Apoptosis (2007) 12:1349–1363
DOI 10.1007/s10495-007-0075-7

ORIGINAL PAPER

Molecular mechanisms of euplotin C-induced apoptosis: involvement of mitochondrial dysfunction, oxidative stress and proteases

Davide Cervia · Mercedes Garcia-Gil · Elisa Simonetti · Graziano Di Giuseppe · Graziano Guella · Paola Bagnoli · Fernando Dini

Published online: 18 April 2007

Abstract The metabolite euplotin C (EC), isolated from the marine ciliate *Euplotes crassus*, is a powerful cytotoxic and pro-apoptotic agent in tumour cell lines. For instance, EC induces the rapid depletion of ryanodine Ca^{2+} stores, the release of cytochrome *c* from the mitochondria, and the activation of caspase-3, leading to apoptosis. The purpose of this study was to gain further insight into the mechanisms of EC-induced apoptosis in rat pheochromocytoma PC12 cells. We found that EC increases Bax/Bcl-2 ratio and that Bax is responsible of the EC-induced dissipation of the mitochondrial membrane potential ($\Delta\psi_m$). In addition, EC induces the generation of reactive oxygene species (ROS) without involvement of p53. The inhibition of ROS generation prevents, at least in part, the pro-apoptotic effects of EC as well as the effects of EC on Bax, $\Delta\psi_m$ and intracellular free Ca^{2+}, indicating a cross-talk between different pathways. However, definition of the effector cascade turns out to be more complex than expected and caspase-independent mechanisms, acting in parallel with caspases, should also be considered. Among them, EC increases the expression/activity of calpains downstream of ROS generation, although calpains seem to exert protective effects.

D. Cervia and M. Garcia-Gil equally contributed to the work.

D. Cervia (✉)
Department of Environmental Sciences, University of Tuscia, largo dell'Università snc, blocco D, Viterbo 01100, Italy
e-mail: d.cervia@unitus.it

D. Cervia · M. Garcia-Gil · E. Simonetti · G. Di Giuseppe · P. Bagnoli · F. Dini
Department of Biology, University of Pisa, Pisa 56127, Italy

G. Guella
Department of Physics, Laboratory of Bioorganic Chemistry, University of Trento, Trento 38050, Italy

Keywords Tumour cells · Cell death · Bax/Bcl-2 · $\Delta\psi_m$ · ROS · Calpains

Introduction

Several molecules of pharmacological interest have been extracted from marine organisms or have been synthesized as a result of knowledge gained from a prototypical marine-derived compound [1–3]. Recently, marine eukaryotic microbes, commonly referred as protistan ciliates, have been found to be a fruitful mine of bioactive secondary metabolites [4–9]. Marine products may have evolved into highly potent inhibitors of physiological processes in the prey, predators or competitors of the marine organisms that produce them [1, 4]. The sesquiterpenoid euplotin C is a lipophilic metabolite isolated from the eukaryotic, unicellular marine ciliate *Euplotes crassus* [4, 10–12] which provides an effective mechanism for damping populations of potential competitors, thus favoring the entrance into new niches and, more generally, adaptative radiation. Indeed, at the functional level, euplotin C kills non-producer *Euplotes* strains whereas at sublethal levels it alters their cell cycle, ciliary motility and cell shape [4, 11]. In addition, euplotin C displays cytotoxic properties against the pathogenic protozoa *Leishmania major* and *Leishmania infantum*, the opportunistic yeast *Candida albicans* and some prokaryotic bacterial strains [13].

Different sesquiterpenoids are effective as cytotoxic agents, possibly because of their apoptosis-inducing properties [14–17]. Apoptosis is the regulated form of cell death utilized by metazoans to remove unneeded, damaged, or potentially deleterious cells [18]. Currently, a great deal of effort is aimed at discovering novel compounds targeting specific regulators of apoptosis, which could set the stage