

Clear cell sarcoma of vulva. A case report

Kristýna Němejcová¹, Pavel Dundr¹, Ivana Krajsová²

¹Department of Pathology, First Faculty of Medicine and General University Hospital, Charles University in Prague, Czech Republic

²Department of Dermatovenereology, First Faculty of Medicine and General University Hospital, Charles University in Prague, Czech Republic

SUMMARY

We report the case of a 67-year-old female with clear cell sarcoma (CCS) of the vulva.

Grossly, the tumor was a partly exophytical vulvar mass, measuring 20 x 15 cm. At the time of presentation, the patient showed metastases to the lung, inguinal and pelvic lymph nodes. Histologically, the tumor consisted of oval or spindle cells with only mild nuclear pleomorphism and rare mitoses (up to 2/10 HPF). The cytoplasm was pale eosinophilic or clear. The tumor cells were arranged in confluent sheets. There were large areas of necrosis and surface ulceration. Immunohistochemically, the tumor cells showed expression of S-100 protein and focal melan A and HMB45 expression. Fluorescent in situ hybridization analysis revealed rearrangement of the EWSR1 gene. To the best of our knowledge, this is the first report of CCS arising in the vulva.

Keywords: clear cell sarcoma – EWSR1 gene – melanoma of soft parts – vulva

Světlobuněčný sarkom vulvy. Kazuistika

SOUHRN

Prezentujeme případ 67 leté ženy se světlobuněčným sarkomem vulvy. Nádor byl zčásti exofytický a dosáhl velikosti 20 x 15 cm. V době klinické prezentace byly prokázány metastázy v plicích a v inguinálních a pánevních lymfatických uzlinách. Mikroskopicky byl nádor tvořený oválnými nebo vřetenitými buňkami s pouze mírným jaderným pleomorfismem. Mitózy byly zastíženy pouze řídce (nejvýše 2/10 HPF). Nádorové buňky měly světle eosinofilní či vodojasnou cytoplasmu a byly uspořádány ve splyvajících plochách. Nádor byl povrchově ulcerovaný, s rozsáhlými ložisky nekrotizace. Imunohistochemicky byl v nádorových buňkách pozitivní průkaz S-100 proteinu a fokálně průkaz Melaninu A a HMB45. Fluorescenční in situ hybridizaci jsme prokázali přestavbu genu EWSR1. Prezentujeme první případ primárního světlobuněčného sarkomu vulvy

Klíčová slova: světlobuněčný sarkom – gen EWSR1 – melanom měkkých částí – vulva

Cesk Patol 2016; 52(4): 215–217

Clear cell sarcoma (CCS), also known as a melanoma of soft parts, is a malignant neoplasm first described by Enzinger in 1965 (1). This tumor is rare and accounts for 1 % of all soft tissue tumors. CCS typically involves deep soft tissues of the extremities, in close proximity to tendons and aponeurotic structures, especially in young adults (2). However, it can arise in other locations including the head and neck or trunk region and retroperitoneum. We report the case of a 67-year-old female with clear cell sarcoma of the vulva. To the best of our knowledge, our case represents the first report of CCS arising in the vulva.

CASE REPORT

Clinical history

A 67-year-old female presented with a one-year history of a slowly growing vulvar mass, finally measuring 20 x 15 cm at its largest size (Fig. 1). At the time of presentation, the patient showed metastases to the lung, inguinal and pelvic lymph nodes. A biopsy of the vulvar tumor was performed.

✉ Correspondence address:

Kristýna Němejcová, M.D., Ph.D.

First Faculty of Medicine and General University Hospital,
Charles University in Prague, Studničkova 2, Prague 2, 12800,
Czech Republic

phone: +420224968632

e-mail: kristyna.nemejcova@vfn.cz

MATERIALS AND METHODS

Immunohistochemical analysis. Sections from formalin-fixed, paraffin-embedded tissue blocks were stained with hematoxylin-eosin. Selected sections were analysed immunohistochemically using the avidin-biotin complex method with antibody directed against following antigens: S-100 protein (1:600, Dako, Glostrup, Denmark), Melan A (clone A103, 1:25, Novocastra, Newcastle, UK), HMB45 (1:50, Dako) and Ki-67 (clone MIB-1, 1:50, Dako), cytokeratin AE1/AE3 (1:50, Dako, Glostrup, Denmark), cytokeratin high molecular weight (clone 34betaE12, 1:200, Dako), estrogen receptors (ER, clone 6F11, 1:50, Novocastra), progesterone receptors (PR, clone 16, dilution 1:200, Novocastra), desmin (clone D33, 1:200, Dako), actin (clone HHH 35, 1:400, Dako), CD34 (clone QBEND 10, 1:50, Dako), CD99 (MIC2, clone 12E7, 1:50, Dako), synaptophysin (clone SY38, 1:25, Dako), chromogranin A (1:50, Dako) and p53 (clone DO-1, 1:50, Bio, Genex).

Fluorescence in situ hybridization. The rearrangement of the EWSR1 gene (22q12) was analyzed using the Fluorescent in situ hybridization method with probe (Dual Color, Break Apart Rearrangement Probe from Abbott Vysis, Downers Grove, IL, USA). The assay procedure was conducted according to the manufacturer's instructions.

Isolation of nucleic acid. Sections of formalin-fixed, paraffin-embedded tissue were used for DNA isolation using standard procedures. Approximately seven 10-mm-thick sections from each sample were deparaffinized in xylene. The DNA was then extracted using the QIAamp DNA mini kit (Qiagen, Hamburg, Germany).

Analysis of the BRAF Gene. BRAF 600/601 mutations were detected by polymerase chain reaction (PCR) or reverse-hybridization.



Fig. 1. Clear cell sarcoma of vulva growing as partly exophytical tumorous mass.

zation (StripAssay, ViennaLab, Austria) according to the manufacturer's instructions.

RESULTS

Five incisional biopsies from the vulva of a 67-year-old female measured 2 mm to 7 x 5 x 3 mm. Histologically, the tumor consisted of oval cells arranged in confluent sheets mostly with only mild nuclear pleomorphism and rare mito-

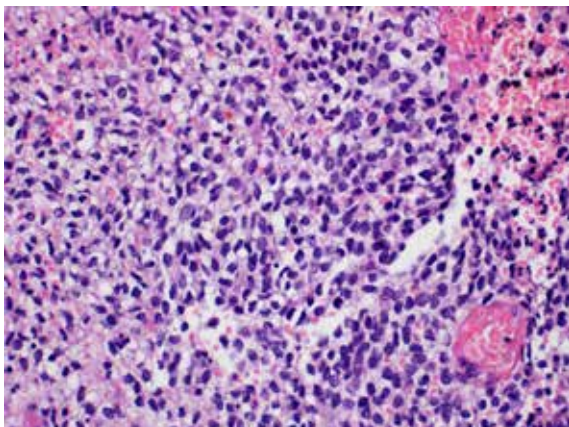


Fig. 2. CCS shows a proliferation of oval cells with pale eosinophilic or clear cytoplasm. (H&E, original magnification 400x).

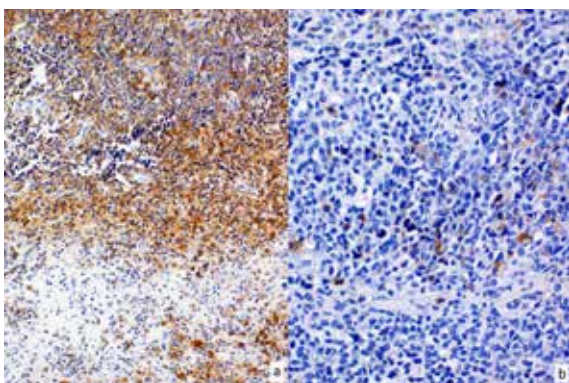


Fig. 3. Immunohistochemical expression of a) S100 protein (original magnification 200x), b) HMB45 (original magnification 400x) in tumor cells.

ses (up to 2/10 HPF) (Fig. 2). The cytoplasm was pale eosinophilic or clear. There were large areas of necrosis and surface ulceration. Immunohistochemically, the tumor cells showed expression of S-100 protein and focal expression of melan A and HMB45. Ki-67 showed positivity in approximately 10 % of tumor cells (Fig. 3). Other markers examined were negative, including cytokeratin AE1/AE3, cytokeratin high molecular weight, estrogen receptors, progesterone receptors, desmin, actin, CD34, CD99, synaptophysin, chromogranin A and p53. Fluorescent in situ hybridization analysis revealed rearrangement of the EWSR1 gene (22q12). BRAF 600/601 mutations were not detected by polymerase chain reaction (PCR) or reverse-hybridization.

DISCUSSION

CCS is a rare tumor that usually presents as a slowly growing mass. Recurrences occur in 14 - 39 % of patients, whereas metastases develop in approximately 50 % of patients. The prognosis of metastasizing CCS is poor with a mean survival of 18.4 months (3). In non-metastasizing cases, unfavorable prognostic factors include tumor size > 5 cm, histological detection of necrosis, and local recurrence (4). Besides soft tissues, CCS can also arise in the gastrointestinal tract. However, it has been a matter of dispute whether CCS of the gastrointestinal tract represents an entity distinct from soft tissue lesions. Recently, a series of 16 cases was published under the term malignant gastrointestinal neuroectodermal tumor (5). CCS shows some phenotypic and immunohistochemical features overlapping with malignant melanoma (MM), which is the most important differential diagnostic consideration (6). However, in contrast to MM, CCS usually arises in deep soft tissues without involvement of the skin. Moreover, neither nuclear pleomorphism nor high mitotic activity are typical features of CCS. However, pleomorphic cases can occur. Immunohistochemically, CCS and MM show the expression of S-100 protein, HMB-45, Melan A and microphthalmia transcription factor (MITF). In contrast to MM, CCS is characterized by a rearrangement of the Ewing's sarcoma (EWS) gene. The most common rearrangement is a balanced translocation (12;22)(q13;q12). This results in the fusion of the EWS gene at 22q12 to the activating transcription factor 1 (ATF1) gene localized at 12q13 (7,8). A translocation (2;22)(q34;q12) resulting in fusion of EWSR1/CREB1 occurs less frequently (9). These changes have not been identified in MM. Moreover, BRAF and NRAS mutations that are commonly found in MM have not been described in CCS (10).

In conclusion, we have described the first reported case of CCS arising in the vulva. The clinician should be aware of the possibility of this unusual primary location of CCS to avoid confusion with other tumors, especially malignant melanoma. In doubtful cases, molecular analysis of the EWSR1 gene will distinguish between these tumors.

ACKNOWLEDGEMENTS

This work was supported by Charles University in Prague (Project PRVOUK-P27/LF1/1, UNCE 204024, Ministry of health, Czech Republic - conceptual development of research organisation 64 165, General University Hospital in Prague, Czech Republic, and by OPBK (Research Laboratory of Tumor Diseases, CZ.2.16/3.1.00/24509).

CONFLICT OF INTEREST

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

REFERENCES

1. **Enzinger FM.** Clear-cell sarcoma of tendons and aponeuroses. An analysis of 21 cases. *Cancer* 1965; 18: 1163–1174.
2. **Weiss SW, Goldblum JR, Folpe AL.** Malignant tumors of the peripheral nerves. In: **Weiss SW, Goldblum JR,** eds. *Enzinger and Weiss's Soft Tissue Tumors* (5th ed). Philadelphia, Pennsylvania: Elsevier Mosby; 2008: 903–944.
3. **Ipach I, Mittag F, Kopp HG, Kunze B, Wolf P, Kluba T.** Clear-cell sarcoma of the soft tissue – a rare diagnosis with a fatal outcome. *Eur J Cancer Care* 2012; 21: 412–420.
4. **Sara AS, Evans HL, Benjamin RS.** Malignant melanoma of soft parts (clear cell sarcoma). A study of 17 cases, with emphasis on prognostic factors. *Cancer* 1990; 65: 367–374.
5. **Stockman DL, Miettinen M, Suster S et al.** Malignant gastrointestinal neuroectodermal tumor: clinicopathologic, immunohistochemical, ultrastructural, and molecular analysis of 16 cases with a reappraisal of clear cell sarcoma-like tumors of the gastrointestinal tract. *Am J Surg Pathol* 2012; 36: 857–868.
6. **Bearman RM, Noe J, Kempson RL.** Clear cell sarcoma with melanin pigment. *Cancer* 1975; 36: 977–984.
7. **Bridge JA, Sreekantaiah C, Neff JR, Sandberg AA.** Cytogenetic findings in clear cell sarcoma of tendons and aponeuroses. Malignant melanoma of soft parts. *Cancer Genet Cytogenet* 1991; 52: 101–106.
8. **Sandberg AA, Bridge JA.** Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: clear cell sarcoma (malignant melanoma of soft parts). *Cancer Genet Cytogenet* 2001; 130: 1–7.
9. **Hisaoka M, Ishida T, Kuo TT et al.** Clear cell sarcoma of soft tissue: a clinicopathologic, immunohistochemical, and molecular analysis of 33 cases. *Am J Surg Pathol* 2008; 32: 452–460.
10. **Yang L, Chen Y, Cui T et al.** Identification of biomarkers to distinguish clear cell sarcoma from malignant melanoma. *Hum Pathol* 2012; 43: 1463–1470.

MONITOR

aneb nemělo by vám uniknout, že ...

■ PATOLOGIE GIT

... byl popsán další typ smíšeného neuroendokrinního nádoru GIT: primární NEC a dlaždicobuněčný karcinom

Nebývá zvykem monitorovat kazuistické sdělení, ale v dubnovém čísle *Histopathology* skupina autorů z Bristolu ve Velké Británii přinesla popis zajímavého nádoru céka u 67letého pacienta. Jednalo se o kombinaci malobuněčného neuroendokrinního karcinomu (NEC G3) a dlaždicobuněčného nerohovějího karcinomu. Přes cílené pátrání nenalezli autoři v nádoru jakákoli ložiska struktur adenokarcinomu (včetně popsaného negativního výsledku histochemického průkazu hlenu v nádoru). V předloženém sdělení popisují autoři detailně imunoprofil obou komponent nádoru: pozitivita CK 5/6 a fokální p63 u dlaždicové komponenty, při negativním barvení neuroendokrinních znaků a Cam5.2 a Ki-67 indexu pod 5 %; „inverzní“ imunoprofil u NEC (pozitivita CD56, synaptofyzinu a Cam5.2, negativita CK 5/6, fokální pozitivita p63, Ki-67 index více než 40 %). Obě komponenty nádoru byly negativní v průkazu chromograninu A, CEA a TTF-1; NEC dále vykazoval silnou jadernou pozitivitu CDX-2. Při genetické analýze vykazoval nádor bodovou mutaci *KRAS* genu v exonu 2: c.35G>A p.(Gly12Asp); analýza kodonů 600 a 601 genu *BRAF* nevykazovala přítomnost mutací.

Klinicky se jednalo o objemný (největší rozměr 60 mm), střevno obturující nádor, s metastázami v 17 z 22 vyšetřených lymfatických uzlin; část metastáz vykazovala morfolologii čistého NEC, část pak smíšenou morfolologii. Pacient zemřel při progresi nádoru 9 měsíců od operace.

Předložený nádor je raritní a ukazuje, že neuroendokrinní nádory nemusí být smíšený pouze s adenomy/adenokarcinomy (MANEC), ale vzácně i s jinými histologickými vidy v nádorové populaci. Na základě zjištěné mutace genu *KRAS* uvažují autoři o možnosti vzniku takového nádoru z pluripotentní kmenové buňky, analogicky s běžnými adenokarcinomy v této oblasti. Určitou slabinu práce spatřuji v málo přesném odhadu proporcí obou nádorových komponent, kdy autoři uvádějí, že převažující komponentou nádoru byl NEC a záchyt dlaždicového karcinomu byl v nádoru „occasional“. Analogicky v MANEC má každá z komponent představovat alespoň 30 % buněčné populace nádoru.

Zdroj:

Hassan U et al. Primary combined neuroendocrine carcinoma (small-cell type) and squamous cell carcinoma of the colon. *Histopathology* 2016; 68: 755–766.

- T. Jirásek -