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Malignant ascites increases the antioxidant ability of human ovarian (SKOV-3) and gastric adenocarcinoma (KATO-III) cells

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Abstract

Objectives. The antioxidant status of cancer cells is an important factor in tumor invasion and metastases. This study investigated whether metastatic cancer cells derive beneficial antioxidant protection from ascitic fluid and are rendered resistant to oxidative stress in the form of a chemically generated free radical insult.

Methods. Human gastric carcinoma (KATO-III) and ovarian adenocarcinoma (SKOV-3) cell lines were cultured and incubated for 24 h with (1) M199 medium; (2) M199 + 20% fetal calf serum (FCS); (3) malignant ascites. All cells were exposed to a hydroxyl radical-generating system for 1 h. Cellular lipid peroxidation was assessed by measuring malondialdehyde (MDA) in cell suspensions. Glutathione (GSH) levels in cell pellet were measured in SKOV-3 cells after 0, 24, 48, and 72 h of incubation with buthionine sulphoximine (BSO). CD44 gene expression of cancer cells was analyzed by Northern blotting.

Results. The results showed that the cancer cells were rendered resistant to oxidative stress and with upregulated CD44 gene expression by components of malignant ascites.

Conclusions. These findings suggest that malignant ascites increases the antioxidant ability of cancer cells and the potential of adhesion and invasion. Thus, determination of the nature of these putative tumor-protective components of ascites may provide targets for therapeutic intervention.

Keywords: Ovarian cancer; Gastric cancer; Malignant ascites; Antioxidant; Glutathione; Metastases; CD44

Introduction

The metastatic spread of cancer is a central characteristic of this group of diseases and is a key target for the development of effective therapies. Metastasis formation is the result of the successful completion of a number of stages in the cancer cell life cycle, each stage being a potential point at which the cell may die or not achieve metastatic capability for some other reason. The ability of cells shed from a primary tumor to penetrate the cellular surface layer of the distant tissue to which they later adhere is a key feature in the formation of metastases. Adhesion molecules such as CD44 have an important role in both the attachment and invasion of cancer cells [1,2], which thus may be used as possible targets for novel anticancer therapies.

In fact, many of the mechanisms believed to be involved in the shedding of cells from the primary tumor have been implicated in the penetration of cellular layers at sites of metastasis formation. In order for secondary tumor deposits to be formed in the peritoneum from cells shed from primaries such as ovarian tumors, it is necessary for cancer cells to anchor to the mesothelial cells that line the peritoneal cavity, penetrate the cellular matrix that surrounds these cells, and also to penetrate the basement membrane to begin growth. The vehicle that facilitates the movement of these cells within the peritoneal cavity is the peritoneal fluid or ascites in the pathological situation.

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During the dissemination of intraperitoneal malignancies, potentially metastatic cells may undergo a period of hypoxia while suspended in ascitic fluid in the peritoneal cavity. Continued normothermic hypoxic metabolism followed by the reestablishment of normoxia upon attachment at a secondary site is likely to cause cell injury such as the free radical-derived damage. Tumor cell viability would thus be diminished, but this does not seem to be reflected by clinical evidence that secondary tumors commonly arise from these metastatic cells. It is therefore likely that the ischemia/reperfusion insult to these cells is not sufficient to cause lethal damage or that these cells benefit from protective "chemical systems" that prevent such damage. These systems, presumably antioxidant mechanisms, are believed to be the reason why some human tumor cells have been shown to be more resistant to hypoxia than those originating in other animals [3]. These mechanisms may also be responsible for the resistance to chemotherapy and radiotherapy inherent in some types of cancers [4]. Our previous study suggested that intracellular levels of oxidants play complex roles in both cytoprotective and cytotoxic pathways, and the net effect may be cell dependent or stimulus dependent. Combination of antioxidants and conventional cytotoxic agents may therefore prove beneficial in cancer therapy [5].

The most ubiquitous antioxidants are the thiol compounds, and in particular, glutathione (GSH). GSH is produced mainly in the cytosol of hepatocytes [6] and is then transported in the plasma. Its passage into tissues is facilitated by the enzyme gamma-glutamyltranspeptidase (γ-GT) [7]. The most important function of GSH is as an antioxidant offering protection against reactive oxygen species, but an equally important role is as a vehicle for the transport of cysteine. The GSH level is a useful measure of oxidative stress and to monitor the effectiveness of antioxidant intervention strategies.

In ovarian cancer, measurement of tumor GSH concentration has been used to predict patients' responses to chemotherapy. There was a significant increase in GSH levels in the tumor cells from patients who had previously received chemotherapy vs. those without receiving chemotherapy. GSH concentration in the tumor was significantly higher in patients with no response to chemotherapy [8–10]. An experimental study showed that coincubation of cisplatin and doxorubicin with buthionine sulphoximine (BSO) resulted in a significant increase in sensitivity to both of these drugs, suggesting that the modulation of GSH using agents such as BSO may be considered in patients with drug-resistant ovarian cancer. In addition, GSH has been shown to have effective protection against chemotherapy-induced side effects in patients with ovarian cancer. The intensity of toxicity was significantly less pronounced in patients treated with GSH than that in the control group [11].

When combined with chemotherapy, antioxidants have been widely used as therapeutic biologic response modifiers. Oral antioxidant therapy such as vitamin C, vitamin E, and β-carotene has shown improved efficacy of chemotherapy [12,13]. In ovarian cancer, vitamins C and K3 have also been used as adjuvant treatment through their cytotoxic effects on cancer cells [14]. Further studies have showed that selenium supplementation significantly influences malondialdehyde (MDA) and the GSH peroxidase system in patients with ovarian cancer undergoing chemotherapy [15]. In those patients receiving selenium, a significant increase in the activity of GSH-P(x) and an increase of the concentration of malondialdehyde have been found after the treatment of supplementation.

Ascitic fluid has been shown to contain a number of physiologically active soluble constituents including molecules such as growth factors that can potentially promote tumor growth and metastasis formation, thereby accelerating disease progression. It is likely that exposure of tumor cells from primaries within the peritoneal cavity to a low-oxygen environment while in suspension in ascitic fluid acts as a priming mechanism for the formation of metastases. Antioxidant activity in human fluids including serum, urine, cerebrospinal fluid, saliva, tears, and ascites may have important biological effects on tumor growth, in particular in ascites where the antioxidant activity measured as uric acid levels is the second highest following saliva [16].

Both in vivo and in vitro studies have shown enhanced effectiveness of standard cancer therapies or a neutral effect on drug action with antioxidant supplementation during cancer therapy [17]. However, any antioxidant found to reduce in vivo toxicity of cancer therapy on healthy tissue has the potential to decrease effectiveness of chemotherapy unless this was specifically studied. In many instances, the effect of an antioxidant compound with a certain therapeutic agent may be specific to a particular tumor type, or may vary with dosage of both antioxidant and chemotherapy [18].

Therefore, the present study was designed to determine whether tumor cells disseminated in ascitic fluid are rendered resistant to oxidative stress in the form of a chemically generated free radical insult with GSH depletion induced by the compound BSO that has been used in a number of studies to deplete intracellular GSH pools [19]. In addition, the effect of malignant ascetic fluid on CD44 gene expression of human gastric and ovarian cancer cell lines was analyzed by Northern blotting to clarify the correlation of antioxidant ability of cancer cells with their adhesion properties.

Materials and methods

Cell lines and cell culture

Two types of cell lines were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, Wiltshire, UK): an ascites-derived ovarian adenocarcinoma cell line