Available online at www.sciencedirect.com

ScienceDirect

Biochimie 90 (2008) 717–725

BIOCHIMIE

www.elsevier.com/locate/biochi

Research paper

RNase-induced apoptosis: Fate of calcium-activated potassium channels

Olga N. Ilinskaya [a,b], Andreas Koschinski [c], Holger Repp [c], Vladimir A. Mitkevich [a,d], Florian Dreyer [c], J. Martin Scholtz [e], C. Nick Pace [e], Alexander A. Makarov [a,*]

[a] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, Moscow 119991, Russia*
[b] *Department of Microbiology, Kazan State University, Kremlevskaya str. 18, Kazan 420008, Russia*
[c] *Rudolf-Buchheim-Institute of Pharmacology, Justus-Liebig-University, Frankfurter str. 107, Giessen 35392, Germany*
[d] *University of Oslo, Centre for Medical Studies in Russia, Vavilov str. 34/5, Moscow 119334, Russia*
[e] *Department of Molecular and Cellular Medicine, Texas A&M University System Health Science Center, College Station, TX 77843, USA*

Received 21 December 2007; accepted 18 January 2008
Available online 2 February 2008

Abstract

The connection between the action of microbial RNases and Ca^{2+}-activated K^+ (K_{Ca}) channels was investigated in human embryo kidney cells HEKhSK4 artificially expressing the channels. These channels protected HEKhSK4 cells from apoptosis induced by binase and 5K charge reversal mutant of RNase Sa. After the first 24 h, potassium current increased without increase in intracellular Ca^{2+}, and mitochondrial potential remained high. After 72 h, the concentration of calcium increased and mitochondria lost their potential. Whole-cell recordings of membrane currents through K_{Ca} channels in RNase-treated cells demonstrated a biphasic pattern: initially their activity in cell population increased, peaked at 24 h, and then gradually decreased. In each individual cell we observed either an increase of the amplitude of K_{Ca} current, or a complete shutdown of the channels. The activity of K_{Ca} channels could be restored by removing RNases from the media. Based on this pattern and especially its timing, we hypothesize that toxic RNases downregulate K_{Ca} channels at the level of transcription or translation. Our results indicate that new anticancer agents could be created on the basis of microbial RNases targeting K_{Ca} channels.

Keywords: Microbial RNases; Cytotoxicity; Human embryo kidney cells; Calcium activated potassium channels; Apoptosis

1. Introduction

Cytotoxic ribonucleases (RNases) represent a novel tool in anticancer therapy. These quite small proteins preferentially attack malignant cells, trigger apoptotic responses, and inhibit protein synthesis [1–3]. RNase from oocytes of *Rana pipiens* (onconase; commercial trademark of Alfacell Inc., USA) induces apoptosis of target cells, most likely through the mitochondrial pathway initiated by caspase-9 [4]. In patients with malignant mesothelioma, onconase is one of the few chemotherapeutic agents studied so far. It has limited side effects [5] and has already revealed a potential survival benefit in a Phase III trial [6]. Bovine seminal (BS) RNase induces apoptosis in ML-2 myeloid cell line and NB-1 and NB-2 neuroblastoma cells [7]. Apoptosis induced by BS RNase is associated with activation of caspase-8 and -9 [8] and coincides with downregulation of Bcl-2 in several carcinoma cell lines [9]. Because of its high selectivity for malignant cells of thyroid origin *in vitro*, BS RNase has been chosen as a treatment of aggressive thyroid cancer [8]. *Bacillus intermedius* ribonuclease (binase) induces apoptosis of human lung carcinoma A549 cells and human myelogenous leukemia K562

Abbreviations: RNase, ribonuclease; binase, RNase from *Bacillus intermedius*; RNase Sa, RNase from *Streptomyces aureofaciens*; 5K, cationic mutant of RNase Sa; K_{Ca} channels, Ca^{2+}-activated K^+ channels.

* Corresponding author. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, Moscow 119991, Russia. Tel.: +7 499 135 4095; fax: +7 499 135 1405.

E-mail addresses: olga.ilinskaya@ksu.ru (O.N. Ilinskaya), aamakarov@eimb.ru (A.A. Makarov).

0300-9084/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2008.01.010