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Research paper

Ribonuclease binase apoptotic signature in leukemic Kasumi-1 cells

Vladimir A. Mitkevich [a,b,1], Olga V. Kretova [a,1], Irina Yu. Petrushanko [a,1], Ksenia M. Burnysheva [a], Dmitry V. Sosin [a], Olga V. Simonenko [a], Olga N. Ilinskaya [a,c], Nickolai A. Tchurikov [a], Alexander A. Makarov [a,*]

[a] Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilova Str 32, 119991 Moscow, Russia
[b] University of Oslo, Centre for Medical Studies in Russia, Moscow, Russia
[c] Department of Microbiology, Kazan Federal (Volga-Region) University, Kazan, Russia

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ABSTRACT

Cytotoxic exogenous RNases triggering apoptotic response in malignant cells have potential as anticancer drugs; surprisingly, detailed characterization of the RNase-induced apoptosis has not been conducted so far. Here we show that a cytotoxic RNase from *Bacillus intermedius* (binase) induces extrinsic and intrinsic apoptotic pathways in leukemic Kasumi-1 cells. The experiments were performed using TaqMan Array Human Apoptosis 96-well Plate for gene expression analysis, and flow cytometry. Cytometric studies demonstrated dissipation of the mitochondrial membrane potential, opening of mitochondrial permeability transition pores, activation of caspases, increase of intracellular Ca^{2+} and decrease of reactive oxygen species levels. We found that expression of 62 apoptotic genes is up-regulated, including 16 genes that are highly up-regulated, and only one gene was found to be down-regulated. The highest, 16 fold increase of the expression level was observed for TNF gene. Highly up-regulated genes also include the non-canonical NF-κB signaling pathway and inflammatory caspases 1,4. The obtained results suggest that binase induces evolutionary acquired cellular response to a microbial agent and triggers unusual apoptosis pathway.

1. Introduction

Ribonucleases (RNases) are promising agents for use in anticancer therapy (For a review see Refs. [1,2]). RNases with potential antineoplastic properties were found in fungi, bacteria, plants and animals [3–5]. An important property for potential medicinal application of antitumor RNases is their ability to induce cell death by apoptosis. The best known antitumor RNase is onconase, an amphibian ribonuclease that currently undergoes clinical trials for the treatment of cancer. Besides own apoptogenic effect, the onconase enhances tumor cells sensitivity to a number of cytotoxic agents [6]. Despite a large number of studies aimed at deciphering the cytotoxic effect of RNases on cancer cells, key elements of the cell signaling network responsible for cytotoxic effects of RNases and the mechanism of programmed cell death triggered by RNase, are poorly understood. In particular not yet defined are the apoptotic pathways of cell death induced by exogenous RNases. The available data is fragmentary and not sufficient for development of the RNases application strategy in oncology, whether on its own or in combination with other cytotoxic agents.

Bacillus intermedius ribonuclease (binase) is cytotoxic for human leukemic K562 [7] and Kasumi-1 cells [8]. Sensitivity of Kasumi-1 cells to the binase toxic action depends on the expression of *KIT* and *AML1-ETO* oncogenes. Furthermore, the mechanism of binase toxic activity toward leukemic cells includes decrease in *KIT* oncogene expression [8,9]. It was shown that binase penetrates cells and reduces total amount of RNA, although this effect does not correlate with the sensitivity of cells to the ribonuclease toxic action [10]. These data led us to the assumption that cleavage by binase of different classes of RNA molecules could trigger a number of cellular regulatory mechanisms, including regulation of apoptosis controlling genes. To study the possible involvement of apoptotic genes in Kasumi-1 cells response to binase action we used Real Time PCR and TaqMan Array Human Apoptosis 96-well Plate. We have observed that expression of 62 apoptotic genes is up-regulated and only one gene was found to be down-regulated. A detailed study of markers of the apoptosis induced by binase

Abbreviations: RNase, ribonuclease; binase, *Bacillus intermedius* RNase; ROS, reactive oxygen species; Ψ, mitochondrial membrane potential; PI, propidium iodide.

* Corresponding author. Tel.: +7 499 1354095; fax: +7 499 1351405.
E-mail address: aamakarov@eimb.ru (A.A. Makarov).

[1] V.A.M., O.V.K. and I.Yu.P. contributed equally to this work.

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