様式３

論　文　内　容　の　要　旨

氏　名　（　稲井瑞穂　）	
論文題名	Cytotoxic mechanism of membrane targeting photosensitizer-incorporated HVJ-E in prostate cancer (膜を標的とした光感受性薬剤含有HVJ-Eの前立腺がんにおける細胞障害機構)

論文内容の要旨

Development of prostate specific antigen screening has resulted in dramatic reduction in overall prostate cancer mortality. Survival improvement of advanced-stage cancer patients, however, is still limited and the establishment of effective modality is eagerly awaited. To tackle this problem, photodynamic therapy (PDT), which bases on light absorption and photochemistry, has emerged as a potential treatment that results in malignant prostatic tissue eradication. PDT has an advantage of high selectivity, yet its limited treatment depth hinders this method to become an integral part of clinical practice. Thus, more potent agents that allow induction of multiple cell death pathways are needed to enhance treatment outcome.

In the previous study, novel photosensitizer named porphyrus envelope (PE) was established by inserting lipidated protoporphyrin IX (PpIX lipid) into the replication-deficient viral particle, hemagglutinating virus of Japan envelope (HVJ-E). Drug release mechanism and its efficacy over conventional photosensitizer 5-aminolevulinic acid (5-ALA) have already been confirmed. This study focuses on uncovering cellular localization of PE and characterizing its ability to induce multiple anti-tumor effects *in vitro* to investigate the effectiveness of PE-mediated PDT against malignant prostate cancer.

Localization and cellular uptake of PE in cells were confirmed via confocal laser scanning microscope and a cell-based fluorescent assay. As well, the effect of direct cytotoxic effect induced by PE was confirmed through analyzing wound-healing ability and colony-forming activity of prostate cancer cells. The effect of PE-mediated PDT was investigated by observing generated reactive oxygen species (ROS) and induced cell death pathway. The combination index (CI) was also calculated to confirm the synergistic activity of HVJ-E and PE-mediated PDT. The results have shown how PE rapidly localizes to the cell membrane after 10 min incubation while ensuring selective uptake of a photosensitizing agent in cancer cells. Direct cytotoxicity induced by PE largely inhibited wound healing and colony-forming activity in all conditions. Furthermore, time-dependent increase in ROS production was observed, and induction of both apoptotic and necrotic cell death pathways was confirmed. PE-mediated PDT was most effective in 5 h sample, which exhibited high fluorescence intensity for ROS. Besides, it is notable that treatment with PE-mediated PDT could result in more rapid cell death than HVJ-E or PpIX lipid alone, suggesting the enhanced therapeutic outcome of PE-mediated PDT. The synergistic activity of HVJ-E and PE-mediated PDT was confirmed with CI of <1.

In all, these results demonstrate the high therapeutic efficacy of PE-mediated PDT with rapid drug delivery to cell membrane and induction of cell death via multiple pathways. Synergistic effect of HVJ-E and photodynamic reactions represents a promising treatment for advanced and metastatic prostate cancer.

様式 7

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

従来の光線力学的療法(PDT)では、悪性度の高い進行前立腺がんに対する十分な治療効果は期待できない。そこで本論文提出者は、腫瘍選択的な免疫原性がん細胞死を誘導する新規光感受性薬剤として複製能欠損型ウイルス粒子HVJ-Eに着目し、これに光感受性薬剤であるPpIX脂質を組み合わせたporphyrus envelope（PE）を開発した。又、PEは膜傷害を起因とした高い殺細胞効果を示しHVJ-E特有の抗がん作用を介してがん細胞死をPDT前後で引き起こすことが可能であることを初めて検証した。さらに、HVJ-E由来の抗がん作用とPDT効果の相乗作用を評価した上で、光照射直後からPEを構成するHVJ-EとPpIX脂質による細胞死が連続的に引き起こされることを確認した。

以上のように、本論文は光感受性薬剤を含むHVJ-Eの前立腺がん細胞膜への障害機構を明らかにしており、学術的・国際的にも新規性が高く評価される。

よって、論文提出者・稲井瑞穂の論文は、博士(工学)の学位に値するものと認める。