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S100A8/9 induces cell death via a novel, RAGE-independent pathway that involves selective release of Smac/DIABLO and Omi/HtrA2

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Abstract

A complex of two S100 EF-hand calcium-binding proteins S100A8/A9 induces apoptosis in various cells, especially tumor cells. Using several cell lines, we have shown that S100A8/A9-induced cell death is not mediated by the receptor for advanced glycation endproducts (RAGE), a receptor previously demonstrated to engage S100 proteins. Investigation of cell lines either deficient in, or over-expressing components of the death signaling machinery provided insight into the S100A8/A9-mediated cell death pathway. Treatment of cells with S100A8/A9 caused a rapid decrease in the mitochondrial membrane potential ($\Delta\Psi_m$) and activated Bak, but did not cause release of apoptosis-inducing factor (AIF), endonuclease G (Endo G) or cytochrome *c*. However, both Smac/DIABLO and Omi/HtrA2 were selectively released into the cytoplasm concomitantly with a decrease in Drp1 expression, which inhibits mitochondrial fission machinery. S100A8/A9 treatment also resulted in decreased expression of the anti-apoptotic proteins Bcl2 and Bcl-X_L, whereas expression of the pro-apoptotic proteins Bax, Bad and BNIP3 was not altered. Over-expression of Bcl2 partially reversed the cytotoxicity of S100A8/A9. Together, these data indicate that S100A8/A9-induced cell death involves Bak, selective release of Smac/DIABLO and Omi/HtrA2 from mitochondria, and modulation of the balance between pro- and anti-apoptotic proteins.

Keywords: Bcl2 protein family; S100/calgranulin; Cancer regression; Drp1; Receptor for advanced glycated endproducts (RAGE); Mitochondrial fission; XIAP

Abbreviations: $\Delta\Psi_m$, mitochondrial membrane potential; AIF, apoptosis-inducing factor; BH3, Bcl2 homology 3; BNIP3, Bcl2/adenovirus E1B 19 kD-interacting protein 3; DD, death domain; DED, death effector domain; DISC, death inducing signaling complex; Drp, dynamin-related protein; DTPA, diethylene triamine pentaacetate; Endo G, endonuclease G; FADD, Fas-Associated Death Domain; FADD-DN, dominant-negative FADD mutant; HtrA2, high-temperature requirement A2; IAPs, inhibitors of apoptosis; IM, inner membrane; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; RAGE, receptor for advanced glycation endproducts; ROS, reactive oxygen species; Smac/DIABLO, second mitochondrial activator of caspases/direct inhibitor of apoptosis binding protein of low PI; XIAP, X-linked inhibitor of apoptosis

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1. Introduction

Polymorphonuclear neutrophils, a vital component of the innate immune response, perform several host-defense functions such as phagocytosis of invading microorganisms and cell debris, release of a number of arachidonic acid-derived eicosanoids, generation of reactive oxygen species (ROS), and release of proteolytic enzymes as well as bactericidal and cytotoxic peptides. A complex, of two S100 EF-hand proteins, S100A8/A9, is one component of this system. S100A8/A9 is released from activated phagocytes and exerts antimicrobial activity as well as cytotoxicity against various tumour cells [1–3].

S100A8 and S100A9 (also known as calgranulins A and B, or MRP8 and MRP14 respectively) are members of the S100 multigene subfamily of cytoplasmic EF-hand Ca^{2+}-binding proteins [4,5]. They are differentially expressed in a wide variety of cell types and are abundant in myeloid cells. High expression of S100A8 and S100A9 has been reported in disorders such as rheumatoid arthritis, inflammatory bowel disease and vasculitis [5]. The S100A8/S100A9 complex is located in the cytosol of resting phagocytes and exhibits two independent translocation pathways when the cells are activated. Therefore, it has been assumed that membrane-associated and soluble S100A8/A9 may have distinct cellular functions. Recent data suggest that intracellular S100A8/A9 might be involved in (phagocyte) NADPH oxidase activation [6], whereas the secreted form exerts antimicrobial properties and induces apoptosis [1–3].

S100 proteins are known to bind to RAGE, and this interaction is considered to represent a novel proinflammatory axis involved in several inflammatory diseases. S100 activation offers an attractive model to explain how RAGE and its pro-inflammatory ligands might contribute to the pathophysiology of such diseases (for review see [7,8]). RAGE is expressed in many cell types, including endothelial cells, smooth muscle cells, lymphocytes, monocytes and neurons. RAGE comprises an extracellular region containing three immunoglobulin-like domains followed by a transmembrane domain and a short cytoplasmic region. Although intracellular binding partners have not yet been identified, the cytoplasmic region appears to be essential for RAGE signaling (for review see [9]). Binding of ligands to RAGE contributes not only to perturbation of cell homeostasis under pathological conditions [7], but also to cell migration and differentiation [10]. Evidence has accumulated that S100A8/A9 induces cell death through a dual mechanism: one associated with zinc extraction from the target cells, the other through binding to the target cell surface, possibly via ligand-induced receptor activation [1]. While the zinc-chelating activities have been characterized [11,12], the S100A8/A9 cell surface receptor and the signaling pathway have not been identified.

In the present study we provide new, important insight into the molecular mechanisms of S100A8/A9-induced cell death. Our data shows that S100A8/A9-triggered cell death, does not involve RAGE, or FADD-dependent death receptors, but is mediated by selected components of the mitochondrial death pathway. We have demonstrated that S100A8/A9-induced cell death is modulated by Bcl2-family members, and also relies on mitochondrial release of OMI/HtrA2 and Smac/DIABLO, but not cytochrome *c*, AIF, or Endo G. These events are concomitant with XIAP cleavage and downregulation of Drp1, that regulates mitochondrial fission.

2. Materials and methods

2.1. Materials

Cell culture media were purchased from Sigma Co. (Canada, Oakville, ON) and Gibco (Canada). Cell culture plasticware was obtained from Nunc Co. (Canada). Diethylene triamine pentaacetate (DTPA) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), monoclonal antibody to human MRP8/14 (FITC-labeled, clone 27 E10, Acris, Germany), rabbit anti-human Bak, mouse anti-human Bax, mouse anti-human Bcl-X_L, rabbit anti-human Mcl-1, and mouse anti-human BNIP3 were obtained from Sigma (Sigma-Aldrich, Oakville, CA), rabbit anti-human/mouse Bcl2, rabbit anti-human/mouse/rat Drp1, anti-human/mouse/rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), rabbit anti-human/mouse/rat Smac/DIABLO, rabbit anti-human/mouse/rat Omi/HtrA2, mouse anti-human/mouse/rat cytochrome *c*, and goat anti-human/mouse/rat endonuclease G (Endo G) were obtained from Santa Cruz Biotechnologies (USA). 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazolylcarbocyanine iodide (JC-1) was obtained from Invitrogen Molecular Probes (Canada). Human RAGE-siRNA and siRNA negative control were obtained from Santa Cruz Biotechnologies (USA). Goat anti-human RAGE blocking antibody was obtained form R&D Systems (Hornby, ON, CA). Anti-CD95 IgM was obtained from Upstate Cell Signaling (CA).

2.2. Purification of S100A8 and S100A9 from human neutrophils

Human neutrophils were prepared from leukocyte-rich blood fractions ("buffy coat"). S100A8/A9 was purified as described earlier [13]. Prior to use, the proteins were re-chromatographed by anion exchange using a UnoQ column (BioRad, Munich, Germany). Recombinant protein was produced by bacterial over-expression as previously described [14]. All experiments were performed using S100A8/A9 purified from human neutrophils and the results were confirmed using recombinant S100A8/A9 [14].

2.3. Cell culture

MCF7 (human, estrogen receptor positive breast cancer), MCF7-Bcl2 over-expressing, MDA-MB231 (human, estrogen receptor negative breast cancer), Jurkat (human T-cell leukemia), Jurkat-Bcl2 over-expressing, Jurkat FADD-DN, BJAB (murine B cell leukemia), BJAB FADD-DN, L929 (murine fibrosarcoma), HEK-293 (human embryonic kidney), and SHEP and KELLY (human neuroblastomas) were cultured in RPMI-1640 or DMEM (MDA-MB231, L929, HEK-293) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Cell cultures were maintained under logarithmic growth conditions.

2.4. MTT-assay

The cytotoxicity of S100A8/A9 and DTPA towards the above indicated cell lines was determined by MTT-assay as previously described [15,16]. Cell viability was calculated as a percentage using the equation: (mean OD of treated cells / mean OD of control cells) × 100 (for each time point the treated cells were compared with control cells which were treated only with solvent of S100A8/A9 and DTPA).

2.5. Measurement of apoptosis by flow cytometry

Apoptosis was measured using the Nicoletti method [17]. Briefly, cells grown in 12-well plates were treated with S100A8/A9 (100 μg/ml) for the indicated time intervals. After scraping, the cells were harvested by centrifugation at 800 g for