ENHANCING HUMAN BREAST CANCER CELLS DESTRUCTION USING COMBINATION OF ADENOVIRUS EXPRESSING P53 AND HYPERTHERMIA TREATMENT

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Specially for my beloved parents, Elengoe and Thavamani

My lovely sister, Suguna and Vaani

&

My wonderful brother, Tevanraj.

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ABSTRACT

In Malaysia, breast cancer is the most common cancer where 1 in 19 Malaysian women will be diagnosed with breast cancer by the age of 85. Moreover, lack of specific symptoms in the early stage of disease leading to delay in diagnosis. Unfortunately, current treatments by chemotherapeutic agents, surgery and radiation are not fully effective for the treatment of breast cancer. Thus, there is an urgency in developing new approaches for the treatment of breast cancer patients. In this study, a novel therapeutic regimen, combining the effects of recombinant adenovirus and hyperthermia was investigated. Firstly, Adenovirus serotype 5 was constructed by cloning of p53 gene into a defective recombinant adenovirus vector, Ad5-p53-DsRed Monomer N1. The Ad5-p53-DsRed Monomer N1 (MOI of 100) was then used to infect breast cancer cells (MDA-MB 231 and MCF-7) with or without combination of hyperthermia treatment (42ºC for 2 hours). The cell killing and viral concentration were then determined by MTT assay and viral plaque formation assay respectively. After that, the heat shock protein (Hsp70) and p53 protein expression in transfected cells were quantitated using ELISA assay. Activated-Caspase 3/7, 8 and 9 were also evaluated to study the apoptotic pathway of cancer cells. Furthermore, the novel protein interaction between nucleotide binding domain (NBD) Hsp70 and human Ad5 E1A 32 kDa motif (PNLVP); and NBD and p53 motif (SCMGGMNR) were investigated through bioinformatics tools such as Gromacs and Autodock softwares. It was found that MDA-MB 231 and MCF-7 cells infected with virus Ad5-p53-DsRed Monomer N1 alone resulted in 46.77±2.74% and 42.26±1.78% cell killing respectively while hyperthermia in combination with virus were 84.82±1.64% and 80.13±3.30% respectively. The Hsp70 expression of both cancer cells was also increased to 170.57% (MDA-MB 231) and 169.83% (MCF-7). Moreover, p53 expression in MDA-MB 231 and MCF-7 cells by virus combined with heat treatment (85.72 ng/L and 79.05 ng/L respectively) could lead to enhanced oncolytic property compared to virus treatment alone (47.82 ng/L and 40.54 ng/L respectively). In addition, caspase activity was first time reported that apoptosis process started at very early stage of infection in breast cancer cells with hyperthermia compared to virus alone. This was due to the evident that the highest kinetic energy was found in caspase 3 whereas virus alone the highest in caspase 8. In conclusion, Hsp70 induction by hyperthermia treatment enhanced Ad5-p53-DsRed Monomer N1 replication and oncolysis in MDA-MB 231 and MCF-7 cells through apoptotic pathway. Besides that, NBD of Hsp70 had the best interaction with PNLVP motif at 42°C. Thus, combining Ad5-p53 with hyperthermia treatment could be a potential approach for breast cancer treatment.

ABSTRAK

Di Malaysia, kanser payudara adalah kanser yang paling umum dimana 1 dalam 19 wanita Malaysia akan didiagnosis dengan kanser payudara menjelang usia 85. Tambahan pula, kekurangan tanda-tanda spesifik di peringkat awal penyakit yang membawa kepada kelewatan dalam diagnosis. Malangnya, rawatan semasa dengan agen kemoterapi, pembedahan dan radiasi tidak berkesan sepenuhnya untuk merawat kanser payudara. Oleh itu, strategi baru diperlukan dengan segera untuk merawat pesakit kanser payudara. Dalam kajian ini, potensi untuk mengabungkan regimen terapeutik novel adenovirus rekombinan dan 'hyperthermia' telah dikaji. Pertamanya, Adenovirus jenis 5 telah dibangunkan dengan pengklonan gen p53 ke dalam vektor adenovirus rekombinan, Ad5-p53-DsRed Monomer N1. Kepekatan 100 PFU bagi Ad5-p53-DsRed Monomer N1 telah digunakan untuk menjangkiti sel-sel kanser payudara (MDA-MB 231 dan MCF-7) dengan atau tanpa digabungkan dengan rawatan hyperthermia (42ºC selama 2 jam). Kemudian, tahap kemusnahan sel dan kepekatan virus telah ditentukan dengan asai MTT dan asai pembentukan plak virus. Selepas itu, pengekspresan protein kejutan haba (Hsp70) dan p53 dalam sel telah dianalisis dengan menggunakan asai ELISA. 'Caspase' teraktif 3/7, 8 dan 9 juga telah dikaji untuk tapak jalan apoptosis sel kanser. Tambahan pula, interaksi protein novel di antara domain pengikat nukleotida (NBD) bagi Hsp70 dan motif Ad5 E1A 32 kDa (PNLVP); dan NBD dan motif p53 (SCMGGMNR) telah dikaji dengan kaedah bioinformatik seperti perisian Gromacs dan Autodock. Kajian ini menunjukkan bahawa MDA-MB 231 dan MCF-7 yang dijangkiti virus Ad5-p53-DsRed Monomer N1 sahaja menyebabkan 46.77 ± 2.74% dan 42.26 ± 1.78% sel musnah manakala 'hyperthermia' dengan virus adalah 84.82 ± 1.64% dan 80.13 ± 3.30% masing-masing. Pengekspresan protein Hsp70 bagi kedua-dua sel kanser juga meningkat kepada 170.57% (MDA-MB 231) dan 169.83% (MCF-7). Selain itu, pengekspresan protein p53 dalam MDA-MB 231 and MCF-7 bagi gabungan virus dan 'hyperthermia' adalah 85.72 ng/L dan 79.05 ng/L masing-masing manakala perlakuan virus sahaja adalah 47.82 ng/L dan 40.54 ng/L masing-masing. Aktiviti 'caspase' telah dilaporkan kali pertamanya bahawa proses apoptotik bermula pada peringkat yang sangat awal bagi gabungan virus dan 'hyperthermia' berbanding dengan virus sahaja. Ini dibuktikan melalui tenaga kinetik yang paling tinggi didapati dalam caspase 3 manakala virus sahaja yang tertinggi dalam caspase 8. Kesimpulannya, induksi Hsp70 oleh perlakuan 'hyperthermia' meningkatkan replikasi Ad5-p53-DsRed Monomer N1 dan 'oncolysis' dalam sel MDA-MB 231 dan MCF-7 melalui proses apoptotik. Selain itu, NBD bagi Hsp70 mempunyai interaksi yang terbaik dengan PNLVP motif pada 42°C. Oleh itu, penggabungan Ad5-p53 dengan 'hyperthermia' mungkin boleh menjadi pendekatan bagi rawatan kanser payudara.