

Original Article

Shadow cell differentiation from squamoid morule in endometrial adenoacanthoma

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Abstract: Shadow cell differentiation (SCD), commonly found in cutaneous pilomatricoma (PMX), has been said to be extremely rare in extracutaneous tumors and its morphogenesis has not been clarified yet. In the present study, 25 cases of endometrial adenoacanthoma were examined with special reference to SCD and with immunohistochemistry for beta-catenin and CD10. Shadow cell nests (SCNs) were observed in 2 out of 5 cases of adenocarcinoma with squamoid morules and all of 4 cases of adenocarcinoma with squamous differentiation and morules, but not in any cases of adenocarcinoma with squamous differentiation. SCNs were just adjacent to morules with or without a mutual transition. Immunohistochemical examination revealed nuclear accumulation of beta-catenin and expression of CD10 in the squamoid morules around SCNs. These results indicate that SCNs are derived from squamoid morules in endometrial adenoacanthoma, and established a link between matrical basaloid cells in PMX and squamoid morules in endometrial adenoacanthoma, as common original tissues, showing nuclear accumulation of beta-catenin and expression of CD10, of SCNs. It seems that SCD is not so uncommon as previously estimated in endometrial adenoacanthoma.

Keywords: Endometrial adenoacanthoma, squamoid morule, shadow cell, beta-catenin, CD10

Introduction

Shadow cells are specialized form of cornified cells differentiating toward hair matrix and show different phenotypes from conventional keratinized cells [1]. Shadow cell differentiation (SCD) is commonly found in cutaneous pilomatricoma (PMX), craniopharyngioma and odontogenic cyst [2], while quite uncommon in extracutaneous tumors. Apart from gonadal teratomas and extragonadal dermoid cysts [3-6], pathogenesis of SCD in visceral carcinoma has not been well examined, because of its rarity. Although there have been only 15 reported cases of visceral carcinoma with SCD except for teratomatous tumors [7-16], we noticed the presence of shadow cell nests (SCNs) in endometrial carcinoma in several cases, all of which were adenoacanthoma (endometrioid adenocarcinoma with squamous metaplasia). In the present study, therefore, we tried to clarify the incidence and pathogenesis of SCD in endometrial adenoacanthoma.

Materials and methods

We checked up all cases of endometrial carcinoma registered on Pathological Database at Suwa Red Cross Hospital (Nagano, Japan) from 2010 to 2014. Twenty-five cases of endometrioid adenocarcinoma with squamous metaplasia were retrieved. These cases were classified into 3 groups: (a) adenocarcinoma with conventional squamous differentiation (ASqD), (b) adenocarcinoma with squamoid morules (ASqM) and (c) adenocarcinoma with both conventional squamous components and squamoid morules (ASqDM). Diagnostic criteria of morule were followed by the description by Nicolae et al. [17]. All cases of endometrial adenoacanthoma were examined histopathologically in the presence or absence of SCNs as well as histological characteristics of squamous/squamoid components around SCNs.

In each case, the representative tissue sections containing SCNs were automatically immunostained for beta-catenin (antibody: clone

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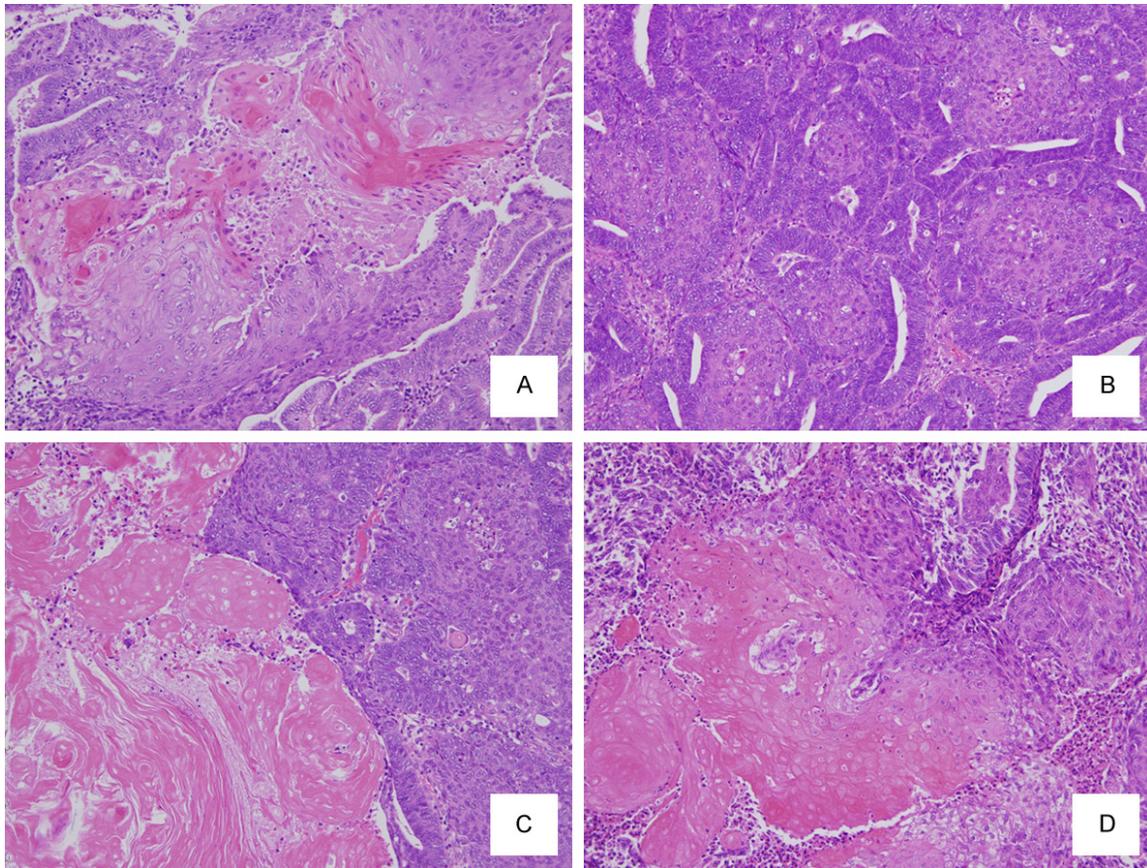


Figure 1. Endometrioid adenocarcinoma with squamous differentiation (A), with squamoid morules (B, D), and with squamous differentiation and morules (C). Shadow cell nests are observed in (C) and (D) with or without interposition of “transitional” cells between shadow cells and morules (hematoxylin and eosin stain). A mutual transition between shadow cell nests and keratinizing foci is observed in (C).

Table 1. Incidence of shadow cell differentiation (SCD) in endometrial adenoacanthoma

	Total No. of cases	No. of cases showing SCD	No of cases showing nuclear accumulation beta-catenin
ASqD	16	0	2
ASqM	5	2	5
ASqDM	4	4	4
Total	25	6	11

ASqD: adenocarcinoma with conventional squamous differentiation; ASqM: adenocarcinoma with squamoid morules; ASqDM: adenocarcinoma with conventional squamous differentiation and morules.

17C2, Leica Microsystems, Newcastle, UK; 1:200 dilution) and CD10 (antibody: clone 56C6, Medical & Biological Laboratories, Nagoya, Japan; 1:150 dilution) by Ventana BenchMark LT (Roche Diagnostics, Basel, Switzerland).

This work was carried out according to the criteria required by the institutional review board on ethical aspects.

Results

Incidence and morphology of SCNs

Out of 25 cases of endometrial adenoacanthoma, 16 cases were classified as ASqD (Figure 1A), 5 cases as ASqM (Figure 1B) and 4 cases as ASqDM (Figure 1C). SCNs were found in 2 cases of ASqM and all of 4 cases of ASqDM, but not in any cases of ASqD (Table 1). SCNs were minute in size in most of the cases and showed the same histological features as those in PMX; i.e., shadow cells preserved cell shape with eosinophilic cytoplasm and ghost-like disappeared nucleus (Figure 1C, 1D). An abrupt transition between squamoid morules and SCNs was usually observed (Figure 1C), while,

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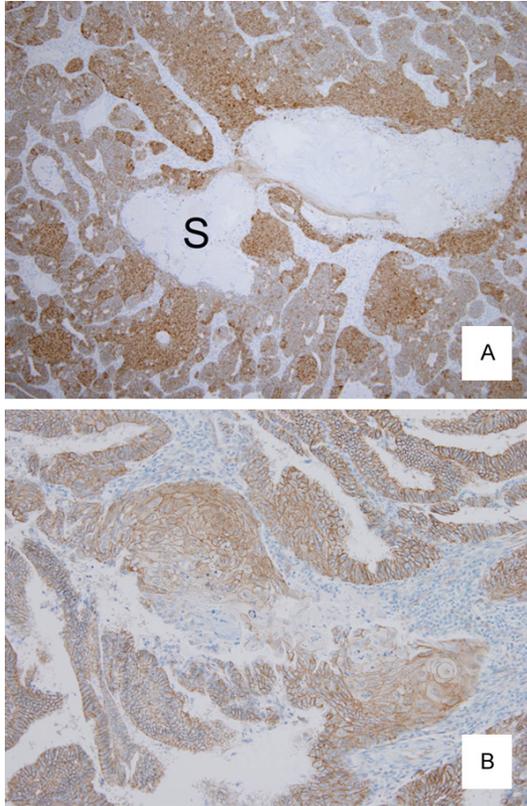


Figure 2. Immunohistochemistry for beta-catenin, showing nuclear accumulation in squamoid morules (A) and membranous localization in the area of squamous differentiation (B). Shadow cell nests (S) are negative for beta-catenin.

in 4 cases, a gradual transition from morule to SCNs was focally observed (**Figure 1D**).

All cases of ASqDM contained both SCNs and keratinized foci, which were just adjacent each other. These two components were histopathologically distinguished each other, while, in part, they showed mutual transition and were somewhat difficult to be differentiated (**Figure 1C**).

Immunohistochemistry

In ASqM and ASqDM, intense nuclear and weak cytoplasmic immunoreaction of beta-catenin was observed in squamoid morules (**Figure 2A**), irrespective of the presence or absence of SCNs, in all cases. Multiple small foci showing the same immunoreaction pattern as that in the morules were also found in the component of adenocarcinoma around the morules. Some “transitional” cells between SCNs and morules also showed nuclear and cytoplasmic localiza-

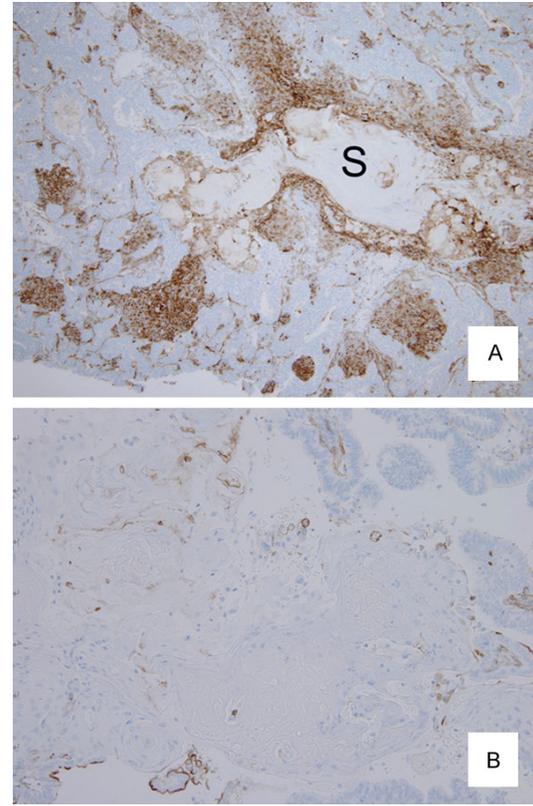


Figure 3. Expression of CD10 in squamoid morules (A), but not in squamous differentiation foci (B). Shadow cell nests (S) are negative for CD10.

tion of beta-catenin. SCNs were completely negative for beta-catenin in all cases examined. In ASqD, on the other hand, adenocarcinoma as well as squamous components showed membranous, but not nuclear, immunolocalization of beta-catenin (**Figure 2B**). Small foci of conventional squamous differentiation showed nuclear accumulation of beta-catenin in 3 cases of ASqDM and 2 cases of ASqD.

CD10 was also immunopositive in the cytoplasm or cell membrane of morules as well as adenocarcinoma component in all cases of ASqM and ASqDM (**Figure 3A**). SCNs *per se* were not immunoreactive for CD10. On the other hand, squamous foci in ASqD were negative for CD10 in any cases (**Figure 3B**), except for focal staining in squamous foci in 2 cases (the same ones as those showing focal nuclear accumulation of beta-catenin).

Discussion

Squamoid morule is found in carcinoma of various organs, particularly of endometrium, and

had been regarded as relatively immature squamous metaplasia or squamous differentiation cell clusters [18, 19]. Recent reports, however, have shown that morule is phenotypically and genotypically different from conventional squamous metaplasia; morules in colonic and endometrial neoplasia were characterized by nuclear accumulation of beta-catenin [20-24] as well as mutation of beta-catenin gene [21], while conventional squamous metaplasia showed membranous, not nuclear, immunolocalization of beta-catenin without genetic mutation [20-24]. Expression of CD10 [20, 23, 24] and CDX2 [24] was also frequent in morules, while rarely observed in conventional squamous metaplasia [20, 23, 24]. Also in the current study, morules and conventional squamous metaplasia were differentiated by the localization pattern of beta-catenin and expression of CD10.

There have been only 5 cases of endometrial carcinoma or atypical endometrial hyperplasia with SCD in the literature [9, 15], while SCNs were found in 6 (24%) out of 25 cases of endometrial adenoacanthoma in the present study. It seems that SCD is not so uncommon as formerly estimated; probably SCNs had been missed, ignored or just recognized as conventional squamous metaplasia in the routine diagnostic practice. Furthermore, all cases of endometrial adenoacanthoma with SCD were ASqM or ASqDM. SCNs were found just adjacent to morules, but were not associated with ASqD with or without keratinization. Such a close relationship between SCNs and morules has not been described so far. It is consequently suggested that SCNs in endometrial adenoacanthoma is derived from squamoid morule, but not from conventional squamous metaplasia. This provides further evidence on difference between SCD and conventional squamous differentiation/keratinization. It should be noted, on the other hand, that SCD and keratinization are not completely different phenomenon but compose a chain of differentiation style, from the following results in the present study: (a) co-existence of SCNs and keratinized components with mutual transition was observed in all of 4 cases of ASqDM and (b) squamous-differentiated cells showed focal nuclear accumulation of beta-catenin and expression of CD10 in 2 cases of ASqD.

SCNs are observed not only in endometrial adenoacanthoma, but also in other tumors such as PMX, ovarian and colorectal carcinoma. In PMX, matrical basaloid cells, the original tissue of SCNs, are characterized by nuclear accumulation of beta-catenin [2] and expression of CD10 [25], as morules are. As for carcinoma with SCD in other sites, we encountered 2 cases of bladder carcinoma [14] and gastric carcinoma (manuscript in preparation), although such cases have been rarely reported. The tumor cells in both cases revealed basaloid appearance with nuclear accumulation of beta-catenin and immunoreaction for CD10, whereas the cases of urothelial carcinoma with conventional squamous differentiation did not show such immunoreactivity [14]. These findings are identical to those in endometrioid adenoacanthoma in the present study, suggesting that nuclear accumulation of beta-catenin and expression of CD10 may be common phenotypic changes in the tumors with SCD regardless of primary site or histological appearance. Further examination of carcinoma showing SCD in other sites is necessary.

In conclusion, the present study established a link between basaloid cells in PMX and morules in endometrial adenoacanthoma, as original tissues of SCNs showing nuclear accumulation of beta-catenin and expression of CD10.

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Disclosure of conflict of interest

None.

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References

- [1] Nakamura T. Comparative immunohistochemical analyses on the modes of cell death in epidermal cyst, trichilemmal cyst and pilomatrixoma. *Am J Dermatopathol* 2011; 33: 78-83.

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- [2] Hassanein AM, Glanz SM, Kessler HP, Eskin TA, Lin C. Beta-catenin is expressed aberrantly in tumors expressing shadow cells. Pilomatricoma, craniopharyngioma and calcifying odontogenic cyst. *Am J Clin Pathol* 2003; 120: 732-736.
- [3] Minkowitz G, Lee M, Minkowitz S. Pilomatricoma of the testicle. An ossifying testicular tumor with hair matrix differentiation. *Arch Pathol Lab Med* 1995; 119: 96-99.
- [4] Zamecnik M, Mukensnabl P, Curik R, Michal M. Shadow cell differentiation in testicular teratomas. A report of two cases. *Ces-slov Patol* 2005; 41: 102-106.
- [5] Ulbright TM, Srigley JR. Dermoid cyst of the testis: a study of five postpubertal cases, including a pilomatricoma-like variant, with evidence supporting its separate classification from mature testicular teratoma. *Am J Surg Pathol* 2001; 25: 788-793.
- [6] Hitchcock MG, Ellington KS, Friedman AH, Provenza JM, McLendon RE. Shadow cells in an intracranial dermoid cyst. *Arch Pathol Lab Med* 1995; 119: 371-373.
- [7] Fang J, Keh P, Katz L, Rao MS. Pilomatricoma-like endometrioid adenosquamous carcinoma of the ovary with neuroendocrine differentiation. *Gynecol Oncol* 1996; 61: 291-293.
- [8] Lalich D, Tawfik O, Chapman J, Fraga G. Cutaneous metastasis of ovarian carcinoma with shadow cells mimicking a primary pilomatric neoplasm. *Am J Dermatopathol* 2010; 32: 500-504.
- [9] Zamecnik M, Michal M. Shadow cell differentiation in tumours of the colon and uterus. *Zentralbl Pathol* 1995; 140: 421-426.
- [10] Nakayama H, Kimura A, Okumichi T, Miyazaki E, Kajihara H, Enzan H. Metaplastic shadow cells in rectal adenocarcinoma: Report of a case with immunohistochemical study. *Jpn J Clin Oncol* 1997; 27: 427-432.
- [11] Garcia-Escudero A, Navarro-Bustos G, Jurado-Escamez P, Rios-Martin J, Gonzalez-Campora R. Primary squamous cell carcinoma of the lung with pilomatricoma-like features. *Histopathology* 2002; 40: 201-202.
- [12] Zamecnik M, Michal M, Mukensnabl P. Pilomatricoma-like visceral carcinomas. *Histopathology* 1998; 33: 395.
- [13] Zamecnik M, Michal M, Mukensnabl P. Shadow cells in extracutaneous locations. *Arch Pathol Lab Med* 1996; 120: 426-428.
- [14] Nakamura T. Bladder carcinoma with shadow cell differentiation: a case report with immunohistochemical analyses. *Int J Clin Exp Pathol* 2012; 5: 840-844.
- [15] Squillaci S, Marchione R, Piccolomini M, Chiudinelli M, Fiumano E, Ungari M. Uterine endometrioid adenocarcinoma with extensive pilomatricoma-like areas. *Pathologica* 2013; 105: 8-10.
- [16] Zamecnik M, Jando D, Kascak P. Ovarian basaloid carcinoma with shadow cell differentiation. *Case Reports Pathol* 2014; ID 391947.
- [17] Nicolae A, Preda O, Nogales FF. Endometrial metaplasias and reactive changes: a spectrum of altered differentiation. *J Clin Pathol* 2011; 64: 97-106.
- [18] Buckley CH. Normal endometrium and non-proliferative conditions of the endometrium. In: Fox H, Wells M, editors. *Haines and Taylor obstetrical and gynecological pathology*. 5th edition. London: Churchill Livingstone; 2003. pp. 391-441.
- [19] Parsons MA. Disorders of growth, differentiation and morphogenesis. In: Underwood JCE editor. *General and systemic pathology*. 3rd edition. Edinburgh: Churchill Livingstone; 2000. pp. 73-99.
- [20] Ueo T, Kashima K, Daa T, Kondo Y, Sasaki A, Yokoyama S. Immunohistochemical analysis of morules in colonic neoplasms: morules are morphologically and qualitatively different from squamous metaplasia. *Pathobiology* 2005; 72: 269-278.
- [21] Saegusa M, Okayasu I. Frequent nuclear beta-catenin accumulation and associated mutations in endometrioid-type endometrial and ovarian carcinomas with squamous differentiation. *J Pathol* 2001; 194: 59-67.
- [22] Makishi S, Kinjo T, Sawada S, Chinen K, Hirayasu T, Hamada T, Saito K, Iwamasa T. Morules and morule-like features associated with carcinomas in various organs: report with immunohistochemical and molecular studies. *J Clin Pathol* 2006; 59: 95-100.
- [23] Chiarelli S, Buritica C, Litta P, Ciani S, Guarch R, Nogales FF. An immunohistochemical study of morules in endometrioid lesions of the female genital tract: CD10 is a characteristic marker of morular metaplasia. *Clin Cancer Res* 2006; 12: 4251-4256.
- [24] Houghton O, Connolly LE, McCluggage WG. Morules in endometrioid proliferations of the uterus and ovary consistently express the intestinal transcription factor CDX2. *Histopathology* 2008; 53: 156-165.
- [25] Yada K, Kashima K, Daa T, Kitano S, Fujiwara S, Yokoyama S. Expression of CD10 in basal cell carcinoma. *Am J Dermatopathol* 2004; 26: 463-471.