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Research Article

Externalization and recognition by macrophages of large subunit of eukaryotic translation initiation factor 3 in apoptotic cells

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Abstract

We previously isolated a monoclonal antibody named PH2 that inhibits phosphatidylserine-mediated phagocytosis of apoptotic cells by macrophages [C. Fujii, A. Shiratsuchi, J. Manaka, S. Yonehara, Y. Nakanishi. Cell Death Differ. 8 (2001) 1113–1122]. We report here the identification of the cognate antigen. A protein bound by PH2 in Western blotting was identified as the 170-kDa subunit of eukaryotic translation initiation factor 3 (eIF3 p170/eIF3a). When eIF3a was expressed in a culture cell line as a protein fused to green fluorescence protein, the fusion protein was detected at the cell surface only after the induction of apoptosis. The same phenomenon was seen when the localization of endogenous eIF3a was determined using anti-eIF3a antibody, and eIF3a seemed to be partially degraded during apoptosis. Furthermore, bacterially expressed N-terminal half of eIF3a fused to glutathione *S*-transferase bound to the surface of macrophages and inhibited phagocytosis of apoptotic cells by macrophages when it was added to phagocytosis reactions. These results collectively suggest that eIF3a translocates to the cell surface upon apoptosis, probably after partial degradation, and bridges apoptotic cells and macrophages to enhance phagocytosis.

Keywords: Apoptosis; eIF3a; eIF3 p170; Translation initiation factor; Macrophage; Monoclonal antibody; Phagocytosis

Introduction

Apoptosing cells are selectively and rapidly cleared from our bodies by phagocytosis ([1] for a review), and this is necessary for the maintenance of tissue homeostasis and many other biological functions ([2–6] for reviews). Selective recognition of dying cells by phagocytes is accomplished through specific interaction between phagocytosis marker molecules present at the surface of target cells and their cognate receptors in phagocytes ([7] for a review). A number of molecules have been proposed as such phagocytosis markers, and many of these molecules change their structure and/or intracellular localization rather than being synthesized de novo and expressed at the cell surface during apoptosis ([8] for a review). The best-characterized phagocytosis marker is the membrane phospholipid phosphatidylserine (PS) ([9,10] for reviews). PS is restricted to the inner leaflet of the membrane bilayer in viable cells, but translocates to the outer leaflet and is thus expressed at the cell surface when cells are induced to undergo apoptosis ([11] for a review). Although the

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involvement of several lipid transporters has been suggested, the precise mechanism of this phenomenon remains unclear ([12,13] for reviews). The intracellular distribution of many other molecules seems to change during apoptosis [3,14–16], but the mechanisms and consequences of this event are largely unknown.

We previously reported that the extent of macrophage phagocytosis of apoptotic cells continues to increase even after the externalization of PS in apoptotic cells reaches a maximal level [17]. This suggested that the expression of another molecule follows externalization of PS in order for cells at late stages of apoptosis to be maximally recognized and phagocytosed by macrophages. To identify such a molecule, we generated monoclonal antibodies by immunizing mice with late apoptotic cells [18]. When we examined the effects of cloned antibodies on phagocytosis, one clone, which we named PH2, was found to inhibit the phagocytosis of cells at late stage of apoptosis [18]. In the present study, we identified and characterized the cognate antigen. The PH2 antigen turned out to be the 170-kDa subunit of eukaryotic translation initiation factor 3 (eIF3 p170, later renamed as eIF3a). eIF3a appeared to translocate from the cytoplasm to the cell surface during apoptosis and help late apoptotic cells to be effectively recognized and phagocytosed by macrophages.

Materials and methods

Antibody and other reagents

PH2 is monoclonal mouse IgM generated by immunizing mice with HF1 cells, HeLa S3 cells overexpressing human Fas [17], at late stage of apoptosis [18] and was used as purified Ig throughout this study. Affinity-purified rabbit IgG antibody specific for eIF3a was prepared as described previously [19]. Monoclonal rat anti-green fluorescence protein (GFP) antibody, clone JFP-K2, was provided by S.C. Fujita of Mitsubishi Kagaku Institute of Life Sciences, Japan. Goat polyclonal anti-glutathione *S*-transferase (GST) antibody was purchased from Amersham Biosciences (Uppsala, Sweden). Antibodies recognizing the following organelle markers were used: GM 130, a marker for *cis*-Golgi [20] (rabbit antiserum provided by Y. Ikehara of Fukuoka University, Japan); β1→4 galactosyltransferase, a marker for *trans*-Golgi [21] (monoclonal mouse IgG clone GTL2) [22]; TGN 46 [21,23] and golgin 97 [24,25], markers for *trans*-Golgi network (rabbit antisera provided by M. Fukuda of Burnham Institute, USA and N. Nakamura of Kanazawa University, Japan, respectively); calnexin, a marker for endoplasmic reticulum [26] (rabbit antiserum purchased from StressGen Biotechnologies, San Diego, CA, USA); Tim 17, a marker for mitochondria [27] (rabbit antiserum provided by K. Mihara of Kyusyu University, Japan); Pex 14p, a marker for peroxisomes [28] (rabbit antiserum provided by Y. Fujiki of Kyusyu University, Japan). Secondary antibodies and fluorescence-labeled reagents used for immunofluorescence were: Cy3-labeled anti-mouse IgM antibody (Jackson ImmunoResarch Laboratories, West Grove, PA, USA) for PH2; biotin-labeled anti-rabbit IgG antibody (Vector Laboratories, Burlingame, CA, USA) and Alexa-Fluor 488-conjugated streptavidin (Molecular Probes, Eugene, OR, USA) for anti-eIF3a antibody; biotin-labeled anti-rat IgG antibody (Vector Laboratories) and Alexa Fluor 546-conjugated streptavidin (Molecular Probes) for anti-GFP antibody; biotin-labeled anti-rabbit IgG (Vector Laboratories) and fluorescein isothiocyanate-labeled avidin D (Vector Laboratories) for anti-GM 130, anti-TGN 46, anti-golgin 97, anti-calnexin, anti-Tim 17, and anti-Pex 14p antibodies. Secondary antibodies used for Western blotting were: peroxidase-conjugated anti-mouse IgM antibody (Jackson ImmunoResearch Laboratories) for PH2; peroxidase-conjugated anti-rabbit IgG antibody (Amersham Biosciences) for anti-eIF3a antibody; alkaline phosphatase-conjugated anti-mouse IgG antibody (Bio-Rad Laboratories, Hercules, CA, USA) for anti-β1→4 galactosyltransferase antibody; alkaline phosphatase-conjugated anti-rabbit IgG antibody (Bio-Rad Laboratories) for anti-GM 130 and anti-calnexin antibodies. Recombinant soluble mouse Fas ligand (WX1) consisting of the signal sequence derived from mouse granulocyte colony-stimulating factor and almost the entire extracellular region of mouse Fas ligand (amino acid 101–279) was prepared as described previously [29] and provided by T. Suda of Kanazawa University, Japan.

Cell culture and induction of apoptosis

HF1 cells were maintained in minimum essential medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum at 37°C in a humidified atmosphere of 5% (v/v) CO_2 in air. HF1 cells were induced to undergo apoptosis by incubation with the anti-Fas monoclonal antibody CH11 (MBL, Nagoya, Japan) (10 ng/ml) and cycloheximide (50 μg/ml) for 24 h. In some experiments, apoptosis was induced in HF1 cells by treatment with WX1 (1000 units/ml) for 24 h. After the induction of apoptosis, cells remaining attached to culture containers and those detached from culture containers were individually recovered and used as cells at early and late stages of apoptosis, respectively, as described previously [18]. Jurkat cells, a human leukemic T-cell line, and THP-1 cells, a human monocyte-derived cell line, were maintained in RPMI 1640 medium containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 in air. For the induction of apoptosis, Jurkat cells were incubated in the same medium containing the anticancer drug doxorubicin (Sigma-Aldrich, St. Louis, MO, USA) (0.3 μg/ml) for 24–36 h as described previously [30]. The occurrence of apoptosis in HF1 cells and Jurkat cells was monitored either by detecting PS externalization in a flow cytometer using fluorescence-labeled annexin V or by determining the extent of chromatin condensation under a