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Direct inhibition of oncogenic KRAS by *Bacillus pumilus* ribonuclease (binase)

Olga N. Ilinskaya [a,1], Indrabahadur Singh [b,1], Elena Dudkina [a,*], Vera Ulyanova [a], Airat Kayumov [a], Guillermo Barreto [a,b,c,d,**]

[a] Institute of Fundamental Medicine and Biology, Kazan Federal (Volga-Region) University, Kremlevskaya str. 18, 420008, Kazan, Russia
[b] LOEWE Research Group Lung Cancer Epigenetic, Max-Planck-Institute for Heart and Lung Research, Parkstr. 1, 61231 Bad Nauheim, Germany
[c] Universities of Giessen and Marburg Lung Center (UGMLC), Germany
[d] German Center of Lung Research (Deutsches Zentrum für Lungenforschung, DZL), Germany

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ABSTRACT

RAS proteins function as molecular switches that transmit signals from cell surface receptors into specific cellular responses *via* activation of defined signaling pathways (Fang, 2015). Aberrant constitutive RAS activation occurs with high incidence in different types of cancer (Bos, 1989). Thus, inhibition of RAS-mediated signaling is extremely important for therapeutic approaches against cancer. Here we showed that the ribonuclease (RNase) binase, directly interacts with endogenous KRAS. Further, molecular structure models suggested an inhibitory nature of binase-RAS interaction involving regions of RAS that are important for different aspects of its function. Consistent with these models, phosphorylation analysis of effectors of RAS-mediated signaling revealed that binase inhibits the MAPK/ERK signaling pathway. Interestingly, RAS activation assays using a non-hydrolysable GTP analog (GTPγS) demonstrated that binase interferes with the exchange of GDP by GTP. Furthermore, we showed that binase reduced the interaction of RAS with the guanine nucleotide exchange factor (GEF), SOS1. Our data support a model in which binase-KRAS interaction interferes with the function of GEFs and stabilizes the inactive GDP-bound conformation of RAS thereby inhibiting MAPK/ERK signaling. This model plausibly explains the previously reported, antitumor-effect of binase specific towards RAS-transformed cells and suggests the development of anticancer therapies based on this ribonuclease.

1. Introduction

The members of the RAS subfamily are guanosine triphosphate (GTP) hydrolyzing proteins (GTPases) that mediate transduction of extracellular stimuli into cellular responses. RAS proteins function as molecular switches that cycle between active, GTP-bound and inactive, GDP-bound forms. Activation of upstream growth factor receptors at the cell membrane results in the recruitment of a preformed complex consisting of Son of Sevenless (SOS) and the adaptor protein GRB2 to the active receptor at the cell membrane. SOS1 and SOS2 are guanine nucleotide exchange factors (GEFs) that facilitate RAS activation by catalyzing the release of GDP from RAS [1,2]. Since cellular concentrations of GTP are approximately 10 fold higher than GDP, GTP re-enters the nucleotide binding pocket of RAS and reloads its active conformation, which has high affinity for various effectors that stimulate specific signaling pathways [3–7]. After carrying out its function, the intrinsic GTPase activity of RAS is enhanced by GTPase-activating proteins (GAPs) converting GTP to GDP and leading to inactive, GDP-bound RAS [8]. Summarizing, the balance between GEF and GAP activity determine the guanine nucleotide status of RAS, thereby regulating RAS activity.

In response to extracellular stimuli that activate cell surface receptors (*e.g.* epidermal growth factor receptor, insulin-like growth factor receptor and platelet-derived growth factor receptor), RAS proteins activate a wide range of signaling pathways [3–7]. This stimulation of specific signaling pathways ultimately results in regulation of gene expression patterns that govern diverse cellular processes like cell growth, proliferation, differentiation, motility, endocytosis and apoptosis. Due to the central roles of RAS proteins in all these processes, it is not surprising that cells use these activities during transformation towards cancer cells. In fact, oncogenic mutations in the *RAS* genes (*HRAS*, *KRAS* and *NRAS*) are present in approximately 30% of all human cancers and

Abbreviations: RAS, Rat sarcoma; GTPase, hydrolase enzyme that can bind and hydrolyze guanosine triphosphate (GTP); Binase, *Bacillus pumilus* ribonuclease; ERK, extracellular signal-regulated kinases.

* Corresponding author.
** Correspondence to: G. Barreto, LOEWE Research Group Lung Cancer Epigenetic, Max-Planck-Institute for Heart and Lung Research, Parkstr. 1, 61231 Bad Nauheim, Germany.
E-mail addresses: lenatimonina@rambler.ru (E. Dudkina), guillermo.barreto@mpi-bn.mpg.de (G. Barreto).

[1] Authors contributed equally.

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