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MUC1 (EMA) expressing plasma cells in bone marrow infiltrated by plasma cell myeloma

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Summary. MUC1 (also called: epithelial membrane antigen, EMA) represents a mucin molecule strongly expressed in various epithelia and epithelial neoplasms. Its expression correlates with clinical and pathological factors as well as prognosis in some tumor types. Additionally, MUC1 was detected in normal haematopoietic cell lines and neoplasms, especially subgroups of human lymphomas including plasma cell myeloma. Therefore, the expression of MUC1 in trephine biopsies exhibiting infiltrates of plasma cell myeloma were investigated immunohistochemically. An immunoreactivity of two monoclonal antibodies (EMA and HMFG-2) was observed in about 50% of the cases. In cases exhibiting a so-called packed marrow, EMA immunoreactivity was reduced. However, MUC1 positivity did not correlate with the cytologic grade of differentiation, the fibre content of the marrow, or survival probability of the patients. However, its strong expression in a certain percentage of cases of plasma cell myeloma may be of therapeutic impact, since new therapeutic strategies include the enrichment of MUC1-specific T cells or MUC1 vaccination.

Key words: Plasma cells, plasma cell myeloma, bone marrow, MUC1, CD138

Introduction

MUC1 represents a mucin molecule expressed in various epithelial tissues and neoplasms as well as normal haematopoietic cell lines. It is identical to numerous mucin antigens that were previously characterized, for example epithelial membrane antigen (EMA) and can be detected by applying various monoclonal antibodies (mabs) recognizing different MUC1-associated epitopes (Baldus et al., 2004). The presence of EMA was demonstrated in subgroups of human lymphomas including plasma cell myeloma in the early 1980s (Sloane et al., 1983; Delsol et al., 1984). An expression of MUC1 has been observed in a certain percentage of normal and neoplastic plasma cells (Delsol et al., 1984; Pinkus and Kurtin, 1985; Boo and Cheng, 1992; Kamoshida and Tsutsumi, 1998; Paydas et al., 2001). MUC1 expression may be induced by dexamethasone (Treon et al., 1999). Furthermore, cell lines of HLA-unrestricted cytotoxic T lymphocytes, which directly recognize the underglycosylated form of MUC1, were established from plasma cell myeloma patients (Noto et al., 1997). In a recently published study, 44% of myeloma patients exhibited elevated frequencies of MUC1-specific CD8 T cells in peripheral blood as well as bone marrow (Choi et al., 2005). Immunohistochemically, about a third of plasmacytoma patients showed MUC1 positivity, which was significantly associated with immature morphology of the plasma cells (Paydas et al., 2001). However, in the latter study biopsies from various localizations were included.

In order to investigate systematically the expression of MUC1 in trephine biopsies containing infiltrates of plasma cell myelomas, we performed a comparative immunohistochemical study concerning the expression of EMA and HMFG-2 epitopes of MUC1 as well as the infiltration by syndecan-1 (CD138) positive plasma cells. CD138 represents an antigen mainly confined to the late stages of B cell differentiation, which is suitable for the assessment of plasma cell infiltration even in patients with minimal bone marrow involvement (Bataille et al., 2006; Ng et al., 2006).

Material and methods

Bone marrow biospies from 105 consecutive patients (61 male, 44 female) suffering from plasma cell myeloma were examined. The mean age of the patients

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was 65.1 years at diagnosis (SD 10.9 years). Eight patients were lost from follow-up. At the end of the study (Nov. 1, 2003), 14 patients were alive. Three μm thick sections of paraffin-embedded tissues were cut and deparaffinized according to standard histological techniques. After microwave treatment (2x5 min at 600 W in citrate buffer, pH 6.0) primary mabs directed against CD138 (1:50, DakoCytomation, Hamburg, Germany), EMA (1:600, DakoCytomation) and HMFG-2 (1:50, Beckman Coulter, Krefeld, Germany), were incubated overnight at 4°C. After washing in tris-buffered saline (TBS, pH 7.6), the EnVision™ system involving alkaline phosphatase-labelled polymers was applied according to the manufacturer's instructions (DakoCytomation). Fast red was used as chromogen and following rinsing in aqua destilled, the nuclei were counterstained with haematoxylin and the tissues were embedded in glycerol jelly.

The sections were completely evaluated at a magnification of x400 and categorized according to the percentage of CD138 immunostained bone marrow plasma cells (0, 0-5 %, 1, >5-35%; 2, >35-65%; 3, >65-100%). Using the same scores, the ratios of EMA/ CD138 and HMFG-2/ CD138 positive plasma cells were calculated. In order to evaluate correlations between the staining results and clinico-pathological variables (cytologic differentiation, fibre content, pattern of infiltration), the chi-square test was applied at a significance level of 5%. Univariate survival analyses were performed according to the Kaplan-Meier product limit method.

Results

In 98 of 105 cases under study more than 5 % of the bone marrow cells were CD138$^+$ plasma cells. However,

Table 1. Correlation of the percentage of CD138$^+$ plasma cell myeloma cells as well as EMA$^+$/ CD138$^+$ and HMFG-2$^+$/ CD138$^+$ ratio with clinico-pathological variables (i = interstitial, n = nodular, d = diffuse).

	Cytologic differentiation			Fiber content			Pattern of infiltration		
	1	2	3	1	2	3	i	i/n	d
CD138$^+$									
Score 0	4	3	0	7	0	0	4	1	2
Score 1+	14	8	0	16	6	0	10	9	3
Score 2+	24	12	6	23	16	3	5	26	11
Score 3+	11	17	6	13	16	5	3	12	19
p	0.106			0.029			< 0.0001		
EMA$^+$/ CD138$^+$									
Score 0	23	17	7	25	17	5	6	20	21
Score 1+	12	4	0	11	5	0	6	9	1
Score 2+	7	10	2	8	8	3	4	7	8
Score 3+	11	9	3	15	8	0	6	12	5
p	0.333			0.318			0.071		
HMFG-2$^+$/ CD138$^+$									
Score 0	29	20	7	29	21	6	9	25	22
Score 1+	10	8	0	13	4	1	7	7	4
Score 2+	8	7	2	9	7	1	3	9	5
Score 3+	6	5	3	8	6	0	3	7	4
p	0.678			0.640			0.497		

Table 2. Correlation of the EMA$^+$/ CD138$^+$ and HMFG-2$^+$/CD138$^+$ ratio (cut-offpoint 35%) with clinico-pathological variables (i = interstitial, n = nodular, d = diffuse).

	Cytologic differentiation			Fiber content			Pattern of infiltration		
	1	2	3	1	2	3	i	i/n	d
EMA$^+$/ CD138$^+$									
<35%	23	17	7	25	17	5	6	20	21
>35%	30	23	5	34	21	3	16	28	14
p	0.602			0.562			0.045		
HMFG-2$^+$/ CD138$^+$									
<35%	29	20	7	29	21	6	9	25	22
>35%	24	20	5	30	17	2	13	23	13
p	0.844			0.372			0.263		

the scores of CD138+ cells did not correlate with any clinico-pathological variables (Table 1). About 55% of the cases showed EMA expression and 47% HMFG-2 immunoreactivity in more than 5% of the myeloma cells in the bone marrow (Fig. 1). However, the scores of MUC1 positivity assessed as a ratio of EMA+/ CD138+ or HMFG-2+/CD138+ cells did not show any statistically significant correlation with the cytologic grade of differentiation, fibre density or the pattern of infiltration, respectively (Table 1). In general, the same results were obtained if the analyses were repeated applying a cut-off point at 35% positivity (Table 2). As the only exception, the EMA/ CD138 ratio was reduced in cases exhibiting a diffuse infiltration (so-called packed marrow). Correlations with survival probability were not be observed either (Table 3).

Discussion

Morphologic characteristics of the bone marrow infiltrates in plasma cell myeloma allow the prediction of prognosis and provide the information required for decisions on treatment modalities (Bartl et al., 1987). Three features have a special importance: The grade of

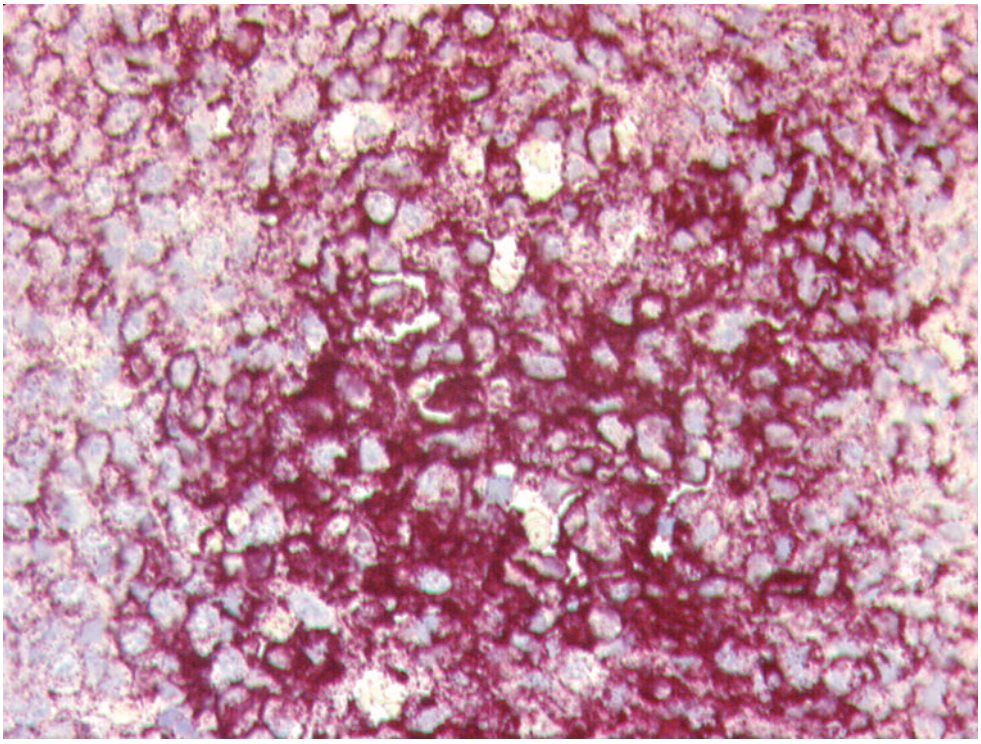

Fig. 1. Plasma cell myeloma cells expressing EMA. x 400

Table 3. Correlation of the EMA+/ CD138+ and HMFG-2+/ CD138+ ratios with overall survival.

	Cases	censored	uncensored	mean survival (days)
EMA+/ CD138+				
0-5%	45	7	38	918
>5-35%	14	2	12	793
>35-65%	17	1	16	857
>65-100%	21	4	17	850
p=0.7998 (Log-rang)				
HMFG-2+/ CD138+				
0-5%	54	8	46	909
>5-35%	15	3	12	1328
>35-65%	16	0	16	778
>65-100%	12	3	9	542
p=0.2990 (Log-rang)				

differentiation results from nuclear configuration, cell size and the ratio of cytoplasmic/nuclear volume. Another criterium is the degree and pattern of bone marrow infiltration. Patients with a mostly discrete interstitial infiltration have the highest survival probability. This is reduced in patients with a nodular pattern of infiltration and even worse in cases with diffuse infiltrates of neoplastic cells (so-called "packed marrow"). An increasing degree of bone marrow fibrosis (fine, patchy, coarse) is also correlated with a worse prognosis. The evaluation of potential new biomarkers for the course of plasms cell myeloma should include a correlation with these prognostically relevant morphologic variables. Up to now, the expression of MUC1 in bone marrow trephine biopsies of patients with plasma cell myeloma is not thoroughly characterized. In our study, about 50% of the cases with plasma cell myeloma involvement of the bone marrow exhibited an expression of MUC1 in more than 5% of the plasma cells. However, the ratio of EMA^{+}/ $CD138^{+}$ or $HMFG\text{-}2^{+}/CD138^{+}$ cells differed considerably, but according to our data, MUC1 expression by bone marrow infiltrating plasma cells did not correlate with most histomorphological parameters or patients' prognosis. The latter result is in contrast to a previously reported observation that elevated serum MUC1 levels in multiple myeloma or plasma cell leukemia patients correlate with anaemia and a shorter survival time (Luminari et al., 2003). Investigating plasmacytomas, Paydas et al. (2001) observed an association of MUC1 expression with immature morphology, but not with other pathological or clinical characteristics.

MUC1 expression in plasma cell myeloma is regulated by dexamethasone as well as various cytokines. Dexamethasone induced MUC1 expression of multiple myeloma cell lines, whereas no changes were observed after treatment with estrogen or progesteron receptor agonists (Treon et al., 1999). TNF-α also induced MUC1 expression of multiple myeloma cells in a dose and time dependent fashion (Hideshima et al., 2001). Furthermore, there was an upregulation of MUC1 mRNA in multiple myeloma cell lines after IFN-γ treatment. IFN-α had a less consistent and potent effect (Reddy et al., 2003). Interleukin-7 (IL-7) stimulation led to an increase of MUC1 on the myeloma cell surface. In addition, IL-7 induced binding of MUC1 to the Lyn tyrosine kinase resulting in an increased tyrosine phosphorylation of the MUC1 C-terminal subunit. Thereby, binding of MUC1 to ß-catenin may be induced (Durum and Aiello, 2003; Li et al., 2003).

Interesting data were published with regard to MUC1-specific cytotoxic T lymphocytes (CTL). Takahashi et al. (1994) observed that MUC1 expressed on multiple myeloma cells is recognized by HLA-unrestricted CTL. Later, Brossart et al. (2001) identified two HLA-A2-restricted T cell epitopes derived from the MUC1 protein. These CTLs efficiently lysed tumor cell lines including multiple myeloma cells. If they were transfected with tumor-derived RNA, dendritic cells induced CTLs lysing myeloma cells in an antigen-specific and HLA-restricted manner (Milazzo et al., 2003).

Additionally, enrichment of MUC1-specific CD8 memory T cells or MUC1 vaccination may represent new aspects in the therapy of plasma cell myeloma (Treon et al., 2000; Choi et al., 2005). In the context of so called targeted therapies, binding of MUC1-specific mab MA5 to multiple myeloma cells as well as uptake of the mab was demonstrated (Burton et al., 1999). In conclusion, multiple myeloma patients could at least in part profit from modern immunotherapeutic approaches focussing on MUC1 glycoprotein, which is expressed in about 50% of the bone marrow infiltrates according to our data. However, it is questionable whether such a therapy would be effective in patients with MUC1 positivity of only 5% or less of the myeloma cells. Therefore, an immunohistochemical determination of MUC1 immunoreactivity may be helpful for the pretherapeutic identification of patients exhibiting a strong MUC1 expression in the neoplastic cell population.

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