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Evidence for CEA release from human colon cancer cells by an endogenous GPI-PLD enzyme

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Abstract

Elevated carcinoembryonic antigen (CEA) blood levels are found in a wide variety of epithelial neoplasms. The precise mechanism of the spontaneous CEA release from normal and cancer cells has not been established yet. In this study we investigated 'in vitro' the role of an endogenous glycosylphosphatidyl inositol phospholipase D (GPI-PLD) in spontaneous CEA release from human colon carcinoma cells. We detected GPI-PLD-specific transcript expression in four human colorectal tumor cell lines, LS180, HT29, HT29/219, and SW742 by RT-PCR. Furthermore, CEA release could be activated and inhibited by incubation of LS180 cells with suramin and 1,10-phenanthroline, compounds known to activate and inhibit GPI-PLD activity, respectively. The results suggest a mechanism for the involvement of an endogenous GPI-PLD in spontaneous CEA release from human colon cancer cells.

Keywords: CEA release; GPI-PLD; Colon cancer cells; Suramin; Phenanthroline

1. Introduction

Carcinoembryonic antigen (CEA) is one of the most extensively used clinical tumor markers. Serum CEA may be elevated in neoplastic diseases of the colon, breast, lung, prostate, bladder and stomach as well as in various gynecological malignancies. The blood CEA levels are highly variable from patient to patient. Some with cancer have normal levels despite the presence of CEA in their tumors [1–3].

The structure of CEA protein includes an N-terminal V-like Ig domain, followed by 6 I-like Ig domains, and a hydrophobic C-terminal domain. The C-terminal domain, consisting of 26 amino acids, is processed so that CEA binds to the plasma membrane through a glycophosphatidyl inositol (GPI) anchor [4].

CEA normal function(s) and its relevance to malignant transformation are not clear. The secretion of CEA by many colorectal tumors is associated with a worse prognosis and a greater likelihood of metastasis [5]. A number of functions that are ascribed to CEA include homophilic and heterophilic intercellular adhesion [6] and a possible instrumental role

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in carcinogenesis [7]. CEA has also been implicated in the process of human colon cancer liver metastasis [8,9]. The latter function has been suggested to be facilitated by soluble CEA through induction of cytokine production by Kupffer cells [8,10].

Although CEA has been for a long time known to be secreted or shed from the cell surface spontaneously, the exact mechanisms by which it is released into serum has not been demonstrated. Triton X-114 partitioning experiments showed that, in contrast to the membrane bound form, circulating CEA is mostly hydrophilic and partitions into the aqueous phase, indicating that it lacks the hydrophobic portion of the GPI moiety [11]. Khan and Hammarstrom demonstrated that CEA on isolated membranes and in cell lysates, but not on intact cells, could be cleaved by fresh human serum or purified GPI-PLD [12]. Studies have also indicated that CEA in normal colonic epithelial cells and colon cancer cells is released by a non-proteolytic cleavage, probably through the action of the endogenous phospholipases [13].

The endogenous GPI-PLD that is responsible for cleavage and release of several GPI-proteins from their anchors at the cell surface has been identified [14–16]. A GPI-specific phospholipase D (GPI-PLD) cDNA has also been cloned [17] and its expression has been shown in a number of human tissues including pancreatic β-cells, macrophages, keratinocytes, and liver [18]. The recent findings that cellular GPI-PLD appears to be involved in hydrolysis and spontaneous release of several GPI-proteins from various cells, including tumor cells [16–18], led us to examine the role of this enzyme in CEA release from colorectal carcinoma cells.

In this report, we investigated the mechanism of spontaneous CEA release in human colon carcinoma cells. We verified GPI-PLD enzyme expression in several human colon carcinoma cells. Using an activator and an inhibitor of this enzyme, we demonstrated that CEA release in LS180 colon cancer cells was readily stimulated or inhibited, respectively, by adding these chemicals to the culture medium. An understanding of the mechanism of CEA release might lead to improved methods of detection and therefore management of cancer patients.

2. Materials and methods

2.1. Materials

Guanidinium thiocyanate, sodium citrate, phenol, 1,10-phenanthroline (PNT) were purchased from Fluka chemical company (Switzerland); L-glutamine, bovine serum albumin (BSA), suramin, Dulbecco's Modified Eagle's Medium (DMEM) were from (Sigma Co., St Louis, USA). Random primers, agarose MP and dNTPs were from Roche (Roche Diagnostics, Mannheim, Germany). Penicillin, streptomycin, ribonuclease inhibitor, M-MuLV reverse transcriptase, Taq DNA polymerase, and DNA markers were supplied and oligonucleotide primers synthesized by Fermentas (MBI Fermentas, Lithuania). Fetal bovine serum (FBS) was from Biochrom (Berlin, Germany). The CEA ELISA kit was from CanAg CEA EIA kit (CanAg Diagnostics AB, Gothenburg, Sweden). Human colon carcinoma cell lines LS180, HT29, HT 29/219, and SW742, were obtained from the National Cell Bank of Iran (NCBI, Pasteur Institute, Tehran). Tissue culture plates and flasks were purchased from Nunc (Denmark). All other chemicals were obtained from Merck (Darmstadt, Germany).

2.2. Cell culture

The human colon carcinoma cell lines LS180, HT29, HT29/219 and SW742 were maintained in T25 or T75 tissue-culture flasks in DMEM containing 10% FBS supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 2 mM L-Gln. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. Measurement of CEA released from various colon carcinoma cells

The human colon carcinoma cell lines were seeded as an aliquot of 40,000 cells per 2 cm well in 4-well tissue culture plates. The cells were grown as monolayers in 250 μl of serum-containing medium as described above until confluence. To study CEA release in the stationary phase, the growth medium at confluence was replenished with serum free medium and the incubation continued for a further 96 h. Culture media were aspirated every 48 h and centrifuged first at 3000×g for 5 min to remove any