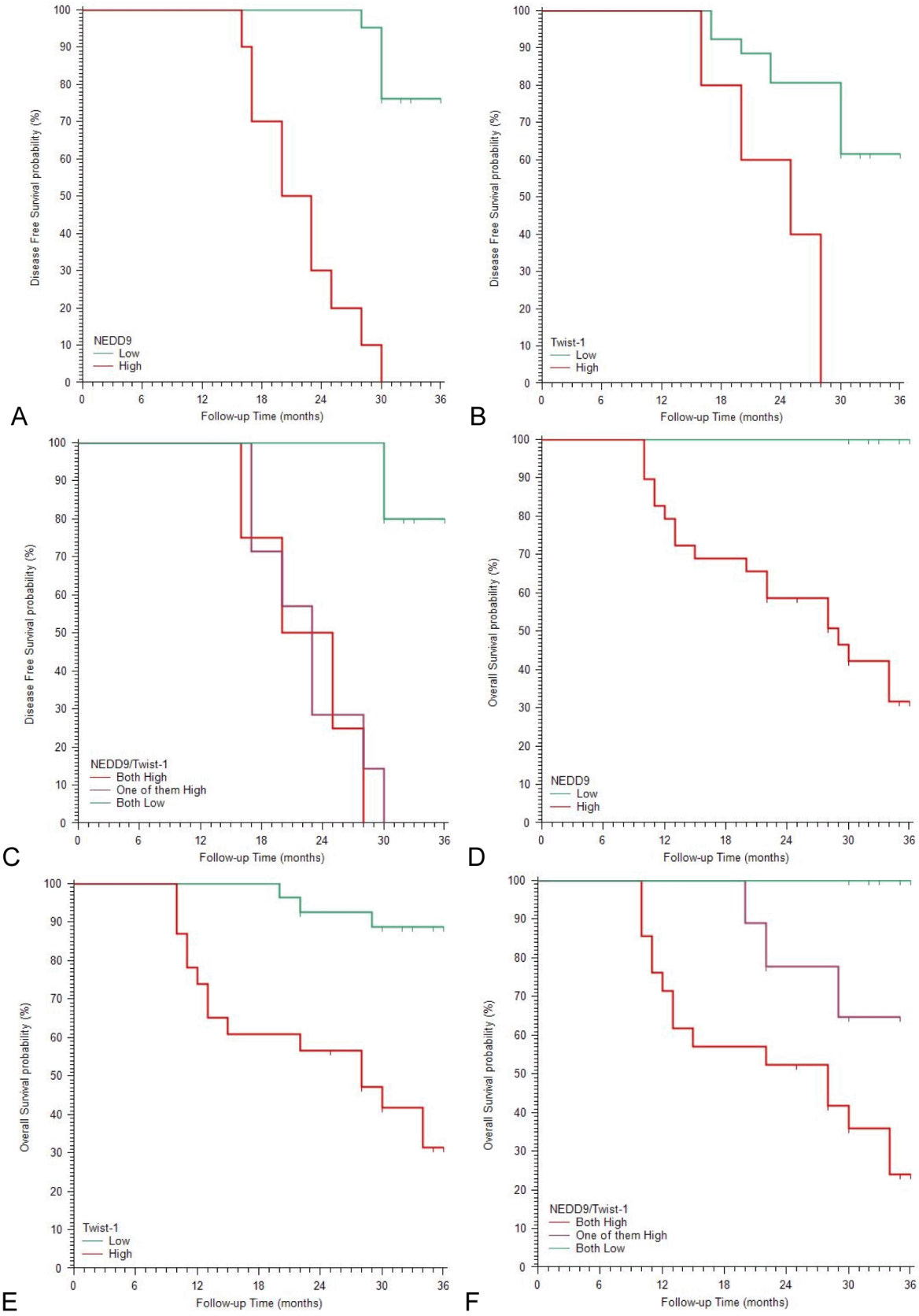

Figure 5: Kaplan Meier Survival plots; (A–C): Disease Free Survival; (D–F): Overall Survival; (A, D) Stratified by NEDD9 IHC staining; (B, E)-Stratified by Twist1 IHC staining; (C, F) Stratified by NEDD9/Twist1 IHC staining.

In summary, NEDD9 is overexpressed in carcinoma of the uterine cervix cells. And its overexpression could stimulate cancer cells migration, invasion and metastases by either controlling FAK tyrosine phosphorylation or the EMT-related protein E-cadherin and vimentin expression. These results did not provide a mechanism for migration, invasion and metastases for NEDD9mediated carcinogenesis only but it could also provide a recent therapeutic target for cervical cancer management. In our study, we confirmed that Twist1 over expression is positively correlated with higher grade and advanced FIGO stage, increasing lymph nodes and distant metastases of carcinoma of the uterine cervix. Moreover, patients with Twist1 would have higher incidence of carcinoma progression, poor response to therapy and higher rate of recurrence.

These results have proved that Twist1 could be a prognostic, predictive and therapeutic target for carcinoma of the uterine cervix, which was in line with Fan et al., who demonstrated similar results in cervical malignancy [28], which explained that Twist1 had mediated cervical carcinogenesis and progression by up-regulating the TGF-β/Smad3 signaling pathways. Results of Shibata et al., have also demonstrated that Twist1 over expression in cervical cancer was associated with increased rate of cancer progression [29].

Also Kyo et al. achieved similar results in endometrial cancer revealing that Twist1 overexpression was significantly associated with deep myometrial invasion, increased lymph node metastasis and worsened patient survival [30].

Similar to the results obtained in our study, Wushou et al. concluded in their meta-analysis of Twist expression in carcinoma that it was related to poor patients' prognosis, worsened survival rates and that Twist1 could be a recent prognostic marker for most types of carcinomas [31]. These results were explained by that Twist1 over expression had an essential role in EMT by making an aberrant expression of E-cadherin [19], that could facilitate cancer growth, while its low expression could suppress cervical cancer occurrence, growth invasion and spread. Another explanation suggests that the transforming growth factor-β (TGF-β) plays a major role in EMT stimulation during carcinogenesis [32], in advanced stages of cancer cervix, TGF-β1 extracellular levels were increased [33], which was indicated in Fan et al., study [28], found that TGF-β stimulated the EMT by Twist1 up regulation by the TGF-β/Smad3 responses. Moreover, the TGF-β induced EMT could be inhibited by Twist and hence proved the EMT influencing role on Twist1 by TGF-β/Smad3 signaling regulation. Shibata et al., achieved different results from ours regarding clinicopathological correlations and comparable results regarding patient survival who studied 101 cervical cancer patients. Twist1 expression levels were not correlated with any of the clinicopathological parameters, also its expression levels were positively related to occurrence of with lymph node metastasis, but that result was not statistically significant. Regarding survival of patient they found similar to us that Twist1 over-expression was correlated to worsened overall survival of patients [29], these differences in the significance between clinicopathological parameters and Twist1 expression in Shibata et al. results [29], may be due to different number of cases or different clone used which may decrease level of significant results. Previous research results have demonstrated Twist1 role as a vital regulator of cancer metastatic process [34–36], and proved the correlations between Twist1 expression levels and clinicopathological parameters in patients with cancer [37–39]. There are many explanations for such association of Twist1 over-expression and poor prognosis of various cancers. One explanation may be an increased cancer metastatic liability by EMT stimulation, also a novel role of Twist1 had been described in the development of chemoresistance that was acquired in many cancer cells as Twist1 overexpression was related to cancer cell resistance to microtube-targeting chemotherapeutics in many cancer types [40], by inhibition of a chemotherapeutics-induced apoptosis by stimulation of Akt-dependent anti-apoptotic pathway [41]. In addition, Stasinopoulos et al. has shown that Twist1 expression levels affected p53-regulated genes expression in cells of breast cancer, so controlling their response to radiotherapy [42]. In our study, we have proved that Twist1 expression levels correlated with poor response to chemoradiotherapy in our patients who needed postoperative adjuvant therapy during their management, so Twist1 expression levels are involved in patient prognosis. Moreover, Twist1 and related signaling pathways played essential roles in cancer progression and could be used as therapeutic target for cancer management [43]. Several studies had correlated Twist1 expression levels and patient survival rates in carcinomas of many organs [44–46] and similar results were obtained regarding Twist1 overexpression positive correlation with worse patient's survival and poor outcome.

We found a significant correlation between NEDD9 and Twist1 expression in carcinoma of the uterine cervix and both of them was associated with poor clinicopathological criteria and poor prognosis that was similar to results of Yang et al. [47] that found that activation of the axis of Twist1_let-7i_NEDD9 in patients' with head and neck cancer was associated with cancer invasiveness and worse outcome and that Twist1_let-7i_NEDD9 axis found in many different cancer types. So our research clarified role of Twist1 and NEDD9-induced cancer cell motility and spread which is essential for understanding the EMT-induced migratory potential of cancer stem cells that could help to detect a suitable target for blocking of Twist1 and NEDD9 induced metastasis by RAC1 activity inhibition.

CONCLUSION

In our study, we found highly significant correlations between NEDD9 and Twist1 expression levels in carcinoma of the uterine cervix and both markers expression was positively related to poor patient outcome suggesting that both of them could be used not only prognostic markers but also novel therapeutic targets in cervical cancer.

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Author Contributions

Ola A. Megahed – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Ola A. Harb – Substantial contributions to conception and design, Acquisition of data, Drafting the articles, Revising it critically for important intellectual content, Final approval of the version to be published

Rham Z. Ahmed – Substantial contributions to conception and design, Acquisition of data, Drafting the articles, Revising it critically for important intellectual content, Final approval of the version to be published

Safa A. Balata – Substantial contributions to conception and design, Acquisition of data, Drafting the articles, Revising it critically for important intellectual content; Final approval of the version to be published

Substantial contributions to conception and design, Acquisition of data, Drafting the articles, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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