Shadow cell differentiation in endometrioid carcinomas of the uterus. Its frequent occurrence and beta-catenin expression

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SUMMARY

Shadow cell differentiation (SCD) is typical for pilomatrixoma and related follicular tumors of the skin. However, it has been described rarely in some extra-cutaneous lesions such as gonadal teratoma, craniopharyngioma, odontogenic cyst, and in rare visceral carcinomas (lung, bladder, gallbladder, uterus, ovary, and colon). In our practice, we have noticed that the occurrence of shadow cells is not very rare in endometrioid carcinoma (EC) of the uterus. For exact determination of SCD in these tumors, we reviewed 59 consecutive cases of uterine EC. The series included curettage and hysteroscopic specimens. We have found SCD in 9 (15.3 %) of the tumors. In these cases, the age of the patients and FIGO grade did not differ significantly from other ECs. Immunohistochemically, all ECs with SCD showed nuclear expression of beta-catenin in areas of SCD, indicating a possible role of the Wnt signaling pathway in tumorigenesis as well as a role of nuclear accumulation of beta-catenin by *trans*-differentiation from glandular toward squamous and shadow cell phenotypes. We have found that the relatively frequent presence of SCD in ECs can assists in the diagnosis of these tumors.

Keywords: beta-catenin – endometrioid carcinoma – shadow cell differentiation – uterus

"Shadow cell" diferenciácia v endometrioidných karcinómoch tela maternice. Jej častý výskyt a pozitivita na beta-katenín

SÚHRN

"Shadow cell" diferenciácia (SCD) je typická pre pilomatrixómy a iné trichogénne kožné tumory. Zriedka bola popísaná v extrakutánnych léziach ako sú gonadálny teratóm, kraniofaryngeóm, odontogénna cysta, niektoré viscerálne karcinómy (pľúc, žlčníka, močového mechúra, hrubého čreva, maternice a vaječníka). V bioptickej praxi sme si všimli, že "shadow" bunky nie sú príliš raritné v endometrioidných karcinómoch dutiny maternice. Pre presnejšie zistenie ich výskytu sme vyšetrili 59 konzekutívnych prípadov endometrioidného karcinómu (hysteroskopické biopsie a kyretáže). SCD sme našli v 9 prípadoch (15,3 %). Vek pacientiek a FIGO grading tumorov sa nelíšili od iných endometrioidných karcinómov. Imunohistochemicky bola u všetkých tumorov so SCD zistená jadrová pozitivita beta-katenínu viazaná na oblasť skvamóznej a "shadow cell" diferenciácie, čo suponuje úlohu mutácie príslušného génu v tumor-genéze (podobne ako u pilomatrixómu) a úlohu nukleárnej akumulácie beta-katenínu v *trans*-diferenciácii od glandulárneho smerom k skvamóznemu a "shadow cell" fenotypu. Vzhľadom k častému výskytu SCD v endometrioidnom karcinóme môže byť jej nález napomocný pri diagnostike tohto tumoru.

Kľúčové slová: beta-katenín – endometrioidný karcinóm – maternica – "shadow cell" differenciácia

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Shadow cells (ghost cells) are a specialized form of keratinized cells. They are typical for pilomatrixoma and other cutaneous lesions with follicular differentiation (1). It was suggested that they represent faulty attempts at differentiation toward hair (1,2). However, the shadow cell differentiation (SCD) was found in non-cutaneous lesions as well, such as gonadal teratomatous tumors (3-7), craniopharyngioma (8), odontogenic cyst (8), and in some visceral carcinomas (9-17). The group of visceral carcinomas, in which SCD was observed, includes carcinomas of the ovary (11,12,15), uterus (9), gallbladder (13), bladder (10,14), colon (9) and lung (16). According to the rarity of reported cases it could seem that SCD in visceral carcinomas represents an unusual finding. However, we noticed in our practice, that SCD is not rare in endometrioid carcinoma (EC) of the uterus. We wanted to ascertain the exact occurrence of this phenomenon, and therefore we searched for SCD in a series of uterine EC. Because cutaneous tumors with SCD are often positive for beta-catenin, indicating a possible role of Wnt signal transduction pathway in their tumorigenesis (18-20), in addition we performed a study of beta-catenin expression in our cases of EC with SCD (to determine whether Wnt signaling pathway may act also in these tumors).

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MATERIALS AND METHODS

Fifty-nine consecutive cases of EC of the uterine corpus were retrieved from routine files of surgical pathology laboratories in Trenčín (Slovak Republic) and Nový Jičín (Czech Republic). The

tumor tissue was obtained by operative hysteroscopy followed by fractional curettage of the uterine cavity and cervical canal. In all cases, the tissue was fixed in 10% formalin and processed routinely. The sections were stained with hematoxylin and eosin, and with a periodic acid-Schiff (PAS) stain. All cases were searched for squamous cell differentiation (including both mature squamous cells and immature-appearing morules) and for shadow cells. A finding of several unambiguous shadow cells was regarded to be positive for SCD. Tumors were graded according to the FIGO system (21). Subsequently, all tumors with SCD were examined immunohistochemically for beta-catenin (clone beta-catenin-1, dilution 1:200, DAKO, Glostrup, Denmark). Some cases retrieved from the files were (for the purposes of a differential diagnosis between endocervical and endometrial carcinoma) had already been stained for estrogen receptors (clone 1D5, dilution 1:40), progesterone receptors (clone PgR636, dilution 1:100), vimentin (clone V9, 1:400), and p16 (clone EGH4, 1:25) (all from DAKO). Immunostaining was performed according to standard protocols. Appropriate positive and negative controls were applied. A statistical analysis was performed to determine whether tumors with SCD and tumors without SCD differ in regard to patient age and tumor grade. A two-sample *t* test was used, with $p < 0.05$ considered statistically significant.

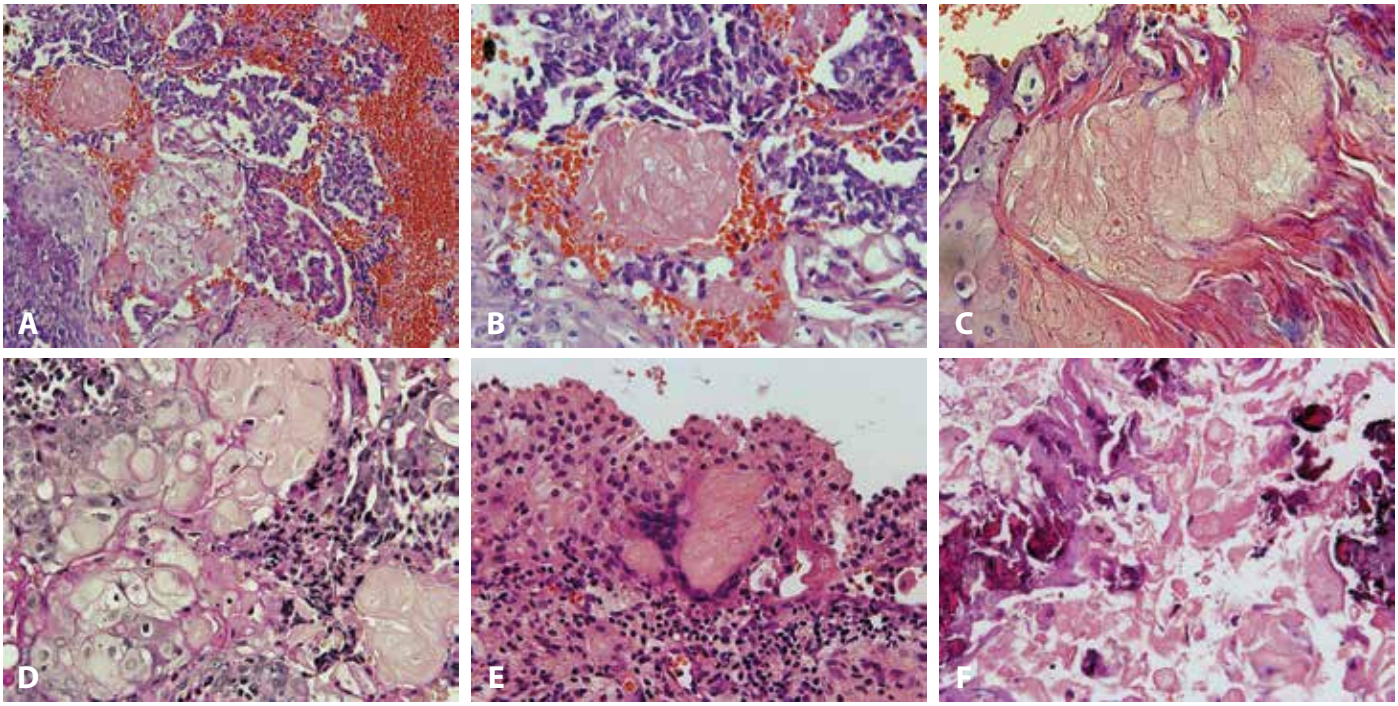

Fig. 1. Endometrioid carcinoma with shadow cells. **A:** the tumor shows endometrioid glandular morphology with foci of squamous and shadow cells. A group of the shadow cells is on the upper left, and isolated shadows cells are visible among the squamous cells. **B and C:** clusters of the shadow cells, with yellowish brown cytoplasmic granules. **D:** in addition to the basaloid and shadow cells, "transitional" squamous cells with PAS-negative eosinophilic to clear cytoplasm are seen. Rare PAS-positive "droplets" highlights minimal glandular differentiation in this tumor focus. **E:** a small group of the shadow cells in superficial low-grade appearing neoplastic epithelium, with secondary giant cell reaction. **F:** isolated shadow cells in disorganized keratin debris with calcifications. A,B,C-E: hematoxylin and eosin, D: PAS stain; original magnifications x200 (A), x600 (B-F).

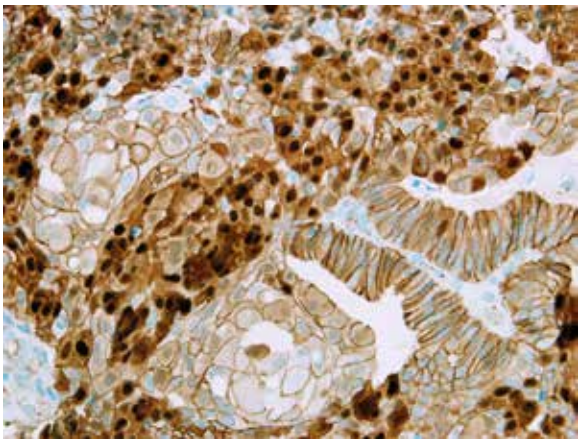

Fig. 2. Endometrioid carcinoma with shadow cells. Immunohistochemically, strong nuclear expression of beta-catenin is seen in basaloid appearing cells, in "transitional" cells and in some squamous cells. In contrast, the nuclei of the cylindrical glandular cells are negative. Original magnification x600.

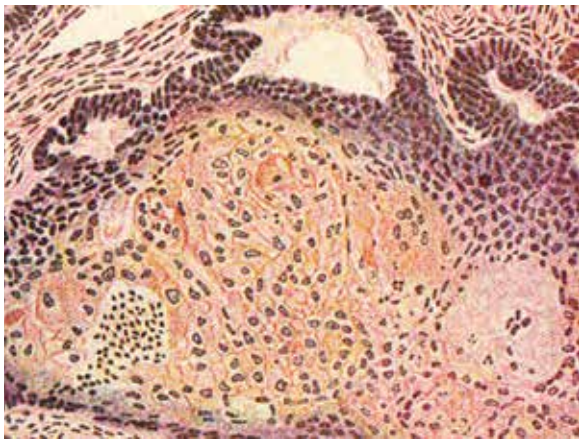

Fig. 3. A part of the color drawing of the uterine tumor labeled as "adenokankroid" in Škorpil's textbook issued in 1950 (22). The pink color of the keratinizing cells, with yellowish amber-like shade is well depicted (and perhaps even enhanced by the painter). A group of the shadow cells with complete karyolysis is seen on the right. Scanned copy of Tab XV, fig. 28 of the textbook.

RESULTS

The series included 59 cases of EC (age range 43 - 94, mean age 65.2, median 65 years). Shadow cells were found in 9 cases (15.3 %). In one case, both a cervical and a corporal fraction of the curettage contained the tumor, and the corporal origin was supported by the discovery of SCD and by expression of sex steroid receptors and vimentin. At least minimal squamous cell differentiation was found in 29 cases of the series (50 %). In cases with SCD, age ranged from 46 to 84 years (mean 64.7 years, median 66 years). All but one of the ECs with SCD were postmenopausal. FIGO grading of EC with SCD was as follows: grade 1 in 3 cases, grade 2 in 4 cases, and grade 3 in two cases. The clusters of shadow cells were numerous in two cases and rare in 7 cases. In all cases, foci of common squamous cell differentiation with focal keratinization were also found. The shadow cells had typical empty spaces after karyolysis, and fine, filamentous or granular cytoplasm, often with yellowish (honey-like) shade (Fig. 1). They were usually associated with basaloid-appearing cells, and often "transitional" cells with PAS-negative clear or eosinophilic cytoplasm were seen between the basaloid and shadow cells (Fig. 1D). Rarely, isolated clusters of shadow cells were visible in fibroblastic stroma, sometimes with giant cell reaction. The shadow cells were often seen intermingled with disorganized keratin debris, sometimes with calcifications (Fig. 1F). Immunohistochemically, all 9 ECs with SCD showed a nuclear expression of beta-catenin (Fig. 2). Beta-catenin was positive in the basaloid and squamous cells near the shadow cells. Interestingly, only rarely were cells of the glandular component of the tumors positive.

For statistical analysis, the group of ECs without SCD included 50 cases, with mean age 65.3 years, median age 64 years, and mean grade 1.48. It was compared with a group of ECs with SCD, which included 9 cases with mean age 64.7, median age 66, and average grade 1.89. A two-sample *t* test showed p-value of 0.853 for the age, and p-value of 0.109 for the grade. Thus, the differences between these groups regarding both age and grade are not significant statistically.

DISCUSSION

Our results show that SCD is quite frequent in EC. It was seen in 15% of all the examined EC cases. Regarding the age of the patients, ECs with SCD did not differ from ECs without SCD (p = 0.853). Also the FIGO grade of ECs with SCD is not different from ECs without SCD (p = 0.109), and both groups appear to show quite an even distribution of all three grades.

Histologically, we have seen that finding of shadow cells is strongly associated with "common" squamous cell differentiation. Taking into consideration only the group of ECs with squamous cell differentiation, shadow cells were found in a full 50% of the cases. In surgical pathology practice, the pathologists usually consider SCD as a part of the squamous cell metaplasia of the tumor cells. We think that this is also apparent in the literature on the topic of EC, and that SCD in EC was pictured in some previous papers or books. SCD in uterine "adenokankroid" appears to be illustrated in color drawing in Škorpil's excellent textbook on tumors which was already published in 1950 (22) (Fig. 3). Other examples include Kim and Scully's paper on peritoneal keratin granulomas in cases of ovarian and endometrial carcinomas (Fig. 4 of this paper) (23) and Fig. 19-9c in Crum and Lee s textbook (24).

A finding of SCD in morphologically otherwise typical EC is usually not important for diagnosis. However, in rare cases the presence of SCD can help by differentiating between corporal and cervical adenocarcinoma in a curettage specimen, as we have observed in one of our cases. Namely, from 1995 when SCD in endometrial carcinoma was firstly published (9), we had never seen SCD in endocervical adenocarcinoma, and therefore the finding of SCD should favor the endometrial origin of the tumor. Findings of SCD can help also through an examination of metastatic adenocarcinoma, because it supports the endometrioid nature of the tumor. In the examination of cutaneous tumors, pathologists should be aware that SCD in poorly differentiated carcinoma does not always indicate malignant pilomatrixoma or a cutaneous origin of the neoplasm. Lalich et al. described cutaneous metastasis of EC with shadow cells, which strongly mimicked malignant pilomatrixoma (15).

In all of our cases, beta-catenin was positive in the tumor cells, like in cutaneous pilomatrixomas (8,10,19,20) and in two previously published visceral carcinomas with SCD (10,12). The expression was strong in basaloid and "transitional" cells of the areas with squamous and shadow cells, and it was only rarely in cylindrical cells of the glandular component. Quite a similar distribution of nuclear positivity of beta-catenin has already been described in ECs and atypical hyperplasias with squamous cell differentiation (25-27). Such expression usually (but not always) reflects mutations of beta-catenin gene (26,27). It can be supposed that nuclear accumulation of beta-catenin (after the mutation of the gene) in association with the Wnt signaling pathway can play a role in the tumor genesis of EC, like it occurs in pilomatrixomas (18-20). This nuclear accumulation can also represent an initial signal for *trans*-differentiation from glandular toward squamous and shadow cell phenotype (26).

In sum, we have described a relatively high frequency of SCD in endometrioid carcinomas of the uterus. The finding of this morphological structure can assist in the diagnosis of EC, because SCD in other carcinomas (including cervical tumors) is very rare. In EC the SCD is associated with nuclear expression of beta-catenin. This expression indicates that the Wnt signaling pathway can play a role in the tumor genesis of some ECs.

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CONFLICT OF INTEREST

The authors dolare that there is no conflict of interest regarding the publication of this paper.

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Jaká je Vaše diagnóza?

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67-ročnej žene bol odstránený nádor v hlbokých mäkkých tkanivách stehna. **Makroskopicky** išlo o tenko opúzdrený tumor, hladkého sivasto-hnedého povrchu, veľkosti 11 x 7 x 4,5 cm. Nádor bol na reze tvorený dvoma zložkami rastúcimi v solídnych uzloch, ktoré boli oddelené väzivovými septami: menšiu časť (cca 1/3) tvorilo tuhoelastické šedo-biele tkanivo; druhá časť bola tvorená o čosi mäkkším ružovo-bielym tkanivom, ktoré bolo ložiskovo myxoidne presiaknuté, žltkasté. Nekróza makroskopicky nebola prítomná (obr. 1).

Mikroskopicky bola tuhšia biela časť reprezentovaná výrazne kolagenizovaným a menej celulárnym nádorovým tkanivom. Nádorové bunky mali malé množstvo nenápadnej eozinofilnej cytoplazmy a oválne až vretenité, variabilne poprehýbané, a pomerne pleomorfné jadrá. Výrazne boli zastúpené bunky s obrovskými hyperchrómnymi „smudgy" jadrami. V niektorých jadrách

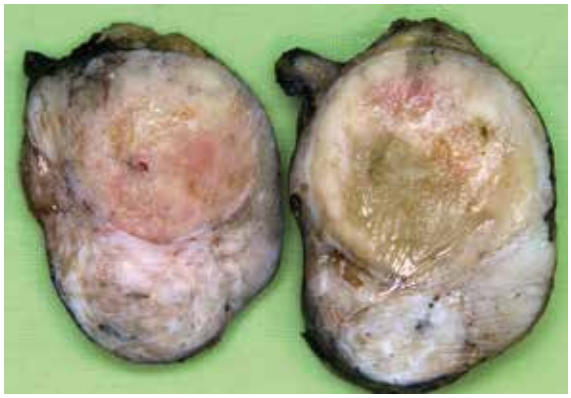

Fig. 1.