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TRAIL and Effect of Irradiation on Apoptosis of Cancer Cells

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ABSTRACT

Tumor using the efficient concomitant radiotherapy and chemotherapy to remove, prior to surgery and, either reduce the size of the tumor after surgery, or was can be made smaller, Or excised tumor, in a way to be removed, most conventional surgical method is surgical excision surgery therapy. And methods reduce or tumor size, or smaller, chemotherapy can kill tumor is administered selectively anticancer agent which increases the radioactive susceptible to tumor cells, sensitive to susceptibility to radiation are those which make it possible to respond to, either TRAIL methods of various biological cytostatic can deform the protein, by deforming the structure of the protein help to cell death it is known. In this paper, the HCT-116 cells thought to be a cancer cell to analyze the interaction of TRAIL and radiation. Experimental results, single use of TRAIL and radiation, results were compared with the control group, it was found to have no significant effect on each cell proliferation and apoptosis. Conversely treated with TRAIL, when treated in parallel radiation, it was possible to know that the HCT-116 cells significantly apoptosis occurs, The proportion of G1 ratio G0 also was found to have increased. TRAIL conclusion is increased apoptosis radiation defensive cells can know that increased radiosensitivity, also possible to alter the cell cycle, reduce cell proliferation ability stepwise it was possible. TRAIL is increased apoptosis, decreased cell proliferative capacity, it is considered to be possible to use as a radiation sensitizer.

Keywords: Proliferation, Apoptosis, Radiosensitizer, HCT 116 cells, TRAIL, Radiation

Ⅰ. INTRODUCTION

HCT-116 cells are frequently used in the cell test cell as colorectal cancer cell line of the human body. [1] Thi s is the cell which is mainly used in research for the pa thogenesis and therapy of human colon cancer. Radioth erapy is used as a mono therapy and in combination th erapy is typically used in combination with the anti-canc er chemotherapy used in the treatment of cancer patient s.[2]

Treatment of locally advanced been rectal cancer, alth ough combination therapy performed radiation therapy a nd chemotherapy after surgery simultaneously have been performed by standard methods of treatment[3-6] In rece nt years, a combination therapy to enforce pre-operative chemotherapy and radiation therapy at the same time te nds to increase.

Apoptosis is a normal physiological phenomenon whi ch plays an important role in the generation and regulat ion of homeostasis object. Recently, apoptosis regulation and has involved many genes, by examining the functio ns and features of these gene products is becoming a p ossible molecular explanation of apoptosis phenomenon. That of tumor necrosis factor (TNF) TNF-related apopt osis-inducing ligand belong to the superfamily (TRAIL) is a kind of transmembrane protein when selectively act on the tumor tissue.[7]

TRAIL is membrane binding of the receptor is that d

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eath (Death receptors, DR4 and DR5) combined with t he intra cellular death domains through an apoptotic sig nal via the outgoing apoptosis of extrinsic (death recept or) pathway, which is known to participate in it. These receptors when coupled with a ligand and activated via a multiplexer (multimerization) of the receptor, thereby various adapter proteins and receptor binding as a resul t. adapter protein are combined to induce apoptosis thr ough the activation of a variety of proteins, such as cas pase that the important action in cell death.[8,9]

Accordingly, the susceptibility to TRAIL in many cell s is dependent on membrane TRAIL receptors and casp ase-8 present in the cell membrane. caspase-8, which is activated by TRAIL is, to start a protease cascade that activates the effector caspase that contains a caspase-3 a nd caspase-7 free in the cell. [10] In particular, TRAIL is very convenient to the treatment strategy of the transfor med cells, such as cancer cells without affecting at all in normal cells, is very high clinical value. [11,12]

Chemotherapy is important to investigate the specific cytotoxicity one has processed a substance which the fo llowing to check whether the cancer cell death is induce d to determine the anti-cancer effects The cancer cell a poptosis and the action associated with the mechanism of the cancer cells and identifying. [13] While there are fe w side effects and the lack of effective anti-cancer thera peutics discovery and development of new therapeutic a gents has been steadily made efforts to increase the ther apeutic effect. In this experiment to investigate the radi ation and then further activated to induce the death of cancer cells by the immune chemicals that act only on cancer cells as the target cells to increase the radiation s ensitivity at the same time evaluate use in cancer cells r emoved.

Cells by a variety of extra cellular stimuli including in flammation adapted to elicit an immune response thereb y producing an immune substance, to further activate th e signaling and immune responses between cells. TRAIL is involved in the immune and inflammatory responses as a typical material in these materials. Also inhibits the apoptosis by controlling a chain reaction of caspase or t he opposite also cause apoptosis. Previous studies have reported that there is induced the different reaction by a variety of factors, including the type of receptor that binds to the type of target cells, the processing time.[14, 15]

In this study, using the TRAIL and radiation, to dete rmine whether the survival of the HCT-116 cells, was tr ying to figure out whether there is effectively anti-cance r effect.

Ⅱ. MATERIAL AND METHODS

1. Irradiation

Irradiated to each of the control group and the cancer g roup 4.0 Gy of gamma radiation and then by replacing wit h fresh medium and cultured. Usiug Gamma kinfe ^{60}Co so urce irradiation time has not been setting. At this time, TR AIL treated group to the 10, 20, 50, 100 ng/ml concentrati ons and TRAIL 20 ng/ml with irradiation.

2. Cell processing

HCT-116 cells is American Type Culture Collection, (Rockville, USA) in the frequency divider receives 90% of the RPMI-1640 medium (Giboco BRL, Grand Island, NY, USA), 10% FBS (fetal bovine serum, FBS) and 1% of penicillin and streptomycin (Biofluids, Rockville, MD, USA) using the medium containing the 37 ℃, and incu bated under 5% CO_2 condition. In order to also solve the congestion of the phenomena of cell proliferation p rocesses the sheet every 48 hours 0.05% trypsin-ethylen ediamine tetraacetic acid (EDTA, Gibco BRL) and incu bated the cells were suspended and then transferred to a Petri dish for cell culture. TRAIL(KOMA Biotech Inc, Seoul, Korea) was diluted in treatment medium to an a ppropriate concentration dissolved in PBS.

3. Cell growth inhibition irradiation by MTT assay

6 well cell culture plate in the frequency divider of 1 $\times 10^5$ cells per well for HCT-116 cell, and cultured for 4 8 hours after the mixing process and the radiation alone

or TRAIL after stabilization for 24 hours. Remove the c ulture medium After 48 hours, dilute the bromide salt t etrazolium (MTT, Sigma, St. Louis, MO, USA) in 0.5 m g / ml concentration was dispensed per 200ml reaction for 3 hours. After 3 hours remove the MTT reagent, which was then compared to the frequency division by 100 ml of DMSO dissolved all of the formazan produc ed in the well using the ELISA reader (moleculer device s, sunnyvale,CA,USA)measured at 540 nm. Experimental results were the mean and standard error of three meas urements. Processes the LmSup, LmE and the cells of t he concentration of 1 × 10^6 cells / ml to 5 μg/ml each of the cultured HCT-116 cells, 24 hours, 48 hours, each was the control group was not treated.

4. Apoptosis measurements

After each incubation time has elapsed, the cells were collected in PBS and then suspended the cells, by the a ddition of annexin V-FITC and propidium iodide(PI) (B D bioscience, San Diego, CA) in the cell suspension wa s measured by flow cytometry(BD bioscience) at room t emperature. Cells were possible staining with Annexin V -FITC is defined as apoptosis occurs cells were analyzed total of 10,000 cells per each sample. and activity measu rement was done.

5. Measuring the cell cycle

In order to examine the changes in the cell cycle,

and LmSup in concentration Cell of 1 × 10^6 cells/ml T he LmE each treated with 5μg / ml, and cultured for 2 4, 48hours. In the control group, and untreated, the HC T-116 cells were cultured, respectively 24, 48 hours.

After the incubation time has elapsed, were each cell, all collected and resuspended in DPPS, centrifuged, the cells were washed 3 times through a process of removin g the top of the liquid.

Washed cells were treated with PI resuspended again DPBS, stained for 10 minutes. It was measured through the flow cytometry cell analyzer. To measure the numb er of cells Sub G0 / G1 phase, it was examined the cel l cycle. The Sub G0 / G1 phase of the cell defined by progress in cell death was analyzed total of 10,000 cells per each sample.

Ⅲ. RESULT

1. The ability of TRAIL treatment and cell proliferation

Along with the results of a study TRAIL of proliferat ive capacity of processing the TRAIL compared to the control group cells to an increase of 10,20,50,100 ng/m l, the cell proliferative capacity stepwise decrease, than 1 00 ng/ml statistically were significant results(Fig. 1).

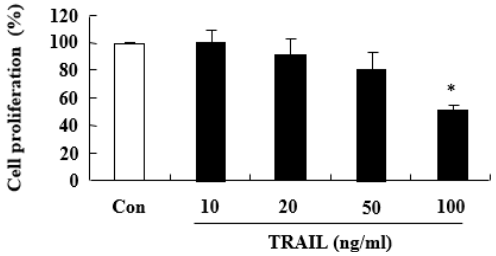

Fig. 1. The effect of HCT-116 cell proliferation on the TRAIL

2. TRAIL treatment and Apoptosis

Depending on TRAIL treatment, results of examining the death of cells, depending on the concentration of th e processing of TRAIL as shown in FIG. 2, showed th e appearance that apoptosis is increased gradually. In p articular, it was statistically significant in the 50 ng/ml a nd 100 ng/ml (Fig. 2).

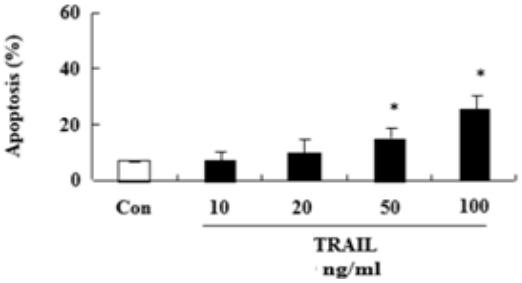

Fig. 2. The apoptosis HCT cell-116 on the TRAIL

3. TRAIL treatment and the radiation environment of the cell proliferation ability

By treating the TRAIL at concentrations of 20 ng / ml, the proliferative capacity of the cells was reduced by about 10% at about 90%. On the other hand, Simply a s a method of line irradiation, decreased cell proliferativ e capacity of about 15% at about 85%, was treated this both, proliferation ability of the cells is reduced by abou t 25 percent, showed a 75% survival rate It was statistic ally significant(Fig. 3).

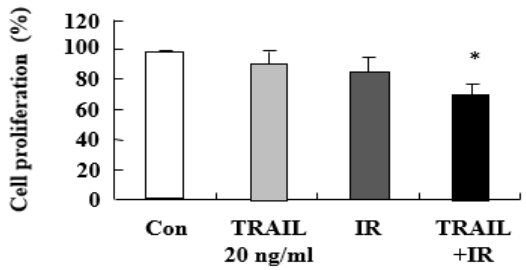

Fig. 3. The cell proliferation of HCT cell-116 on the TRAIL and IR(irradiation) and TRAIL+IR

4. TRAIL treatment and the radiation environment with apoptosis

When treated with TRAIL at concentrations of 20 ng /ml, apoptosis, although the difference between the con trol group at approximately 10% had little, the frequenc y of apoptosis when used with the irradiation was incre ased to 25%. If who underwent only irradiated showed apoptosis rate of about 17%(Fig. 4).

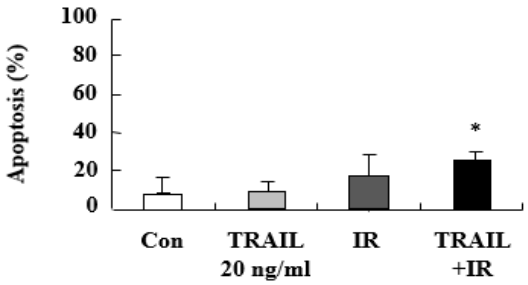

Fig. 4. The apoptosis HCT cell-116 on the TRAIL, IR and TRAIL+IR

5. The proportion of cell cycle G1 ratio G0 phase

When treated with TRAIL at concentrations of 20 ng/ml, when the percentage of G0 corresponding to the resting phase of the cell division cycle has been used in combination if the TRAIL and radiation research alone and enforcement when treated with TRAIL, respectively, had a higher percentage in the case of at the same time implementing the TRAIL and radiation at 20%, 30%, 40% or more.(Fig. 5).

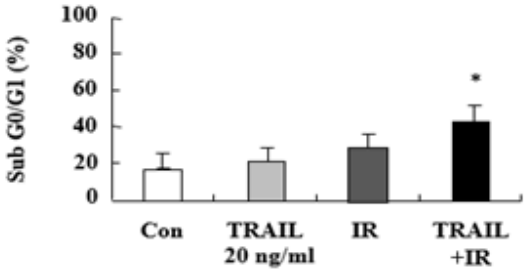

Fig. 5. The TRAIL, IR and TRAIL + IR on the G0/G1

Ⅳ. CONSIDERATION

As colorectal cancer cell lines, the cells used in cell e xperiments have HCL-116 cells. On the other hand, rad iation therapy, may be mentioned is typically possible to chemotherapy is largely divided into monotherapy and c ombination therapy applied to the patient in combinatio n therapy. Apoptosis is a normal physiological phenom enon which plays an important role in the generation a nd regulation of homeostasis object tumor necrosis fact or(TNF) TNF-related apoptosis-inducing ligand(TRAIL) belongs to the superfamily is typically a type of transme mbrane protein. Susceptibility to TRAIL in a number of cells depends on the cell membrane TRAIL receptors a nd caspase-8, caspase-8 activated by TRAIL, the activity of the effector caspase containing caspase-3 and caspase -7 free in the cell start the protease cascade which of, T RAIL is usefully employed in the treatment of transfor med cells such as cancer cells without affecting at all on normal cells. Materials used in chemotherapy, it is impo

rtant to clarify in the study of cancer cells apoptosis an d its associated mechanism of action, should effective e ven though there is no side effects. Over TRAIL was c an affect the tissue to be adversely affected. TRAIL is i nvolved in the immune and inflammatory responses as a typical material in these materials. Also inhibits apopto sis by controlling the chain reaction of the anti-caspase or may induce apoptosis. Previous studies have been pr oposed in many papers that induce different responses by a variety of factors, including the type of receptor th at bindsto the type of target cells, the processing time. Research results of this paper, the proliferative capacity of cells treated with TRAIL as compared to the control group as the concentration of TRAIL to increase, and d ecreased cell proliferative capacity in stages. This is, it ca n be determined that the TRAIL used alone gave me to some extent reduce the cell proliferative capacity. Result of observation of the Apoptosis, according to TRAIL tr eatment Apoptosis show aspects that gradual increase (t han 50 ng/ml of 100 ng/ml was statistically significant), which is the process of TRAIL can be viewed as induc ed Apoptosis. By the Comparing TRAIL and irradiation TRAIL processing (20 ng/ml), the proliferative capacity of the cells was reduced by about 10%, radiation alone enforcement is proliferative capacity of about 15% cells were decreased. When treated with both showed a reduc tion in cell proliferative capacity of approximately 25%, it was possible to know the Additive effect of radiation TRAIL. Further, the ratio of G0 Phase corresponding t o the resting phase of the cell division cycle, if used in combination with TRAIL and radiation, than when carri ed out singly, increase the proportion of more display r esting cells it TRAIL and radiation irradiation, it was po ssible to know the revive the action at the same time w ith each other. Through this experiment, when each trea tment alone to TRAIL and irradiation, both were able to confirm the apoptosis, it showed Additive effect if th e probability of such apoptosis is was applied to the tw o conditions at the same time, in the G1 phase S phase rather than move to, a change in the resting stage to th e G0 phase of cell is increased cell division was found to aborted.

Ⅴ. CONCLUSION

Using the two methods of TRAIL and irradiation, res ult of observation of the death of cells, than single treat ment of TRAIL or irradiation, treating both simultaneou sly have found that promoted apoptosis.

Based on this, it is determined that it is possible to t ake advantage of the application of TRAIL as a future radiosensitizers.

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TRAIL과 방사선 조사가 암세포의 사멸에 미치는 효과

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요 약

종양을 효율적으로 적출, 제거하기 위해서는 부수적으로 방사선 치료 및 항암화학요법을 이용하여 수술 전이나, 수술 후 종양의 크기를 줄이거나 작게 할 수는 있었으나, 종양을 적출하거나 제거하는 방법으로 외과적 절제수술요법이 가장 재래적인 수술 방법이다. 종양의 크기를 줄이거나 작게 하는 방법과, 종양을 사멸시킬 수 있는 항암화학요법은 방사성 감수성을 증가시키는 항암약제를 종양세포에 선택적으로 투여하여 방사선에 대한 감수성에 민감하게 반응 할 수 있도록 한 것이며, 다양한 생물학적인 세포증식억제 방법 중 TRAIL은 단백질을 변형 시킬 수 있으며 단백질 구조를 변형시켜 세포의 사멸에 일조를 하는 것으로 알려져 있다. 본 논문에서는 HCT-116세포를 암세포로 간주하여 TRAIL과 방사선과의 상호관계를 분석하였다. 실험결과 TRAIL과 방사선의 단독사용은 대조군과 비교해 본 결과 각각 세포증식과 세포자멸사에 유의적인 영향을 끼치지 않은 것으로 나타났다. 반대로 TRAIL로 처리하고, 방사선 조사를 병행해서 처리한 경우 HCT-116세포가 유의적으로 세포자멸사가 발생되었음을 알 수 있었고, G1대비 G0의 비율도 증가한 것으로 나타났다. 결론적으로 TRAIL은 방사선 방어적인 세포의 세포자멸사를 증가시켜 방사선 감수성을 증가시켰음을 알 수 있었으며, 또한 세포주기를 변화시켜 세포 증식 능력을 점진적으로 감소시킬 수 있었다. TRAIL은 세포자멸사를 증가시키고 세포증식 능력을 감소시켜 방사선 증감제로서 사용이 가능하다는 것으로 사료된다.

중심단어: 세포증식 능력, 세포자멸사, 방사선 증감제, HCT-116 세포, TRAIL, 방사선 조사