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Internalization of *Bacillus intermedius* ribonuclease (BINASE) induces human alveolar adenocarcinoma cell death

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ABSTRACT

Ribonuclease (RNase) treatment represents a novel mechanism based approach to anticancer therapy as an alternative to the DNA damaging drugs commonly used in clinical practice. Apart from their ribonucleolytic activity, cytotoxic effects have attracted a considerable attention to RNases because of their potential as selective agents for treatment of certain malignancies. Among these enzymes, Binase, an RNase from Bacillus intermedius, has shown promising results. Here, we have found that binase selectively attacked human A549 alveolar adenocarcinoma cells to trigger an apoptotic response, whereas normal lung epithelial cells LEK were not affected by the ribonuclease. The tumor transformation led to the modification of certain cellular characteristics causing cell sensitivity to binase. Although a general mode for RNases cytotoxicity includes their penetration into the cell, translocation to the cytosol and degradation of ribonucleic acid, many aspects of this process have not been fully elucidated. Our data revealed the following time-dependent changes induced by binase in A549 cells: (a) fast permanent internalization of the enzyme during the first hours of treatment; (b) temporary increase in cellular permeability for macromolecules during the 4–6 h of treatment; (c) apoptotic alterations in population after 24 h and (d) DNA fragmentation and cell death after 72 h of treatment with binase. Elucidation of these molecular strategies used by this promising toxin provides us essential information for the development of new anticancer drugs.

1. Introduction

Ribonucleases (RNases) are ubiquitously distributed enzymes that catalyze the cleavage of phosphodiester bonds in RNA. They possess a broad spectrum of biological activities, demonstrating cell stimulating properties at low concentrations (Leschinskaya et al., 1995; Kipenskaia et al., 1998) as well as cytotoxicity (Fang and Ng, 2011; Makarov and Ilinskaya, 2003; Makarov et al., 2008; Ulyanova et al., 2011) and genotoxicity (Ilinskaya et al., 1995, 1996) at high concentrations. RNases are promising antitumor and antimicrobial agents based on their concrete cytotoxic activities *in vitro* and *in vivo*. Although binding to cell membrane, followed by translocation to the cytosol, intracellular RNA degradation is considered the main cause of the exogenous RNase cytotoxic effects, yet the detailed mechanism of action remains elusive (Ardelt et al., 2009).

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