Therapeutic potential of cold atmospheric plasma in cancer eradication and its possible application as an adjuvant anti-cancer therapy

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研究分野 Research area

放射線科学 (Radiological Sciences)

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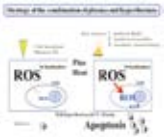
研究内容 Research content

Cold atmospheric plasmas (CAPs) have been proposed as a novel therapeutic method for its anti-cancer potential and have gained increased attention for its biological and medical purposes. The cellular effects of CAP are mainly mediated via generation of reactive oxygen species (ROS). The CAP induced huge amount of ROS in the liquid phase than intracellular, and it seems that CAP-induced extracellular ROS is not completely recognized intra-cellularly. Therefore, it is worthy to investigate the effects of CAP in combination with other physical modalities such as hyperthermia (HT) or ionizing radiation. Considering the fact, this study was aimed to determine the effects of cold atmospheric helium plasma (He-CAP) in combination with mild HT and discussed the effects of combined treatment on enhanced cancer cell killing.
The synergistic effects were accompanied by increased ROS production. Hydrogen peroxide (H2O2) and superoxide (O2•−) generation was increased immediately after He-CAP treatment, but fails to initiate cell death process. Interestingly, at late hour`s He-CAP-induced O2•− generation subsides, however the combined treatment showed sustained increased intracellular O2•− level, and enhanced cell death than either treatment alone. He-CAP caused marked induction of ROS in the aqueous medium, but He-CAP-induced ROS seems insufficient or not completely incorporated intra-cellularly to activate cell death machinery. The observed synergistic effects were due to the HT effects on membrane fluidity which facilitate the incorporation of He-CAP-induced ROS into the cells, thus results in the enhanced cancer cell death following combined treatment. These findings would be helpful when establishing a therapeutic strategy for CAP in combination with HT or radiation.

研究のポイント Research point

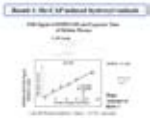
Cancer is still the leading cause of deaths worldwide, and remains very hard to treat. Despite, recent advancements in cancer biology, therapy resistance and non-selectivity are the main issues associated with the currently available treatments. Therefore, search for more selective anti-cancer strategy should be urgently required. Cold atmospheric plasma (CAP) is an ionized low temperature gas, produced by applying a high voltage electric field at normal or atmospheric pressure. The most increasingly important focus of CAP research is on the development of new therapeutic approaches based on its anti-cancer potential. CAP has a distinctive feature to selectively kill cancer cells, while sparing healthy cells. However, one of main hindrance in the development of CAP device for clinical application is lack of standardization in between CAP devices because the anti-cancer activity of CAP is directly linked with its ability to produce reactive oxygen species, which can enormously vary in between CAP devices. For clinical application of CAP it is necessary to develop one standardized therapeutic strategy based on common aspect of CAP models. Therefore, in this study we have demonstrated a useful strategy by combining He-CAP with HT and radiation, in which HT or radiation facilitates the incorporation of CAP-induced extracellular ROS inside the cells and enhances its efficacy. HT and radiation, alone or in combination with chemotherapy have shown promising anti-cancer effects for various cancer and the effects of these combination therapies have been well documented.

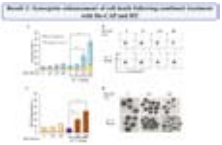


研究への取組、今後の展望

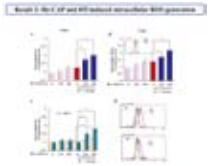
This study provides the initial piece of evidence regarding the combined used of CAP with other physical modalities. The synergistic enhancement in apoptosis with He-CAP and HT was not only confined to human lymphoma U937 cells, rather it was also observed in other cell lines harboring different p53 status such as human lymphoma MOLT-4 , and human colon carcinoma HCT-116 cells. Interestingly, more profound synergistic effects were observed in U937, which are p53 mutant cells. In addition, the combined treatment showed no toxicity towards normal HaCaT cell line. These findings emphasize the efficiency of combined treatment with HT, as synergistic effects were achieved when cancer cells were exposed, irrelevant to p53 status. We have demonstrated the strategy for possible future clinical application of CAP with HT or radiation. This plasma-thermia or plasma-hyperthermia strategy would help to overcome the barrier regarding CAP clinical application, such as limited penetration of ROS, variance in CAP devices and its induced effects.

研 究 REPORT

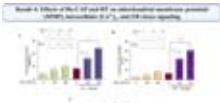




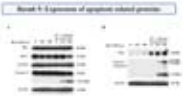
(A) Cells were treated with He-CAP and exposed to mild HT at 42 oC 20 min. After 6 h of incubation cells were subjected to annexin V-FITC/PI double staining. Flowcytometry revealed that the apoptotic features induced by He-CAP and HT alone were not observed as compare to combined treatment. However, no enhancement was detected with 60 s in combination with HT. (B) Representative flow cytometry histograms of Annexin V-FITC/PI staining are shown. (C) Further, cell death was measured by DNA fragmentation, significant increase in the percentage of DNA fragmentation was observed following combined treatment compared to HT treatment alone. (D) Giemsa staining showed that typical morphological changes associated with apoptosis were more prominent in the combined treatment than treatment alone. One representative photomicrograph is shown here, arrow head shows apoptotic cells. All the data are presented as mean ± SD. ***p<0.005, vs. HT alone.



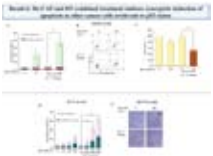
Cells were harvested immediately after combined treatment or at indicated time. Elevated level of ROS species were analyzed by flow cytometry. (A) Superoxide generation was immediately increase following He-CAP treatment and significantly enhanced in the combined treatment. (B) The generation of H2O2 was markedly increased immediately after He-CAP treatment, which was further enhanced in the combined treatment. Upper small panel shows the representative histogram of DCFH-DA staining. (C) Interestingly, at 1 h and 3 h the He-CAP induced superoxide generation subsides, however it remains elevated in the combined treatment. (D) Representative histogram of HE staining at 1 and 3 h are shown. Data are presented as the mean ± SD. *p<0.05, **p<0.01, than the HT alone.



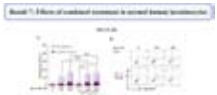
(A, B) Cells were treated with HT and the combination, changes in the loss of MMP and [Ca2+]i were detected by flow cytometry using TMRM staining and Flou-3 AM, respectively. The combined treatment significantly increased the loss of MMP and [Ca2+]i concentration, while no such change was observed in with either treatment alone. (**p<0.01 vs HT, *p<0.05 vs HT). (C) Further, to investigate the reason of this [Ca2+]i increase, effects on ER stress were determined. Bip/GRP78 and CHOP/GADD153 are considered as the main regulator of ER-stress and their activation is the major indication for ER stress. BIP and CHOP expression was markedly increased in the combined treatment. β-actin was used to normalize the expression level in each sample.



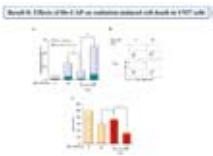
Cells were harvested 6 h post-treatment following combined treatment with He-CAP and HT and subjected to Western blot (A) Western blot analysis revealed that the expression of anti-apoptotic Bcl-2 was decreased following combined treatment. While the expression of pro-apoptotic Bax remained unchanged. In addition, expression of caspase-3 was increased. (B) To investigate the involvement of extrinsic apoptotic pathway, effects of combined treatment on Fas externalization and caspase-8 activation were studied. The protein expression of Fas and caspase-8 was markedly increased following combined treatment. β-actin was used to normalize the expression level in each sample.



(A) Evaluation of apoptosis in MOLT-4 cells. (B) Representative flow cytometric histogram of Annexin V-FITC/PI staining in MOLT-4 cells. (C) Cell survival was assayed 6 h post-treatment following combined treatment by cell counting kit-8. Data are presented as the mean ± SD . **p<0.01, ***p<0.005, than HT alone. (D) Annexin V-FITC/PI assay was carried out at 24 h following combined treatment in HCT-116 cells. Dose dependent increased in the apoptosis was observed. Data are presented as the mean ± SD. ***p<0.005, than HT alone (E) DIC live images of HCT-116 cells. Cell proliferation 24 h after combined treatment of He-CAP 180s and HT.



(A) Annexin V-FITC/PI assay was carried out at 24 h following combined treatment in HaCaT cells. (B) Representative flow cytometric histogram of Annexin V-FITC/PI staining in HaCaT cells. No significant increased in the apoptosis was observed following combined treatment than He-CAP treatment alone. Data are presented as the mean ± SD . *p<0.05, than HT alone, N.S (not-significant).



(A) Annexin V-FITC/PI assay following treatment of He-CAP 60 s and radiation 5 Gy. (B) Representative flow cytometric histogram of Annexin V-FITC/PI staining with He-CAP and radiation. (C) Cell survival assay by cell counting kit-8 was carried at 6 h post-treatment. Data are presented as the mean ± SD. *p<0.05, ***p<0.005, vs. treatment alone.

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