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RESEARCH ARTICLE

RRBP1 Expression in wild and mutated p53 immunophenotype endometrioid endometrial carcinoma: Relation to ER-α and clinicopathologic factors

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ABSTRACT

Background: Endometrial carcinoma (EC) is the most common gynecologic tumor in the developed world and ranks second in Egypt. Ribosome-binding protein 1 (RRBP1) is a membrane protein of rough endoplasmic reticulum essential for stabilization of endoplasmic reticulum. Overexpression of RRBP1 was detected in several malignant tumors. p53 is a tumor suppressor transcription factor encoded by TP53. The mutation of TP53 is considered the most common significant molecular alteration in half of human cancers. **Aim**: This study aimed to explore the immunohistochemical expression of RRBP1 in wild and mutated p53 immunophenotype endometrioid EC in relation to estrogen receptor alpha (ER-α) status and clinicopathological factors. **Materials and Methods:** Fifty-six endometrioid EC paraffin blocks were collected. Sections were stained with anti-RRBP1, anti-p53, and anti-ER-α antibodies. **Results:** RRBP1 overexpression was significantly related to high tumor grade, presence of lymphovascular invasion (LVI), advanced TNM staging, and negative ER-α. Mutated-type p53 expression was associated with high grade, LVI, advanced TNM staging, and negative ER-α. A Significant difference in p53 expression patterns was detected in relation to clinicopathological prognostic factors. Studied tumors with positive ER-α nuclear expression significantly showed wild-type p53 expression patterns more frequently compared to EEC with negative ER-α expression. Furthermore, a positive correlation was detected between RRBP1 overexpression and mutated-type p53. **Conclusion**: RRBP1 is considered a bad prognostic indicator in EEC and together with mutated p53 expression could be beneficial as a potential therapeutic target for EC.

Keywords: Endometrioid endometrial carcinoma; ER-α; immunohistochemistry; p53; RRBP1.

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INTRODUCTION

Endometrial carcinoma (EC) is the most common invasive female genital tract tumor in the developed world (Siegel et al., 2021). The annual diagnosis shows an increased number of new cases, as the estimated number of EC cases in 2020 was 417 367 (Ferlay et al., 2019). In Egypt, EC is the second most common gynecologic tumor (after ovarian) with an incidence rate of 3.9%. The number of Egyptian EC cases recorded in 2020 was 1694 and the number of deaths was 350 (21%) (Sung et al., 2021). The Incidence of EC is rising as a result of the growing expectancy of life, the prevalence of obesity, and other metabolic disorders such as diabetes mellitus. Furthermore, a surge in the mortality rate of ECs has been observed over the past few years (Yang and Wang, 2019).

Endometrial carcinoma is classified into two distinct types: I and II with different genetic pathways and prognoses. Type I is the most common subtype constituting more than 80% of EC (Felix et al., 2010). Although most type I EC cases are low-grade well-differentiated endometrioid carcinoma, a considerable percentage of type I tumors have high grades

with adverse outcomes (Singh et al., 2019; Koskas et al., 2021).

The recognition of biomarkers that are involved in EC development and progression is crucial for evaluating the prognosis, especially for patients with advanced or high-grade tumors and for developing innovative therapies that target the molecular pathways, hence preventing tumor progression and metastasis and increasing the overall survival (Kolehmainen et al., 2020; Jamieson and Bosse, 2021).

Ribosome-binding protein 1 (RRBP1) is a membrane protein of the rough endoplasmic reticulum, it is pivotal for the secretion and intracellular transport of proteins. RRBP1 stabilizes the endoplasmic reticulum preventing the accumulation of unfolded proteins in the endoplasmic reticulum, which would otherwise cause endoplasmic reticulum stress (ERS), through unfolded protein response (UPR) (Tsai et al., 2013). UPR is an adaptive response that aims to increase the expression and function of endoplasmic reticulum companions to raise endoplasmic reticulum-folding capacity and eliminate ERS preventing cell death and apoptosis (Kaufman et al., 2002). RRBP1 overexpression has been detected in the lung (Tsai et al., 2013), colorectal (Pan et al., 2015), esophagus (Wang et al., 2018), breast (Liang et al., 2015), and EC (Liu et al., 2019). It is associated with enhancement of the growth and invasion of these tumors and can predict the prognosis.

The p53 protein is a tumor suppressor transcription factor encoded by TP53. It controls the cell cycle through G_1/S phase arrest if there is damaged DNA and directs the cell to repair mechanisms. If the DNA damage is too severe to be repaired, p53 stimulates the transcription of apoptotic genes (Vousden and Lu, 2002; Reinhardt and Schumacher, 2012). Mutations of TP53 are largely missense mutations, that lead to p53 nuclear protein overexpression, and to some extents are splice site mutations leading to cytoplasmic staining, while deletion mutations with complete absence of p53 are rare (Köbel et al., 2019). The missense (point) mutations may not only cause loss of wild-type p53 function but may also acquire new activities that alter the cellular homeostasis, this is called the gain of function (GOF) (Liu et al., 2014; Schulz-Heddergott and Moll, 2018; Alvarado-Ortiz et al., 2021).

The immunohistochemical expression pattern of p53 protein has been used as a substitute for molecular testing for detecting TP53 mutation since the sequencing of TP53 is not available to most laboratories (Singh et al., 2020). Since detecting TP53 mutation is not only important for diagnostic and prognostic purposes being used in distinguishing between type I and type II EC and in molecular subtyping of EC (Nakamura et al., 2019), but also it is of great value in planning the treatment options and predicting response to anticancer therapy as TP53 mutation and loss of normal wild p53 function may result in chemoresistance (Bykov et al., 2018).

During the process of EC development, the level of estrogen receptor alpha (ER-α) expression becomes lower when compared to that of normal endometrium and endometrial hyperplasia (Li et al., 1996). ER-α expression in EC has been associated with high tumor grade, poor response to hormonal therapy, and distant metastasis possibilities (Gul et al., 2010; Bartosch et al., 2015). To our knowledge, the RRBP1 expression in different p53 immunophenotype expression pattern in EC and its relation to ER-α expression has not been previously investigated. Thus, this work aims to study the immunohistochemical expression of RRBP1 in wild and mutated p53 immunophenotype endometrioid EC (EEC) in relation to ER-α status and clinicopathological factors.

MATERIAL AND METHODS

Clinicopathological data

After the research approval by the Ethical Committee of the Faculty of Medicine, Tanta University, (approval code: 34376/1/21), fifty-six paraffin blocks of selected primary EEC were collected from the archives of the pathology departments of Tanta and Aswan Faculties of medicine. Clinical data including the patient's age, the status of metastasis, preoperative treatment, and primary or recurrent tumor were obtained from the pathology record files.

Inclusion criteria were: cases with primary EEC underwent total hysterectomy with pelvic lymphadenectomy; did not receive neoadjuvant chemotherapy; had complete clinical data. Exclusion criteria were: patients with incomplete clinical data; non-representative tumor tissues; extensive or total tumor necrosis; recurrent tumors. All cases were anonymous and handled according to ethical standards.

Histopathological evaluation

Sections stained with H&E were prepared to confirm the diagnosis, assess tumor grade, and presence of tumor necrosis (recognized as an area of necrotic tumor cells adjacent to viable tumor tissue) as it has been associated with increased cellular proliferation and advanced TNM staging (Bredholt et al., 2015). The depth of myometrial invasion, lymphovascular invasion, cervical involvement, and pelvic lymph node infiltration was also evaluated. Tumors were graded according to the binary grading system as recommended by WHO. Grade 1 and 2 tumors were categorized as low-grade EEC and grade 3 tumors as high-grade (Soslow et al., 2019). EEC cases were staged according to the TNM staging system (Brierley et al., 2017).

Immunohistochemical staining

Sections of 5-µm thickness were cut from the paraffin-embedded tissue blocks followed by deparaffinization with xylene, and rehydration using descending grades of ethanol. Incubation with sodium citrate buffer (pH 6.0) was done for retrieving antigen. Sections prepared from each case were incubated overnight at 4°C with the following antibodies: anti-RRBP1 polyclonal antibody (1:100; ABclonal, USA), anti-p53 polyclonal antibody (1:100; ABclonal, USA) and anti-ER-α polyclonal antibody (1:150; ABclonal, USA). The slides were then counter-stained with hematoxylin. The immunohistochemical stained slides were examined by two pathologists blinded to patients' clinicopathological characteristics.

Evaluation of immunohistochemical staining

RRBP1: Tumor cells that showed cytoplasmic expression were regarded as positive cells. The percentage of positive cells was determined as follows: 1 (<25%), 2 (25%–50%), 3 (>50%). Staining intensity was divided into negative (0), weak (1), moderate (2), and strong (3). The total score was obtained by multiplying the number of positive cells and staining intensity. RRBP1 expression was graded as low-expression (<4) and high-expression (≥4). Prostatic carcinoma was used as an external positive control (Li et al., 2019).

p53: Immunostaining pattern of p53 was classified into two groups: wild-type p53 pattern which showed scattered nuclear staining in 1-80% of tumor cells with staining intensity compared with that of stromal cells (fibroblasts and lymphocytes) which were used as an internal positive control. Wild-type staining pattern is characterized by an admixture of staining intensities which varies from weak or moderate in most tumor cell nuclei to strong staining in a few nuclei. The mutated p53 pattern showed nuclear overexpression (strong nuclear staining in 80-100% of tumor cells) or cytoplasmic expression (Köbel et al., 2019).

ER-α: The percentage of ER-α positive cells (nuclear expression) was graded as follows: 1 (≤ 25%), 2 (26–75%), 3 (>75%). The staining intensity was scored as 1: absent or weak, 2: moderate and 3: strong. The total score was obtained by adding percentage and intensity. Tumors were categorized as negative if the total score was 2 or positive if the total score was 3-6 (Chambers et al., 1990).

Statistical Analysis

Statistical analysis was conducted using SPSS (version 20) (Chicago, IL, USA). Median was calculated for patients' age. The relation between biomarkers' expression and clinicopathological factors was analyzed using Chi-square (x^2) test and post hoc analysis. Spearman correlation (r) was performed to assess the correlation between RRBP1 and p53-immunophenotype expression pattern. The results were considered statistically significant if the p value was < 0.05.

RESULTS

Clinicopathological characteristics of studied cases

Among 56 studied cases of primary EEC, 12 cases (21.4%) were < 60-year-old and 44 cases

(78.6%) were ≥ 60 years, the age of the patients ranged from 52-78 years with a median age of 69. Thirty-seven EEC (66.1%) were of low-grade and 19 EEC (33.9%) were of high-grade.

Necrosis was appreciated in 26 tumors (46.4%). Lymphovascular invasion (LVI) was found in 16 tumors (28.6%). Twenty-five tumors (44.7%) were of T_1 stage, 16 cases (28.6%) with T_1 tumors showed less than half of myometrial thickness invasion (T_{1a}) and 9 tumors (16.1%) invaded ≥ half of the myometrial thickness (T_{1b}), 24 tumors (42.8%) showed cervical involvement (T_2) and 7 tumors (12.5%) invaded the serosa and/or adnexa (T_{3a}). Metastasis to pelvic lymph nodes (N_1) was identified in 23 cases (41.1%) and distant metastasis in 14 cases (25%). Positive nuclear ER-*α* expression (Figure 1) was detected in 38 tumors (67.9%) (Table 1).

Table 1: Clinicopathologic characteristics of studied cases

Clinicopathologic Characteristics	Number (%)
Age	
Median	69
< 60	12(21.4)
≥ 60	44(78.6)
Tumor grade	
Low grade	37(66.1)
High grade	19(33.9)
Tumor necrosis	
Absent	30(53.6)
Present	26(46.4)
LVI	
Absent	40(71.4)
Present	16(28.6)
T stage	
T_1	
T_{1a}	16(28.6)
T_{1b}	9(16.1)
T_2	24(42.8)
T_{3a}	7(12.5)
N stage	
N_0	33(58.9)
N_1	23(41.1)
M stage	
M_0	42(75)
M_1	14(25)
ER- α	
Negative	18(32.1)
Positive	38(67.9)

LVI: Lymphovascular invasion,
T stage: primary tumor,
N stage: regional lymph node invasion,
M stage: distant metastasis,
ER-α: Estrogen receptor alpha

Immunohistochemical expression of RRBP1 in EEC cases

Out of 56 cases of EEC, high cytoplasmic RRBP1 expression was detected in 25 tumors (44.6%). Tumors of the age group ≥ 60 years exhibited a slightly higher frequency of RRBP1 overexpression than tumors of <60 years (47.7% and 33.3% respectively). High-grade EEC tumors (63.2%) significantly showed higher RRBP1 expression compared to low-grade tumors (35.1%) (p=0.046). No statistically significant difference was found between RRBP1 expression and the presence of tumor necrosis (p=0.197). RRBP1 overexpression was recognized more often in tumors with LVI (75%) than those without (25%) with a significant statistical difference between the two groups (p=0.004) (Table 2).

Table 2. Immunohistochemical expression of RRBP1 in the EEC cases

	RRBP1 expression		Chi-square x^2	*p* value
	Low n (%) 31(55.4)	High n (%) 25(44.6)		
Age				
< 60	8(66.7)	4(33.3)	0.790	0.516
≥ 60	23(52.3)	21(47.7)		
Tumor grade				
Low grade	24(64.9)	13(35.1)	3.989	0.046*
High grade	7(36.8)	12(63.2)		
Tumor necrosis	19(63.3)	11(36.7)	1.663	0.197
Absent	12(46.2)	14(53.8)		
Present				
LVI				
Absent	27(67.5)	13(32.5)	8.353	0.004*
Present	4(25)	12(75)		
T stage				
T_{1a}	13(81.25)	3(18.75)		
T_{1b}	6(66.7)	3(33.4)	8.659	0.034*
T_2	10(41.6)	14(58.3)		
T_{3a}	2(28.6)	5(71.4)		
N stage				
N_0	24(72.7)	9(27.3)	9.810	0.002*
N_1	7(30.4)	16(69.6)		
M stage				
M_0	27(64.3)	15(35.7)	5.419	0.020*
M_1	4(28.6)	10(71.4)		
ER-α				
Negative	5(27.8)	13(72.2)	8.164	0.004*
Positive	26(68.4)	12(31.6)		

EEC: endometrioid endometrial carcinoma**, LVI:** lymphovascular invasion, T stage: primary tumor, N stage: regional lymph node invasion, M stage: distant metastasis **ER- α=**Estrogen receptor alpha, *: Statistically significant at p <0.05

Similarly, high RRBP1 immunolabeling was significantly detected in EEC with advanced T stage (p =0.034). Post hoc analysis revealed that higher rates of RRBP1 overexpression were significantly seen among T_2 and T_{3a} tumors (p=0.013 and 0.018 respectively) compared to T_{1a} (Table 3). Also, tumors associated with nodal (69.6%) and/or distant metastasis (71.4%) significantly expressed higher RRBP1 immunostaining (p=0.002 and p=0.020 respectively) than those without. Tumors with negative ER-α expression showed a significantly higher rate of RRBP1 overexpression than those with positive ER-α expression (72.2% and 31.6% respectively) (p=0.004) (Table 2,3 & Figure 2).

Table 3. Post hoc analysis of RRBP1 expression in relation to tumor stage (T stage)

		p value
T_{1a}	T_{1b}	0.464
	T_2	0.013*
	T_3	0.018*
T_{1b}	T_{1a}	0.464
	T_2	0.183
	T_3	0.117
T_2	T_{1a}	0.013*
	T_{1b}	0.183
	T_3	0.523
T_{3a}	T_{1a}	0.018*
	T_{1b}	0.117
	T_2	0.523

*: Statistically significant at p < 0.05

Immunohistochemical expression patterns of p53 in EEC cases

Wild-type p53 expression pattern was detected in 43 (76.8%) cases, while p53 mutated-type expression pattern either nuclear overexpression (12 cases) or cytoplasmic expression (only in one case) was detected in (23.2%) of EEC. A statistically significant difference was observed between wild- and mutated-type p53 expression pattern in relation to tumor grade as the percentage of high-grade tumors (42.1%) showed mutated-type p53 expression pattern was more than the percentage of low-grade ones (13.5%), whereas the majority of low-grade tumors (86.5%) displayed wild-type p53 expression pattern (p=0.016). Likewise, a significant distinction in p53 expression pattern was detected in relation to LVI, in which 7 tumors (43.8%) with LVI expressed mutated-type p53 compared to 26 tumors without LVI (86.7%) that showed wild-type immunostaining pattern (p=0.021). Examination of p53 expression pattern in relation to TNM staging revealed a high frequency of wild-type p53 expression pattern in T_{1a} (100%), T_{1b} (88.9%), and T_2 (70.8%) compared to T_{3a} tumors which showed an increased incidence of mutated-type p53 expression (71.4%) (p=0.002) (Table 4). Also, a significant statistical difference was observed between p53 expression pattern in relation to regional lymph node invasion and distant metastasis (p=0.003 and p=0.001 respectively). The majority of tumors with positive ER-α immunostaining (86.8%) showed a wild-type p53 expression pattern with a statistically significant relation (p=0.017). No significant relationship was detected between either wild or mutated-p53 expression pattern and the age of patients or the presence of tumor necrosis (p=0.869 and 0.060 respectively) (Table 4 & Figure 3).

Table 4. Immunohistochemical expression pattern of p53 in EEC cases

	p53 expression		**Chi-square x^2**	***p* value**
	Wild pattern n (%) 43(76.8)	Mutated pattern n (%) 13(23.2)		
Age				
< 60	9(75)	3(25)	0.027	0.869
≥ 60	34(77.3)	10(22.8)		
Tumor grade				
Low grade	32(86.5)	5(13.5)	5.757	0.016*
High grade	11(57.9)	8(42.1)		
Tumor necrosis				
Absent	26(86.7)	4(13.3)	3.539	0.060
Present	17(65.4)	9(34.6)		
LVI				
Absent	34(85)	6(15)	5.299	0.021*
Present	9(56.2)	7(43.8)		
T stage				
T_{1a}	16(100)	0(0)		
T_{1b}	8(88.9)	1(11.1)	15.183	0.002*
T_2	17(70.8)	7(29.2)		
T_{3a}	2(28.6)	5(71.4)		
N stage				
N_0	30(90.9)	3(9.1)	8.991	0.003*
N_1	13(56.5)	10(43.5)		
M stage				
M_0	37(88.1)	5(11.9)	12.055	0.001*
M_1	6(42.9)	8(57.1)		
ER-α				
Negative	10(55.6)	8(44.4)	6.707	0.017*
Positive	33(86.8)	5(13.2)		

EEC: Endometrioid endometrial carcinoma**, LVI**: lympho-vascular invasion, T stage: primary tumor, N stage: regional lymph node invasion, M stage: distant metastasis **ER- α=**Estrogen receptor alpha, *: Statistically significant at p < 0.05

Table 5. Correlation between RRBP1 and p53 immunohistochemical expression in the EEC cases

	***RRBP1* expression**		***r***	***p value***
	Low n (%)	High n (%)		
p53 expression pattern				
Wild-type p53	28(65.1)	15(34.9)	0.357	0.007*
Mutated-type p53	3(23.1)	10(76.9)		

EEC: endometrioid endometrial carcinoma, **r:** Spearman correlation test, ***:** Statistically significant at $p < 0.05$

Correlation between RRBP1 and wild and mutated p53 expression patterns in the studied cases

A significant positive correlation was recognized between RRBP1 overexpression and mutated-type p53 expression pattern, as 76.9 % of EEC with mutated-type p53 displayed high RRBP1 immunostaining, and only 34.9% of tumors with wild-type p53 expression pattern showed RRBP1 overexpression. Whereas 23.1% of mutated-type p53 EEC showed low RRBP1 immuno-labelling (r=0.357, p=0.007) Table 5.

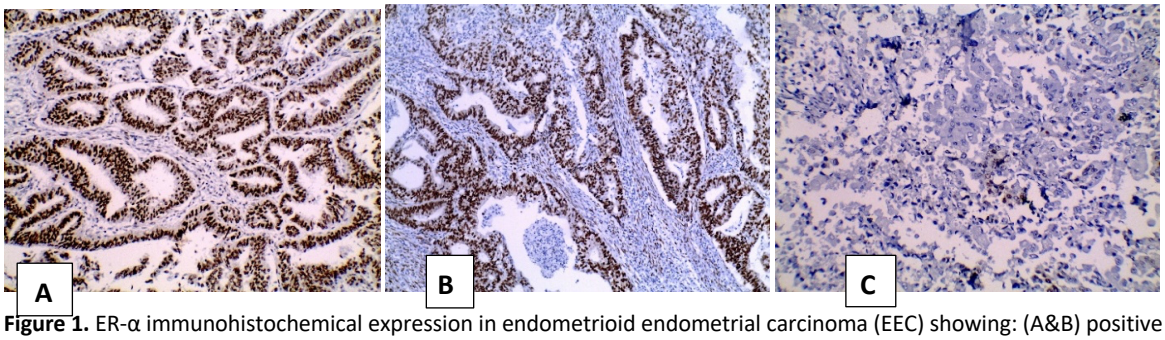

Figure 1. ER-α immunohistochemical expression in endometrioid endometrial carcinoma (EEC) showing: (A&B) positive expression in low-grade EEC (×200); (C) Negative expression in high-grade EEC (×200).

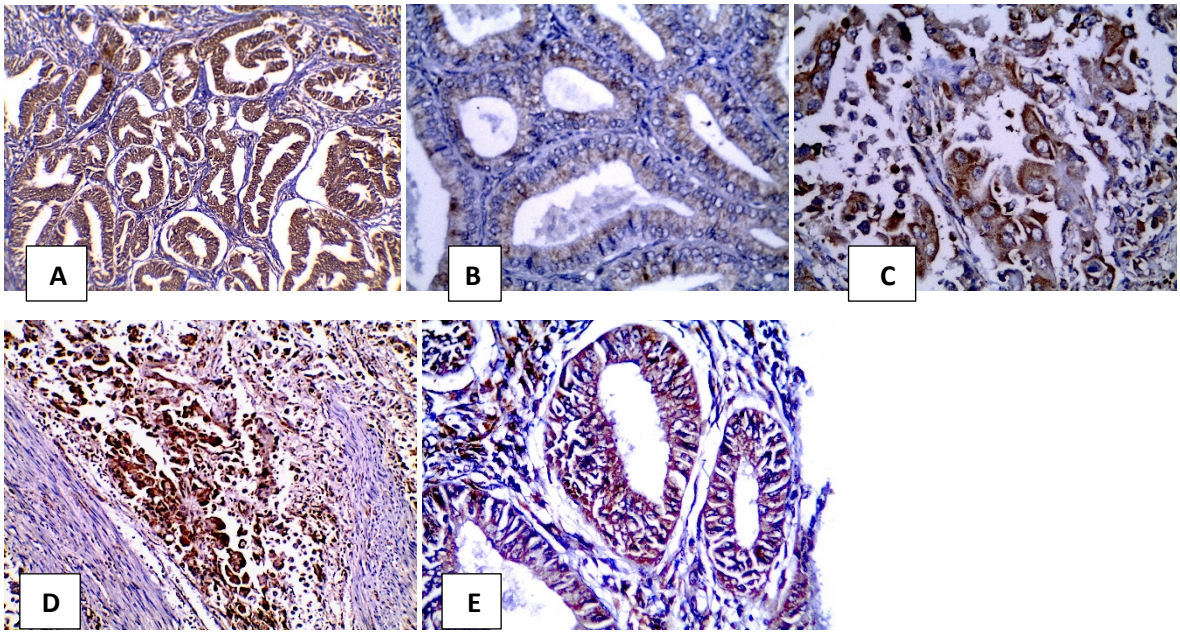

Figure 2. Immunohistochemical expression of RRBP1 showing: (A) High RRBP1 expression in high-grade EEC (×400); (B) High RRBP1 expression in high-grade EEC with myometrium invasion (×200); **(C)** Low RRBP1 expression in low-grade EEC (×400); (**D**& **E)** high RRBP1 expression in low-grade EEC (×200 & ×400 respectively).

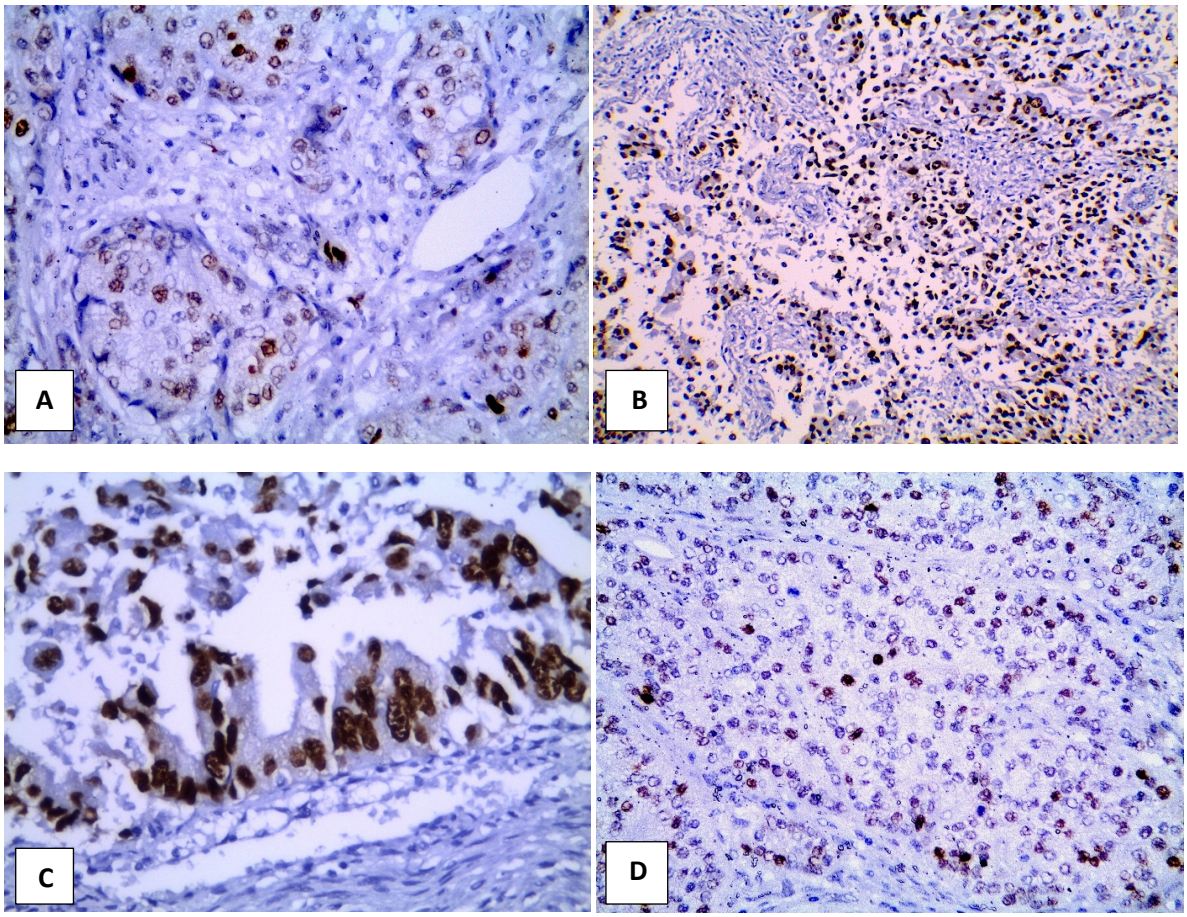

Figure 3. Immunohistochemical expression pattern of p53 in EEC showing: (**A** & **B)** p53 mutated-type nuclear overexpression in high-grade EEC (×400 & ×200 respectively); (**C)** p53 wild-type expression pattern in low-grade EEC in the form of nuclear staining varied from weak-to-moderate in most of the tumor cells to strong staining in few nuclei (×400); (**D)** p53 wild-type expression pattern in high-grade EEC (×200).

DISCUSSION

Classification, prognosis, and treatment decisions of EC are now becoming dependent basically on the molecular characteristics and abnormalities of tumor cells rather than the morphology alone (Baiden-Amissah et al., 2021). The RRBP1 protein is located in the endoplasmic reticulum where it is involved in protein transport and secretion in the cells. Previous studies demonstrated its role in ERS improvement through UPR (Spear and Ng, 2001; Tsai et al., 2013). Thus, the present work aimed to study the immunohistochemical expression of RRBP1 in wild and mutated p53 immunophenotype EEC. To our knowledge, this is the first work to explore the relation between RRBP1 expression in wild- and mutated-type p53 EEC in relation to ER-α expression and clinicopathological data.

The expression of RRBP1 in EEC was investigated in relation to clinicopathological and prognostic factors. It was found that RRBP1 overexpression was significantly associated with unfavorable prognostic factors namely: high tumor grade, presence of LVI, advanced tumor stage, regional lymph node invasion, and distant metastasis compared to tumors with low RRBP1 expression. Our findings confirmed the relation between high expression of RRBP1 and adverse prognostic factors (high grade and advanced TNM staging) which supported the proposed role of RRBP1 in tumor progression and invasion. In concordance with our results, Liu et al. (2019) showed that RRBP1 overexpression in EEC was linked to advanced FIGO stage, presence of lymph node metastasis, and increased depth of myometrial invasion, and shorter duration of overall survival. Also, it was reported that RRBP1 overexpression in colorectal carcinoma and esophageal carcinoma

respectively was related to high TNM staging and short disease-specific survival (Pan et al., 2015; Wang et al., 2018). Furthermore, Wang et al. found that RRBP1 is overexpressed in urothelial carcinoma compared to normal tissues and RRPB1 knockdown inhibited cancer cell migration and invasion (Wang et al., 2020). Similarly, Tsai et al. (2013) suggested that RRBP1 overexpression in lung cancer improves stress of the endoplasmic reticulum and increases the survival of cancer cells.

EEC tumors with negative ER-α immunostaining significantly exhibited higher RRBP1 expression in tumor cells compared to ER-α immune-positive tumors. This result could be explained by the fact that the occurrence of ERS as a result of tumor development and proliferation stimulates the UPR. UPR protects the tumor cells from ERS and guarantees their survival through several cellular responses such as protein degradation, changing the rates of transcription and translocation, and decreased lipid synthesis. Thus, leading to loss of ER-α expression in tumor cells (Clarke and Cook, 2015). Accordingly, this clarifies why tumors with RRBP1 overexpression were associated with ER-α negative expression. Loss of ER-α expression was associated with poor prognostic parameters in EEC in previous studies (Guan et al., 2019).

The transcriptional p53 protein plays an important role in tumor suppression. It is encoded by the TP53 gene, the guardian of the genome. The function of wild-type p53 is to arrest the cell cycle and initiate apoptosis in response to DNA damage through transcriptional activation of apoptosis genes (Beckerman and Prives, 2010). Mutated-type p53 was found to interact with other transcription factors, increasing or suppressing their normal function, thus changing the expression of their target genes (Sobhani et al., 2020).

Wild- and mutated-type p53 expression pattern was examined in EEC in relation to clinicopathological and prognostic factors. It was observed that high-grade tumors significantly expressed mutated-type p53 compared to low-grade ones. Similarly, the rate of mutated-type p53 expression pattern was significantly high in tumors with LVI and advanced TNM staging. A significant difference in wild- and mutated-type p53 expression pattern was found in relation to tumor grade, LVI, T stage, lymph node invasion, and distant metastasis. In agreement with our findings, several studies reported the association between p53 mutation and poor prognostic parameters in EC (Alkushi et al., 2004; Garg et al., 2010; Schultheis et al., 2016; Huvila et al., 2018; Shivkumar et al., 2020). Moreover, high-grade EEC with mutated-type p53 carries an adverse prognosis than EEC of the same grade exhibiting wild-type p53 (Kobel et al., 2017). Depending on EEC molecular subtypes by the Cancer Genome Atlas Group (TCGA), mutated-type p53 immune-labeling is considered as a sign of adverse prognosis (Talhouk et al., 2017).

Regarding p53-immunoexpression pattern and ER-α status in EEC, it was found that tumors with positive ER-α nuclear expression significantly showed wild-type p53 expression pattern more frequently compared to EEC with negative ER-α expression. This could be related to the theory of positive loop response between ER-α and p53 expression in which mutated-type p53 can suppress ER-α transcription and hence its expression (Berger et al., 2012; Berger et al., 2013). Besides, another mechanism was reported showing that ER-α can bind MDM2 preventing wild-type p53 from degradation (Tackmann and Zhang 2017).

Subsequently, the correlation between RRBP1 and p53 expression pattern in EEC was investigated. A significant positive correlation was found between RRBP1 overexpression and mutated-type p53 expression pattern whether nuclear overexpression or cytoplasmic. Contrarily, only 23.1% of mutated-type p53 tumors showed low RRBP1 expression.

The results of the present work can be explained by the following facts. First, during tumor growth and proliferation, cancer cells require the increased activity of endoplasmic reticulum protein folding, assembly, and transport leading to ERS. An adaptive stress response is initiated to protect cancer cells from ERS to improve ERS and restore the endoplasmic reticulum function which is essential for malignant cells' survival and

proliferation. This adaptive response is known as the unfolded protein response (UPR).

UPR increases the endoplasmic reticulum transmembrane stabilizing proteins which stimulates the degradation of unfolded proteins to prevent the ERS-mediated cell apoptosis (Corazzari et al., 2017). Wild-type p53 is activated during ERS leading to inhibition of UPR and activation of cell apoptosis. However, it has been found that cancer cells with mutant-type p53 overcome the ERS by stimulating the ATF6, one of the important endoplasmic reticulum transmembrane stabilizing proteins, that regulates the UPR to maintain the survival of cancer cells (Sicari et al., 2019).

Second, mutated-type p53 protein enhances carcinogenesis not only by deleting the tumor suppressor effect of wild-type p53 protein but also by new oncogenic GOF actions. These oncogenic effects of mutated-type p53 are mainly related to its ability to form complexes with other transcriptional factors. These complexes are involved in DNA transcription or signal transduction leading to gene activation and protein expression (Di Agostino et al., 2016). Mutated-type p53 GOF was proved by Román-Rosales et al. (2018) who reported that p53 mutated type induced HER2 overexpression through increased HER2 transcription in breast cancer cell lines and other cancers.

Further, the mutated-type p53 GOF was found to be due to its ability to merge with several transcriptional factors such as SMADs, NF-kB, and Sp1 which lead to malignant cell survival, invasion, and metastasis (Kim and Lozano, 2018). These findings in addition to ours supported that mutated-type p53 GOF activity may differ according to changes within tumor cells or in the tumor microenvironment (D'Orazi and Cirone, 2019; Zhang et al., 2020; Alvarado-Ortiz et al., 2021).

Thereby, the positive correlation between RRBP1 and mutated-type p53 expression found in the current work could be attributed to RRBP1 transcriptional activation by mutated-p53 GOF to maintain the survival of cancer cells despite ERS due to insufficient nutrients, oxygen deprivation, and accumulation of mutations forming abnormal proteins that cannot be adequately folded.

In conclusion, RRBP1 overexpression in EEC is associated with unfavorable prognostic factors including high tumor grade, LVI, advanced TNM staging, and negative ER-α. Also, the finding of a strong positive correlation between RRBP1 overexpression and mutated-type p53 expression pattern in ECC suggests that the future therapeutic approaches would be directed to disturb mutated-type p53, restore wild-type p53 functions and downregulate RRBP1 expression to prevent its accumulation that leads to alteration of the normal regulatory anti-cancer cellular mechanisms.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval by the Local Ethical Committee, Faculty of Medicine, Tanta University, Egypt (34376/1/21)

AVAILABILITY OF DATA AND MATERIALS

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

COMPETING INTERESTS

The authors state that there were no commercial or financial relationships that may be considered a potential conflict of interest during the research.

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AUTHORS' CONTRIBUTIONS

The authors confirm contribution to the paper as follows:

- Study conception and design: Marwa A. Abd El-Azeem, Shaimaa M. M. Bebars
- Analysis and interpretation of results: Marwa A. Abd El-Azeem
- Draft manuscript preparation: Marwa A. Abd El-Azeem, Shaimaa M. M. Bebars
- Data collection: Marwa A. Abd El-Azeem, Shaimaa M. M. Bebars
- All authors reviewed the results and approved the final version of the manuscript.

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