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Mitochondria-related oxidative stress contributes to ovarian cancer-promoting activity of mesothelial cells subjected to malignant ascites

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ABSTRACT

Very little is known about the mechanisms by which malignant ascites modulates the cancer-promoting activity of human peritoneal mesothelial cells (HPMCs). Because malignant ascites induces pro-tumoral senescence in HPMCs, here we examined if this effect could be driven by oxidative stress. The study showed that malignant ascites generated by serous ovarian tumors induced oxidative damage to the DNA (γH2A.X, 53BP1, 8-hydroxy-2′-deoxyguanosine) and lipids (8-isoprostane) in HPMCs as well as increased the production of mitochondrial superoxides and cellular peroxides in these cells. This activity coincided with increased activity of two enzymes involved in the mitochondrial production of oxidants, i.e. cytochrome c oxidase and NADH dehydrogenase, decreased mitochondrial inner membrane potential, increased mitochondrial mass, and increased the activity of peroxisome proliferator-activated receptor gamma coactivator-1 alpha. Increased production of superoxides and peroxides in cells subjected to the malignant ascites was effectively reduced when the fluid was pre-incubated with neutralizing antibodies against hepatocyte growth factor. Moreover, when HPMCs subjected to the malignant ascites were protected against oxidative stress with a spin-trap scavenger of reactive oxygen species, they displayed decreased expression of senescence-associated β-galactosidase and their potential to stimulate cancer cell adhesion, proliferation, and migration was significantly diminished. Collectively, our findings indicate that improved ovarian cancer cell progression in response to HPMCs exposed to malignant ascites may be associated with the development of profound oxidative stress in these cells.

1. Introduction

Although a plethora of evidence has emerged that malignant ascites contributes to progression of ovarian cancer, more knowledge is being gained about the mechanistic aspects of its activity. In general, it is believed that malignant ascites creates a hospitable peritoneal milieu that helps ovarian cancer cells survive, invade, and disseminate (Ahmed and Stenvers, 2013). This is linked, in turn, with the ascites' ability to suppress peritoneal inflammation (Simpson-Abelson et al., 2013) and with the presence of large amounts of agents that support angiogenesis and cancer cell motility in this fluid (Kipps et al., 2013). Last but not least, malignant ascites modulates the behavior of normal peritoneal cells, e.g. by inhibiting the cancer cells' apoptosis driven by the peritoneal mesothelium (Matte et al., 2014).

It has been shown that human peritoneal mesothelial cells (HPMCs), which constitute the largest fraction of cells within the peritoneal cavity, support the progression of ovarian cancer *in vitro* and *in vivo* when they become senescent (Mikula-Pietrasik et al., 2016b). Interestingly, recent findings indicate that malignant ascites accelerates the senescence of HPMCs via the activity of growth-related oncogene 1 (GRO-1) and hepatocyte growth factor (HGF) (Mikula-Pietrasik et al., 2016a). The intracellular mechanism underlying this activity of the ascites remains, however, unknown.

Taking into account that the senescence of normal somatic cells is often causatively linked with deleterious activity of reactive oxygen species (ROS) (Davalli et al., 2016), the goal of this study was to examine if the pro-senescence activity of malignant ascites and concomitant pro-tumoral effects of HPMCs may be caused by oxidative stress. To this end, several parameters associated with oxidative cell status were examined, including oxidative DNA damage, lipid peroxidation, production of ROS, activity of enzymes involved in ROS release, mitochondrial membrane potential, and mitochondrial

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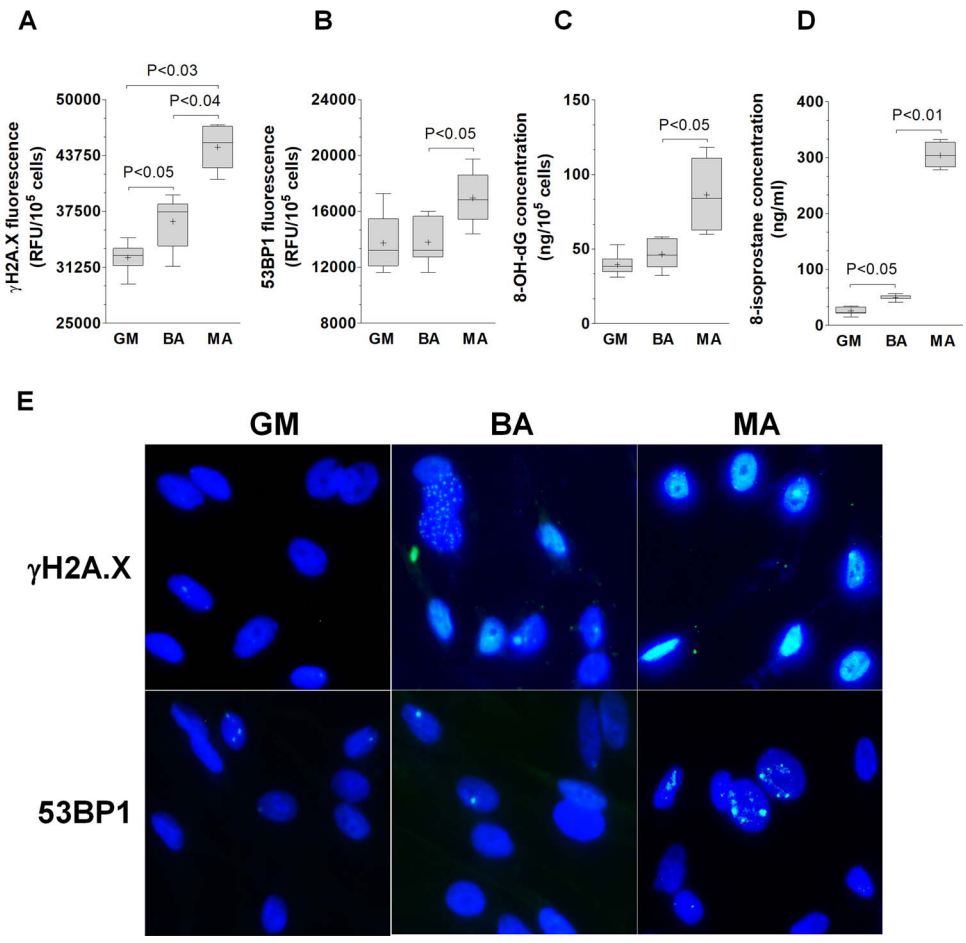

Fig. 1. Effect of growth medium (GM), benign ascites (BA) and malignant ascites (MA) on activation of DNA damage response (DDR) and magnitude of lipid peroxidation in cultured HPMCs.
Quantification of fluorescence of γH2A.X (A) and 53BP1 foci (B) was used as a measure of DDR. Quantification of 8-OH-dG was treated as a measure of nonspecific DNA injury (C). Quantification of 8-isoprostane was used to estimate the magnitude of lipid peroxidation (D). Representative pictures showing γH2A.X and 53BP1 foci (green dots) in the nuclei (blue DAPI staining) of cells exposed to growth medium, benign and malignant fluid (E). The results derive from experiments performed with HPMCs obtained from 8 different donors, and with benign and malignant fluids collected from 8 different patients. (+) in the boxes indicate means, while the horizontal lines indicate medians. RFU – relative fluorescence units. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

biogenesis. These tests were followed by intervention studies in which HPMCs treated with ascites were protected against oxidative stress by using a spin-trap ROS scavenger, then their senescence and their impact on cancer cell progression were evaluated.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals were from Sigma (St. Louis, MO) and culture plastics were from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Ascitic fluids

Malignant ascites were obtained during cytoreductive surgery from patients with high-grade serous ovarian cancer at stage IV (n = 8). The histopathology, grade, and stage of the tumors were assigned in keeping with the criteria of the International Federation of Gynecology and Obstetrics. Benign fluids were obtained from age-matched patients with cystadenoma mucinosum multiloculare (n = 8). After collection in sterile conditions, the fluids were centrifuged at 2500 rpm for 5 min and the acellular supernatants were stored at −20 °C until required. The study was approved by an institutional ethics committee (consent number 543/14).

2.3. Cell cultures

Human peritoneal mesothelial cells (HPMCs) were isolated from fragments of the omentum derived from 8 non-oncological patients undergoing abdominal surgery (institutional consent number 187/14), as described in detail elsewhere (Ksiazek, 2013). Cells were identified as pure mesothelium by their epithelial-like morphology at confluency and uniform positive staining for cytokeratins and the HBME-1 antigen. During the experiments only HPMCs obtained from the first two passages, which corresponds to about 25% of their replicative lifespan, were used. The viability of these cells exceeded 98%.

Ovarian cancer cells, SKOV-3, were obtained from the ECCC (Porton Down, UK) and maintained in RPMI 1640 medium with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 g/ml), and 10% FBS. Primary epithelial ovarian cancer cells (EOCs) were isolated from tumors excised during cytoreductive surgery from patients with serous ovarian cancer (stage IV). Briefly, the tumors were cut into pieces of equal weight and then placed in a solution of 0.05% trypsin and 0.02% EDTA for 20 min at 37 °C with gentle shaking. After resuspension in RPMI1640 containing 20% FBS, the cells were probed with an antibody directed against the epithelial-related antigen (MOC-31; Abcam, Cambridge, UK) to confirm their cancerous nature. Finally, the ovarian cancer cells were cultured in RPMI 1640 supplemented with L-glutamine (2 mM) and 20% FBS.

2.4. Experimental conditions

HPMCs were placed onto culture dishes and allowed to grow until reaching sub-confluency. Then the cells were exposed to standard growth medium (GM), 10% malignant ascites (MA), and 10% benign ascites (BA) for 72 h. In some experiments, the HPMCs were pre-incubated for 6 h with a spin-trap ROS scavenger, N-tert-butyl-alpha-phenylnitrone (PBN, Sigma; 800 μM), before the addition of malignant and benign ascites. Starting from pre-incubation, PBN was constantly