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Invited paper

Possible therapeutic option of aqueous plasma for refractory ovarian cancer

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ABSTRACT

Based on a number of recent reports, plasma exerts anti-proliferative and apoptotic effects on various cancer cells. Besides a direct effect on the cells, the proliferation of normal and tumorous mammalian cells was reported to be downregulated by indirect plasma-exposed medium through the generation of reactive oxidative species. We demonstrated that plasma-activated medium (PAM) also had an anti-tumor effect on even acquired chemoresistant OC cells. Furthermore, the aqueous plasma displayed an anti-tumor effect against clear-cell carcinoma, which is natively chemo-refractory OC, and we confirmed that the efficacy is selective for tumor cells rather than normal mesothelial cells. The adhesion potency to type-I-collagen-coated dishes was significantly lower in both chemosensitive and chemoresistant cells pretreated with PAM than that of non-stimulated cells ($P < 0.0001$). In particular, the adhesion potential of acquired chemoresistant cells was more evident than that of the original chemosensitive cells. Taken together, PAM reduced not only the proliferative activity but also attachment to extracellular matrix components. Here, a possible application of this technologic modality as a therapeutic target for OC is proposed.

1. Introduction

Plasma, commonly is essentially an ionized gas in which a fraction of the atoms or molecules is ionized [1]. Recently, owing to technical developments, new-generation plasma, called non-thermal plasma, therapy has been focused on as a novel medical practice, including tissue sterilization, blood coagulation, wound-healing promotion, and dental bleaching [2–5]. On the other hand, there has been a number of reports showing the direct effect of non-thermal plasma on cell death of various tumors, including skin, colon, pancreatic, lung, breast, brain, and uterine cervical cancer cells [6–12]. Previous studies reported that plasma generates a large amount of reactive oxidative species (ROS), resulting in DNA damage and cell death [13–15]. Besides a direct effect on the cells, the growth of normal and tumorous mammalian cells was also downregulated by plasma-exposed medium through the generation of ROS [14,16]. Actually, in several cancer cells, there has been a number of evidence showing those indirect effect of plasma on cell death [10,16,17].

Ovarian cancer (OC) is the most lethal gynecologic malignancy, and it is the leading cause of cancer-related mortality in females in Western countries. In 2015, it was estimated that 21,290 women were diagnosed with OC, and 14,180 died of the disease in the United States [18]. Due to the establishment of a treatment strategy that consists of maximum cytoreductive surgery followed by front-line chemotherapy, complete clinical remission can be achieved in approximately 80% of these patients [19]. Although the short-term prognosis of patients with OC appears to be favorable, the majority of those clinical complete responders experience recurrence. Moreover, OC has a variety of histological types. It is well-known that clear-cell carcinoma (CCC), which is the second most frequent subtype in Japan, is one of the most aggressive and malignant tumors in OC [20–22]. CCC is associated with potential resistance to conventional platinum-based chemotherapy, compared to other types of OC such as serous adenocarcinoma.

Here, we wonder whether the plasma-activated medium (PAM) has an anti-tumor effect in advanced or chemorefractory OC without damaging normal peritoneal cells. In the present study, we overviewed the aqueous plasma therapy instead of direct plasma, considering intraperitoneal administration in the future.

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2. Clinical background of ovarian cancer

Since OC frequently remains clinically silent, the majority of patients have advanced intraperitoneal metastatic disease at diagnosis (Fig. 1A). Once recurrence occurs, complete cure becomes very difficult, and patients experience repeated salvage treatments until the last day of their life (Fig. 1B). The prognosis of patients with OC is related to the degree of peritoneal dissemination. Fig. 1C shows the overall survival curve of OC patients, who were registered and analyzed by Nagoya University Hospital and affiliated hospitals, with or without peritoneal dissemination. Patients with peritoneal dissemination showed a much poorer prognosis than those without it ($P < 0.0001$).

The peritoneal mesothelium is the main metastatic site of OC, besides the direct extension of tumors into neighboring tissues or lymph nodes. In the initial step of peritoneal dissemination, carcinoma cells detach from the primary lesions and are released in ascites. Once the OC cells attach to the mesothelium, the cells invade local tissues with increased neovascularization [23]. According to our prior report, OC cells that have acquired chemoresistance may have hallmarks that favor easily spreading into the peritoneal cavity, resulting in an increased chance of adhering to the mesothelium and the enhanced formation of microscopic or macroscopic peritoneal metastasis *in vivo* [24]. If all tumor cells are sensitive to chemotherapy, all disseminated tumors may disappear. Nevertheless, tumor sensitivity is actually heterogeneous. Therefore, recurrence will be inevitable due to the remaining of resistant tumor cells. Thus, the prognosis of advanced or recurrent cases remains poor as most mortality cases result from metastasis that is refractory to these chemotherapeutic agents.

3. The effect of aqueous plasma on oc cells

3.1. Effect of PAM on OC cells

In our earlier investigation, two independent human OC cell lines and normal fibroblasts treated with high-flux non-thermal plasma were evaluated by toxicity and proliferation assays. Consequently, both types of OC cell were discriminately killed through inducing enhanced apoptosis, while plasma-treated fibroblasts were not damaged [25]. However, when we consider the intraperitoneal treatment for numerous micrometastatic disseminations of OCs, direct plasma irradiation therapy is not able to target all the numerous tumors spreading throughout the peritoneal cavity. In this context, plasma-activated medium (PAM) would be more practical since it is thought that aqueous ROS permeate peritoneal fluid.

We first evaluated the anti-tumor potential of PAM to inhibit the growth of human OC cell lines using the cell viability assay [26]. Detailed information about the experimental system specification and production of PAM was described previously [26]. Fig. 2A shows cell viability of K2 cells after exposure to PAM for the indicated times. PAM was used immediately after plasma irradiation (incubation time: 48 h). In K2 cells, the viability decreased by approximately 5% when cells were treated with PAM for 3 h, compared to cells without PAM treatment (representing images were shown in Fig. 2B and C). However, such a marked growth-inhibitory effect was not observed in K2 cells exposed to PAM 19 h after irradiation (data not shown). The growth-inhibitory effects of PAM were completely blocked on being pretreatment and co-incubation with N-acetyl cysteine (final concentration: 4 mM) (Fig. 2D). These results indicate that the treatment efficiency was gradually decreased in accordance with ROS inactivation by intrinsic scavengers.

3.2. Effect of PAM on intrinsic and acquired chemoresistant OC cells

To assess whether PAM treatment could affect chemoresistant cell lines, we examined this effect in paclitaxel-resistant OC cells (K2R100) [24,26]. Cell viability was evaluated 24 h after PAM treatment (Fig. 3). The viability rate of K2R100 cells was higher than that of K2 cells with an exposure time of less than 1 h. However, for longer than 2-h exposure, the growth-inhibitory effect by PAM was reversed between the two lines. The results indicate that even acquired chemoresistant cells may be more sensitive to PAM with a longer exposure time.

On the other hand, there are two types of chemoresistance: intrinsic and acquired [24]. Actually, K2R100 cells have the latter type. Regarding intrinsic chemoresistant cells, ovarian clear-cell carcinoma (CCC) is thought to be representative. We confirmed the anti-tumor effect of PAM on the growth of CCC cell lines (TOV-21G, ES-2). Cell morphological changes, such as shrinking, rounding up, and detachment from dishes are typical of apoptosis [26]. Actually, PAM treatment caused these morphological changes. Subsequently, we assessed whether the cytotoxic effect of PAM against EOC cells was associated with the induction of apoptosis by TUNEL assay [27]. Compared with control cells, both TOV21G and ES-2 cells treated with PAM showed more TUNEL-positive staining, indicating that PAM induced apoptosis in both cell lines (Fig. 4). Aqueous plasma also exhibited cytotoxic activity against CCC cells which were intrinsically resistant to chemotherapy.

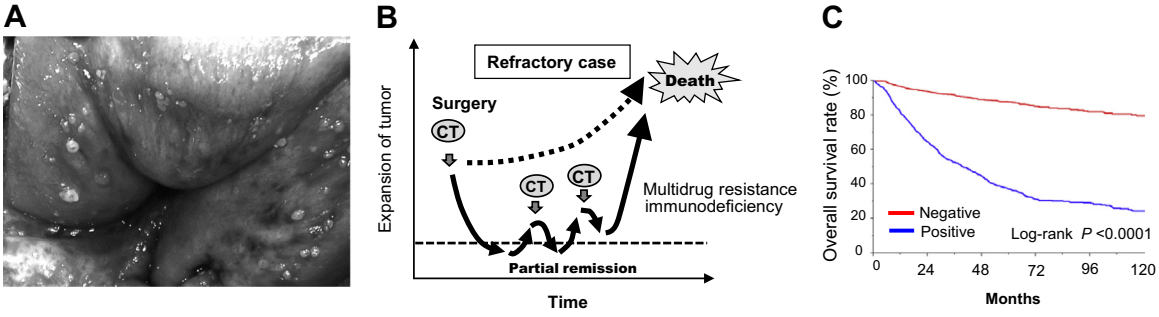

Fig. 1. Clinical characteristics of ovarian cancer (OC). A: Representative macroscopic appearance of OC and peritoneal disseminated tumors. B: Representative clinical course of patients with OC who experience partial remission and recurrence despite repeat salvage treatments. C: Survival curves of patients with OC. Kaplan–Meier overall survival curves in patients with OC with or without peritoneal dissemination which were registered and analyzed by the Tokai Ovarian Tumor Study Group. Red line: without peritoneal dissemination (FIGO stage I: $N=872$), blue line: with peritoneal dissemination (FIGO stage III-IV: $N=982$). Patients with peritoneal dissemination showed a much poorer prognosis than those without it (Log-rank: $P < 0.0001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).