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BRIEF REPORT

Negative E-cadherin expression on bone marrow myeloma cell membranes is associated with extramedullary disease [version 1; peer review: 2 approved]

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Abstract

Background: The loss of E-cadherin expression and the induction of N-cadherin are known as hallmarks of the epithelial-to-mesenchymal transition, an essential initial step in the process of metastasis in solid tumors. Although several studies have reported expressions of these cadherins in patients with multiple myeloma (MM), their clinical significance is unknown as MM cells are non-epithelial.
Methods: In this study, we examined the expression of E- and N-cadherins by immunohistochemistry using bone marrow (BM) biopsy specimens from 31 newly diagnosed MM patients and in subsequent biopsy specimens from six of these.
Results: Negative E-cadherin expression on BM myeloma cell membranes was significantly associated with the presence of soft-tissue masses arising from bone lesions and breaking through the cortical bone, referred to as extramedullary disease (EMD).
Conclusions: Given the aggressive nature of EMD, our study suggests that screening for E-cadherin using BM immunohistochemistry is one measure that could predict the development of EMD in patients with MM.

Open Peer Review

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	1	2
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Any reports and responses or comments on the article can be found at the end of the article.

Keywords

E-cadherin, extramedullary disease, multiple myeloma, immunohistochemistry
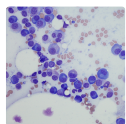
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Introduction

The loss of E-cadherin expression and the subsequent induction of N-cadherin are known as hallmarks of the epithelial-to-mesenchymal transition, an essential initial step in the process of metastasis in solid tumors.[1] Although multiple myeloma (MM) cells are non-epithelial cells, Roccaro *et al.* and Azab *et al.*[2,3] suggested that reduced E-cadherin expression in MM cells can promote extramedullary disease (EMD) *in vitro* and in an animal model, partly due to epithelial-to-mesenchymal-transition-like features. EMD is defined as the presence of an MM-cell tumor outside the bone marrow (BM), either in the form of a soft-tissue mass spreading contiguously from the bone and breaking out of the cortical bone (osseous) or arising in an isolated organ not contiguous with bone lesions (extraosseous).[4] Epithelial-to-mesenchymal transition-like features in MM patients can be used as biomarkers for EMD; however, the clinical significance of E- and N-cadherin expressions, especially in BM specimens of patients with MM, is still largely unknown and thus needs to be elucidated.

In this article, we examined E- and N-cadherin expression on MM cells obtained from patients using immunohistochemistry. We tried to clarify whether the expression of these cadherins is associated with the presence of EMD and other clinical parameters.

Methods

Study group

This study was approved by the ethics committee of Saiseikai Central Hospital (Rin 28-66) and Keio University Faculty of Pharmacy (190605-3). Written informed consent for publication of the patients' details and their images was obtained from the patients. This retrospective study included 89 consecutive patients (49 men and 40 women) between the ages of 36-94 years old (mean: 70 ± 13 years) between January 2009 and December 2016 who were newly diagnosed as having MM at Saiseikai Central Hospital. All eligible study patients with newly diagnosed MM did not receive any therapy at the time of BM biopsy. Inclusion criteria were provision of full data available on EMD involvement (yes or no) at time of diagnosis, its location, and the number of EMDs. After the exclusion of cases who were lost at follow-up or lacked indispensable medical documents, 31 patients (18 men and 13 women) between the ages of 46-88 years old (mean: 67 ± 11 years) were selected for this study. A total of 31 BM samples were collected at diagnosis and five BM samples and one surgically removed extraosseous EMD sample were collected during disease progression. The median observation period was 46 months (range 3-93).

Disease stage for each patient was based on the International Staging System.[16] Serum concentrations of albumin, β2 microglobulin, calcium, creatinine, lactate dehydrogenase and hemoglobin were measured as a part of routine medical care.

Immunohistochemistry

Immunohistochemistry for E- and N-cadherin was performed on BM and EMD biopsy specimen in 31 patients. Tissue sections were cut from formalin-fixed, paraffin-embedded blocks containing MM cells and were processed for immunohistochemistry using an automated staining system (BOND-III, Leica Microsystems, Wetzlar, Germany). For assessment of E- and N-cadherin and CD138 expressions, the E-cadherin rabbit monoclonal antibody (Cell Signaling Technology Cat# 3195, RRID:AB_2291471), N-cadherin mouse monoclonal antibody (Abcam Cat# ab98952, RRID: AB_10696943) and CD138 mouse monoclonal antibody (Agilent Cat# M7228, RRID:AB_2254116) were applied at 1:600, 1:4,000 and 1:50 dilutions, respectively, followed by detection using the BOND Polymer Refine Detection kit (Leica Biosystems Cat# DS9800, RRID:AB_2891238). Antigen retrieval was performed with BOND Epitope Retrieval Solution 2 (EDTA, pH=9.0). Positive staining of MM cells was independently evaluated by two pathologists, K.M. and S.H., without any clinical information (Cohen's κ score 1.0). At a magnification of 200, one field with the highest cadherin positive myeloma cell count on each slide was selected. The percentage of E- or N-cadherin-positive MM cells was calculated using the equation E- or N-cadherin-positive MM cells/total BM nucleated cells, regardless of the number of hematopoietic cells. A score >0.5% was used to determine positivity (yes vs. no) (Figure 1). The percentage of BM plasma cells was calculated using the equation CD138-positive cells/total BM nucleated cells, regardless of the number of hematopoietic cells.

Imaging of EMD

The presence of EMD was evaluated by well-trained radiologists. 18F-fluorodeoxyglucose positron emission tomography (PET)/computed tomography (CT) was used in 16/31 cases (51.6%) in total at diagnosis. For the remaining 15 cases, only CT, magnetic resonance imaging (MRI) or both was used in five, four, and six cases, respectively. For the five patients from whom second BM samples were taken during progression, PET/CT, only CT, and both CT and MRI were used in two, two and one cases, respectively. For the patient whose EMD sample was taken during progression, CT was used.
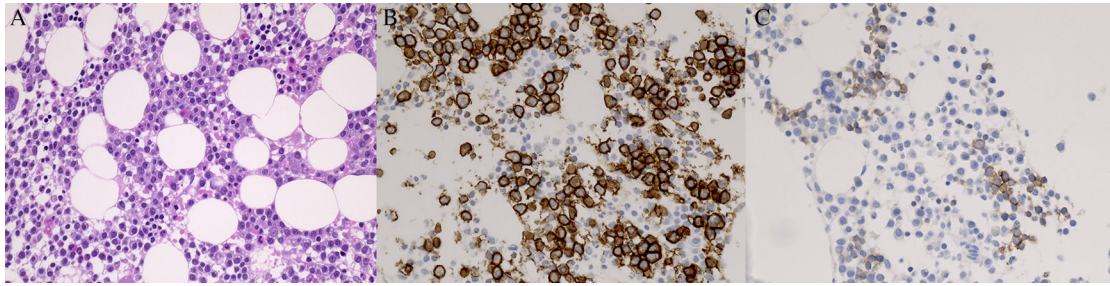
Figure 1. Typical immunophenotype of E-cadherin-positive MM cells (original magnification ×400). (A) BM biopsy with MM cell infiltrate. Hematoxylin and eosin stain. Consecutive sections of BM biopsies were stained with antibodies against (B) CD138 and (C) E-cadherin. Note that E-cadherin was clearly stained in the membrane of MM cells. MM, multiple myeloma; BM, bone marrow.

Statistics

Statistical analysis was performed with SAS 9.4 software (SAS institute, Cary, NC, USA; http://www.sas.com; Statistical Analysis System, RRID:SCR_008567). To compare clinical parameters between cadherin-positive and -negative MM patients, for continuous variables (i.e., age, serum concentrations of albumin, β2 microglobulin, calcium, creatinine, lactate dehydrogenase, hemoglobin, and BM plasma cell %), we performed pooled t-test for equal variances using the t-test analysis. Furthermore, for categorical variables we used the FREQ procedure to perform Fisher's exact test for the data frequency table (i.e., sex and presence of EMD) because of the large number of cells with count less than five, and chi-square test for monoclonal protein subtypes and the International Staging System. The Kaplan–Meier method was used to analyze the survival probability distribution of the groups accounting for the E-cadherin expression. The statistical significance level was given at $p < 0.05$ (two-sided).

Results

Relationship between cadherin expression and presence of EMD

Figure 1 shows typical E-cadherin positivity by immunohistochemistry in a patient with MM. Table 1[15] depicts the results of E-cadherin expression in conjunction with various clinical parameters at diagnosis. E-cadherin expression was positive in 25 (80.6%) of the initial 31 BM samples. At initial screening, EMD was found in 14 (45.2%) cases. Four additional patients developed EMD on follow-up from 17 patients who had no EMD at diagnosis. Negative E-cadherin expression at diagnosis (found in six patients) was significantly associated with the presence of EMD ($p=0.0041$). At diagnosis, all 14 (100%) of the patients with EMD had osseous lesions; none (0%) had extraosseous lesions (Table 2). No association was found between N-cadherin expression and the presence of EMD.

In five (patients #25, 28, 29, 30 and 31) of the 31 newly diagnosed patients, four of whom (#25, 28, 29 and 31) were initially positive for E-cadherin and three of whom (#25, 28 and 30) had EMD, we were able to investigate E-cadherin status in an additional BM sample, later in disease progression. In two patients (#25 and 28), the second BM sample became negative for E-cadherin after the first had been positive; in both cases, new EMDs (two additional osseous EMDs in #25 and pleural and chest wall EMDs in #28) were present at the time of the second sample. The two (patients #29 and 31) who were positive for E-cadherin at both biopsies did not show EMD, but patient #30 who was negative for E-cadherin at both biopsies showed osseous EMD throughout the course of the disease, with an increase from one to more than 20. Furthermore, in patient #24, who developed testicular EMD during disease progression, E-cadherin expression was negative both in the BM sample at diagnosis and in the EMD sample at the time of disease progression.

Relationship between cadherin expression and clinicopathological parameters

Patients with E-cadherin-positive cells had significantly higher serum albumin concentrations ($p=0.0221$), and hemoglobin values ($p=0.0268$) (Table 1). Figure 2 shows the overall survival (OS) probability in all enrolled patients. The median OS values of positive and negative E-cadherin patients were 51.5 months (range 3-93) and 25.8 months (range 6-62), respectively (Log-rank $p=0.0644$, Wilcoxon $p=0.0682$, -2Log (LR) $p=0.0443$). By contrast, N-cadherin expression was not significantly associated with any clinical parameters.

Discussion

Our present report suggests that the loss of E-cadherin expression in MM cell membranes in BM is involved in the pathogenesis of osseous EMD. Negative E-cadherin expression was significantly associated with the presence of osseous EMD ($p=0.0041$) (Table 1). Few studies so far have specifically addressed whether epithelial-to-mesenchymal-transition-like features predict EMD. One experimental study demonstrated that cell lines overexpressing CXCR4

Table 1. Relationship between E-cadherin expression and clinicopathological parameters at diagnosis.

Clinicopathological parameters	E-cadherin Negative (n=6)	E-cadherin Positive (n=25)	p value
Age, years (range)	67 (55-82)	67 (46-88)	0.8862
Sex[#]			1.0000
Male	4	14	
Female	2	11	
Subtype[♭]			0.4655
IgG	4	12	
IgA	2	8	
Light chain disease	0	5	
ISS[♭]			0.3232
I	0	5	
II	5	13	
III	1	7	
Median Alb, g/dl (range)	2.9 (2.2-3.7)	3.6 (2-4.9)	0.0221*
Median β2MG, mg/l (range)	3.4 (2-5.5)	4.7 (2-15.3)	0.3067
Median Ca, mg/dl (range)	9.9 (8.1-11.8)	9.6 (7.4-12)	0.5797
Median Cr, mg/dl (range)	1.00 (0.7-1.4)	1.18 (0.5-2.89)	0.4588
Median LDH, U/l (range)	190 (132-295)	186 (99-370)	0.8936
Median Hb, g/dl (range)	8.4 (6.8-10)	10.8 (6.5-17.7)	0.0268*
Median BM PC, % (range)	66.6 (40-90)	62 (10-90)	0.6893
EMD[#]			0.0041*
Present	6	8	
Absent	0	17	

ISS, International Staging System; Alb, albumin; β2MG, β2 microglobulin; Ca, calcium; Cr, creatinine; LDH, lactate dehydrogenase; Hb, hemoglobin; BM, bone marrow; PC, plasma cell; EMD, extramedullary disease; IgG, immunoglobulin G; IgA, immunoglobulin A.
*Statistically significant.
[#]Fisher's exact test.
[♭]chi-square test.

Table 2. The EMD sites involved and the number of EMD sites per patient at initial diagnosis.

| Patient	EMD sites	EMD number
1	sacral bone, rib bones	6
8	thoracic spine, vertebral canal	2
11	iliac bone	1
13	thoracic spine, rib bone	2
17	humerus bone, rib bones	4
18	thoracic spine	1
22	thoracic spine	2
23	lumbar spine	1
24	thoracic spine, lumbar spine	2
25	lumbar spine	1
26	iliac bone	1
27	iliac bone, cervical spine, rib bone	3
28	sacral bone, iliac bone, lumber spine	5
30	rib bone	1

EMD, extramedullary disease.
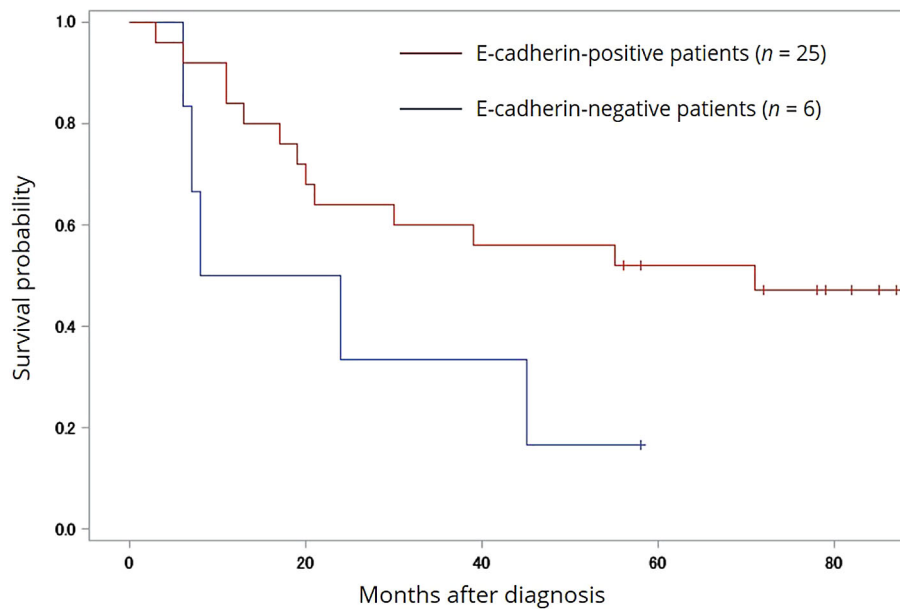
Figure 2. Survival analysis of E-cadherin in univariate analysis. OS stratified by the E-cadherin negativity of MM cells. A statistical significance in OS was found between the E-cadherin-positive and -negative groups (Log-rank *p*=0.0644, Wilcoxon *p*=0.0682, -2Log (LR) *p*=0.0443). Hashmarks indicate surviving patients at last visit. OS, overall survival; MM, multiple myeloma.

showed decreased E-cadherin.[2] This study demonstrated progression to EMD in a mouse model subjected to the injection of this cell line. One clinical study provided evidence that the expression frequency of Twist1, which suppresses E-cadherin, in EMD samples from MM patients, is higher than that in BM samples from these patients or MM patients without EMD.[5] In another study, the process of EMD formation has been associated with the upregulation of CXCR4 in EMD specimens of patients with MM.[6] Notably, our study is the first to examine the E-cadherin expression of MM cells using BM biopsy specimens from patients.

In agreement with the hypothesis that loss of E-cadherin indicates development of EMD, in two out of five patients with follow-up BM samples (#25 and #28), E-cadherin was found to be positive at the time of diagnosis but negative at the time of disease progression. Interestingly, at progression, the number of osseous EMDs increased in patient #25 and new extraosseous pleural and chest wall EMDs developed in patient #28. In other cases, E-cadherin positivity (#29 and 31) or negativity (#30) was observed in both the first sample and that taken later in the course of the disease; one of these (#30) had EMD. These findings are consistent with the association of negative E-cadherin expression with the higher incidence of EMD, which seemed to be related to the shorter OS. In another patient with sequential samples, #24, a testicular EMD sample during disease progression was investigated. E-cadherin was found to be unexpressed in both the BM at diagnosis and in the EMD at the time of disease progression, possibly indicating the outgrowth of clones without E-cadherin during disease evolution. Taken together, these results suggest that clones without E-cadherin expression in the BM are "fit" under pressure and are assumed to be able to produce specific tropic growth to the EMD site.

The incidence of osseous EMD in this study was 45.2%. In the literature, osseous EMD is only present in up to 32.5% of newly diagnosed MM cases.[7] The higher frequency in our patients is probably explained by the fact that our cohort was enriched by patients using PET/CT (51.6%) at initial staging. Indeed, PET/CT has shown superior potential to regular CT in detecting osseous EMD.[8]

Another objective of the current study was to investigate whether expression of E-cadherin was associated with clinicopathological parameters, and particularly OS, in these patients. In addition to the significant difference in OS (25.8 vs. 51.5 months; *p*=0.0443), it is noteworthy that negative E-cadherin expression in MM patients in this study was associated with lower serum albumin and hemoglobin levels, suggesting that the loss of this molecule is either a deteriorating factor or a marker for disease severity and shorter OS. Several pieces of circumstantial evidence support the loss of E-cadherin being closely associated with the progression of MM. A study using the MM mouse model showed that decreased E-cadherin expression was associated with tumor progression.[3] In a clinical study using DNA-methylation

analysis, the E-cadherin gene was shown to be silenced with increased frequency in MM than in patients with monoclonal gammopathies .[9] Another clinical study using immunohistochemistry in spinal plasmacytoma, including 28 MMs with osseous EMD and 13 solitary plasmacytomas of bone, showed that patients with negative E-cadherin expression had a significantly shorter OS than those with positive (26.0 vs. 48.7 months; p <0.05).[10] Although the immunohistochemistry samples were taken from surgically removed spinal masses in their study, their finding was in good accordance with our results in which the immunohistochemistry samples were taken from BM. However, the role of E-cadherin in disease progression and severity is still a matter for debate because the research results are not always consistent.[11] As to the relationship between N-cadherin and patient prognosis, Vandyke $et\ al.$[12] revealed an inverse relationship between circulating N-cadherin and patient OS. Contrarily, in our study, no statistically significant relationship was observed between N-cadherin expression in MM cells and patient prognosis.

There are some limitations in this study. The relatively small number of patients and incomplete data on cytogenetics and molecular biology make it difficult for us to assess the direct impact of E-cadherin expression of MM cells on OS. Therefore, further studies with a larger sample size and more consistent follow-up are required to confirm the association of negative E-cadherin expression with poor survival in MM patients with EMD. From the therapeutic viewpoint, preventive strategies to inhibit EMD have been proposed.[13,14] Given the aggressive nature of EMD, biomarkers for patients at risk of EMD will help develop precision medicine for patients with MM.

Taken together, our results suggest that further investigation into the usefulness of E-cadherin expression in simple and routine BM as a biomarker for EMD is warranted.

Data availability
Underlying data
Dryad: Negative E-cadherin expression on bone marrow myeloma cell membranes is associated with extramedullary disease. https://doi.org/10.5061/dryad.ns1rn8pvn.[15]

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Acknowledgements

References

1. Loh C-Y, Chai JY, Tang TF, *et al*.: **The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges.** *Cells.* 2019; **8**(10): 1118.
   PubMed Abstract | **Publisher Full Text**

2. Roccaro Aldo M, Mishima Y, Sacco A, *et al*.: **CXCR4 Regulates Extra-Medullary Myeloma through Epithelial-Mesenchymal-Transition-like Transcriptional Activation.** *Cell Reports.* 2015 2015/07/28; **12**(4): 622–635.
   PubMed Abstract | **Publisher Full Text**

3. Azab AK, Hu J, Quang P, *et al*.: **Hypoxia promotes dissemination of multiple myeloma through acquisition of epithelial to mesenchymal transition-like features.** *Blood.* 2012 Jun 14; **119**(24): 5782–594.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

4. Bansal R, Rakshit S, Kumar S: **Extramedullary disease in multiple myeloma.** *Blood Cancer J.* 2021 Sep 29; **11**(9): 161.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

5. Yang JZ, Lian WG, Sun LX, *et al*.: **High nuclear expression of Twist1 in the skeletal extramedullary disease of myeloma patients predicts inferior survival.** *Pathol Res Pract.* 2016 Mar; **212**(3): 210–216.
   PubMed Abstract | **Publisher Full Text**

6. Weinstock M, Aljawai Y, Morgan EA, *et al*.: **Incidence and clinical features of extramedullary multiple myeloma in patients who underwent stem cell transplantation.** *Br J Haematol.* 2015; **169**(6): 851–858.
   PubMed Abstract | **Publisher Full Text**

7. Varga C, Xie W, Laubach J, *et al*.: **Development of extramedullary myeloma in the era of novel agents: no evidence of increased risk with lenalidomide-bortezomib combinations.** *Br J Haematol.* 2015 Jun; **169**(6): 843–850.
   PubMed Abstract | **Publisher Full Text**

8. Tian C, Wang L, Wu L, *et al*.: **Clinical characteristics and prognosis of multiple myeloma with bone-related extramedullary disease at diagnosis.** *Biosci Rep.* 2018 Jun 29; **38**(3).
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

9. Seidl S, Ackermann J, Kaufmann H, *et al*.: **DNA-methylation analysis identifies the E-cadherin gene as a potential marker of disease progression in patients with monoclonal gammopathies.** *Cancer.* 2004 Jun 15; **100**(12): 2598–606.
   PubMed Abstract | **Publisher Full Text**

10. Wang L, Liu H, He J, *et al*.: **E-cadherin expression and its clinical significance in 41 cases of spinal plasma cell myeloma.** *Journal of Medical Colleges of PLA.* 2009 2009/02/01; **24**(1): 56–62.
    Publisher Full Text

11. Bi E, Li R, Bover LC, *et al*.: **E-cadherin expression on multiple myeloma cells activates tumor-promoting properties in plasmacytoid DCs.** *J Clin Invest.* 2018 Nov 1; **128**(11): 4821–4831.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

12. Vandyke K, Chow AW, Williams SA, *et al*.: **Circulating N-cadherin levels are a negative prognostic indicator in patients with multiple myeloma.** *Br J Haematol.* 2013 May; **161**(4): 499–507.
PubMed Abstract | **Publisher Full Text**

13. Usmani SZ, Weiss BM, Plesner T, *et al*.: **Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma.** *Blood.* 2016 Jul 7; **128**(1): 37–44.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

14. Gagelmann N, Ayuk F, Atanackovic D, *et al*.: **B cell maturation antigen-specific chimeric antigen receptor T cells for relapsed or refractory multiple myeloma: A meta-analysis.** *Eur J Haematol.* 2020 Apr; **104**(4): 318–327.
PubMed Abstract | **Publisher Full Text**

15. Hirao M: **Negative E-cadherin expression on bone marrow myeloma cell membranes is associated with extramedullary disease, Dryad, Dataset.** 2022.
Publisher Full Text

16. Greipp PR, San Miguel J, Durie BG, *et al*.: **International staging system for multiple myeloma.** *J Clin Oncol.* 2005 May 20; **23**(15): 3412–3420. Erratum in: J Clin Oncol. 2005 Sep 1; 23(25):6281. Harousseau, Jean-Luc [corrected to Avet-Loiseau, Herve].
PubMed Abstract | **Publisher Full Text**

Open Peer Review

Current Peer Review Status: ✅ ✅

Version 1

Reviewer Report 18 May 2022

https://doi.org/10.5256/f1000research.121064.r134806

✅ **Makoto Sasaki**

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The authors analyzed E-cadherin expression of clonal plasma cells in bone marrow using the immunohistochemistry staining and revealed the significant association between lack of expression of E-cadherin and osseous extramedullary disease (EMD) in patients with multiple myeloma (MM). Furthermore, they exposed the association of negative expression of E-cadherin with poor survival in patients with MM. Although the sample size is relatively small, this study provides essential information for physician-scientists in the hematological field.

1. There is some evidence of the association between CXCR4 and EMD. In some mouse myeloma models, low expression of CXCR4 is associated with progression of EMD[1], and CXCR4 overexpression enhances epithelial-to-mesenchymal transition (EMT) like features with upregulation of Snail, Slug, and Twist, and downregulation of E-cadherin[2]. It would be more informative if the authors could evaluate and discuss the expression of CXCR4 in their patients.

2. In this report, EMD was observed in 14(45%) of 31 cases at diagnosis. Although, the incidence of EMD in myeloma is around 5% at diagnosis and 20-30% in relapsed/refractory patients [3,4].What is the cause of the extremely high prevalence of EMD in this study group?

3. It is better to add each patient's type of light chain in table 2. In the previous report cited by the authors, the expression of E-cadherin had a strong association with the type of light chain[5]

References
1. Stessman H, Mansoor A, Zhan F, Janz S, et al.: Reduced CXCR4 expression is associated with extramedullary disease in a mouse model of myeloma and predicts poor survival in multiple myeloma patients treated with bortezomib. *Leukemia*. 2013; **27** (10): 2075-2077 Publisher Full Text
2. Roccaro AM, Mishima Y, Sacco A, Moschetta M, et al.: CXCR4 Regulates Extra-Medullary Myeloma through Epithelial-Mesenchymal-Transition-like Transcriptional Activation.*Cell Rep*. 2015; **12** (4): 622-35 PubMed Abstract | Publisher Full Text

3. Jagosky MH, Usmani SZ: Extramedullary Disease in Multiple Myeloma.*Curr Hematol Malig Rep*. **15** (2): 62-71 PubMed Abstract | Publisher Full Text
4. Touzeau C, Moreau P: How I treat extramedullary myeloma.*Blood*. 2016; **127** (8): 971-6 PubMed Abstract | Publisher Full Text
5. Bi E, Li R, Bover L, Li H, et al.: E-cadherin expression on multiple myeloma cells activates tumor-promoting properties in plasmacytoid DCs. *Journal of Clinical Investigation*. 2018; **128** (11): 4821-4831 Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Multiple Myeloma, Clinical Trials

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 19 April 2022

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Antonio Bedalov
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Epithelial to mesenchymal transition (EMT) and resulting alterations in cell adhesion, cell polarity, and cytoskeleton remodeling have been associated with the acquisition of invasive and metastatic

properties of cancer cells. EMT is orchestrated by a well-characterized set of transcription factors (e.g. SNAIL, TWIST) which change the expression of several adhesion proteins, including downregulation of E-cadherin and upregulation of N-cadherin. Even though the hematopoietic system is mesoderm-derived, an EMT-like transcriptional cascade has been described in hematologic cancers, including multiple myeloma (MM) as shown in a mouse model of MM progression [1].

A major finding of the study is that the lack of expression of E-cadherin in diagnostic bone marrow biopsy of 31 multiple myeloma patients is associated with the presence of extramedullary disease. All 6 patients that lack E-cadherin expression exhibit extramedullary disease (EMD) 6/6=100%), while among the 25 patients that express E-cadherin EMD is found at diagnosis in only 8 patients (8/25=32%). Even though the sample size is small, this association is significant and strong, which strengthens the argument that the EMT might be involved in the acquisition of invasive phenotype in MM. The authors have also observed that the absence of cadherin E expression is associated with reduced survival, however, it is presently not clear whether the lack of cadherin E expression was an independent prognostic marker or just a marker of more severe/advanced disease, given that other markers of disease severity such as reduced albumin and hemoglobin level are also associated with the absence of cadherin E expression. While limitations of the studies are mentioned. I believe that additional points that should be brought up in the discussion are:

1. Less than half of the patients with EMD exhibit loss of cadherin E expression at diagnosis (does this reflect MM heterogeneity, sampling error, or do the authors believe that invasive phenotype occurs in EMT-independent fashion);

2. Does the prognosis of the patients with EMD but without loss of cadherin E differ from that in patients with EMD and loss of cadherin E;

3. Are there patients for which the biopsy of both EMD and bone marrow biopsy have been obtained, and if so, what was the concordance in cadherin E expression between the two?

If unable to address these points directly because of the small sample size, this limitation should be acknowledged and discussed.

References

1. Azab AK, Hu J, Quang P, Azab F, et al.: Hypoxia promotes dissemination of multiple myeloma through acquisition of epithelial to mesenchymal transition-like features.*Blood*. 2012; **119** (24): 5782-94 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer biology, hematologic malignancies, epigenetics, DNA replication

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 09 May 2022

Maki Hirao, Saiseikai Central Hospital, 1-4-17 Mita, Minato-ku, Japan

Thank you for your useful comments and suggestions. The reviewer has asked for a description of why loss of cadherin E expression at diagnosis was observed only in the 43% of the EMD observed cases (6 out of 14). MM is a clinically as well as biologically heterogeneous disease. Thus, we think loss of E-cadherin expression is not only the mechanism for EMD formation, but diverse molecular mechanisms exist for it. Currently, the small number of patients has only been used in assessment of the direct impact of E-cadherin expression of MM cells on OS, and we are unable to rule out the possibility of sampling errors. Therefore, our results require further studies with a larger sample size and more consistent follow-up. We will include this explanation in the discussion of the revised manuscript.

Additional data analysis was performed to address potential roles of E-cadherin. Among the 14 patients with EMD, no correlation was found between E-cadherin expression and the clinical outcome. The median OS values of positive and negative E-cadherin patients were 34.9 months (range 5-87) and 25.8 months (range 6-62), respectively (Log-rank p=0.8459, Wilcoxon p=0.6507, -2Log (LR) p=0.6663). Further studies should be addressed to investigate the potential role of cadherin E in EMD patients for prognostic prediction.

We note that the reviewer is concerned about the correlation in cadherin E expression between BM staining and EMD lesion. To address this issue, we have validated the concordance in cadherins E expression between the testicular EMD and BM biopsies only in patient #24. E-cadherin was found to be unexpressed in both the BM and the EMD. The findings are described on line 27 to 28 of page 4 and line 13 to 16 of page 6 of the manuscript. We are planning to carry out further studies on the EMD biopsies.

Competing Interests: No competing interests were disclosed.

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