Exploring marine-derived fungal preussin toxicity on MDA-MB-231 cells cultured in 2D and 3D

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Triple-negative breast cancer (TNBC) presents poor prognosis, early recurrence, limited therapeutic options, and high drug resistance, calling for the search of molecules that may serve as new drugs, drug adjuvants, or scaffolds for drug development. Preussin is a naturally derived compound that reduces cell viability and proliferation and promotes cell death and cell cycle arrest in 2D cultured cell lines. In this study, we investigated the effects of the preussin isolated from the marine sponge-associated fungi *Aspergillus candidus* on the TNBC cell line MDA-MB-231, comparing 2D and 3D cell culture models. 3D cultures typically better simulate the tumour behaviour in vivo and were generated here using ultra-low adhesion U bottom plates. Preussin at 25 µM or higher decreased cell viability in 2D (72 h exposure) and 3D cultured cells (96 h exposure), assessed with the MTT assay. These effects align with the observed decrease in cell proliferation (above 25 µM of preussin) in both culture models, evaluated with the BrdU assay, as well as the increase in late apoptosis and necrosis at 35 µM for 2D culture and over 25 µM in 3D culture, as measured by flow cytometry using Annexin V-PI staining. In agreement, characteristics of apoptosis and necrosis were observed in the cells' ultrastructure after exposure to preussin. With standard and enzymatic versions of the comet assay, we excluded the genotoxic effects of preussin at 25 or 35 µM after 2 h and 24 h of exposure. In an in vitro scratch assay, preussin decreased cell migration in the MDA-MB-231 cell line at a starting concentration of 5 µM. This study shed light on the mechanisms underlying the preussin effects on a TNBC cell line, supporting its value as an antiproliferative, cytotoxic, and anti-migratory agent in a dose-dependent manner in both culture models.

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Keywords: triple-negative breast cancer; cell culture; cell death; cell migration; preussin.