学位論文内容の要旨
（Abstract of thesis）

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Enhancement of the activity of oncolytic adenovirus by augment HuR export of host cells
(RNA 結合タンパク HuR の核外輸送活性化による腫瘍溶解アデノウイルスの効果増強)

Background and objectives

Cancer is the second leading cause of human death. For cancer treatment various treatment options has been developed. Among those, some are widely using clinically and some are using clinical and preclinical trial state. Oncolytic virus is also a fascinating anticancer tool due to capability of replication selectively in cancer cells without harming normal cells. Various oncolytic virus has been developed and showed their effectiveness in clinical and preclinical trials.

In this study, we used E4or6-deleted adenovirus as an oncolytic virus. This virus replicates selectively in cancer cells where AU-rich element (ARE) containing mRNA is stabilized mediated by binding with RNA binding protein HuR. ARE is RNA element that enhances the rapid decay of mRNA. HuR stabilizes ARE-mRNA by exporting it to the cytoplasm. In the vast majority of cancer cells, HuR is constitutively exported to the cytoplasm and ARE-mRNA is stabilized.

Adenovirus gene product E4orf6 exports HuR to stabilize ARE-mRNA in adenovirus-infected cells and the stabilization is required for full virus replication. The previous report showed that E4orf6-deleted mutant dl355 has great replication capability and oncolytic activity in cancer cells without harming the normal cells. In this study, we examined whether the further enhancement of HuR export by exogenous regulators can stimulate the replication and the oncolytic activity of dl355.

Material and Methods

To evaluate exogenous up-regulators as HuR export enhancer, we used ethanol, X-Ray radiation and Ultraviolet (UV) radiation. For this study, we used cancer cell line HeLa (human cervical cancer cells), A549 (human lung adenocarcinoma cells) and a normal cell line BJ (human foreskin fibroblast cells). We identified the most effective dose of these up-regulators for cytoplasmic HuR exportation by western blotting. We also checked the virus replication and cell death mechanism with these HuR stimulators combine with dl355 and dl355 alone in cancer cells. Viral replication has been evaluated by virus titer assay and cytolytic activity was detected by cytopathic effect assay (CPE) and XTT assay. E1A and Hexon expression also checked by western blottig to find out the mechanism of ethanol induced viral replication. To find out another potential candidate to viral replication induced by ethanol, IVa2 mRNA expression was examined by q-RT PCR analysis.

Results

All of three exogenous HuR up-regulators promoted the cytoplasmic relocalization of HuR in cancer cells. By the CPE assay, we confirmed that MOI 100 vp/cell was the most potent dose to see the effect of dl355. Western blotting result revealed that

0.3% ethanol treatment is more reliable for HuR export and it was non-toxic for cells as well. Replication efficiency of dl355 was much higher in ethanol-treated cells than virus infection alone. In response with ethanol combination, the cytolytic activity of the virus was significantly higher for cancer cells and showed greater apoptosis-inducing ability of dl355. Increased level of cleaved PARP by both dl355 and ethanol treatment in cancer cells indicate the cell apoptosis in cancer cells but not in normal cells. E1A expression was enhanced in ethanol treated cancer cells until 24 hours of infection and Hexon expression was found in 48 hours without any dissimilarity between ethanol combination and dl355 infection alone. Beside these, q-RT PCR analysis showed the quantities of IVa2 mRNA were markedly higher in ethanol treated cells compared to dl355 infected cells in absence of ethanol in 24 hours. Taken together, these results suggest that ethanol can augment the cytolytic activity and oncolytic activities of dl355 in cancer cells.

On the other hand, we fixed 1 Gray/min for X-Ray and 10 J/m2 for UV radiation for combination treatment. We observed that dl355 has higher replication tendency and cytolytic activity in cancer cells with X-Ray combination. With UV radiation, viral replication tendency and cytolytic activity were quite higher but almost same manner compared to only dl355 infection.

Discussion

In this study, we found the activity of dl355 was enhanced by increasing HuR export in the presence of ethanol. And ethanol was able to activate the viral replication to facilitate apoptosis mediated cytolytic activity of dl355 in cancer cells. We detected that IVa2 mRNA, which is the only adenovirus transcript that contains ARE, rather than E1A was enhanced in response to ethanol in dl355 infected cells. These results indicate that, ethanol is capable of augmenting the oncolytic activity of viruses regulated by ARE-mRNA stabilization system.

Beside we found that both X-Ray and UV radiation increased the HuR translocation in cytoplasm. With X-Ray radiation, dl355 showed propensity of virus replication in cancer cells and more cytolytic activity compared to dl355 infection alone. Though UV radiation combination treatment did not augment viral replication and the cytolytic effect was almost the same manner of dl355 alone infection. It is striking that HuR, RNA binding proteins, respond to radiation therapy, by shifting their location to the cytoplasm where their targeted mRNAs can be stabilized and translated. To date, HuR promotes the ARE-mRNA stabilization but no evidence was found the actual links in between stabilization and translation of target transcript in cancer cells with both X-Ray and UV radiation. Collectively our results revealed that in cancer cells with radiation therapy, dl355 showed enhanced potential of oncolytic activity compared to dl355 infection alone.

Conclusion

In this study, the enhancement of HuR export using exogenous regulators such as ethanol and X-Ray can inspire dl355 oncolytic activity. dl355 did not show significant replication and cytolytic effect with UV radiation, although it can export HuR to the cytoplasm of cancer cells. This oncolytic virus also relatively safe for normal cells even infection alone or with ethanol combination. These combinations might be one of the effective cancer treatment options in near future.