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学位論文内容の要旨
（Abstract of thesis）

博士の専攻分野の名称　博士（医理工学）　氏 名　ホサイン エロラ
（Degree conferred: Doctor of Philosophy）　（Name of recipient:　Hossain Elora　）

学 位 論 文 題 名
（Title of thesis）

Oncolytic Adenovirus Utilizing RNA Stabilization Mechanism Can Synergize with Chemotherapy

（RNA 安定化メカニズムを応用した腫瘍溶解性アデノウイルスとそのがん化学治療法との相乗効果）

Background: Cancer therapy remains a challenge despite of an increased number of therapeutic strategies. A better understanding about the underlying biological process of cancer will enable the enhancement and development of therapeutic strategies for an effective battle against cancer. Oncolytic virotherapy is a rejuvenation in the therapeutic approach for cancer therapy. Ad-fosARE is a newly developed conditionally replicative adenovirus engineered by inserting AU-rich element (ARE) in the 3'-untranslated regions of the E1A gene. ARE enhances the rapid decay of mRNAs and the fate of ARE-mRNA is controlled by ARE-binding proteins HuR. In cancer cells, HuR constitutively relocates to the cytoplasm, resulting in the stabilization of ARE-mRNA. In this study, we examined the oncolytic activity of Ad-fosARE . For better combatting with the tumor microenvironment chemotherapeutic agent used for combination therapy with the Ad-fosARE virus and considered a synergistic combination for treating cancer using this virus and chemotherapeutic agent Paclitaxel (PTX). Paclitaxel is the most common anticancer agent used against many cancers, including　breast cancer, lung cancer, Kaposi sarcoma, cervical cancer and pancreatic cancer but the emergence of drug resistance is a major drawback.

Objective: To trear cancer effectively and reduse the burden of chemotherapy by minizing the dose.

Methods: Ad-fosARE was constructed using a pXhoIC plasmid and the c-fos gene was inserted into the HpaI site in the 3'-UTR of the E1A gene of pXhoIC. We used six cancer cells (HeLa, C33A, A549, H1299, U2OS and HepG2) and normal cell line (BJ and HGF1) to evaluate selectivite replication and cytolytic activity of the virus. To evaluate dependency on ARE mRNA stabilization by cytoplasmic HuR, halflife of E1A mRNA was measured in HuR depleted contions such as heat-shoched or siRNA treated cells. Efficacy of Ad-fosARE was also compared with dl1520 and WT300 virus by virus production and cytolytic assay.For the effect of combination treatment with Ad-fosARE and PTX; HeLa, A549 and BJ cells were used in vitro. Treatment induces replication and cytotoxicity were assessed by western blot analysis, virus titer, CPE, cell viability assay, immunocytochemistry. Virus and drug synergism was calculated by Chou-Talalay combination indices.For in vivo experiment human cervical cancer mouse model was used to evaluate efficacy using female BALB/c　nu/nu mice.

Results: The expression of viral early protein E1A and E1B 55kDa and late viral protein was detected only in cancer cells but not in normal cells. Viral propagation in cancers cells were

also significantly higher in cancers cell. In terms of cytotoxicity, the results of cytopathic assay and cell viability assay showed significant cancer selectity. The expression of viral early gene E1A, which is encoded in ARE including mRNA, was significantly high in cancer cells due to be stabilized in it, as a result, the efficiencies of replication and cytolytic activity of this virus with cancer cells were higher than those of normal cells. In HuR depleted cells the half-life of E1A mRNA decreases and as a result in HuR deplete cells virus produnctoin reduced.In comparison with dl1520 equvalent to H101 virus we found that, the virus production in cancer cell by Ad-fosARE was higher. In case of cytolytic activity Ad-fosARE causes less damage to the normal cells. These results demonstrated oncolytic potentials of Ad-fosARE.

From western blot and confocal microscopy results we found that PTX can upregulate cytoplasmic HuR export only in cancers cells. Which resulted in enhancing virus replication through elevate E1A expression and stabilize E1A mRNA and also increased half-life of E1A mRNA. We found that PTX upregulated CAR (coxsackie adenovirus receptor) required for viral uptake both in vitro and in vivo. Thus the virus propagation and cytolytic activity of Ad-fosARE increases with combination with PTX. PTX also demonstrated synergistic cytotoxicity when applied together, with Chou-Talalay combination indices ranging from 0.5 to 0.85 for HeLa cells and 0.4 to 0.52 for A549 cells. Furthrmore, PTX altered the dynamic instability of microtubules which is essential for viral internalization and trafficking to the nucleus.Hence, PTX significantly influences the replication and spread of Ad-fosARE, and additionally, the virus may also enhance the antitumor activity of PTX through the modification of MTs.

In vivo results shows marked tumor regression by combination treatment of Ad-fosARE and PTX compared to only virus or PTX or PBS treated cells. Our data demonstrate not only the effectiveness of the combination but also their synergistic effects.

Discussion:In the vast majority of cancer cells, ARE-mRNAs relocate to the cytoplasm with HuR and are constitutively stabilized. We investigated the requirement of mRNA stabilization system for Ad-fosARE replication. Since Ad-fosARE possesses an ARE downstream of E1A gene, an increased expression level of E1A mRNA was expected in cancer cells, where ARE-mRNA is stabilized, thereby promoting virus growth which makes this virus cancer selective. We also investigated the effect of PTX in E1A mRNA stabilization. After treatment with PTX, the half-life of E1A mRNA was increased, and these results indicate that Ad-fosARE grows in an ARE-mRNA stabilization-dependent manner, thus increasing the level of cytoplasmic HuR in cancer cells by PTX enhances the oncolytic activity of Ad-fosARE. We found that the ability of this virus to replicate was markedly high in cancer cells compared with that in normal cells. The propagation and cytolytic activity of this virus in cancer celss were similar to dl1520 (equivalent to H101), which is clinically applied. Our results showed that PTX treatment synergistically increased the oncolytic activity of Ad-fosARE both in vitro and in vivo. To the best of our knowledge, this is the first study to show that PTX activates oncolytic adenovirus function by promoting HuR cytoplasmic translocation.

Conclusions: To conclude, we showed that a conditionally replicative Ad-fosARE is a potential oncolytic virus. In combination with PTX, had a synergistic effect for cancer cells both in vitro and in vivo. PTX did influences the replication and spread of Ad-fosARE, and Ad-fosARE may enhance the antitumor activity of PTX. Ad-fosARE and PTX combined therapy appears to be an safe and effective therapy for the treatment for cancer. This synergism would be promising gene therapy for a patient with cancer, which can minimize the burden of chemotherapy. These data provide a base for designing clinical trials, which holds hope for patients with cancers.