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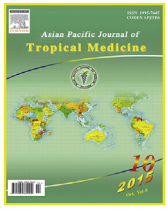

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Potential of four marine-derived fungi extracts as anti-proliferative and cell death-inducing agents in seven human cancer cell lines

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ABSTRACT

Objective: To evaluate the *in vitro* anticancer activity of crude ethyl acetate extracts of the culture of four marine-derived fungi *Aspergillus similanensis* KUFA 0013 (E1), *Neosartorya paulistensis* KUFC 7897 (E2), *Neosartorya siamensis* KUFA 0017 (E4) and *Talaromyces trachyspermus* KUFC 0021 (E3) on a panel of seven human cancer cell lines.

Methods: Effects on cell proliferation, induction of DNA damage and cell death were assessed by MTT and clonogenic assays, comet assay and nuclear condensation assay, respectively.

Results: The proliferation of HepG2, HCT116 and A375 cells decreased after incubation with the extracts E2 and E4. The anti-proliferative effect was confirmed by morphologic alterations and by clonogenic assay. Both extracts also induced cell death in HepG2 and HCT116 cells. Doxorubicin was used as a positive control and showed *in vitro* anticancer activity.

Conclusions: This study demonstrated, for the first time, that extracts of *Neosartorya paulistensis* and *Neosartorya siamensis* have selective anti-proliferative and cell death activities in HepG2, HCT16 and A375 cells. The bioactivity of these extracts suggests a potential for biotechnological applications and substantiates that both should be further considered for the elucidation of the molecular targets and signal transduction pathways involved.

1. Introduction

Cancer is one of the main causes of death worldwide. In the following decades, the number of people with cancer will continue to increase, largely due to lifestyle, nutrition and environmental conditions in developed countries [1–3]. During cancer development, cells acquire several genetic and epigenetic changes. These changes result in the progressive acquisition of biological characteristics such as sustained proliferative signaling, insensitivity to growth suppressors, evading apoptosis, increasing genomic instability, activating mobility, invasion, metastasis and angiogenesis that may thus evolve into a malignant phenotype [4].

Advances in cancer biology knowledge have allowed the development of new treatment strategies, including new anticancer drugs that may act in one or more of the hallmarks described above. In fact, compounds that reactivate cell death and/or decrease proliferative ability in cancer cells show a potential anticancer activity. However, most of the anticancer drugs currently used, such as doxorubicin, give rise to undesirable side effects, such as cardiotoxicity and tumor drug-resistance [5]. Therefore, new anticancer drugs with more efficiency and ability to mitigate side effects are in need.

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It is interesting to note that more than 50% of the drugs used in cancer treatment are from natural origin, mainly from plant sources [6]. Nonetheless, the marine environment represents about 95% of the world's biosphere and is an important source of bioactive compounds to be explored [7,8]. As terrestrial microorganisms have proved their value as sources of bioactive compounds, the focus has also turned to marine microorganisms [9]. Marine fungi have remained until recently much less studied than terrestrial fungi, nevertheless, novel metabolites have been found in marine fungi that greatly differ from those found in terrestrial counterparts [6]. The production of secondary metabolites by marine fungi can be influenced by the combination of the unique conditions of marine environment, such as variations in temperature, light, water current, salinity, and nutrient availability, all of which create a highly competitive environment and thus force marine organisms to evolve complex chemical adaptations, many of which developed under a symbiotic relationship with other species [10]. In fact, recent research has exploited these symbiotic relationships in marine ecosystems as a source for bioactive compounds. This is particularly pertinent when analyzing the case of microbe-sponge relationships, and whereas sponges are known to be notable sources for bioactive compounds, where origin has also been attributed to the sponge's microbial associates, namely fungi and bacteria [11]. Furthermore, compounds are frequently produced as a chemical manner of defense by many marine organisms, and are released into the water and thus diluted. Consequently, these compounds must be extremely efficient in order to produce their effect in spite of their dilution in the water. Hence, these metabolites seem to have interest as novel lead structures for the synthesis of new bioactive compounds [9,12,13].

In regard to biological activity, several metabolites produced by marine-derived fungi have been reported as antibacterial, antiviral, antifungal, antioxidant and anticancer agents [14–17]. Notwithstanding the increasing interest in these bioactive compounds, there is frequently an effective difficulty in extracting these compounds from nature since their source organisms are often hard to reproduce and manipulate in laboratorial conditions, limiting their availability and use. Interestingly, some marine fungi may grow efficiently under laboratory conditions, which may therefore enable the use of biotechnological tools for a massive production of the compounds of interest [18,19]. In summary, marine-derived fungi seem to be good candidates as a source of new bioactive compounds, thus making them a pivotal part of the emergent marine biotechnology applications.

Our present purpose was to assess the *in vitro* anticancer activity of crude ethyl acetate extracts of the sponge-derived fungi *Aspergillus similanensis* (*A. similanensis*) KUFA 0013 (E1), *Neosartorya paulistensis* (*N. paulistensis*) KUFC 7897 (E2) and *Talaromyces trachyspermus* (*T. trachyspermus*) KUFC 0021 (E3), and the sea fan-derived fungi *Neosartorya siamensis* (*N. siamensis*) KUFA 0017 (E4), on a panel of seven human cancer cell lines, namely, colorectal carcinoma (HT29 and HCT116), hepatocellular carcinoma (HepG2), breast adenocarcinoma (MCF-7), malignant melanoma (A375), non-small cell lung carcinoma (A549) and glioblastoma (U251) cells. The chemical composition of the ethyl extracts E1, E2 and E3 has been previously analysed by members of our research team [20–22].

2. Materials and methods

2.1. Chemicals

Doxorubicin, DMEM, MEM, RPMI-1640, sodium pyruvate, sodium bicarbonate, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid), penicillin/streptomycin, trypsin solution, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum was purchased from Biochrom KG (Berlin, Germany). All other reagents and chemicals used were of analytical grade.

2.2. Fungal material

A. similanensis KUFA 0013 was isolated from the marine sponge *Rhabdermia* sp., as described by Prompanya *et al* [22], *N. paulistensis* KUFC 7897 was isolated from the marine sponge *Chondrilla australiensis*, as previously reported [20], and *T. trachyspermus* KUFC 0021 was isolated from the marine sponge *Clathria reianwardii*, as reported by Kumla *et al* [21].

N. siamensis KUFA 0017 was isolated from sea fan (*Rumphella* sp.), collected from the coral reef at Similan island, Phang Nga province, Southern Thailand, in April 2010. Briefly, the sea fan tissue was cut into a piece of 0.5 cm × 0.5 cm, placed on the malt extract agar (MEA) with 70% sea water and incubated for 28 °C for 7 d. The fungus was identified by one of us (T. Dethoup) by morphological features, including the characteristic of ascospores and colonies, and by sequence analysis of the β-tubulin gene [23]. The pure cultures were deposited as KUFA0017 at Kasetsart University Fungal Collection, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

2.3. Preparation of crude ethyl acetate extracts from marine-derived fungi

A. similanensis KUFA 0013, *N. paulistensis* KUFC 7897 and *T. trachyspermus* KUFA 0021 were cultured as described by Prompanya *et al* [22], Gomes *et al* [20] and Kumla *et al* [23], respectively. Briefly, the fungi were cultured for one or two weeks in Petri dishes with malt extract agar. Erlenmeyer flasks, containing rice and water, were autoclaved at 121 °C for 15 min and then inoculated with mycelia plugs of the fungi and incubated at 28 °C for 30 d, after which the moldy rice was macerated in ethyl acetate and filtered. The two layers were separated, and the ethyl acetate solution was concentrated at a reduced pressure to yield 97.51 and 102.00 g of the crude ethyl acetate extracts, respectively [21,22].

N. siamensis KUFA 0017 was cultured for one week in Petri dishes with 25 mL of potato dextrose agar per dish. Thirty-five Erlenmeyer flasks containing 200 g of rice and 100 mL of water were autoclaved at 121 °C for 15 min, and then inoculated with 10 mycelium plugs of the fungus. The culture was incubated at 28 °C for 30 d. To each flask with the mouldy rice was added 500 mL of ethyl acetate and the content was left to macerate for 7 d. The content of the flasks was filtered by filter paper and the filtrate was evaporated under reduced pressure to give 1000 mL of the solution and then anhydrous sodium sulphate was added and filtered. The ethyl acetate solution was evaporated under