Clear cell myeloma. Report of two cases with comments on morphogenesis and ubiquitin expression

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Received: 9 October 2010 / Accepted: 3 November 2010 / Published online: 17 November 2010
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Abstract Three bone marrow trephine biopsies from two multiple myeloma patients were involved by a diffuse infiltrate of microvacuolated plasma cells with a variable number of signet ring cells, CD138, κ -light chains, and ubiquitin positive. The vacuoles remained clear on PAS and in May–Grünwald–Giemsa-stained bone marrow aspirates. Differential diagnosis on conventional stains included malignant epithelial and mesenchymal tumors, non-neoplastic histiocytes, and fixation artifacts. Electron microscopy revealed translucent vacuoles with no apparent membrane and a fuzzy internal surface occasionally merging with smaller membrane-delineated spaces filled with granular material, without communication with cisternae of rough endoplasmic reticulum. Rare multivesicular bodies with dilated intraluminal vesicles were noted. The findings concur with the presumed derivation of the vacuoles from the endolysosomal compartment. Strong ubiquitin expression was encountered in neoplastic plasma cells but not in non-neoplastic plasma cells, presumably due to the endoplasmic reticulum-associated degradation, triggered by excessive production of misfolded immunoglobulins in the former.

Keywords Bone marrow · Clear cell myeloma · Pseudo-Gaucher cells · Signet ring cells · Ubiquitin

Introduction

A diagnosis of plasma cell myeloma is established by integration of clinical, radiographic, and biochemical findings and confirmed by bone marrow trephine biopsy and/or aspirate [1]. Sheets or a diffuse infiltrate of neoplastic plasma cells is recognized with confidence on hematoxylin and eosin (H&E) or Giemsa-stained sections in most of the cases. However, cytological variants imitating other neoplasms, such as metastatic carcinoma, lymphoma, myeloid or monocytic leukemia, sarcoma or non-neoplastic lesions, such as storage diseases or inflammatory conditions, can cause a diagnostic dilemma [2]. A neoplastic plasma cell infiltrate obscured by abundant pseudo-Gaucher cells has been recently reported as a diagnostic pitfall [3]. Herein, we are reporting on two cases of clear cell myeloma (CCM) masquerading as a histiocytic infiltrate and a metastatic signet ring cell carcinoma. We also comment on morphogenesis of the vacuolar change and expression of ubiquitin in myeloma cells.

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Case 1

A 58-year-old male presented with a compressive fracture of the thoracic spine in June 2005 followed by progressive fatigue. Clinical investigations revealed evidence of plasma cell myeloma with plasmablastic leukemia, clinical stage IIIB (serum M-protein IgA κ (42.76 g/l), Bence-Jones protein κ (1.99 g/l), 80% of plasma cells in bone marrow aspirate analyzed by FACS and cytogenetics (CD138+,

CD38+, CD56+, CD45–, del17p, and monosomy13), 20% of plasma cells in the peripheral blood and multiple osteolytic lesions in the axial skeleton and upper extremities). Treatment with 4 cycles of VAD (vincristine, doxorubicine, dexamethasone), autologous stem cell transplantation, Thalidomide and Velcade achieved only a transient episode of partial remission. The patient died 36 months after the diagnosis.

Case 2

A 59-year-old male underwent medical examination in 2008 for a 3-month history of back pain. This revealed evidence of multiple myeloma clinical stage IIIA (serum M-protein IgG κ (60,5 g/l), Bence–Jones protein (0,671 g/l), 74% plasma cell population (CD138+, CD38+, CD56+, CD45–) on FACS analysis of bone marrow aspirate and multiple osteolytic lesions in the axial skeleton, lower and upper extremities.

Treatment with 4 cycles of Velcade, thalidomide and dexamethason) and autologous stem cell transplantation induced complete remission (FW 7, Hb 143g/l, serum protein 74 g/l, no paraproteinemia or proteinuria). The patient is alive and well 24 months after the diagnosis.

Material and methods

Both cases were identified in the course of routine diagnostic work. Two sequential trephine biopsies and bone marrow aspirates were available from [Case 1](#) and one biopsy and aspirate from [Case 2](#).

Bone marrow trephines were fixed and decalcified in formic acid–formalin solution for 24 h. Upon routine processing, the paraffin-embedded trephines were stained with H&E, PAS, Giemsa, reticulin and Sudan ([Case 2](#)) stains. An antigen retrieval step by heating in citrate buffer pH 6.0 at 98°C for 40 min preceded application of primary antibodies (clone, dilution, source): CD138 (M115, 1:50, DAKO), κ -light chains (polyclonal, 1:50 000, DAKO), λ -light chains (polyclonal, 1:50 000, DAKO), CD45 (2B11+ PD7/26, 1:100, DAKO), CD68 (PGM1, 1:100, DAKO), pancytokeratins (AE1/3, 1:50, DAKO), and ubiquitin (polyclonal, 1:1500, DAKO). The reaction products were detected by Histofine (Nichirei Biosciences, Japan) and diaminobenzidine as chromogen.

Samples for electron microscopy were extracted from the paraffin-embedded tissue, deparaffinized and postfixed overnight in 1% osmium tetroxide and embedded in epoxy resin. Ultra thin sections were double stained with uranyl–acetate/lead citrate. The bone marrow aspirates were air dried and stained with May–Grünwald–Giemsa.

Results

Histopathological and immunohistochemical findings

The initial bone marrow trephine biopsy of [Case 1](#) (Fig. 1a) and the bone marrow trephine of [Case 2](#) (Fig. 1e) showed similar histological findings dominated by high cellularity but faint appearances on low-power magnification. On high-power magnification, diffuse infiltration by uniform population of medium-sized foam cells (FC), possessing eccentric round nuclei with dense homogenous or finely granular chromatin and no or inconspicuous nucleoli was observed. Signet ring cells (SRC) with compressed nuclei by a single water-clear vacuole were occasionally noted, as well as occasional mature plasma cells, forming a single ill-defined aggregate in [Case 2](#) (Fig. 2). Hematopoiesis was reduced to individual hematopoietic cells, or their clusters scattered in the neoplastic background in [Case 1](#), and to a sharply demarcated area with myelodysplastic features in [Case 2](#).

The follow-up bone marrow trephine biopsy of [Case 1](#) (Fig. 1c) showed a persistent neoplastic infiltrate with a marked shift towards large SRC at the expense of FC. Immunohistochemical studies in all three biopsies demonstrated expression of CD138, κ -light chain (Fig. 2), and ubiquitin (Fig. 3), whereas λ -light chain (Fig. 2 inset), CD68 (PGM1), and pancytokeratins (AE1/3) yielded negative results. The ubiquitin and κ -light chain reaction products gave the FC speckled appearances and created a fuzzy line along the inner surface of SRC, leaving the vacuoles water clear. Notably, four cases of bone marrow plasma cell myeloma and one solitary bone plasmacytoma gave a strong ubiquitin reaction, whereas plasma cells in two inflammatory infiltrates and one case of rheumatoid lymphadenitis were weakly positive or negative.

The vacuoles remained unstained on PAS, Sudan, and May–Grünwald–Giemsa (bone marrow aspirates). Although FC were predominantly microvacuolated, a spectrum of vacuoles of larger size was apparent, suggesting evolution into the final stage of SRC. Moreover, ordering of all three biopsies and aspirates by the number of SRC revealed a morphological continuum from predominantly FC differentiation, imitating accumulation of histiocytes 1 (Fig. 1a, b, e, f), to predominantly SRC differentiation, imitating SRC carcinoma (Fig. 1c, d).

Electron microscopic findings

The follow-up bone marrow trephine biopsy of [Case 1](#) and [Case 2](#) were studied. Neoplastic plasma cells were identified by nuclei with clumped chromatin, often possessing a small nucleolus, and/or an abundant rough endoplasmic reticulum (RER). The SRC cells contained a

Fig. 1 **a, b.** Case 1-initial biopsy. **c, d.** Case 1-follow-up biopsy. **e, f.** Case 2. Bone marrow trephine biopsies (**a, c, e**) and bone marrow aspirates (**b, d, f**) showing numerous vacuolated neoplastic plasma

cells. These imitate histiocytes in **a, b** and **e, f** and signet ring cell adenocarcinoma in **c, d, h, e**. May–Grünwald–Giemsa. Magnification $\times 600$

single translucent vacuole compressing a sickle-shaped nucleus, while FC possessed centrally placed nuclei. Their cytoplasm was fenestrated by multiple translucent vacuoles, some delineated by incomplete, poorly preserved membranes, with residues of fluffy granular material in places. The vacuoles occasionally merged with small membrane-delineated spaces filled with granular material of moderate density (Fig. 4, asterisk). A multivesicular body with dilated intraluminal vesicles was noted (Fig. 5). RER

formed distinct narrow cisternae without any evidence of communication with the vacuoles (Fig. 4, arrow).

Discussion

Chen et al. were the first to introduce a descriptive term CCM for a myeloma characterized by FC and SRC differentiation in 1985[4] having been reportedly preceded

Fig. 2 Case 2. Immunohistochemical detection of light chains, κ positive and λ negative (inset). Unstained microvacuoles give the cells speckled appearances. A cluster of well-differentiated neoplastic plasma cells is shown in the right-lower quadrant. Magnification $\times 600$

only by an abstract publication ‘Signet ring cell myeloma’ presented by Jacot-des-Combes and Kapacini in 1984 [5].

Searching the PubMed database, we identified only six myeloma cases [4, 6–10] and one monoclonal gammopathy of uncertain significance [11] displaying a vacuolar change. This was in stark contrast to over 100 reported cases of SRC malignant lymphomas. The clinicopathologic data, including the presented two cases, are summarized in Table 1. Briefly, all nine patients were males aged from 44 to 73 years at presentation (mean 62, median 61). Monoclonal IgG:IgA ratio was 4:3 (seven cases compared), and paraprotein κ : λ ratio was 7:2. The values are skewed towards male predominance and a high proportion of IgA,

Fig. 3 Case 2. Immunohistochemical detection of ubiquitin, giving a strong reaction similar to κ -light chains in neoplastic plasma cells and a weak-to-no reaction in plasma cells of chronic inflammatory infiltrate (inset). Magnification $\times 600$

Fig. 4 Case 1. Follow-up biopsy. Electronogram showing an immature plasma cell with translucent membrane-free large vacuoles and occasional small membrane-bound spaces with granular, moderately dense contents, rarely merging with the large vacuoles (*asterisk*). RER is compressed between the vacuoles (*arrow*). Bar 2 μ m Uranyl acetate. Magnification $\times 4000$

but the significance is unclear due to the small number of cases.

Despite its rarity, awareness of FC and SRC differentiation in multiple myeloma is important in individual

Fig. 5 Case 1. A multivesicular body with dilated intraluminal vesicles and a margin of a large vacuole with a fuzzy internal surface. Bar 1 μ m. Uranyl acetate. Magnification $\times 5000$

Table 1 Clinicopathologic data

Year of publication Reference	Age	Sex	Diagnosis	M-protein paraprotein	Material	Light microscopy proportion of PLC and vacuolated cells	Electron microscopy membrane	AP
1985/4	70	M	clear cell myeloma	IgA κ	bone tumor BM smear BM trephine	40% PLC, most vacuolated	Incomplete occasional	nd
1986/9	61	M	multiple myeloma	IgG κ	BM smear	77% MM cells, practically all vac.	not done	pos
1989/10	60	M	multiple myeloma	no spike BJP κ	BM smear	marked proliferation of PLC, most with few vacuoles	membrane demarcated vacuoles	pos
1990/7	44	M	multiple myeloma with SRC	IgG κ	BM trephine	dense infiltrate of PLC, many solitary or multiple vacuoles	limiting membrane	nd
1992/8	67	M	multiple myeloma PLC leukemia	IgG λ	BM trephine PB	90% PLC, most vac. vac.PLC in PB	distinct membrane	pos
1997/6	69	M	BJM with SRC	not specified λ	BM smear BM trephine	several larger cells with single vacuoles	not done	nd
1997/11	73	M	MGUS	IgA κ	BM smear	some	not done	nd
Case 1	58	M	clear cell myeloma	IgA κ	BM trephine	90–95% MM cells, practically all vac.	indistinct	nd
Case 2	59	M	clear cell myeloma	IgG κ	BM trephine	80% MM cells, practically all vac.	indistinct	nd

Ref reference number, *PLC* plasma cells, *BJP/M* Bence–Jones protein/myeloma, *SRC* signet ring cells, *PB* peripheral blood, *vac* vacuolated/vacuoles, *AP* acid phosphatase, *nd* not done

cases, as the appearances both in bone marrow trephines and aspirates lead to a broad differential diagnosis, including metastatic SRC adenocarcinoma, liposarcoma, Langerhans cell histiocytosis, pseudo-Gaucher cells or fixation artifacts. Our observations suggest that nuclear appearances, scattered mature plasma cells, and transitional forms of FC to SRC are signs which should raise suspicion of CCM and prompt immunohistochemical investigations. Comparison of the herein presented figures to those shown in Ref. 3, documents striking morphological similarity between histiocytes and neoplastic FC of clear cell myeloma.

In 1990, Eyden et al. published an ultrastructural study providing evidence that vacuoles in SRC B- and T-cell lymphomas arise from multivesicular bodies (MVB) and suggested that their genesis is related to altered membrane recirculation [12]. MVB represent late endosomes lacking recirculation-related enzymes and carrying ubiquitinated cargo, destined for degradation upon merging with lysosomes [13]. Indeed, acid phosphatase was identified in all three tested cases of vacuolated myeloma cells [8–10], and experimental manipulation of MVB induced a vacuolar change in cell culture [14]. Our findings of empty vacuoles on conventional stains and no-reaction products on immunohistochemical detection of light chains, no evidence of communication with RER, and a finding of MVB harboring

dilated microvesicles on ultrastructural studies, lend further support to the origin of the vacuoles from the endolysosomal compartment.

Finally, we are not aware of any previous reports on differences in ubiquitin expression in neoplastic and non-neoplastic plasma cells detected by immunohistochemistry. Obviously, our observation is preliminary and needs verification in a robust study. However, it concurs with recent experimental data [15, 16] and biological theory, which offers a good explanation based on the unfolded protein response to massive production of misfolded immunoglobulins and/or their subunits in neoplastic, but not reactive plasma cells. This triggers the endoplasmic reticulum-associated degradation, which utilizes cytoplasmic ubiquitylation for targeting the misfolded proteins for degradation in the proteasome [17]. We hypothesize that upregulation of ubiquitin synthesis in myeloma cells explains the observed differences.

In conclusion, CCM is a rare differentiation pattern of multiple myeloma, which should be included in the differential diagnosis of disorders dominated by the presence of histiocytes or SRC in bone marrow trephine biopsies and aspirates. SRC represent the final evolutionary step in the vacuolar change, which in one of the presented cases, correlated with clinical progression. The vacuoles originate in the endolysosomal compartment.

Experimental and theoretical data, concurrent with the presented observation, suggest that ubiquitin could become an immunohistochemical adjunct in detection of neoplastic plasma cells.

Acknowledgments The authors are grateful to Mrs Blanka Veghova and Mrs Eva Kirchmanova for their excellent technical assistance and Mrs Denise Davison for editing the English language.

Conflict of interest The authors declare that they have no conflict of interest.

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