Coincidence of chronic lymphocytic leukaemia with Merkel cell carcinoma: deletion of the RB1 gene in both tumors

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SUMMARY

The authors report a case of a 64-year-old man with chronic lymphocytic leukaemia (CLL) diagnosed 5 years ago. Recently, the patient was admitted with a tumour of the skin in the left lumbar region. Histological and immunohistochemical examinations established the diagnosis of Merkel cell carcinoma (MCC). Electron-microscopic examination revealed the formation of spherical aggregates of intermediate-sized filaments in the perinuclear region. The coincidence of MCC and CLL is rather rare and in published cases, no cytogenetic examinations were performed. We examined the RB1 gene using the interphase FISH method. A biallelic deletion in CLL tumour cells was detected; in MCC tumour cells, biallelic deletion was found in 33 % of the cells and monoallelic deletion in 57 % of the cells. In addition, chromosome 6 trisomy and 1p36 deletion were detected. Examination of non-neoplastic cells of the patient's skin showed a biallelic presence of the RB1 gene. According to the relevant literature, examination of the RB1 gene in CLL has informational value as a prognostic factor. The relationship between deletion of the RB1 gene and prognosis in MCC has not yet been determined and needs more research.

Keywords: Merkel cell carcinoma – chronic lymphocytic leukaemia – immunohistochemistry – RB1 gene

Koincidence chronické lymfatické leukémie a karcinomu z Merkelových buněk: delece RB1 genu v obou nádorech

SOUHRN

Autoři popsali případ 64-letého muže, u kterého byla zjištěna chronická lymfatické leukémie (CLL) před pěti lety. V současné době byl pacient přijat pro nádor kůže v lumbální oblasti vlevo. Histologické a imunohistologické vyšetření ukázalo, že jde o karcinom z Merkelových buněk (MCC). Elektronově mikroskopické vyšetření ukázalo charakteristické paranukleární globule tvořené intermediálními filamenty. Koincidence MCC a CLL je poměrně vzácná a cytogenetická vyšetření zde nebyla publikována. Vyšetřovali jsme RB1 gen pomocí interfázové FISH metody. Cytogenetické vyšetření RB1 genu ukázalo bialelickou deleci u nádorových buněk CLL; u MCC byla bialelická delece u 33 % a monoalelická delece u 57 % buněk. Současně byla prokázána trizomie 6 a delece 1p36. Vyšetření nenádorové kůže ukázalo přítomnost RB1 genu v obou alelách. Podle literárních údajů má vyšetření RB1 genu u CLL význam při stanovení prognózy onemocnění. Vztah mezi delecí RB1 genu a prognózou onemocnění nebyl dosud u MCC stanoven a vyžaduje vyšetření dalších případů.

Klíčová slova: karcinom z Merkelových buněk – chronická lymfatická leukémie – imunohistochemie – RB1 gen

The coincidence of Merkel cell carcinoma (MCC) and chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL) was described in isolated cases (1–3). To distinguish the tumours, immunohistochemical or electron-microscopic examination is required in most cases. In cases where MCC occurs subsequently after the formation of CLL, it is necessary to eliminate the possibility of a transformation to lymphoma of a high grade of malignancy.

Cytogenetic examination of MCC reveals a deletion of the RB1 gene (location 13q14), trisomy 6 occurs in about 50 % of cases and distal deletion involving chromosome 1p35-36 is common; in CLL, 13q14 deletion is rather frequent as well (4,5). The goal of this study was to examine both tumours in histological, immunohistochemical and cytogenetic terms. The interphase FISH method was used to prove the presence of the tumour suppressor gene RB1.

MATERIALS AND METHODS

In a 64-year-old patient, CLL was diagnosed 5 years ago by flow cytometry of peripheral blood – the lymphocytes were CD5 and CD19 positive. The disease did not progress, and the patient was followed in clinical conditions, but he was not treated (watch and wait management). Neither the lymph nodes, spleen nor liver were enlarged. During the examination, the number of lymphocytes in the peripheral blood was 47.2 x 10^9/L, erythrocytes 2.52 x 10^{12}/L,

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Fig. 1. Merkel cell carcinoma of the skin in the lumbar area.

thrombocytes 98.4 x 10⁹/L and the concentration of haemoglobin 86g/L.

A hemispherical tumour sized 4.0 x 6.0 cm occurred on the skin in the left lumbar region with a 4-month history (Fig. 1). The tumour was removed surgically and no relapse was found in the next 8 months of clinical follow-up. Standard histological, immunohistochemical, electron-microscopic and cytogenetic examinations using the FISH method were performed.

The tumour of the skin was fixed by 4% neutral formalin and processed using a standard paraffin technique. Histological sections were stained with haematoxylin-eosin.

Immunohistochemical examination. All immunohistochemical examinations were performed using the avidin-biotin complex method according to the manufacturer's data sheets with positive and negative controls. The following antibodies were used (dilution in parenthesis): AE1-AE3, clone AE1-AE3 (1:50); CK20, clone Ks 20.8 (prediluted); CK7, clone OV-TL 12/13 (1:50); NSE, clone 2F11[1] (1:50); LCA, clone 2B11+PD7/26 (1:100); CD20 clone L26 (1:100); CD45 RO, clone UCHL 1 (1:100); Bcl-2, clone 124 (1:25); CD117 (1:25); CD99, clone 12E7[1] (1:25); vimentin, clone 3B4 (1:50); Ki-67, clone MIB-1, all from DAKO Glostrup, DK; synaptophysin, clone 27G12 (1:100); chromogranin, clone 5H7 (1:100); CD56, clone 1B6 (1:100); TTF-1, clone SPT24 (1:50), from Novocastra, Newcastle-upon-Tyne, UK; CAM 5.2 (1:200) produced by Becton Dickinson, USA.

Electron-microscopic examination. Small pieces of tumour tissue were prefixed in 3% glutaraldehyde in a 0.2 M phosphate buffer pH 7.4 for 24 h at 4 °C and postfixed in a 2% osmium tetroxide in phosphate buffer for 2 h at 4 °C, followed by dehydration and embedding in an Epon-Durcupan resin. Ultrathin sections were stained with uranyl acetate and lead citrate. The FEI Morgagni 268(D) electron microscope was used for the examination.

Interphase fluorescence in situ hybridization (FISH). Paraffin sections with a thickness of 5μm were heated overnight at 56 °C. After the paraffin was removed, paraffin-free sections were permeabilized using HCl and incubated in sodium thiocyanate (NaSCN). Then, proteolytic predigestion with protease at 37 °C for 30–35 minutes followed. The sections were postfixed in a 10% buffered formalin and, after the probe was applied, co-denatured at 85 °C for 1 minute. Hybridization was carried out overnight at 37 °C. After the probe was washed out, the nuclei were additionally stained with DAPI II. The result was read in a fluorescent microscope. The following probes were used for the hybridization: LSI 13(RB1) 13q14 Spectrum Orange Probe and Spectrum Green Probe, LSI 1p36/1q25, LSI 13q34; CEP 6 made by Vysis, Abbott Laboratories Inc., Des Plaines, IL, USA.

According to a standard method, cultured lymphocytes from the patient's peripheral blood were processed as well. The LSI 13(RB1) 13q14 Spectrum Orange Probe was used together with the LSI 13q34 Spectrum Green Probe (Vysis, Abbott) for verification purposes.

RESULTS

Histological examination of the skin tumour showed a malignant neoplasm consisting of medium-sized cells having large nuclei with homogeneous chromatin (Fig. 2). Necrosis was observed in some nodules. The tumour extended from the upper dermis to the subcutis; angioinvasion was evident in some areas. The mitotic rate was high (50 mitoses/10HPF).

The immunohistochemical examination revealed that the tumour cells reacted positively with the following markers: CK20 (dot-like paranuclear positivity) (Fig. 3), synaptophysin, Bcl-2, CD99, CD56, proliferation marker Ki-67 was positive in about 40 % of cells. Negative results were found with the following markers: TTF1, LCA, CD20, CD45RO, CD10, CK7, AE1-AE3, CAM 5.2, vimentin, CD117.

Electronmicroscopic examination showed mostly spherical formations consisting of intermediate filaments in the cytoplasm of
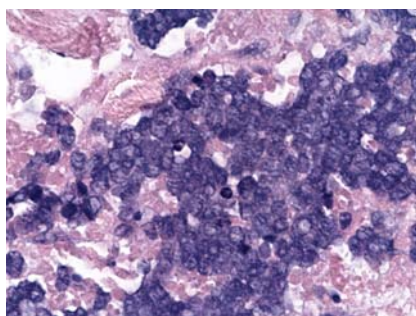

Fig. 2. Merkel cell carcinoma consists of medium-sized tumour cells containing rather large, oval, hyperchromatic nuclei and a small amount of cytoplasm. Haematoxylin-eosin, magnification 400x.
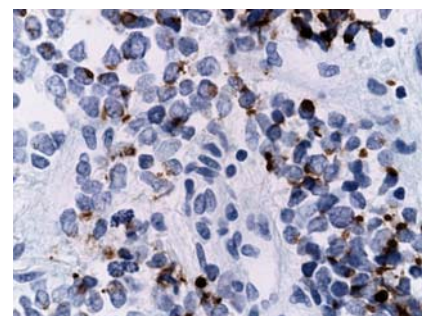

Fig. 3. Merkel cell carcinoma – paranuclear dot-like positivity with an antibody against CK20. Immunohistochemistry CK20, magnification 400x.
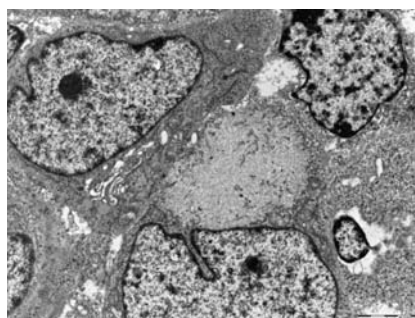

Fig. 4. Merkel cell carcinoma – paranuclear aggregates of intermediate-sized filaments. Electron-microscopy, magnification 7100x
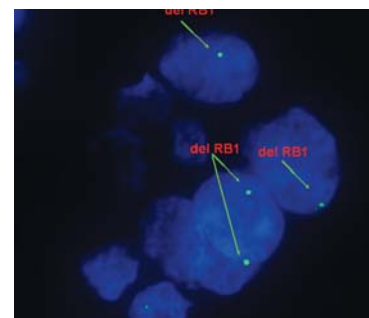

Fig. 5. Biallelic deletion of the RB1 gene in Merkel cell carcinoma. Interphase FISH method with a 13q14 probe, magnification 5000x.

many cells in the paranuclear area (Fig. 4). The presence of neurosecretory granules was minimal. Cell junctions were formed by desmosomes.

Using the interphase FISH method in MCC, monoallelic deletion of the RB1 gene was shown in 57 % of tumour cells and biallelic deletion in 33 % (Fig. 5). Examination of the tumour-free cells of the patient's skin revealed the presence of the RB1 gene in both alleles. In the neoplastic lymphocytes of CLL, deletion of the RB1 gene was found in both alleles. In addition, chromosome 6 trisomy and 1p36 deletion were found in MCC.

DISCUSSION

MCC is a rather rare tumour that occurs predominantly at the age of about 68 years (4,6). It is derived from Merkel cells dispersed between basal cells of the epidermis and hair follicles.

On the contrary, CLL is one of the most frequent leukaemias/lymphomas of the western world with incidence increasing with age. The mean age of diagnosis is 65 years (7).

Brenner et al. (1) found, in a group of 67 patients diagnosed with MCC, that a second neoplasia occurred in up to 25 % of the patients; in 63 % of cases the second tumour occurred before the formation of MCC, 2 % contemporaneously and 26 % subsequently. The average time of formation of the secondary neoplasia was 4 years. In our case, CLL preceded and it was not treated. MCC occurred 5 years later.

Two main causes play a role in the carcinogenesis of these two tumours:
a) an immune system defect with various immunocompromised conditions (8,9).
b) cytogenetic changes; particularly 13q losses with deletion of the RB1 locus are very important in most cases (10). Protein of the RB1 gene (pRB1) reacts with the family of transcriptional factors involved in cell cycle regulation. If pRB1 is bound to the transcription factor E2F, the cell does not enter the cell cycle (11).

Another cause of carcinogenesis in MCC is Merkel cell polyomavirus, found in about 80 % of cases (12,13). After viral episome disruption and integration into the cell genome, the process of carcinogenesis can be initiated.

Although the suppressor gene RB1 was discovered in retinoblastoma, its mutations and deletions are associated with other types of tumours as well, such as small-cell lung cancer, bladder cancer, cervical cancer and prostate cancer.

In CLL, deletion of the RB1 gene (13q14) can be found frequently. Using the FISH method, the deletion was found in 40–50 % of

patients (14). Other frequent cytogenetic aberrations are: IG genes are rearranged in 40–50 % of cases, non-mutated (>98% homology with the germline) and showing somatic hypermutation in 50–60 % of cases (7).

If deletion of the RB1 gene occurs in both alleles, it usually results in a loss of pRB1 formation or function (15). In our case, the biallelic deletion was found in lymphocytes of CLL while it was shown in 33 % of MCC tumour cells. Examination of tumour-free skin revealed a biallelic presence of the RB1 gene. Therefore, the genetic changes are supposed to occur subsequently during the patient's life. Deletion of the RB1 gene in lymphocytes of CLL is relatively frequent (in 40–50 % of cases) (14). In MCC, the data indicate 13q losses as the most common chromosomal abnormalities and the likely target of these deletions is the RB1 locus (10). According to our opinion, deletion of the RB1 gene in both tumours is an incidental event having no connection with mutation mosaicism.

Chromosome 6 trisomy occurs in almost 50 % of cases in patients diagnosed with MCC, and deletions of 1p35-36 are frequent as well (4). Both cytogenetic abnormalities were present in our case.

Using immunohistochemical methods, MCC must be distinguished particularly from lymphomas, metastasis of small-cell lung cancer, Ewing's sarcoma/PNET and melanoma. By immunohistochemical examination, dot-like positivity with an antibody against CK20 is found. The finding is typical of MCC and it was noted in our case as well. Small-cell lung cancer expresses the CK20 marker in only 0.03 % of cases (16). On the contrary, positivity with an antibody against CK7 occurs in up to 43 % of small-cell lung cancer cases. The TTF-1 marker is typical of small-cell lung cancer, and is positive in more than 90 % of cases (16). In our case, the CK7 and TTF-1 markers were negative.

MCC can be distinguished from lymphomas by immunohistochemical examination with the LCA, CD20, CD45RO and CD10 antibodies. A positive finding including markers CD99, synaptophysin and CD56 would favour Ewing's sarcoma/PNET. This diagnosis is also disproved by the findings of dot-like positivity with an antibody against cytokeratin CK20 and of intermediate filaments by electron-microscopic examination (16).

A coincidence of CLL and MCC can probably occur accidentally in a patient's life. Since the number of tumours increases substantially in later life, the probability of their concurrent or subsequent occurrence increases as well. Deletion of the RB1 gene occurs frequently in the two tumours as well. Results of the RB1 gene examination in progenitor cells would also be interesting. Deletion of the RB1 gene adversely affects the prognosis of certain tumours such as plasmacytoma and CLL (5,17).

Relationship between deletion of the RB1 gene and prognosis in MCC has not been defined.

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MONITOR
aneb **nemělo by vám uniknout, že ...**

■ **ORTOPEDICKÁ PATOLOGIE** ▶

… *téměř 70 % angiosarkomů kosti je cytokeratin pozitivních s možnou snadnou záměnou za metastatický karcinom*

Vaskulární nádory kostí (kromě hemangiomů) jsou vzácné a jejich klinické příznaky a histologické znaky jsou variabilní – od snadno rozpoznatelné vazoformativní proliferace po nediferencovaný nádor. Angiosarkomy (AS) kostí jsou známy svým nepředvídatelným chováním a mohou být extrémně agresivní.

Autoři ze dvou kontinentů se pokusili na sestavě 42 angiosarkomů kosti od 38 pacientů definovat histologické znaky primárního kostního angiosarkomu, které zatím nejsou nikde v literatuře jednoznačně vymezeny. Podle některých je AS kosti definován high-grade cytologickými atypiemi a vysokou mitotickou aktivitou, jiní doporučují jako kritérium jadernou hyperchromázii, pleomorfismus a vysokou mitotickou aktivitu (> 5 mitóz/10 HPF).

Dalším cílem studie byla eventuální identifikace intermediární kategorie vaskulárních nádorů kosti ležící mezi epiteloidním hemangiomem a AS kosti. Bylo naznačeno, že endoteliální malignity kosti by měly být považovány za součást spektra, jehož limitem na jedné straně jsou zcela benigní afekce, na druhé high-grade endoteliální malignity (AS). Klasifikace nádorů s morfologií mezi běžným hemangiomem a angiosarkomem je poněkud kontroverzní. Dobře popsanou jednotkou je epiteloidní hemangiom, který může být multifokální, může destruovat kortex a šířit se do měkkých částí, a recidivuje v 8 % případů a ve 2 % metastazuje do lymfatických uzlin. Má značné podobnosti s nádorem, který byl popsán Evansem et al. jako hemangioendoteliom kosti s intermediárním biologických chováním. Nicméně kritéria odlišující epiteloidní hemangiom a hemangioendoteliom nejsou v literatuře uváděna, snad kromě lobulární architektury u epiteloidního hemangiomu. Dalším maligním tumorem je epiteloidní hemangioendoteliom kosti s charakteristickou morfologií, identickou jako u měkkotkáňových případů.

Pro tyto dva cíle bylo vybráno 80 tumorů od 74 pacientů diagnostikovaných jako AS kosti, a vybrány byly nádory vykazující atypie, hyperchromazii jader, mitózy a/nebo atypické mitózy, a ty, které byly pozitivní v imunoreakci nejméně pro jeden endoteliální marker.

Histologicky AS kosti nejčastěji vykazovaly solidní růst, v 50 % případů byly přítomné zralé cévy, vždy vystlané atypickými endoteliemi. Většina angiosarkomů měla epiteloidní morfologii. Častým histologickým znakem byla přítomnost intracytoplazmatických vakuol s erytrocyty nebo prázdných, extravazace erytrocytů s depozicí Fe. Všechny nádory vykazovaly cytonukleární atypie, od lehkých až po těžké. U většiny angiosarkomů byla jádra nádorových buněk vezikulární, a ve více než polovině případů tato jádra obsahovala 1 – 3 jadérka, a asi ve třetině makronukleoly (pokud > 75 % nádorových buněk obsahovalo jadérko, které mělo rozměr poloviny lymfocytu, tj. 4 μm či větší). V polovině nádorů byly nekrózy, ve všech případech byl přítomen zánětlivý infiltrát různé intenzity, obvykle lymfocytární, ale eozinofilní infiltráty (> 4 eozinofily/10 HPF) byly téměř v 50 % případů.

Imunohistochemicky bylo 95 % případů CD31+, 39 % CD34+ a 60 % vWF+. Všechny nádory byly pozitivní nejméně pro jeden endoteliální marker, což však bylo selekční kritérium. 2/3 vykazovaly pozitivitu izolovaných buněk pro hladkosvalový aktin, D2-40+ byla 1/3. 69 % bylo cytokeratin (CK) pozitivních (všechny s epiteloidní morfologií). Solidní růst, epiteloidní buňky, pozitivita CK a multifokalita tak může vést ke snadné záměně s metastatickým karcinomem a na tuto možnost je třeba myslet při vyšetřování podezřelé kostní „metastázy karcinomu".

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– K. Veselý –