

# The Selective Expression of Ret Finger Protein in Endometrial Cancer: Can RFP be a Marker of Serous Carcinomas?

## Ret Finger Proteininin Endometrium Kanserlerinde Seçici Ekspresyonu: RFP Seröz Kanserlerin Belirteci Olarak Kullanılabilir mi?

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### ABSTRACT

**Objective:** Endometrial cancer is a common malignancy of the gynecological system and has been classified into two major groups, Types I and II. Type I tumors are estrogen-related, low-grade endometrioid tumors, whereas type II tumors are aggressive, high-grade non-endometrioid tumors. Ret finger protein is a nuclear transcription factor with a tripartite motif that is highly expressed in different tumor cells.

**Material and Method:** To analyze the expression of ret finger protein in endometrial tissues and cancer, 18 cases of secretory and proliferative endometrium, endometrial polyp, endometrial hyperplasia and endometrial intraepithelial neoplasia and 21 cases of types I and II endometrial carcinoma were evaluated immunohistochemically.

**Results:** Although rare cases of secretory endometrium showed a weak focal nuclear positivity, remaining proliferative endometrium, endometrial hyperplasia and type I endometrioid cancer cases were negative. In contrast, all cases of serous cancers showed strong nuclear positivity. After these strong positive results for serous endometrial cancer, 12 more cases of ovarian and endometrial serous carcinoma cases were added to the study. All of the additional cases were also strongly positive for ret finger protein.

**Conclusion:** We suggest that ret finger protein might play a role in the carcinogenesis of the serous tumors of gynecological system and can be used to differentiate serous carcinomas from other epithelial tumors.

**Key Words:** Endometrial cancer, Serous carcinoma, Ret finger protein

### ÖZ

**Amaç:** Endometrium kanseri, kadın genital sisteminin en sık görülen kanserlerinden biri olup, tip I ve tip II olmak üzere iki ana grupta sınıflandırılmaktadır. Tip I tümörler genellikle östrojen hormonu ile bağlantılı olup, düşük dereceli, endometrioid morfolojiye sahipken; tip II tümörler genellikle agresif davranışlı ve yüksek dereceli, endometrioid dışı morfoloji sergilemektedir. Ret finger protein üç parçalı motif yapısında nükleer bir transkripsiyon faktörü olup birçok tümörde yüksek miktarda eksprese edilmektedir.

**Gereç ve Yöntem:** Tip I ve II endometrial karsinom örnekleri (toplam 21 adet) ile sekretuar endometrium, endometrial polip, endometrial hiperplazi ve endometrial intraepitelial neoplazi tanısı almış dokular (toplam 18 adet) immünohistokimyasal teknikle boyanarak ret finger protein ekspresyonu açısından karşılaştırmalı olarak değerlendirildi.

**Bulgular:** Sekretuar endometriumda çok nadir olarak zayıf nükleer ekspresyon saptanırken, tüm proliferatif endometrium, endometrial hiperplazi ve tip I endometrium karsinom olguları ret finger protein ile negatif boyanma gösterdi. Buna karşın, tüm endometrial seröz karsinom dokuları ile daha sonra ek olarak boyanan 12 adet over ve endometrial seröz karsinom örneğinin ret finger protein ile kuvvetli pozitif nükleer ekspresyon gösterdiği saptandı.

**Sonuç:** Ret finger proteininin over ve endometrial seröz karsinomlardaki seçici ekspresyonu, hem bu proteinin seröz karsinomların patogenezindeki rolünün ortaya çıkarılmasında hem de seröz tümörlerin kadın genital sisteminde gelişen diğer epitelyal tümörlerden ayrılmasında faydalı olabilir.

**Anahtar Sözcükler:** Endometrial neoplazi, Seröz adenokarsinom, Ret finger protein

## INTRODUCTION

Endometrial cancer is one of the common malignancies of the female genital tract. This cancer is the seventh most common cancer of women worldwide and has the highest incidence in Western countries (1). Almost 90% of endometrial cancers are sporadic, whereas the remaining 10% of cases are hereditary (2). Based on the etiology, clinical behavior and pathological characteristics, a dualistic model of endometrial tumorigenesis has long been recognized and broadly termed as types I and II (3).

Type I endometrial cancers are histologically low-grade endometrioid carcinomas with a favorable prognosis and represent about 70–80% of sporadic endometrial carcinomas. These tumors are associated with unopposed estrogen exposure and usually develop in perimenopausal women, with the risk factors of obesity, anovulation, nulliparity, and exogenous estrogen exposure. They arise in a background of complex and atypical endometrial hyperplasia and commonly express estrogen and progesterone receptors (ER and PR). Although they are rare, mucinous adenocarcinomas are also considered within this group since they have low histopathological grade and usually express ER and PR (3, 4).

On the other hand, type II cancers are histologically high-grade non-endometrioid carcinomas, most frequently serous papillary, have an aggressive clinical behavior and represent about 10-20% of endometrial cancers. These tumors are unrelated to estrogen excess, and usually develop in older postmenopausal women, without any hormonal risk factors. They arise in a background of atrophic endometrium with the putative precursor lesion being endometrial intraepithelial carcinoma and occasionally endometrial polyps. Although the histological prototype for type II tumors is serous carcinomas, the less frequent clear cell carcinoma is also considered within this group (3, 4).

Ret finger protein (RFP) is a nuclear protein that belongs to B-box RING finger family and consists of a tripartite motif including RING finger, B-box zinc finger and coiled coil domain (5-8). The interest in RFP was originally arose from its oncogenic activity when fused with the RET tyrosine kinase (9). RFP mRNA is highly expressed in various human and rodent tumor cell lines (10). RFP expression is detected in male germ cells, peripheral and central neurons, hepatocytes, adrenal chromaffin cells, as well as breast cancer and seminomas (11-13). Although RFP is highly expressed in different tumor types, the exact function of RFP in cancer remains unclear.

To our knowledge, there are only two studies related with the expression of RFP in endometrial cancer in the literature. Zhang et al. investigated the levels of RFP expression among cervical squamous cell carcinomas, endometrial adenocarcinomas, normal cervix and endometrium (14). Tsukamoto et al. examined the clinical significance of RFP expression in endometrial cancer (15). However, neither of these two studies compared RFP expression in endometrial cancer with respect to morphological characteristics.

Herein, the expression of RFP in benign, hyperplastic and neoplastic endometrium, as well as type I and type II endometrial cancer cases was analyzed to understand its morphological selectivity.

## MATERIAL and METHOD

**Tissue samples:** The paraffin blocks of randomly chosen cases included in this study were selected from the archives of the pathology department. Two pathologists (GGT and AU) re-evaluated the morphologic features. Of the 39 cases, 18 cases were benign (3 cases with secretory endometrium, 2 cases with proliferative endometrium, 5 endometrial polyp, 3 benign endometrial hyperplasia and 5 endometrial intraepithelial neoplasia [EIN]); and 21 cases were type I (16 endometrioid cases including high grade tumors) and type II (3 serous, 2 clear cell) endometrial carcinoma. After the strong positive results selectively for serous endometrial cancer cases, 12 more cases of ovarian (9 cases) and endometrial serous (3 cases) carcinoma were added to the study to analyze organ and/or morphology specificity.

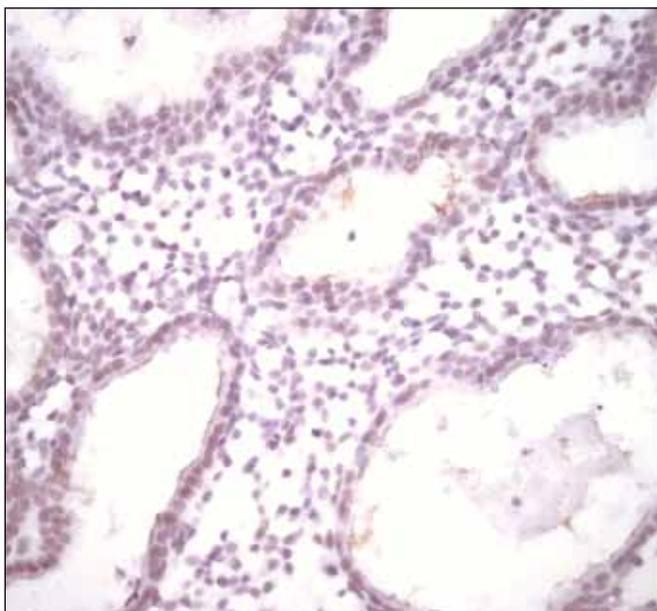
**Immunohistochemistry:** Four micron sections obtained from the selected cases were stained for RFP. Immunohistochemical staining was carried out by the strepto-avidin biotin method, using a commercially available kit (UltraTek HRP Anti-Polyvalent Lab Pack, ScyTek Laboratories). Anti-RFP antibody (12) was used as a primary antibody. Briefly, deparaffinized sections were treated with methanol containing 0.3% H<sub>2</sub>O<sub>2</sub> for 15 min for endogenous peroxide blockage. After washing with PBS, blocking solution was applied for 5 min. All slides received pretreatment with citrate for antigen retrieval. Then, RFP primary antibody was allowed to react at room temperature for 60 min in dilution of 1:750. After washing in PBS, secondary antibody was applied for 10 min, followed by horseradish peroxidase-marked strepto-avidin for 10 min. Peroxidase was visualized by diaminobenzidine tetrahydrochloride containing 0.3% H<sub>2</sub>O<sub>2</sub>. Nuclei were stained with Harris' hematoxylin. Appropriate controls were included for this study. The RFP expression was evaluated as positive when there was nuclear staining in more than 5% of nuclei.

## RESULTS

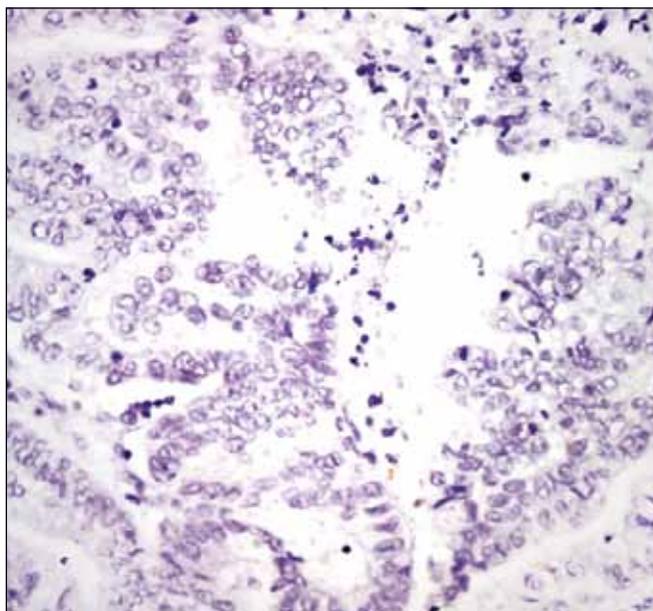
RFP expression was negative in 18 benign cases (secretory endometrium [1 cases], proliferative endometrium [2 cases], endometrial polyp [5 cases], benign endometrial hyperplasia [3 cases] and EIN [5 cases]) except for 2 secretory endometrium cases showing weak focal nuclear positivity (Figure 1). Among 21 cases of endometrial carcinoma, all type I cases were negative (Figure 2). Interestingly all of the serous carcinoma cases (3 cases) showed diffuse strong nuclear positivity (Figure 3). Of

note, one of the two endometrial clear cell carcinoma cases showed intermediate nuclear positivity.

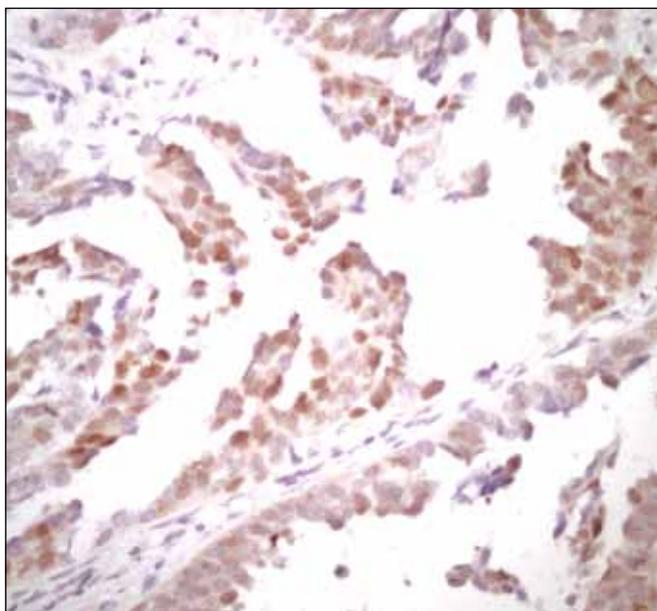
After the strong nuclear positive results for only endometrial serous adenocarcinoma cases, 12 cases including 9 ovarian and 3 endometrial serous carcinoma cases were added to the study to analyze tissue and/or morphology specificity. All of these additional 12 cases; including 9 ovarian and 3 endometrial serous carcinoma cases were also showed strong and diffuse positivity for nuclear RFP expression (Figure 4).



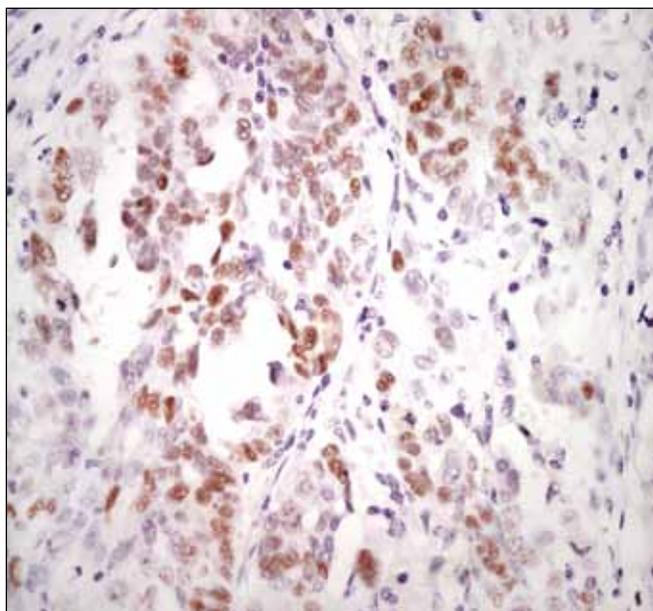
**Figure 1:** Immunohistochemical staining for RFP expression in secretory endometrium (x200).



**Figure 2:** Immunohistochemical staining for RFP expression in endometrioid type endometrial carcinoma (x200).



**Figure 3:** Immunohistochemical staining for RFP expression in endometrial serous carcinoma (x200).



**Figure 4:** Immunohistochemical staining for RFP expression in ovarian serous carcinoma (x200).

## DISCUSSION

In the present study, we evaluated the expression of RFP in non-neoplastic endometrium samples as well as type I endometrial cancer in comparison to Type II endometrial cancer and found that nuclear expression of RFP was strongly correlated with serous carcinoma morphology. Due to strong positive results for serous endometrial cancer, we also included ovarian serous adenocarcinoma cases, all of which were stained strongly for RFP. This is the first report to demonstrate the differential expression RFP in 2 major subtypes of endometrial carcinoma as well as ovarian serous carcinoma.

RET finger protein, which belongs to the large B-box RING finger protein family, is widely expressed in various normal and tumor tissues, including liver, kidney, testis, breast, endometrium and germ cell tumors, namely seminomas (11-13, 15-17). There are over 200 members of the RING finger protein family reported to date, including PML, BMI1/Mel-18, RING1, and KAP-1 (8, 18, 19).

Apart from histological and clinical features, Type I and Type II endometrial cancers are further distinguished by genetic alterations since they are associated with mutations of independent sets of genes. While Type I tumors show microsatellite instability and involve mutations in PTEN (17, 20-23), K-ras (17), and  $\beta$ -catenin (20), as well as defects in DNA mismatch repair (24, 25); Type II tumors frequently exhibit aneuploidy and p53 mutations (26-29), as well as overexpression of p16 (29, 30), insulin-like growth factor II messenger RNA-binding protein (31), HER-2/neu (c-ErbB2) (32, 33) and have alterations of E-cadherin (34, 35) and claudins (36-40).

In contrast to significant advances in the study of type I (mainly endometrioid carcinomas) endometrial carcinogenesis, studies on type II (mainly ESC) carcinogenesis have been limited. The higher case-fatality rate associated with type II cancers is probably at least in part a function of the advanced stage at which a significant proportion of patients with type II cancers present (41). Zheng et al. recently proposed a model in an effort to understand endometrial serous carcinogenesis so that its precancers can be delineated and treated, stating that endometrial serous carcinoma starts from latent precancer (p53 signature glands), develop into precancerous lesions (endometrial glandular dysplasia), then into early serous cancer (serous endometrial intraepithelial carcinoma), and finally to fully developed endometrial serous carcinoma (42).

Currently, how RFP contributes to carcinogenesis is unclear. However, RING finger proteins are thought to play roles in the formation and architecture of large protein complexes that contribute to diverse cellular processes such as oncogenesis, apoptosis, development, and ubiquitination (8, 18, 19, 43-45). We had previously reported a significant correlation between RFP staining and overexpression of ErbB2, which is a well defined poor prognostic factor for invasive breast carcinoma, both at protein and gene level (11). Townson et al. previously reported that; although RFP did not interact with estrogen receptor- $\alpha$  (ESR1) directly, that RFP is a component of an ESR1 regulatory complex (46). RFP was also shown to make complexes with histone deacetylase 1 (HDAC1) and transcription factor NF-Y (15). This protein complex is reported to confer resistance to anticancer drugs by decreasing the expression of thioredoxin binding protein-2 (TBP-2); which is an inhibitor of thioredoxin, a scavenger of reactive oxygen species (ROS); and sensitizes cells to oxidative stress and cisplatin (47-50).

Tsukamoto et al. had previously reported RFP expression as a predictive marker for an unfavorable clinical outcome in patients with endometrial cancer irrespective of histological type and proposed positive RFP expression as an independent prognostic factor for survival (15).

The mechanism by which RFP plays a role in development of serous carcinoma is yet to be investigated. Tsukamoto et al. had found that integrin  $\beta$ 1 and integrin  $\alpha$ 2 expressions were significantly decreased in RFP knockdown cells, suggesting that importance of these integrins might play a role in the progression of endometrial cancer (15). They also suggested that RFP expression might be associated with the poor clinical outcome of endometrial cancer by regulating cancer cell resistance to oxidative stress and resistance to anticancer drugs such as cisplatin (15). The role of RFP in estrogen signaling pathways might also be of importance regarding the frequent loss of expression of the estrogen and progesterone receptors in serous endometrial carcinomas (26, 51, 52) and RFP being proposed a component of an ESR1 regulatory complex (46).

In this study, we found that RFP stains specifically the serous type endometrial carcinomas and none of endometrioid type cancers, irrespective of grade. Although the assessment of cell type is straightforward in most cases and there is a reported reproducibility of 0.62 to 0.87, determining the cell of origin can become problematic; especially in high grade tumors (53-56). According to World Health Organization definitions, the diagnosis of primary endometrioid adenocarcinoma should be given

where the tumor is composed of glands resembling those of the normal endometrium, generally in a background of atypical hyperplasia, whereas serous carcinoma is characterized by a complex pattern of papillae with cellular budding and not infrequently psammoma bodies, typically in an atrophic endometrium or with accompanying high-grade in situ carcinoma (endometrial intraepithelial carcinoma) (57). However, endometrioid carcinomas showing papillary growth or slit-like glandular spaces and serous carcinomas having a predominantly or exclusively glandular architecture, or areas of solid growth exist in real life (58, 59). This is where immunohistochemistry is being more and more frequently used as an adjuvant to H&E diagnosis. The markers most commonly used currently in clinic are ER, PR, p53, p16, proliferation index and PTEN, with varying sensitivity and specificities (60). Approximately 70-90% of serous carcinomas express strong and diffuse nuclear p53 staining; however p53 is overexpressed in 10-35% of endometrioid carcinomas, and majority of these are the high grade ones (28, 58, 61-63). Almost all low-grade and most of high grade endometrioid carcinomas show ER and/or PR expression and pure serous carcinomas are expected typically to be negative for ER and PR. However, serous carcinomas associated with low-grade endometrioid carcinoma or atypical hyperplasia often show ER and/or PR expression (26). A high proliferation index is 'typical' of serous carcinoma (mean labelling index of 50% positive nuclei versus 20%), although there is no real cut-off value (64). Serous carcinomas should demonstrate diffuse and strong p16 staining and endometrioid carcinomas may show patchy weak to even moderate staining with p16 regardless of grade (30, 65). Loss of expression PTEN is also reported to be characteristic of endometrioid carcinoma, especially of high grade endometrioid compared to serous histology (58). Currently a panel of at least 3-4 markers are needed to aid in the separation of endometrioid versus serous type, especially in cases with atypical morphological and high grade features. The strong correlation of RFP expression with serous morphology in our study not only in carcinomas of endometrium but also of ovarian origin might greatly ease this distinction as more is found out about the molecular role of RFP in serous tumor carcinogenesis.

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