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Villin immunohistochemical expression in endometrial carcinoma

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Summary

Introduction: Villin is a highly specialised protein and is expressed in intestinal epithelium. It was detected in colorectal carcinomas and other non-gastrointestinal tumours. The aim of the current study was to investigate the immunohistochemical expression of villin in endometrial carcinoma and normal endometrium. *Materials and Methods:* Tissue microarray blocks were prepared from 73 archival of endometrial carcinomas obtained from the Department of Pathology at King Abdulaziz University Jeddah, Saudi Arabia. Tissue sections were stained using anti-human villin monoclonal antibody. The extent of immunostaining results were recorded and categorised. *Results:* Positive immunostaining for villin was observed in 12.3% of endometrial carcinoma and was negative in all non-neoplastic endometrial tissues. Villin staining was observed in the apical surface cytoplasm and nucleus. Villin expression showed no age preference. All positive tumours are of the endometrioid type with no preference to histological grade or stage. *Conclusion:* In summary, the authors observed the immunostaining in a small number of endometrial carcinomas in the apical border, cytoplasm, and for the first time in nucleus. Endometrial carcinomas should be considered when evaluating patients with villin positive adenocarcinoma of unknown origin. The mechanism and the importance of villin immunostaining and its expression pattern in endometrial carcinoma needs further investigations.

Key Words: Endometrium; Tissue microarray; Immunohistochemistry; Antibody.

Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract [1]. Endometrial carcinoma represents a health problem worldwide as it has increased in incidence 21% since 2008 and has doubled its death rate [2]. In Saudi Arabia, endometrial carcinoma constitutes the sixth most common cancer in females (4.1%) [3]. Endometrial carcinoma is classified into two major groups, types I and II, according to clinicopathological features and molecular pathogenetic mechanisms [4].

Villin protein is one of the gelsolin family of calcium-regulated actin-binding proteins [5]. Villin was first isolated and characterised in the microvilli of intestinal epithelium and later on was found in many absorptive epithelia [6, 7]. Under normal physiological conditions, villin is primarily expressed in epithelial cells of the digestive tract, renal proximal tubules, and hepatic bile ducts, with no expression being reported in normal lung cells, such as the bronchiolar epithelium, alveolar cells, or bronchial gland cells [8-10]. The intestinal microvilli in the apical membrane (brush border) are maintained by bundles of parallel actin filaments that are organised by multiple actin-binding proteins including villin [11].

Villin was shown to be expressed in colorectal carcinoma and correlated with differentiation [12]. Accordingly, it has been used to differentiate neoplasms of intestinal origin from non-intestinal neoplasms [9, 10]. Villin is also expressed in gastrointestinal neuroendocrine tumours [13]. Villin in endometrial carcinoma has not been intensively investigated. The aim of this study is to explore the occurrence and distribution of villin immunostaining in endometrial carcinoma.

Materials and Methods

The study included paraffin wax tumour blocks from 73 patients diagnosed with endometrial carcinoma in the period from 2003-2012. Also paraffin blocks from non-neoplastic endometria of 30 patients in the period from 1995-1998 were included (20 proliferative endometrium and ten secretory endometrium). All blocks were retrieved from the archives of the Department of Pathology at King Abdulaziz University, Jeddah, Saudi Arabia. Some clinicopathological characteristics of patients are listed in Table 1. The study was performed in accordance with the ethics committee of Faculty of Medicine, King Abdulaziz University, Saudi Arabia, and declaration of Helsinki.

Archival paraffin-embedded endometrial carcinoma samples and neoplastic tissues were selected and representative areas were

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Table 1. — *Clinicopathological features of patients.*

	Parameter	Number (%)
Age	Below 50 years	21 (28.7%)
	50 years and more	52 (71.3%)
Histological type	Endometrioid	68 (93.1%)
	Serous	5 (6.9%)
Tumour grade	Well differentiated	44 (60.3%)
	Moderately differentiated	18 (24.7%)
	Poorly differentiated	11 (15%)
FIGO staging	IA	21 (28.7%)
	IB	12 (16.4%)
	II	4 (5.5%)
	IIIB	1 (1.4%)
	IIIC	6 (8.2%)
	IVA	2 (2.7%)
	IVB	1 (1.4%)
	Unstaged	29 (39.7%)

FIGO (International Federation of Gynaecology and Obstetrics)
Stage I: The tumour is confined to the uterine fundus.
Stage IA: The tumour is limited to the endometrium.
Stage IB: The tumour invades through less than 50% of the myometrial thickness.
Stage IC: The tumour invades through greater than 50% of the myometrial thickness.
Stage II: The tumour invades the cervical stroma.
Stage III: There is regional tumour spread.
Stage IIIA: The tumour penetrates the uterine serosa, involves adnexa.
Stage IIIB: The tumour involves the vagina.
Stage IIIC: The tumour involves regional lymph nodes.
Stage IV: The tumour invades contiguous organs or has metastasized to remote organ sites.
Stage IVA: The tumour invades the bladder or rectum.
Stage IVB: Distant metastases are present.

marked on haematoxylin and eosin (H&E)-stained slides. Two tissue cylinders (cores) with a diameter of 1.5 mm were punched from morphologically representative tissue areas of each 'donor' tissue block and brought into new recipient paraffin blocks by using an automated tissue microarray instrument (TMA Master 1.14 SP3). Placental tissue was used for orientation [14].

TMA blocks were cut at four µm, and mounted on positive-charged slides. Sections were deparaffinised in xylene and rehydrated in an automated immunostainer. Pre-treatment was done using CC1 (prediluted cell conditioning solution) for 60 minutes. Anti-human rabbit polyclonal anti-villin antibody (E1664) was used at dilution 1:50 with incubation time 30 minutes. DAB detection kit was used according to kit manufacturer instructions. Subsequently, slides were washed, counterstained with Mayer's haematoxylin and mounted. Negative control and positive control slides were included.

In order to evaluate villin immunostaining, the subcellular localisation of villin in tumour cells and non-neoplastic endometrial cells was observed as previously described [9, 10]. To assess the extent (%) of villin expression (apical membranous and cytoplasmic), all available tumour cells and non-neoplastic endometrial cells in each section were counted at microscope magnification of 200×. Positive cells were then counted. The mean values of positivity were calculated and expressed as a percentage of the total number of normal and tumour cells. The extent of villin immunostaining was categorised as negative expression (0 = 0%), low expression (1 = 1–9%), moderate expression (2 = 10–50%), and high expression (3 ≥ 50%) [12].

Results

In the current study, a positive immunostaining for villin was observed in nine out of 73 patients with endometrial carcinoma (12.3%). While in non-neoplastic endometrial tissues, no staining for villin was observed. The pattern of staining in endometrial carcinoma was in the apical surface of malignant glands (a brush border like pattern) (1/73 = 1.4%), diffuse cytoplasmic staining (7/73 = 9.6%), and nuclear staining (2/73 = 2.7%) (Figure 1). The extent of villin immunostaining was as follows; moderate apical staining pattern (21%), the diffuse cytoplasmic staining (five moderate staining and two low staining), while for nuclear staining moderate staining in one occasion and low occasion in the other. Villin expression showed no age preference. All positive tumours are of the endometrioid type with no preference to histological grade or stage.

Discussion

Villin is primarily a protein of intestinal epithelium and later on was found in many absorptive epithelia [5-7]. It is not expressed in normal bronchiolar epithelium, alveolar cells, bronchial gland cells, or endometrial cells [8-10]. The expression of villin was detected in different malignant tumours [9-12, 15-17]. Villin expression was described in gastrointestinal, non-gastrointestinal carcinomas, and in neuroendocrine carcinoma from different organs [13].

In the current study, a larger number of endometrial carcinomas and non-neoplastic endometria were used to investigate the immunolocalisation of villin. Villin immunoexpression was detected in 12.3% of endometrial carcinomas studied and none of the non-neoplastic endometrial tissues showed staining for villin. The current cohort is far larger than shown previously by Moll *et al.*, who revealed villin expression in 4/11 of endometrial carcinoma and none of normal tissues [9].

In the present study, there were three patterns of villin subcellular localisation; apical, cytoplasmic, and nuclear. The apical localisation corresponded to the gastrointestinal brush border localisation. Villin brush border staining was described to be exclusive for gastrointestinal adenocarcinoma [10, 18, 19]. However, the apical brush border staining was lost in some of cases of colorectal carcinoma [11, 12]. Abnormal localisation has been described in neuroendocrine carcinoma from different organs [13]. Cytoplasmic villin staining was described in 31% of hepatocellular carcinoma, while in cholangiocarcinoma cases it demonstrated cytoplasmic and apical staining [15, 17].

In the current study, the extent of expression was either low or moderate. The authors did not find any correlation of villin staining (apical, cytoplasmic, or nuclear) with clinicopathological features of tumours. Villin staining was

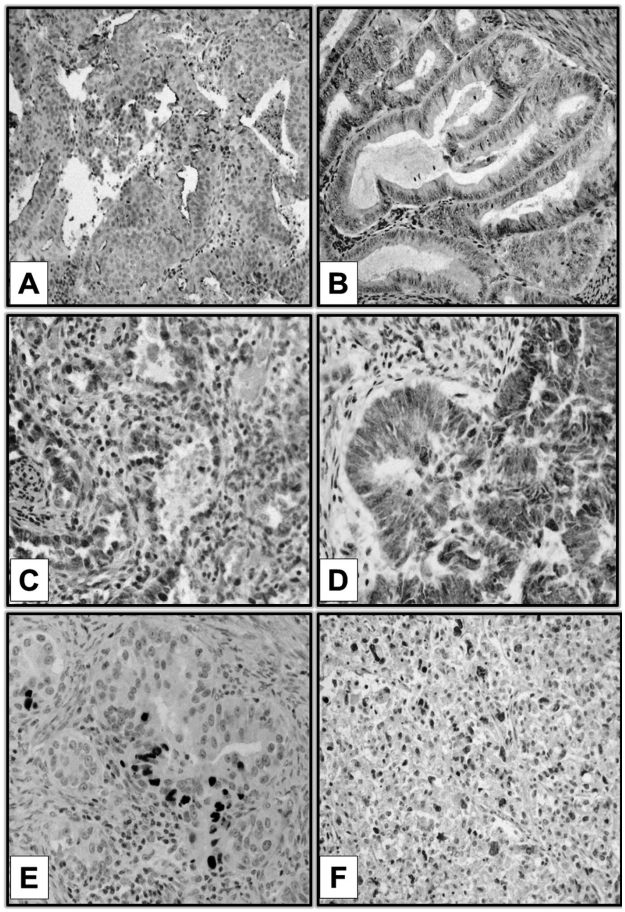

Figure 1. — Immunostaining of villin in endometrial carcinoma. Apical staining in the border of malignant cells (A) (100×). Focal villin immunostaining within the cytoplasm of malignant cells in a well differentiated endometrial carcinoma (B), a moderately differentiated endometrial carcinoma (C, D) (200×). Strong nuclear villin immunostaining in a well differentiated endometrial carcinoma (E) (200×). Negative immunostaining of villin in a poorly differentiated endometrial carcinoma (F) (200×). Immunohistochemical labelling was done using the anti-villin antibody and diaminobenzidine used as the chromogen and haematoxylin as counterstain.

lacking in normal endometrium, which is similar to previous findings [9] and contrary to colon [12]. The presence of villin in endometrial carcinomas and complete negativity in normal endometrium may indicate novel expression of villin in premalignant endometrial lesions or appears only in malignant endometrium.

Another finding in the present study is the nuclear localisation of villin. This is novel for the current study. Although the number of cases was very low, the staining extent and intensity may indicate the importance of nuclear appearance of villin.

The present results are more difficult to explain. All cases were proven to be primary endometrial in origin. The presence of original intestinal metaplasia may be considered. Mucinous differentiation in endometrioid adenocarcinomas is not uncommon and pure mucinous adenocarcinomas of the endometrium comprise 1-10% of all endometrioid adenocarcinomas. However the presence of intestinal differentiation and of goblet cells in particular, within the endometrium, is rare. [20]. The presence of intestinal-type differentiation in endometrial carcinoma may occur in primary endometrial carcinoma and should also warrant a metastatic carcinoma from the gastrointestinal tract. Intestinal endometrial metaplasia may be a precursor lesion leading to progression to the rare gastrointestinal-type adenocarcinoma. [21]. Also the presence of intestinal metaplasia possibly raises the association between intestinal metaplasia at different sites in the female genital tract [22]. Rare variants of mucin-producing endometrial carcinomas include those with gastric or intestinal differentiation [23].

In summary, in this study the authors evaluated villin in a large number of endometrial carcinomas. They observed the immunostaining in a small number of endometrioid carcinoma. Endometrial carcinomas in the apical border, cytoplasm, and for the first time, in nucleus. The mechanism of translocation of villin to the cytoplasm and nucleus of malignant cells has not yet been clarified and needs to be addressed. Endometrial carcinomas should be considered when evaluating patients with villin positive adenocarcinoma of unknown origin. When the differential diagnosis is between intestinal carcinoma and endometrial carcinoma, villin is recommended to be added to the usual immunohistochemistry panel, taking in consideration the frequent expression of villin in the former and the rarity of the expression in the latter.

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References

[1] Burke W.M,. Orr J., Leitao M., Salom E., Gehrig P., Olawaiye A.B., *et al.*: "Endometrial cancer: a review and current management strategies: part I". *Gynecol. Oncol.*, 2014, *134*, 385.
[2] Sorosky J.I.: "Endometrial cancer". *Obstet. Gynecol.*, 2012, *120*, 383.
[3] The Saudi Cancer Registry: "Cancer Incidence Report 2014".
[4] Yoo S.H., Park B.H., Choi J., Yoo J., Lee S.W., Kim Y.M., Kim K.R.: "Papillary mucinous metaplasia of the endometrium as a possible precursor of endometrial mucinous adenocarcinoma". *Mod. Pathol.*, 2012, *25*, 1496.
[5] Athman R., Louvard D., Robine S.: "The epithelial cell cytoskeleton and intracellular trafficking. III. How is villin involved in the actin cytoskeleton dynamics in intestinal cells?" *Am. J. Physiol. Gastrointest Liver Physiol.,* 2002, *283*, G496.
[6] Bretscher A., Weber K.: "Villin is a major protein of the microvillus cytoskeleton which binds both G and F actin in a calcium-dependent manner". *Cell*,1980, *20*, 839.

[7] Braunstein E.M., Qiao X.T., Madison B., Pinson K., Dunbar L., Gumucio D.L.: "Villin: A marker for development of the epithelial pyloric border". *Dev. Dyn*., 2002, *224*, 90.
[8] Robine S., Huet C., Moll R., Sahuquillo-Merino C., Coudrier E., Zweibaum A., Louvard D.: "Can villin be used to identify malignant and undifferentiated normal digestive epithelial cells?" *Proc. Natl. Acad. Sci. U. S. A.,* 1985, *82*, 8488.
[9] Moll R., Robine S., Dudouet B., Louvard D.: "Villin: a cytoskeletal protein and a differentiation marker expressed in some human adenocarcinomas". *Virchows Arch. B. Cell. Pathol. Incl. Mol. Pathol.,* 1987, *54*, 155.
[10] Bacchi C.E., Gown A.M.: "Distribution and pattern of expression of villin, a gastrointestinal-associated cytoskeletal protein, in human carcinomas: a study employing paraffin-embedded tissue". *Lab Invest*., 1991, *64*, 418.
[11] Arango D., Al-Obaidi S., Williams D.S., Dopeso H., Mazzolini R., Corner G., *et al.*: "Villin expression is frequently lost in poorly differentiated colon cancer". *Am. J. Pathol.,* 2012, *180*, 1509.
[12] Al-Maghrabi J., Gomaa W., Buhmeida A., Al-Qahtani M., Al-Ahwal M.: "Loss of villin immunoexpression in colorectal carcinoma is associated with poor differentiation and survival". *ISRN Gastroenterol.,* 2013, *2013*, 679724.
[13] Zhang P.J., Harris K.R., Alobeid B., Brooks J.J.: "Immunoexpression of villin in neuroendocrine tumors and its diagnostic implications". *Arch. Pathol. Lab. Med.,* 1999, *123*, 812.
[14] Al-Maghrabi J., Gomaa W., Buhmeida A., Qari Y., Al-Qahtani M., Al-Ahwal M.: "Prognostic significance of VEGFR1/Flt-1 immunoexpression in colorectal carcinoma". *Tumour Biol.,* 2014, *35*, 9045.
[15] Al-Muhannadi N., Ansari N., Brahmi U., Satir A.A.: "Differential diagnosis of malignant epithelial tumours in the liver: an immunohistochemical study on liver biopsy material". *Ann. Hepatol.,* 2011, *10*, 508.
[16] Grone H.J., Weber K., Helmchen U., Osborn M.: "Villin—a marker of brush border differentiation and cellular origin in human renal cell carcinoma". *Am. J. Pathol.,* 1986, *124*, 294.
[17] Lau S.K., Prakash S., Geller S.A., Alsabeh R.: "Comparative immunohistochemical profile of hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma". *Hum. Pathol.,* 2002, *33*, 1175.
[18] Yoshino N., Kubokura H., Yamauchi S., Ohaki Y., Koizumi K., Shimizu K.: "Mucinous carcinoma identified as lung metastasis from an early rectal cancer with submucosal invasion by immunohistochemical detection of villin". *Jpn. J. Thorac. Cardiovasc. Surg.,* 2006, *54*, 328.
[19] Nambu Y., Iannettoni M.D., Orringer M.B., Beer D.G.: "Unique expression patterns and alterations in the intestinal protein villin in primary and metastatic pulmonary adenocarcinomas". *Mol. Carcinog.,* 1998, *23*, 234.
[20] Nieuwenhuizen L., Khalil M.K., Naik V.R., Othman N.H.: "Prevalence of goblet cell metaplasia in endocervical and endometrial adenocarcinoma: a histochemical study". *Malays J. Med. Sci.,* 2007, *14*, 56.
[21] Rubio A., Schuldt M., Guarch R., Laplaza Y., Giordano G., Nogales F.F.: "Pseudomyxoma-type invasion in gastrointestinal adenocarcinomas of endometrium and cervix: a report of 2 cases". *Int. J. Gynecol. Pathol.,* 2016, *35*, 118.
[22] Nicolae A., Goyenaga P., McCluggage W.G., Preda O., Nogales F.F.: "Endometrial intestinal metaplasia: a report of two cases, including one associated with cervical intestinal and pyloric metaplasia". *Int. J. Gynecol. Pathol.,* 2011, *30*, 492.
[23] Zheng W., Yang G.C., Godwin T.A., Caputo T.A., Zuna R.E.: "Mucinous adenocarcinoma of the endometrium with intestinal differentiation: a case report". *Hum. Pathol.,* 1995, *26*, 1385.

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