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ORIGINAL ARTICLE

Bone Marrow Urokinase Plasminogen Activator Receptor Levels are Associated with the Progress of Multiple Myeloma△

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Key words: multiple myeloma; urokinase-type plasminogen activator receptor; bone marrow; urokinase

Objective To determine the mRNA and protein levels of urokinase plasminogen activator receptors (uPAR) in bone marrow fluid and bone marrow tissue from multiple myeloma (MM) patients and assess association of uPAR level with prognosis of MM.

Methods uPAR levels in bone marrow fluid of 22 MM patients at the stable and progressive stages and 18 iron deficiency anemia patients with normal bone marrow (control) were examined by ELISA. Furthermore, uPAR expression in bone marrow tissue was investigated by RT-PCR and Western blot, respectively. The distribution of uPAR in MM cells was examined using immunofluorescence staining. The pathological changes in different stages of MM patients were studied by HE staining.

Results uPAR level in bone marrow fluid of MM patients (1.52±0.32 μg/ml) was found to be higher than that in the control group (0.98±0.15 μg/ml). Interestingly, uPAR protein (0.686±0.075 *vs.* 0.372±0.043, $P<0.05$) and mRNA (2.51±0.46 *vs.* 4.46±1.15, $P<0.05$) expression levels of MM patients at the progressive stage were significantly higher than those at the stable stage. The expression of uPAR in MM bone marrow was confirmed by immunofluorescence staining. Moreover, HE staining revealed a great increased number of nucleated cells and severe impairment of hematopoietic function in the bone marrow of patients with progressive-stage myeloma.

Conclusion Our study reveals that uPAR expression is positively correlated with the development and progress of MM.

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MULTIPLE myeloma (MM) is a fatal hematologic malignancy characterized by a neoplastic proliferation of plasma cells and the secretion of monoclonal Ig.[1, 2] The presenting symptoms of MM includes pathologic fractures, recurrent infection, anemia, and renal failure. It accounts for 1% of all malignant tumors and 13% of all hematological malignancies.[3] MM is commonly found in people aged between 50 and 70 years regardless of gender. It is estimated that, with the aging of population the number of MM patients in China will rise within the next few years,[4] which could aggravate the financial burden due to the medical care and compromise the quality of life of suffered people as well. Early diagnosis of MM is believed to be the key to treat and cure this devastating disease.

The urokinase type plasminogen activation (uPA) system, consisting of uPA and its receptor uPAR, plays a key role in tissue remodelling and extracellular matrix (ECM) degrading.[5, 6] uPA has been demonstrated as a prognostic marker for breast cancer.[7, 8] Moreover, the essential role of both uPA and uPAR in the evasion and metastasis of cancers has been confirmed,[5, 6] which made this system an attractive target for cancer treatment. The value of uPAR as a prognostic marker was also demonstrated in various types of cancers including acute leukemia, breast cancer, and MM.[8, 9] Intriguingly, it has been showed that the plasma level of uPAR was positively correlated with the prognosis of MM.[10] However, the level of uPAR in the bone marrow of MM patients and its association with MM prognosis are not reported. In fact, bone marrow, the hematopoietic center in our body, is closely related to pathogenesis of MM, as the presence of large numbers of neoplastic plasma cells is one of the characters of MM. Importantly, the microenvironment of bone marrow is critical to the growth and survival of bone marrow cells.[11] Therefore, it will be valuable to investigate whether uPAR level in bone marrow can serve as a new prognostic marker for MM patients compared with that in plasma. In this study, we measured the level of uPAR in both bone marrow and its fluid from MM patients and further assessed the association of uPAR with the prognosis of MM progression and its chemotherapeutic treatment.

PATIENTS AND METHODS

Patient eligibility

The bone marrow samples were collected from 22 patients who were diagnosed with MM between years 2009 and 2014 at Huzhou Central Hospital. All patients met the diagnostic criteria described in Standard of Diagnosis and Therapeutic Effect of Hematopathy by Zhang and Shen.[12] The median age at diagnosis was 57 (range 39-79) years and 13 (59%) patients were male. Eight patients were newly diagnosed with MM and 14 were retreated including 5 relapsed/refractory cases, 6 cases who achieved partial remission, and 3 cases with complete response. The duration of the disease was from 1 to 67 months with an average of 16.4 months. Informed consent was obtained from all subjects before the study.

The clinical staging of patients was determined based on treatment response.[13] Patients were classified as being in the stable stage if they are responsive to therapies (10 cases, 45%), or being in progressive stage if they do not respond to treatment (12 cases, 55%).

In addition, uPAR levels of 5 newly diagnosed patients after chemotherapy were compared with before therapy.

Eighteen iron deficiency anemia patients with normal bone marrow were served as the control. Median age of the control group was 52 (range 38-76) years and 11 (61%) patients were male.

ELISA

An aliquot of 2 ml bone marrow fluid was mixed within 5% EDTA under sterile condition and centrifuged at 4500×*g* for 10 minutes. The supernatant was then preserved at −80°C for future use. uPAR level was evaluated with human uPAR ELISA kit (R&D system, Minneapolis, USA) according to manufacturer's protocol. Colour developed was read at 450 nm and the optical density value is directly proportional to the concentration of uPAR in the samples (μg/ml).

Western blot

Bone marrow tissue was homogenized and lysed in RIPA buffer (Beyotime Institute of Biotechnology) containing protease inhibitors. After centrifugation (30000×*g*, 4°C) for 10 minutes, the supernatants were collected and denatured by SDS sample buffer. Equal amounts of protein samples were separated by SDS-PAGE gel and transferred onto a nitrocellulose membrane. After blocking in 10% milk for 1 hour at room temperature, the membranes were incubated with rabbit anti-human uPAR or mouse anti-human β-actin antibody (Santa Cruz, USA, 1:1000 dilution) at 4°C overnight. The band was detected after the membrane was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (Santa Cruz) for 2 hours at room temperature by using NBT/BCIP (Beyotime Institute of Biotechnology). The blot was photographed with a fluorescence microscope (Carl Zeiss, German) and the density of the bands on the membrane was quantified by Image J software (gel