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Coibamide A Induces mTOR-Independent Autophagy and Cell Death in Human Glioblastoma Cells

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Abstract

Coibamide A is an *N*-methyl-stabilized depsipeptide that was isolated from a marine cyanobacterium as part of an International Cooperative Biodiversity Groups (ICBG) program based in Panama. Previous testing of coibamide A in the NCI *in vitro* 60 cancer cell line panel revealed a potent anti-proliferative response and "COMPARE-negative" profile indicative of a unique mechanism of action. We report that coibamide A is a more potent and efficacious cytotoxin than was previously appreciated, inducing concentration- and time-dependent cytotoxicity (EC_{50}<100 nM) in human U87-MG and SF-295 glioblastoma cells and mouse embryonic fibroblasts (MEFs). This activity was lost upon linearization of the molecule, highlighting the importance of the cyclized structure for both anti-proliferative and cytotoxic responses. We show that coibamide A induces autophagosome accumulation in human glioblastoma cell types and MEFs via an mTOR-independent mechanism; no change was observed in the phosphorylation state of ULK1 (Ser-757), p70 S6K1 (Thr-389), S6 ribosomal protein (Ser-235/236) and 4EBP-1 (Thr-37/46). Coibamide A also induces morphologically and biochemically distinct forms of cell death according to cell type. SF-295 glioblastoma cells showed caspase-3 activation and evidence of apoptotic cell death in a pattern that was also seen in wild-type and autophagy-deficient (ATG5-null) MEFs. In contrast, cell death in U87-MG glioblastoma cells was characterized by extensive cytoplasmic vacuolization and lacked clear apoptotic features. Cell death was attenuated, but still triggered, in Apaf-1-null MEFs lacking a functional mitochondria-mediated apoptotic pathway. From the study of ATG5-null MEFs we conclude that a conventional autophagy response is not required for coibamide A-induced cell death, but likely occurs in dying cells in response to treatment. Coibamide A represents a natural product scaffold with potential for the study of mTOR-independent signaling and cell death mechanisms in apoptotic-resistant cancer cells.

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Introduction

There is high demand for new small molecules that can strategically target the dysregulated signaling pathways that underlie aggressive solid cancers such as glioblastoma. Glioblastoma multiforme (GBM), classed by the World Health Organization (WHO) as a high-grade IV astrocytoma-like tumor, is the most common malignant primary tumor of the central nervous system (CNS) and is associated with a particularly poor prognosis. Present therapeutic strategies have had little impact on the overall survival rate, with median patient survival times remaining at 14 to 19 months depending on the treatment regimen [1,2,3]. Collective efforts to classify the pathogenesis of gliomas have shown that GBM frequently harbors a signature of mutations that tend to attenuate the function of tumor suppressor genes, such as p53 and PTEN, or enhance activation of receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) (reviewed in [3,4]). In turn, cell signaling driven by growth factors, such as the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, is dramatically enhanced. Together these aberrant signaling networks tend to promote cell survival and lend GBM a natural resistance to apoptosis, rendering conventional chemotherapeutic drugs that typically induce apoptosis ineffective for the treatment of this condition [3]. Consequently, there is a great need for new pharmacologic tools that cause cell death in glioblastoma and other apoptosis-resistant cancer cells.

As part of the ICBG program based in Panama, we previously reported the discovery of the *N*-methyl-stabilized cyclopeptide natural product coibamide A [5], named for its isolation from a marine cyanobacterium collected from the Coiba National Park, Panama. Coibamide A displayed an unprecedented profile in the National Cancer Institute 60 (NCI-60) human cancer cell line panel, showing sub-nanomolar potency as a growth inhibitory agent in many of the cell lines tested. Coibamide A was deemed "COMPARE-negative", in that the pattern of cellular responses to

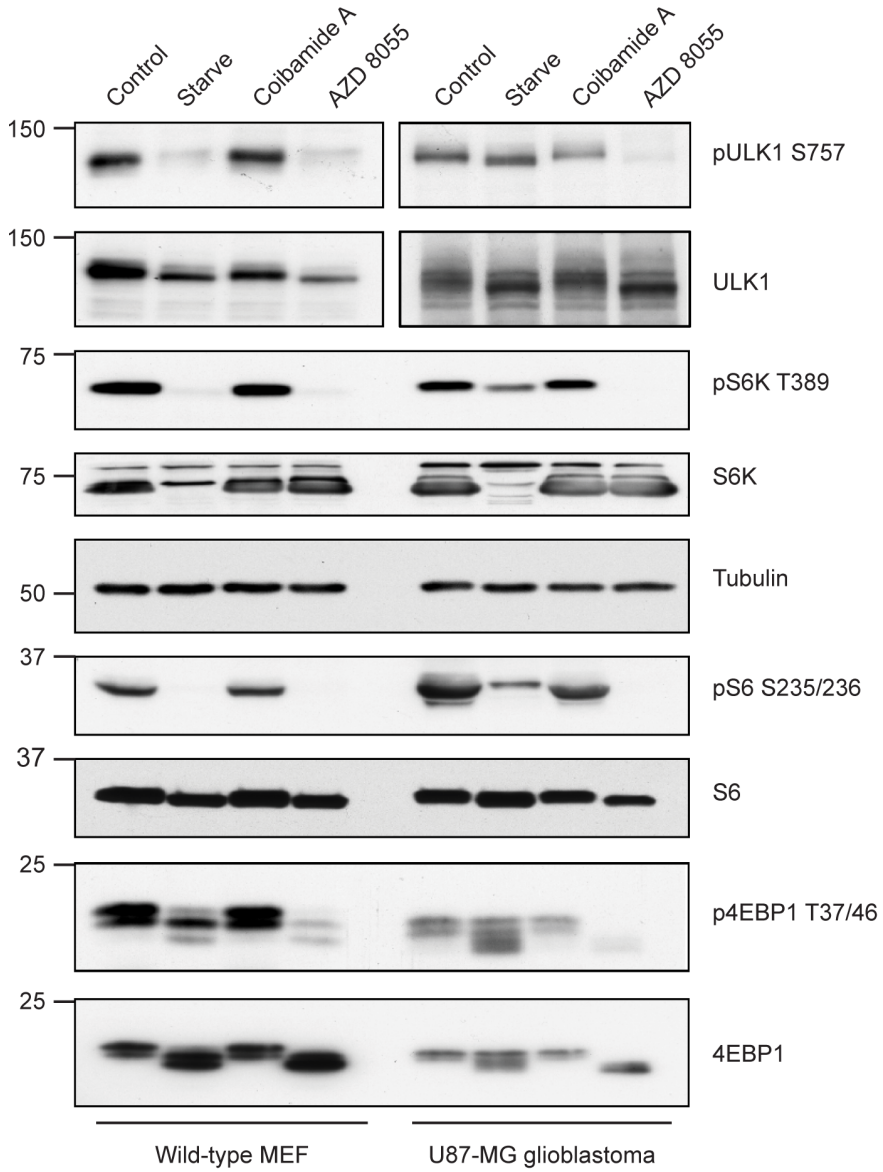

Figure 10. Coibamide A induces mTOR-independent autophagy. Coibamide A-induced signaling is mTOR-independent and can be distinguished from starvation- and AZD 8055-induced autophagy in MEF and U87-MG glioblastoma cells. Cells were incubated in EBSS starvation medium, or treated with or without coibamide A (30 nM) or the dual mTOR inhibitor AZD 8055 (300 nM) in standard nutrient-rich medium for 4 h. Following treatment cells were lysed and subjected to immunoblot analysis. The phosphorylation status of residues considered to be major targets of the mTOR pathway was assessed: phospho-ULK1 (Thr-757), phospho-p70 S6 Kinase (Thr-389), phospho-S6 ribosomal protein (Ser-235/236), and phospho-4E-BP1 (Thr-37/46) are shown relative to total ULK1, S6K1, S6 ribosomal protein and 4-E binding protein, respectively. Tubulin served as a loading control. Results are representative of at least three independent experiments.
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migrates more slowly than LC3-II, which is recruited to the membrane of autophagosomes. We analysed the expression of endogenous LC3 in lysates derived from adherent U87-MG cells that had been treated with coibamide A for up to 48 h. By 1 h we observed a clear increase in LC3-II expression in coibamide A-treated cells relative to control (Fig. 6A). This increase in LC3-II expression was generally sustained through 48 h and comparable to that observed with the macrolide natural product rapamycin (Fig. 6A), a known inducer of autophagy in glioblastoma cells [12]. We next stained treated cells with MDC, a fluorescent marker used as a probe for mature autophagosomes and lysosomes [17]. Vital staining of coibamide A-treated U87-MG cells with MDC revealed increased accumulation of the dye relative to control cells (Fig. 6B, panels a and b). Furthermore, we found this effect to be qualitatively indistinguishable from cytosolic MDC accumulation observed for cells treated with rapamycin (Fig. 6B, panels b and c). We also utilized TEM to visualize the ultrastructure of adherent U87-MG cells following exposure to coibamide A at 16 and 48 h (Fig. 6C). These studies revealed no evidence of nuclear condensation and segregation, or cytoplasmic shrinking expected of cells undergoing apoptosis. The nuclear membrane frequently had an undulating character to it, but was discernable as an intact double-membrane. Cells exposed to coibamide A for 16 h showed small electron lucent vacuoles that were absent in control cells (Fig. 6C, panels a and b). Empty vacuoles were also observed at 48 h by TEM, with 20% of cells containing one or more large