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Expression and clinical significance of ARHI and p130 in endometrial carcinoma

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Summary

Background: To evaluate the expression status of ARHI protein and pRb2/p130 in endometrial cancer (EC), while analyzing the relevance in EC simultaneously. *Materials and Methods:* Surgical resection of 20 tumor samples from primary EC patients, 20 atypical hyperplasia endometrial samples, and ten normal endometrial samples from benign disease such as uterine fibroid after surgery were randomly chosen for co-immunoprecipitation assays, which was used to explore the expression of ARHI and p130. *Results:* The positive status of ARHI protein in dropped gradually from normal endometrial tissues at proliferative stage (90%), endometrial tissues of atypical hyperplasia (50%) to EC tissues (30%); statistically significant differences were evaluated among the groups ($p < 0.05$). Meanwhile The positive rate of pRb2/p130 decreased gradually, which had a apparent difference among groups ($p < 0.05$). There were correlations between lack of positive statue of ARHI and the degree of EC and clinicopathological classification ($p < 0.05$). However the positive expression statue of ARHI protein was not associated with the histological type ($p > 0.05$). However, there were correlations between lack of positive expression of pRb2/p130 and the degree of EC, classification of surgical pathology, and histological type ($p < 0.05$). *Conclusions:* The drop or lack of the positive expression status of ARHI protein and pRb2/P130 result in the occurrence and worsening of EC.

Key words: ARHI; p130; Immunohistochemical; Endometrial carcinoma; Endometrial cancer.

Introduction

Endometrial cancer (EC) is a common malignancy among in Western countries. Currently, in European countries, including Hong Kong, EC is fairly common. The Hong Kong Cancer Association data of 2004–2014 shows that EC has risen up to third place from the ninth place as the most common cancer in women, although EC mortality is still about 1%. However, in the past decade, the EC incidence has increased significantly [1]. Now, EC has become a relatively common malignancy among Hong Kong women, while cases of EC have also spread in China and throughout the rest of the world, seriously affecting women's health and quality of life, and increasing the burden on family and society. Therefore, early detection and early treatment is essential for EC prognosis. Understanding the molecular mechanisms will be conducive to clinical management of the disease. Currently, research has continued to explore the related factors, the corresponding proteins, and involved functions and relevance, dedicated to a better detection and treatment of the tumorigenesis and its development. The present authors have adopted the immunohistochemical method to detect the ARHI protein and pRb2/p130 positive expression situation in EC, analyzed the relationship between two factors, and discussed their occurrence and the developmental functions of EC.

Materials and Methods

Fifty cases of archival paraffin at the Fourth Affiliated Hospital of Harbin Medical University ranging from January 2012 and January 2014 were included in this study. All the biopsies were confirmed by two pathologists with high qualifications by reading the pieces, including 20 EC cases, aged from 28-78 years, with an average age of 53 years. Clinical staging was based on the Association of Obstetrics and Gynecology (FIGO 1988). There were seven cases in GI period, seven cases in G2 period, six cases in G3 period, 15 cases of EC, and five cases of EC in other types. There were 20 cases of atypical hyperplasia of endometrium, aged from 35-87 years, with an average age of 57 years, among which there were ten cases of mild atypical hyperplasia of endometrium and ten cases of moderate or severe atypical hyperplasia. Meanwhile, there were ten cases of normal endometrial tissue sections of uterine leiomyoma, aged from 41-75 years, with an average age of 53 years. None of the patients received any treatment, such as radiation and chemotherapy.This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of First Affiliated Hospital of Xi-An Medical University. Written informed consent was obtained from all participants.

Experimental procedure was combined with instructions and routine immunohistochemical steps for operation; each group and each batch had a separate negative control experiment (PBS buffer alternating the primary antibody)

Cytoplasm of ARHI protein in normal cells and cancer cells or the brown particles appearing in the membrane was set as a positive performance. Rb2 / p130 protein of nucleus or cytoplasm of normal cells and cancer cells appearing with brown particles was

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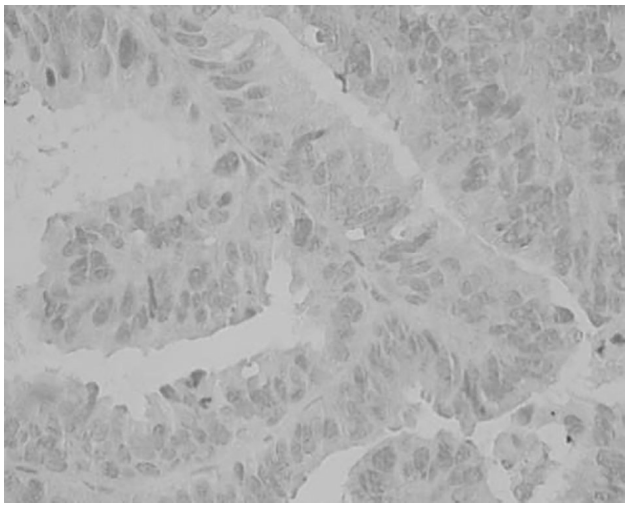

Figure 1. — The expression of ARHI in endometrial carcinoma (×400).

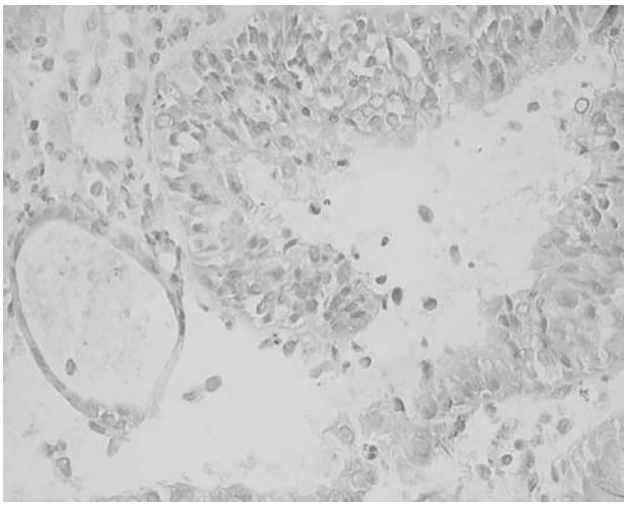

Figure 2. — The expression of ARHI in endometrial tissues of atypical hyperplasia (×400).

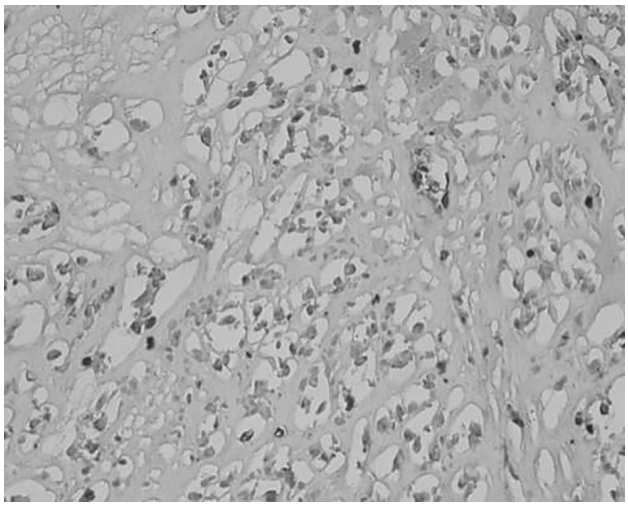

Figure 3. — The expression of ARHI in normal endometrium (×400).

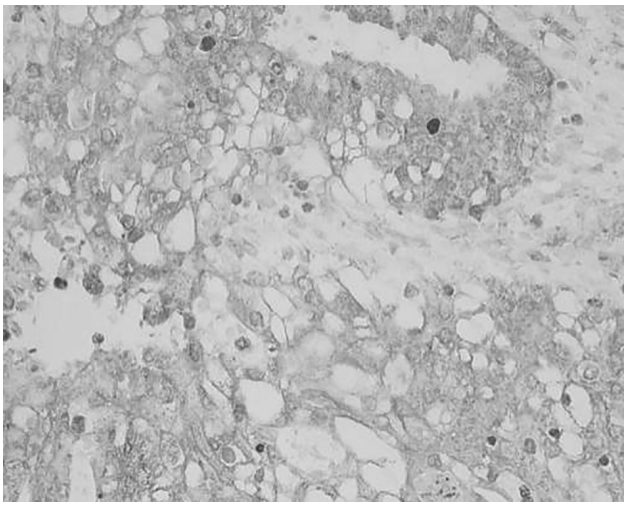

Figure 4. — The expression of p130 in endometrial carcinoma (×400).

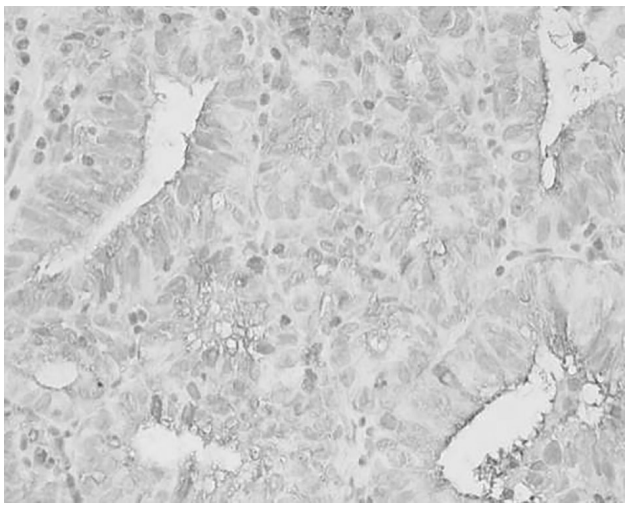

Figure 5. — The expression of p130 in endometrial tissues of atypical hyperplasia (×400).

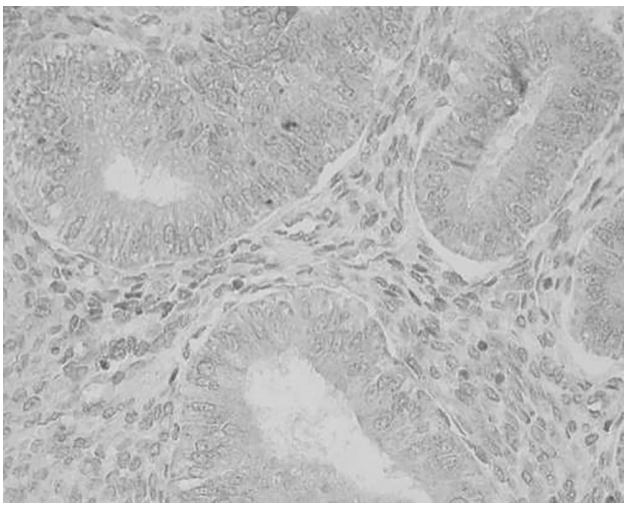

Figure 6. — The expression of ARHI in normal endometrium (×400).

Table 1. — *The expression levels of ARHI in different types of endometrial tissues.*

Groups	Cases	Expressions of ARHI				Positive rate	*p*
		-	+	++	+++		
Endometrial cancer	20	13	3	2	2	0.38	0.02A
Endometrial atypical hyperplasia	20	10	4	3	3	0.50	0.02B
Normal endometrium	10	1	1	3	5	0.90	0.01C

A: Comparison between normal endometrial group and endometrial cancer group.
B: Comparison between endometrial of atypical hyperplasia group and endometrial cancer group.
C: Comparisons among three groups.

Table 2. — The expression levels of p130 in different types of endometrial tissues

Groups	Cases	Expression of p130				Positive rate	*p*
		-	+	++	+++		
Endometrial cancer	20	16	2	2	0	0.2	0.02D
Endometrial atypical hyperplasia	20	14	2	2	2	0.3	0.01E
Normal endometrium	10	0	2	4	4	1	0.02F

D: Comparison between normal endometrial group and endometrial cancer group.
E: Comparison between endometrial of atypical hyperplasia group and endometrial cancer group.
F: Comparisons among three groups.

Table 3. — *Relationship between endometrial cancer and pathological features.*

	cases	ARHI		*p*	p130		*p*
		+	-		+	-	
Tissue differentiation							
G1	7	3	4		2	5	
G2	7	2	5		2	5	
G3	6	1	5	<0.05	1	5	<0.05
Organization type							
Endometrial carcinoma	15	6	9	>0.05	3	12	<0.05
Other types	5	2	3		1	4	
Surgical Stage							
I	37	15	22		11	26	
II	20	7	13		11	9	
III	15	5	10		4	11	
IV	8	2	6	<0.05	3	5	<0.05

set as a positive performance. A ten high-power microscope (×400) was utilized at random and statistics for 100 normal cells or cancer cells within each corresponding field were recorded, with semi-quantitative analysis to determine the statistical results. Thee score according to the dye strength was as follows: 0 score: no dyeing, 1 score: light yellow, 2 score: brown yellow, and 3 score: brown. The dyeing scores of pathological staining were compared with the background color of the slice, and then score was based on the proportion of positive cells. A 0 score was negative, 1 score: positive proportion less than 25%, 2 score: positive proportion 26-50%, 3 score: positive proportion 51-75%, and a 4 score: positive proportion more than 76%. Then, both scores were be multiplied. 0-1 score indicated immune reaction as (-), 2-4 score as (+), 5-7 score as (++), and a score of above 7 as (+++).

The relevance statistics of clinical data was assessed using SPSS 16.0 software for its processing. The statistics comparison among the groups relied on the X^2 test, and the statistical description on correlation between two factors adopted a Spearman *p* value of < 0.05 with statistical significance.

Results

ARHI protein was located in the cytoplasm and/or membrane of normal cells and cancer cells, while pRb2 / p130 was mainly located in the nuclei and cytoplasm of normal cells and cancer cells. The positive rate (38%) of ARHI protein of endometrial carcinoma group (Figure 1) was significantly lower than that in the group of atypical endometrial hyperplasia type (50%) (Figure 2) and in normal endometrium (90%) (Figure 3), therefore the difference among the groups had a statistical significance (p <0.05) (Table 1). The positive rate (20%) of Rb2/p130 protein in the endometrial carcinoma group (Figure 4) was significantly lower than that in the group of atypical endometrial hyperplasia type (30%) (Figure 5) and in normal endometrium (100%) (Figure 6); therefore the difference among the groups also had a statistical significance (p < 0.05) (Table 2).

In endometrial carcinoma, the degree of malignancy was higher, the pathological grade was lower, and the positive expression of ARHI protein protein decreased or disappeared, hence there was no relationship between the positive expressions of ARHI protein and the histological type of endometrial carcinoma. The degree of malignancy was higher, the surgical pathology grade was lower, and Rb2/p130 protein expression decreased or disappeared. The positive expression rate of ARHI protein in endometrial adenocarcinoma was higher than that in other endometrial carcinomas (Table 3).

Correlation analysis of Spearman rank showed that the expression of ARHI protein and Rb2/p130 protein in endometrial carcinoma was positively correlated ($p < 0.05$. r = 0.51).

Discussion

ARHI is a tumor suppressor gene, which is located on chromosome 1p31, encoded with a 26kd small GTP associated protein, and is highly homologous with RAS and Rap [2]. It is tumor suppressor gene, instead of a proto oncogene, but a tumor suppressor gene. It leads to programmed cell death by regulating the cell cycle and signal transduction pathway so as to inhibit cancer cell growth [3]. It showed a high expression in normal breast and ovarian tissue, however, it showed low expression in more than 60% of breast and ovarian cancer tissues [4]. Meanwhile, Yang *et al.* [5] found that ARHI was highly expressed in pancreatic duct and alveolar tissues, but low or missing expression in almost 50% of pancreatic cancer tissues was consistent. Currently, there have been few studies on endometrial carcinoma. This experiment mainly explored the expression situation of ARHI protein in endometrial carcinoma. There was a positive expression of ARHI in normal endometrium, atypical hyperplasia endometrium, and endometrial carcinoma are decreased gradually or in shortage, consistent with the expression trend of the cancerous tissue of the ARHI in other corresponding organs throughout the body.

In the mechanism of ARHI in the development of cancer, in the signal transduction pathway, ARHI plays a role in the regulation of autophagy. These primarily are: 1) to participate in signal transduction and transcription factor 3 (STAT3) in order to inhibit the proliferation and differentiation of cancer cells. The pathway of Ra s/MAPK Ra and PI3K-AKT can be inhibited by JNK, and ARHI gene inhibits this pathway indirectly [6]. 2) By acting on dynein CyclinD1 components within the cell cycle or on a higher expression of induced p21WAFI/CIP, thereby inhibiting CKD physiological activity and cell growth [7]. 3) Relying on two types of calpain and apoptosis protein, apoptosis signal transduction is triggered, initiates apoptosis effector, and induces cell apoptosis or spontaneous programmed death. In addition, CPG island of ARHI features aberrant methylation. There are three CPG islands in ARHI, among which CPG I and CPG II are located in the promoter region, and CPG III is located within the coding area. Methylation occurs in CPG Island I and II of the promoter, and the third CPG Islands within the second exon coding region [8]. Including the loss of heterozygosity, Melcher *et al.*[9] adopted an analysis technique of a single nucleotide polymorphism microarray to conduct a research in 32 patients with sporadic colorectal cancer, to prove that 8p22 exists in the microsatellite instability with loss of heterozygosity, which is a common genetic change in a human cancer genome. In addition, regulation of E2F1 and E2F4 at the transcriptional level causes mRNA half-life to shorten [10]. The role of ARHI in the occurrence and development of cancer has been further improved.

The Rb2/p130 gene is a member of the RB family, which is located on the human chromosome 16q12.2. PRB2/p130 is a protein product of inhibition oncogene, with a similar or even the same structural domain that plays a role in PRB and p107, which has become a “pocket structure”, so RB family proteins are called “pocket protein” families. Among these, Rb2 / p130 protein consists of 1,139 amino acid residues, with a functional pocket domain (known as the A / B structural domain), composed by exons 10-13 (A structural domain) and exon 17-20 (B structural domain) [11]. The structure has a decisive effect on its function. The structural changes within such region were found in many human tumors. In this experiment, the expression of p130 in normal endometrium, atypical hyperplasia, and EC tissues gradually decreased, which was similar to the findings of Susi *et al.* [12]. Their study showed that the positive expression of RB2/P130 protein decreased gradually in endometrial tissue, endometrial atypical hyperplasia tissue, and EC within the normal proliferative phase. From the research of foreign scholars, such as Mas *et al.* [13], we know that p130 expression was lower in the malignant tumor, which can effectively evaluate the prognosis of the tumor .

The function mechanism of the action of p130 has received much attention. Experimental studies show that the pRb2/p130 protein is related to cell cycle. During the process of cell cycle regulation, pRb2/p130 proteins interact with cellular proteins, such as transcription factor E2F, and cell cycle protein and cyclin dependent kinase experienced interactions. Among these, the combination and the separation of E2F and pRb 2/p130 play a role in G0 period, G0/G1 transformation period, and G1/S transformation period, while in G0 stage, the combination of pRb2/p130 and E2F formed p130/E2F-4/DP complex, so that the transcriptional activity adjusted by E2F decreased or disappeared, making the cells remain in G0 phase. When pRb2 / p130 is phosphorylated, the complex of p130 and E2F can be decomposed, causing exposure to the binding sites of gene transcription. Freedom E2F and a special gene pair shall begin a sub-region combination, so that the transcription gene can begin to transcript and express, in turn caus-

ing the cell cycle to be transferred into G1 phase from G0 phase, and into S phase [14], which indicates that pRb2 / p130 protein play a regulatory role in cells of early stage [15].

In summary, the positive expression of ARHI protein and RB2/P130 protein has the same trend in normal endometrium, atypical hyperplasia endometrium, and EC. Through the mechanism of a signal pathway, we know that they have their own regulation mechanism, but they have mutual communication in the regulation pathway. For example, in the transformation process within the cell cycle G1/S, RB protein is the control center of cell cycle G1/S. During the cell cycle, an upstream component of the regulation pathway dominated by RB includes dynein cyclinD1-CDKs complex, CKI, and CDK activated kinase (CAK), which plays an important role in regulation function. Meanwhile, ARHI can also regulate the activity of cyclinD1-CKDs by $P21^{WAF/CIP1}$, so as to regulate the cell cycle [16], but the specific regulation still needs further research. Furthermore, it is known that CyclinD1 and CDK family members form the CyclinD1/CDKs complex. E2F factor was released from the family protein of the phosphorylation of the mother cell tumor. Freedom E2F and a special gene pair may begin the region combination, so that such genes may start to transcript and express [5], in order to promote the transformation from G1 to S phase in the cell. Meanwhile, E2F1 as the family member of E2F plays a role in the process of the tumor development resulting from ARHI. From the research, it is known that E2F1 mediated apoptosis plays the role of p53 dependence or p53 independence way [17]. The p53 pathway plays a role in the autophagy regulation process of ARHI. The p53 pathway interacts with the media of E2F1 via the autophagy pathway [18], however, how it is achieved during the regulation process of the whole cell cycle still requires further understanding.

Signal transducer and transcription activator3 (STAT3) inhibited the proliferation and differentiation of cancer cells through RAS/MAPK and PI3K-AKT pathway in the autophagy pathway. In serous ovarian cancer expressions, the expression STAT3 and E2F1 were increased, and E2F1 protein overexpression inhibited the expression of ARHI, caused the loss of the negative regulation of STAT3, and activated the downstream to increase the function of value-added inhibition apoptosis [19]. However, there is a relationship between E2F1 and p130. At the same time, 3 CPG islands demonstrated methylation except within the ARHI gene, resulting in an abnormal regulation of cell cycle. However, related research shows that commencing sub-regions of gene RB2/p130 causes a CPG island methylation exception, resulting in the inhibiting of its combination with related factors (such as RBF1 and ATF1), thereby impeding the activity of RB gene promoter [20], affecting the cell cycle progression, and resulting in the deterioration of normal cells.

The occurrence and development of EC is a complicated and uncoordinated regulation process. The signal transduction pathway goes through the whole process of regulation. Any abnormal changes in the pathway may play a role to a certain degree, such as a substitute injury or permanent injury. From the experiment, it is known that the expression of ARHI and p130 are abnormal in EC. Two kinds of factors regulate the microenvironment of the cell through the signal transduction pathways, respectively, and the two factors also have a certain degree of connection and interaction. The combination of the two factors could have a certain significance in screening EC at an early stage.

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