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Effect of Amniotic Membrane Proteins in Human Cancer Cell Lines: An Exploratory Study

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Abstract Human amniotic membrane (hAM) has recently drawn attention as an upcoming anti-cancer therapy. Regarding the strategies which have already investigated, little is known about hAM protein extracts (hAMPE) effect on cancer. So, this work aims to study the effect of hAMPE in metabolic activity of several human cancer cell lines. hAMPE were mechanically obtained, thus avoiding the effect of detergents and other reagents commonly used in protein extraction under the cell lines studied. After quantification of proteins in hAMPE, their effect on the metabolic activity of 21 human cancer cell lines was assessed by 3-(4,5-dimethylthia-zolyl-2)2,5-diphenyltetrazolium bromide (MTT) assay. Our results indicate that there is an inhibition of metabolic activity until 25 and 50 % in two and seven cell lines, respectively. Five cell lines proved to be very sensitive to hAMPE, being its metabolic activity more than 50 % inhibited. Our results show that hAMPE can inhibit the metabolic activity of some human cancer cell lines. However, research about this cell line-dependent response to hAMPE becomes indispensable.

Keywords Amniotic membrane · Cancer cell lines · Metabolic activity · MTT assay

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Introduction

The inner layer of the placenta is called amniotic membrane (hAM), a structure that completely surrounds the embryo/fetus during its development (Mamede et al. 2012a). hAM is a thin and avascular membrane composed by three main layers: an epithelial layer that delimits the amniotic cavity, a basement membrane and a mesenchymal layer that is closely in contact with maternal cells (Marongiu et al. 2010; Miki et al. 2010).

In 1910, Davis used hAM as a wound dressing for burned patients. Since then, hAM has been recognized as an attractive biomaterial for several medical purposes associated with genito-urinary tract, oral cavity, skin, stomach, larynx, head, and neck (Mamede et al. 2012a, b; Niknejad et al. 2008; Parolini and Soncini 2006). In ophthalmology, hAM has been widely studied and clinically applied (Rahman et al. 2009). In fact, although many of the properties and functions of the hAM are already known, its protein content is still poorly characterized. Concerning

this, few scientific publications related to this subject identified some hAM proteins through mass spectrometry, two-dimensional electrophoresis, immunoblotting, and MALDI-TOF/TOF (Hopkinson et al. 2006; Park et al. 2006).

Regarding the effects of hAM proteins in cancer cells, almost nothing is known until now. So, we intend to evaluate the effects of hAM protein extracts (hAMPE) on metabolic activity of human cancer cell lines.

Materials and Methods

Cell Culture

In this study, we used the following human cancer cell lines: prostate cancer (PC3 and LNCaP), breast cancer (HCC1806, MCF7, and HCC1954), colon cancer (WiDr, LS1034, and C2BBe1), hepatocarcinoma (HuH7, HepG2, and Hep3B2.1-7), lung cancer (A549 and H1299), pancreatic cancer (MIA PaCa-2 and PANC-1), bile ducts cancer (TFK-1), melanoma (A375), endometrial cancer (ECC-1), bladder cancer (HT-1376), osteosarcoma (MNNG/HOS), and esophagus cancer (OE19). All cells lines were purchased from ATTC (USA), except TFK-1 cell line that was obtained from DSMZ (Germany), HuH7 cell line that was purchased from JCRB (Japan) and OE19 cell line from ECACC (UK). All cells lines were cultured in accordance with repository instructions, cultured until 30 passages and maintained at 37 °C with 5 % CO_2.

Protein Extraction from hAM

hAM was obtained with informed consent according to the guidelines of Ethical Committee of the Coimbra Hospital and University Centre (Coimbra, Portugal). Placentas were obtained from healthy women after cesarean section and washed with a phosphate buffered solution with 2 % antibiotic/antimycotic (Gibco 15140-122). hAMPE were obtained through a protocol based on Shao et al. (2004) work. hAM was mechanically separated from chorion, placed on ice and subjected to homogenization and sonication. At last, hAM was centrifuged at 14,000×*g* for 15 min, and the supernatant was collected and stored at −80 °C. Proteins were quantified using NanoDrop® (ND-1000 Spectrophotometer, USA). Protein extraction procedure was carried out in PBS and without the use of detergents that could affect the results.

Evaluation of Metabolic Activity

To evaluate hAMPE effect on metabolic activity, we used the colorimetric test 3-(4,5-dimethylthia-zolyl-2)2,5-diphenyltetrazolium bromide (MTT). Metabolically active cells have dehydrogenase enzymes that can cleave tetrazolium ring of MTT and form dark blue formazan crystals that can subsequently be solubilized and quantified by spectrophotometry (Berridge et al. 2005). For each experiment, cells were incubated with 1 μg/μL of hAMPE. After 72 h, MTT assay was performed as described by Mamede et al. (2012b). Metabolic activity was calculated based on the ratio between the value obtained for hAMPE treated cells and control cells.

Results and Discussion

Several scientific studies point to a potential anti-cancer effect of hAM (Jiao et al. 2012; Kang et al. 2012a, b; Magatti et al. 2012; Niknejad et al. 2012; Seo et al. 2008). However, and despite the growing scientific publications, nothing has been published so far about the effect of hAMPE in human cancer. To evaluate the effect of hAMPE in metabolic activity of human cancer cell lines, we resorted to MTT assay. Figure 1 represents the percentage of metabolic activity of several cancer cell lines after incubation with 1 μg/μL of hAMPE during 72 h.

As can be seen in Fig. 1, three types of response after treatment with hAMPE were registered: stimulation, inhibition and no effect on metabolic activity. Colon cancer cells C2BBe1 are stimulated by hAMPE. In fact, metabolic activity increased 71 %. Also, metabolic activity of colon LS1034, breast HCC1954, and bile ducts TFK-1 cancer cell lines are enhanced by hAMPE. Prostate LNCaP, breast MCF7 and lung A549 cancer cell lines not seem to be sensitive to treatment, being its metabolic activity unaltered after treatment. On the other hand, the remaining fourteen cell lines appear to be sensitive to treatment. A decrease in metabolic activity until 25 % was registered in lung H1299 and endometrial ECC-1 cancer cell lines. There was a decrease of 25–50 % in metabolic activity in breast HCC1806, pancreatic MIA PaCa-2, melanoma A375, liver HuH7, osteosarcoma MNNG/HOS, esophageal OE19, and bladder HT-1376 cancer cell lines. Prostate PC3 and colon WiDr cancer cell lines decreased its metabolic activity in 54 and 51 %, respectively. The three cell lines most susceptible to hAMPE were pancreatic cancer PANC-1 (74 % of reduction) and hepatocarcinoma HepG2 and Hep3B2.1-7 (reduction of 64 and 83 %, respectively).

According to results, we can verify that inhibition of metabolic activity with hAMPE differs between different cell lines of the same type of cancer, being dependent of the particular genetic profile of each cell line. Thus, it is urgent to study individual response of each cell line. These results contribute to the study of cancer therapy with

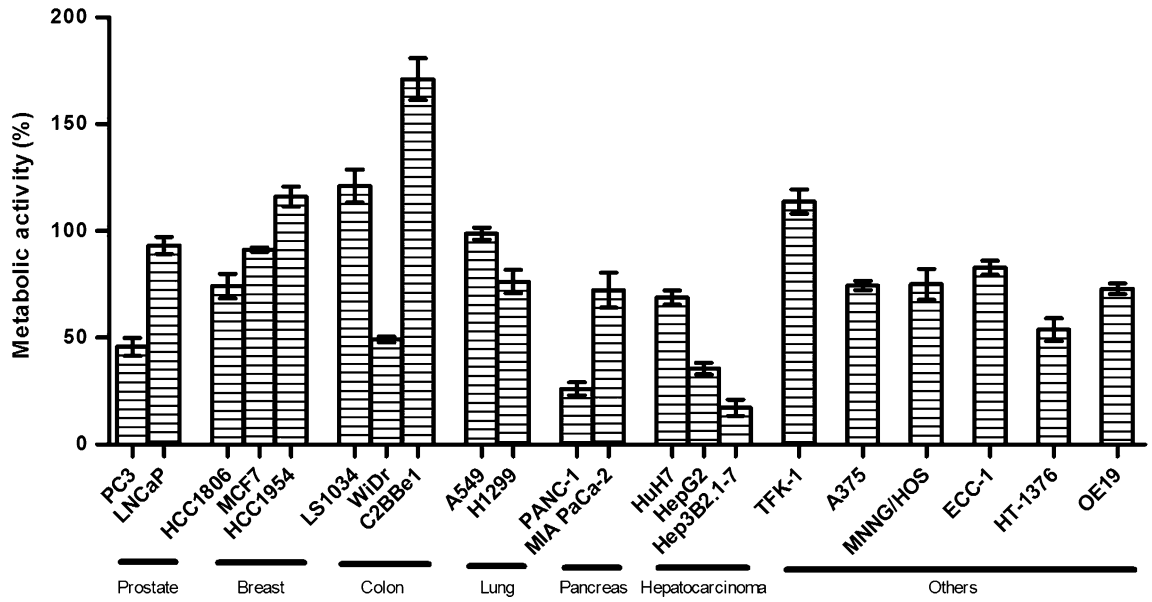

Fig. 1 Metabolic activity (%) of several human cancer cell lines evaluated after 72 h of incubation with 1 μg/μL of hAMPE. For each cell line, results were obtained with a minimum of four hAM and eight experiments. Values represent mean ± SEM

resource to the embryonic membranes, opening new challenges for the study of hAM anti-cancer effects.

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Conflict of interest The authors declare that they have no conflicts of interest.

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