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The Apoptosis according to the Processing Irradiation and The Tumor Necrosis Factor

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ABSTRACT

Acute promyelocytic leukemia(APL) is not just the poor grades of treating a type of blood cancer hayeoteul combination with chemotherapy despite concurrent radiation therapy are known to exhibit a greater effect and also works on normal cells to result in side effects. In this study, when after treatment with gamma rays, such as TNF-α in order to reduce these side effects was confirmed how affected the cell death of normal cells and cancer cells. HL-60 cells were used as the APL cell line HL-60 cells were differentiated with DMSO for treatment are shown the properties of normal granulocytes was used as a control group. As a result, HL-60 cells treated with TNF-α and gamma rays with only showed a cytotoxic effect by inducing the apoptosis cells were put to death. Consequently, TNF-α is thought to active substances that can increase the efficiency of cancer treatment to increase the removal of cancer cells when used with low-density gamma-ray treatment in order to eliminate the side effects of chemotherapy.

Keywords: Acute promyelocytic leukemia, gamma ray, Tumor Necrosis Factor, Apoptosis,

Ⅰ. INTRODUCTION

Radiation therapy is maintaining the dose of the nor mal tissue to a minimum, and may be said that at the same time give it the appropriate dose to the tumor tiss ue.[1] The radiation in order to increase the effectiveness of treatment In order to increase the therapeutic ratio (t reatment ratio, TR) and to reduce the lethal dose of the tumor tissue parallel to anti-cancer agents that induce ap optosis and cell death in tumor cells leading to elevate the therapeutic effect ratio It is known as the method.[2]

Acute Promyelocytic leukemia (acute promyelo-cytic le ukemia, APL) often HL-60 cells used in the study for t he pathogenesis and treatment of cells commonly used f or cancer of pot leukemia research generated in the blo od and bone marrow to be. Because the primarily treate d with chemotherapy rather than surgery, the HL-60 is mainly used. Chemotherapy is to study the specific cells to anti-cancer effects to ensure that the cancer cell apop tosis is induced after the treatment a material containing a toxic and in particular, the apoptosis of cancer cells, t he associated mechanism of action to respond only to c ancer cells, and identified It is important.[3]

Treatments with acute Promyelocytic leukemia Althou gh primarily by inducing differentiation and apoptosis of leukemia cells used in the treatment using the derivative of ATRA (all-trans retionic acid) of vitamin A, ATRA is a difficult part of breath, fever, low blood pressure, etc. it is known to induce the RAS (retionic acid syndrome) accompanied by. Photon including gamma rays are bein g actively used in cancer treatment, but the effects of th

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e acute pot leukemia cells low bar been clearly identifie d irradiation, even the most effective therapeutic effects of cancer treatments height but normal tissues and cells in addition to cancer at the same time affecting someti mes lead to various side effects. There is currently no e ffective anti-cancer treatments consistently lacking the si de effects require more effort to discovering and develo ping novel therapeutic substance. Anti-cancer agents use d in cancer treatment is raised, the commercial treatmen t accompanied by radiation therapy beyond a simple tre atment.[4]

Therefore, the present experiment was to evaluate by using as the immunosorbent material of low dose of ga mma radiation acting irradiating only the target cells, an d then the cancer cells to increase the radiation sensitivi ty further activated at the same time the use of the cell death of cancer cells using a tumor removal.

Ⅱ. MATERIAL AND METHODS

1. Irradiation

Irradiated to each of the control group and the cance r group 0.1, 0.5, 1,5 Gy of gamma radiation and then b y replacing with fresh medium and cultured. At this tim e, TNF-α treated group to the TNF-αtreatment of 10 n g / ml concentrations, respectively before gamma irradia tion 3 hours incubation 12, 24hours.

2. Cell processing

HL-60cells(American Type Culture Collection, Rockvil le,USA) is 10% heat-inactivated fetal bovine serum(FB S), 100U/ml of penicillin, 100μg/ml of streptomycin(Lif e technologies, Inc.,USA) is RPMI 1640 medium was in cubated at(Life technologies,Inc.) contained. Cells we re cultured in 37℃, 5% CO_2 conditions. HL-60 cells we re induced by treatment of DMSO in HL-60cells differe ntiated into granulocytes in order to prepare a normal c ell of the same line were used as Promyelocytic leukemi a cells. So for 3 days 1.3% DMSO-treated HL-60 cells are used as the control group and DMSO-untreated HL -60 cells were used as cancer cells, the group.[4]

3. MTT measurements

Using the MTT assay kit (Roche, Penzberg, German y) was confirmed cell viability. By gamma radiation by i rradiating the suspended cells and then concentration to a concentration of 1 × 10^5 cells / 50μl was dispensed in 96 well plate. Passed 16 hours, respectively, MTT (3-4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromid e) was added to a 10μl solution incubated for 4 hours at 37℃, 5% CO_2 incubator and then 100μl of the solu bilization solution was added further. Allowed to stand for one day at 37℃, 5% CO_2 incubator and then the a bsorbance was measured at 550 nm.

4. Apoptosis measurements

Subjected to annexin-V and propidium iodide (PI) (B D bioscience, San Diego, CA)to determine the dyeing A poptosis was measured and then the number of annexin -V staining on cells by flow cytometry. All cells are defi ned as cells that caused apoptosis-stained for Annexin V and a total of 10,000 cells was analyzed for each sample.

Ⅲ. RESULT

1. Check cytotoxicity by Gamma

Processing the DMSO in HL-60 cells were differentia ted into cells with characteristics of normal granulocytes. Consider normal and tumor cell population was used as a conventional HL-60 cells. Processing of the gamma ra ys to each of the cells is examined by each concentratio n of the gamma ray in 0.1, 0.5, 1, 5Gy to ensure that any result indicating the following was confirmed in cell viability Cell viability by MTT assay.

As a result, significantly inhibited cell viability by DM SO concentration in all treatment groups and the untrea ted group.(Fig. 1). In addition, this cell death is irradiat ed with gamma rays to each of the cells as above to de termine whether the following were confirmed by the a poptosis of cells stained with annexin-V and PI by flow

cytometry. Annexin-V is regarded that all of the cells st ained with the cell apoptosis took place. As a result, Fi g. 1 reduction in cell viability due to the gamma irradiat ion appeared it in was confirmed that appeared due to an increase in apoptosis.(Fig. 2).

The apoptosis was induced by gamma irradiation as s tatistically significant. But the cell death caused by gam ma irradiation, as shown in Fig. 1 and Fig. 2 could be confirmed that almost similarly derived from DMSO-tre ated group and the untreated group.

2. Gamma irradiation at a low concentration without cytotoxic substance identification to induce apoptosis only in cancer cells

For 3 hours handle 10 ng / ml TNF-α in each cell, and then irradiated with 0.5Gy of gamma radiation and cultured for 24 hours. As a result it in DMSO treated HL-60 cells were irradiated with gamma radiation of 0.5 Gy TNF-α treatment did not have a significant effect o n apoptosis as a shown in Fig. 3. However, significantl y it showed that the apoptosis is increased to about 2 5%, as shown in Fig. 3 was made to TNF-α treatment in a DMSO untreated HL-60 cells. 0.5Gy of gamma-ray s in low concentration through this result it can be seen that the induction part to apoptosis of tumor cells in D MSO untreated HL-60 cells by treating sex as the TNF- α does not affect the cytotoxicity.

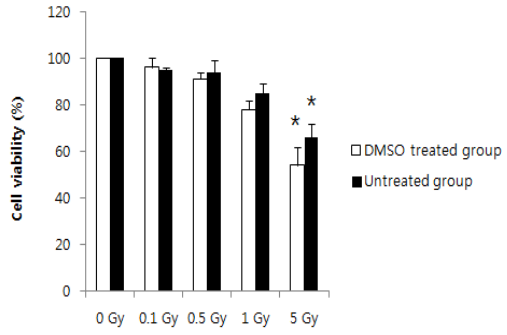

Fig. 1. The effect of gamma radiation on the cell viability of DMSO-treated/untreated HL-60 cells.

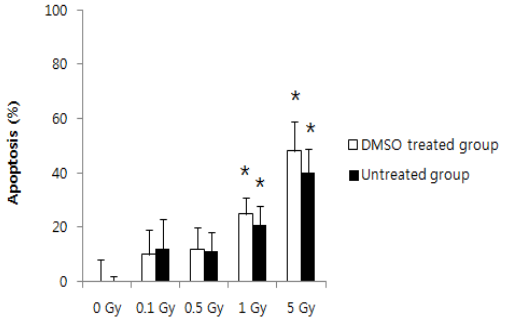

Fig. 2. The effect of gamma radiation on apoptosis of DMSO-treated/untreated HL-60 cells.

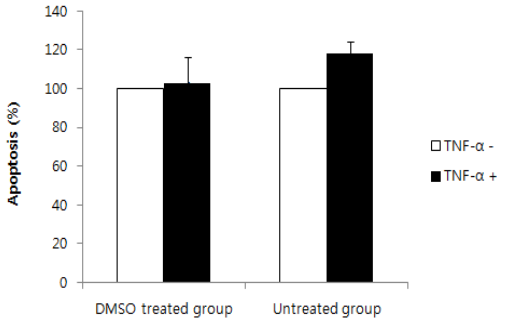

Fig. 3. The differential effect of TNF-α on apoptosis of DMSO-treated/untreated HL-60 cells.

Ⅳ. CONSIDERATION

TNF-α is extracellular stimuli by the cells(a variety of stimuli, such as inflammation) is displayed an immune r esponse and serves to making the material more immun e activation related to the systematic immune response s ignaling between cells.[5]

TNF-α will bind to the receptor as one of the major pro-inflammatory cytokines involved in the immune and inflammatory response are known to pass into the cell s timulation. In particular, the combination of TNF-α wit h its receptor will also inhibit induced apoptosis by ind ucing the activity of, or vice by the key transcription fac tor NF-κB (nuclear factor-kappa B) adjusting a chain re action of a caspase. In addition, TNF-α concentration is high is known to cause symptoms of acute shock in the human body. Based on this study, even if hayeoteul co mbination with radiation techniques to determine if thes

e facts were more effective anti-cancer effect.[6]

DMSO treated HL-60 cells were irradiated by gamma rays at different concentrations cell death caused by ga mma irradiation results confirm the viability can be conf irmed that there was almost similarly derived from DM SO-treated group and the untreated group. This means that the gamma ray irradiation for the treatment of acut e myelogenous leukemia before the cancer cells, as well as almost the same as abnormal cell function.

In particular, using methods to increase only in canc er cells, the target cell susceptibility to radiation radiothe rapy in normal cells, to make sure that you can remove it by inducing apoptosis only in cancer cells without aff ecting DMSO treated HL-60 cells and DMSO untreated it only the cancer cells with 0.5Gy of gamma-rays that are not cytotoxic in the HL-60 cells were specifically to find a method of inducing apoptosis.[7]

As a result, though a little effect of apoptosis apopto sis in DMSO untreated cells by accompanying handle T NF-α in the low dose gamma-ray showed that the signi ficant apoptosis is increased to about 25% it did not pr ocess the DMSO at the same conditions It was found to partially induce.[8]

TNF-α appears as a variety of effects to the opposite action, depending on the type of cell. This may appear different by the time the receptor that binds to TNF-α cause irritation to the cells. That of TNFR1 are cells ou t of TNF-α and the coupling when the cell interior of the TNFR1 and bond together with TNF receptor - ass ociated death domain (TRADD), receptor interacting pr otein-1 (RIP-1), TNFR-associated factor 2 (TRAF2) of t he complex comes off RIP-1 is a mitogen-activated prot ein kinase kinase kinase(MAP3K) and the action is stim ulated MEKK 3 IKK, IκBα order of activation of NF-κB activation and finally to the induction. In addition, TNF-α is the FADD is separated out and then combin ed with the TNFR it will stimulate the caspase cascade via the death-inducing signaling complex (DISC) activity [9,10]. In a direction for future research in the developme nt of blood cancer therapy using gamma-rays and TNF-α on the basis of the results identified in this study that if further verify the specific mechanisms to identify and efficacy of the cancer cells induced while reducing the s ide effect efficient tumor suppression It is believed to b e applicable.[11]

Ⅴ. CONCLUSION

DMSO untreated HL-60 was made handle TNF-α in the cell gamma rays of about 25% was significantly bea m that the apoptosis is increased to lower the concentra tion 0.5Gy is by treatment as a TNF-α does not affect the cytotoxicity DMSO untreated cells, the cancer cells were partially induced apoptosis in HL-60 cells Provinc e. Based on this, even if the specific mechanisms to ide ntify and verify the efficacy of the cancer cells is less eu myeonseo side effects is thought to be applicable to the development of blood cancer therapy to induce efficient tumor suppression.

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종양괴사인자와 방사선이 세포자멸사에 미치는 영향

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요 약

급성전골수구성 백혈병(Acute promyelocytic leukemia, APL)은 혈액암의 일종으로 치료의 성적이 좋지 않을 뿐 아니라 항암요법과 병행 하였을 경우 큰 효과를 보이는 것으로 알려져 있는 방사선 치료를 병행함에도 불구하고 정상세포에도 작용하여 부작용을 초래한다. 본 연구에서는 이러한 부작용을 감소시키기 위하여 감마선을 TNF-α와 같이 처리하였을 경우 정상세포와 암세포의 세포 죽음에 어떠한 영향을 미치는지 확인하였다. HL-60 세포는 APL 세포주로서 사용하였고 DMSO를 처리하여 분화시킨 HL-60 세포는 정상과립구의 성질을 나타내어 정상대조군으로 이용하였다. 그 결과 TNF-α와 함께 감마선을 처리한 HL-60 세포에서만 세포독성효과를 나타내었고 세포자멸사를 유도하여 세포가 죽음에 이르게 하였다. 결론적으로 TNF-α는 항암치료의 부작용을 없애기 위해 저농도 감마선 치료 시 함께 사용하여 암 세포의 제거를 증가시켜 암의 치료효율을 높일 수 있는 유효물질로 사료된다.

중심단어 : 급성전골수구성 백혈병, 세포자멸사, 감마선, 종양괴사인자