Human Gen Genom. 2021 December; 5(2): A-10-353-1 doi: 10.52547/jhgg.5.2.74
Published online 2023 January 20 Research Article

The Effects of the Engineered Stem Cells Containing Plasmid Encoding IL-12 on Colon Cancer Cell Line-in vitro study

Mohammad Amin Azimifar [1], Maryam Hashemi [2,3*], Nahid Babaei [1], Zahra Salmasi [2], Abbas Doosti [4]
1Department of Cell Molecular Biology, Bushehr Branch, Islamic Azad University, Iran.
2Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
3Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
4Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

*Corresponding author: Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Fax: +98 51 37112470; Tel: +98 5137112471. E-mail: hashemim@mums.ac.ir

Received 2022 November 26; **Revised** 2022 December 10; **Accepted** 2022 December 20.

Abstract

Background: Colon cancer is one of the common malignancies in the world, which its treatment with chemotherapy are associated with many side effects. Recently, interleukin 12 (IL-12) has shown anticancer effects in studies. In the present study, the effect of the engineered stem cells containing plasmid encoding IL-12 on colon cancer cell line was studied.

Methods: HT-29 colon cancer cell line were treated with stem cells containing plasmid encoding IL-12 and IL-12. Cell survival was studied using MTT test at 0, 24, 48 and 72 hours. Also, in these time intervals, the amount of apoptosis and necrosis of cells were studied using flow cytometry technique. Data were analyzed using two-way analysis of variance by GraphPad prism V.8 software.

Results: The results of the present study showed that exposure of HT-29 cell line with stem cells containing plasmid encoding IL-12 leads to a severe decrease in cell viability compared to IL-12, alone. Also, the death of HT-29 cells as a result of exposure to stem cells containing plasmid encoding IL-12 occurred more as a result of apoptosis.

Conclusion: Stem cells containing plasmid encoding IL-12 have the potential to be used in the treatment of colon cancer. However, more studies are needed to confirm.

Keywords: Colon Cancer, Apoptosis, Necrosis, Stem cell, Recombinant

Background

Colon cancer is the leading cause of death in the world and about 8% of cancer deaths occur due to this malignancy (1). This malignancy affects one million people every year (1) and in recent years, the prevalence of this disease has been increasing in Iran (2).

Chemotherapy is the first line of treatment for colon cancer, but this treatment method is associated with many side effects and damage to the patient's health (3). Immunotherapy is one of the novel methods in the treatment of all types of cancers, and in this therapeutic approach, the immune system causes specific activation of immune factors against cancer cells, which ultimately leads to the identification and removal of tumor cells by the individual's own immune system (4). In recent year, this approach has attracted the attention of researchers, because it has much less side effects than other cancer treatment approaches such as radiation therapy or chemotherapy (5).

One of the factors of the immune system involved against cancer cells is Interleukin 12 (IL-12), which prevents the spread of cancer cells by stimulating the production of interferon gamma (IFN-γ) (6). This has caused the systemic administration of IL-12 to be considered as one of the cancer treatment approaches (7). However, the main limitation of this therapeutic approach is the low half-life of this cytokine (8). To overcome this problem, gene therapy can be a suitable solution, because this approach can lead to an increase in the levels of this protein and cause the death of cancer cells (9).

Recently, our research group based on poly-(amidoamine) (PAMAM) (G5) introduced a plasmid encoding Il-2 into stem cells and showed that these recombinant cells have a high

Copyright© 2022, Journal of Human Genetics and Genomics. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

[Downloaded from humangeneticsgenomics.ir on 2026-11-24]

[DOI: 10.52547/jhgg.74]

ability to penetrate into cancer cells, which can be used as an effective carrier for cancer treatment (10). In the present study, considering the increasing trend of colon cancer in different societies, the effect of these recombinant stem cells on the colon cancer line HT-29 was studied.

2. Materials and Methods

2.1. Cell line preparation

HT-29 cell line was obtained from Institute Pasteur-Tehran, Iran. The cells were cultured in RPMI medium supplemented with fetal bovine serum (10%), penicillin and streptomycin antibiotics (0.01µg/L) and finally incubated (temperature: 37°C).

2.2. Preparation of engineered stem cell

In our previous work, we delivered IL-12 encoding plasmid to stem cells (10). We used the engineered cells in current study. Briefly, 30% and 5% of the surface amines of generation-five poly-(amidoamine) (PAMAM) was substituted with 10-bromodecanoic acid and cell-penetrating peptide (low molecular weight protamin), respectively. Physicochemical properties of this modified PAMAM including size, surface charge, plasmid DNA condensing ability, transfection ability to deliver reporter GFP gene and IL-12 gene into MSCs, toxicity of prepared nanoparticles, and finally the migration potential of the engineered stem cells into cancer and normal cells (HepG2 and NIH/3T3) were evaluated (10).

2.3. MTT assay

To perform this test, 5 × 104 HT-29 cells were cultured in a 96-well plate. Then, the cells were treated with 50 ng/ml IL-12 (Sigma, USA) and 1×106 engineered stem cells and incubated for one day. Then, 20 microliters of MTT solution was added to each plate and incubated for 180 minutes. Finally, the intensity of light absorption at 570 nm was read by a spectrophotometer.

2.4. Flowcytometry

We used this method to measure cells' apoptosis and necrosis. Annexin 5-propidium iodide was used to perform this test; for this purpose, 24 hours after the cell treatment, the cells adhering to the bottom were separated from the bottom of the plate and transferred into the eppendorf, and then centrifuged. Buffer Binding is added and then 5 microliters of annexin 5 and propidium iodide were added to each sample. Then, the samples were incubated for 5 minutes in the dark and checked with a flowcytometer (FACS Calibur instrument (Becton Dickinson)).

2.5. Statistical analysis

After ensuring data normal distribution by Kolmogorov–Smirnov test, all data were analyzed using one-way analysis of variance (ANOVA) by GraphPad Prism V.8 software.

3. Results

3.1. Cell Viability

The viability of colon cancer cell line HT-29 treated with IL-12 and engineered stem cells containing plasmid encoding IL-12 was measured at 0, 24, 48 and 72 hours, and the results are shown in Figure 1. As can be seen, there is no significant change in cell viability up to 24 hours after treatment. Nevertheless, significant changes in cell viability were observed at 48 and 72 hours. At these times, treatment of the HT-29 cell line with engineered stem cells containing plasmid encoding IL-12 resulted in a significant decrease in survival compared to the control. In the period of 72 hours, an almost 50% decrease in the viability of HT-29 cells was seen. A strong effect of engineered stem cells containing plasmid encoding IL-12 was seen in reducing cell viability compared to IL-12 alone.

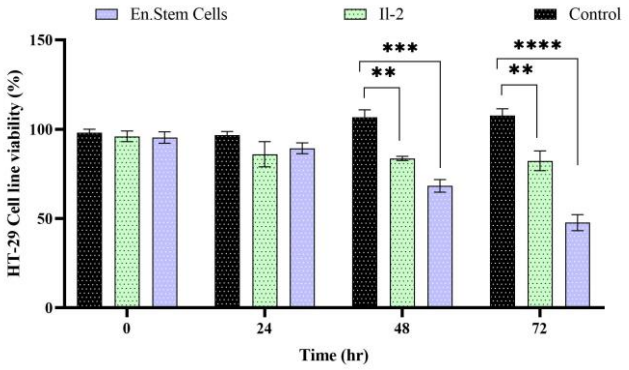

Figure 1. *The HT-29 cell line viability after treatment with IL-12 and engineered stem cells containing plasmid encoding IL-12 at 0, 24, 48 and 72 hours. **P<0.01vs. control. *** P<0.001vs. control. **** P<0.0001vs control .*

[Downloaded from humangeneticsgenomics.ir on 2026-11-24]

[DOI: 10.52547/jhgg.74]

3.2. Cell apoptosis

The results of the present study showed that the death of HT-29 cells as a result of treating cells with IL-12 and engineered stem cells containing plasmid encoding IL-12 is more due to apoptosis (Figure 2). Significant differences in the mean percentage of cell apoptosis were observed one day after the treatment. The highest amount of cells apoptosis was seen after 72 hours as a result of treatment of cells with engineered stem cells containing plasmid encoding IL-12 (Figure 2).

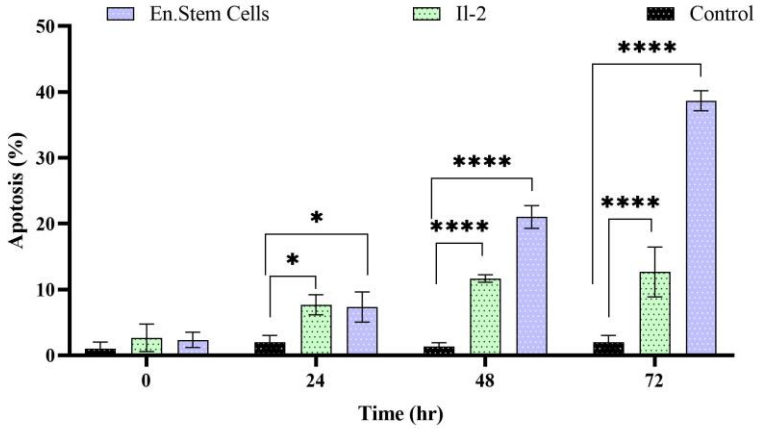

Figure 2. *The HT-29 cell line apoptosis after treatment with IL-12 and engineered stem cells containing plasmid encoding IL-12 at 0, 24, 48 and 72 hours. *P<0.05vs. control, **** P<0.0001vs control .*

3.3. Cell necrosis

No significant difference was observed in the necrosis of HT-29 cells up to 24 hours after treating the cells with IL-12 and engineered stem cells containing plasmid encoding IL-12 compared control. Nevertheless, in the period of 48 and 72 hours, a significant increase in the necrosis of cells treated with engineered stem cells containing plasmid encoding IL-12 was seen. Interestingly, the cells treated with IL-12 did not show any significant difference in terms of cell necrosis with the control at the studied times (Figure 3).

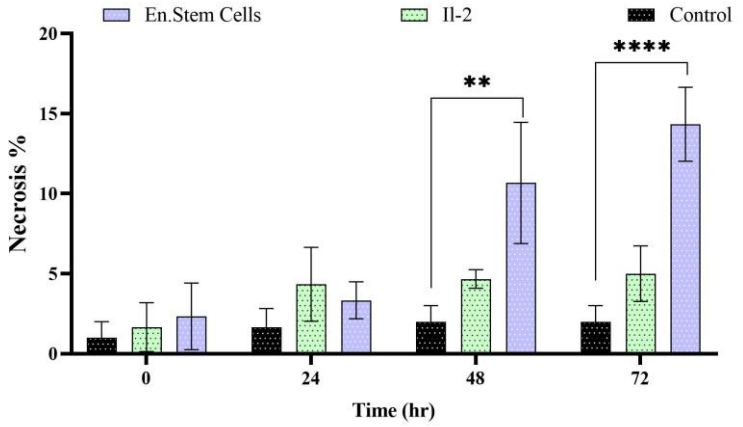

Figure 3. *The HT-29 cell line necrosis after treatment with IL-12 and engineered stem cells containing plasmid encoding IL-12 at 0, 24, 48 and 72 hours. **P<0.01vs. control, **** P<0.0001vs control .*

4. Discussion

Colon cancer is one of the most common malignancies in the world, and its treatment with chemotherapy has severe and harmful side effects for the patient's health (3). In recent years, immunotherapy has attracted the attention of researchers as a cancer treatment approach with low side effects (11). It has been found that IL-12 can help in the treatment of cancer by killing cancer cells. However, one of its disadvantages is the limited half-life of IL-12 and its systemic administration may be associated with cytotoxic

[Downloaded from humangeneticsgenomics.ir on 2026-11-24]

[DOI: 10.52547/jhgg.74]

Recently, our research group designed engineered stem cells containing plasmid encoding IL-12 and showed that these recombinant cells have high invasiveness to cancer cells, which was proposed as an effective carrier for cancer treatment (10). In this study, the effects of engineered stem cells containing IL-12 encoding plasmid on HT-29 colon cancer cells were studied and these effects were compared with exposure of cells to IL-12. The results of the present study showed that engineered stem cells containing plasmid encoding IL-12 have a high ability to kill HT-29 cells compared to IL-12 alone, which indicates its potential application in the treatment of colon cancer. This ability can be attributed to the continuous production of IL-12 by these recombinant stem cells, and therefore, it seems that the engineered stem cells containing the IL-12 encoding plasmid designed by our research group were able to overcome the limitation of the low half-life of IL-12 in cancer treatment.

In the present study, the results of flow cytometry test showed that the death of HT-29 colon cancer cells due to the treatment of cells with engineered stem cells containing plasmid encoding IL-12 is more as a result of apoptosis. The anticancer effects of Il-12 have been studied on a variety of malignancies (12-15), and the results have shown that this cytokine can be a candidate for cancer immunotherapy treatment, which is in accordance with the findings of the present research. In the present study, it has been shown that engineered stem cells containing plasmid encoding IL-12 have the ability to kill HT-29 colon cancer cells, which indicates its high potential in the treatment of this malignancy.

However, the present study was done in vitro and needs to confirm in in vivo and clinical conditions. Therefore, in future research, it is recommended that the effect of these recombinant stem cells be studied in animal model and the mechanism of anticancer effects be investigated at the molecular level.

5. Conclusion

In general, it is concluded that the engineered stem cells containing IL-12 encoding plasmid by our research group have the potential to be used in the treatment of colon cancer. However, more studies are needed in this field.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Jensen OM. Colon cancer epidemiology. Experimental colon carcinogenesis: CRC Press; 2019. p. 3-24.
2. Maajani K, Khodadost M, Fattahi A, Shahrestanaki E, Pirouzi A, Khalili F, et al. Survival Rate of Colorectal Cancer in Iran: A Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev. 2019;20(1):13-21.
3. Wiela-Hojeńska A, Kowalska T, Filipczyk-Cisarż E, Łapiński Ł, Nartowski K. Evaluation of the toxicity of anticancer chemotherapy in patients with colon cancer. Advances in Clinical and Experimental Medicine. 2015;24(1):103-11.
4. Xu Z, Zeng S, Gong Z, Yan Y. Exosome-based immunotherapy: a promising approach for cancer treatment. Molecular Cancer. 2020;19(1):160.
5. Wang C, Qiao W, Jiang Y, Zhu M, Shao J, Wang T, et al. The landscape of immune checkpoint inhibitor plus chemotherapy versus immunotherapy for advanced non-small-cell lung cancer: a systematic review and meta-analysis. Journal of cellular physiology. 2020;235(5):4913-27.
6. Nguyen H-M, Guz-Montgomery K, Saha D. Oncolytic Virus Encoding a Master Pro-Inflammatory Cytokine Interleukin 12 in Cancer Immunotherapy. Cells [Internet]. 2020; 9(2).
7. Mirlekar B, Pylayeva-Gupta Y. IL-12 Family Cytokines in Cancer and Immunotherapy. Cancers (Basel). 2021;13(2).
8. Jung K, Ha J-H, Kim J-E, Kim J-A, Kim Y-J, Kim C-H, et al. Heterodimeric Fc-fused IL12 shows potent antitumor activity by generating memory CD8+ T cells. OncoImmunology. 2018;7(7):e1438800.
9. de la Torre P, Pérez-Lorenzo MJ, Alcázar-Garrido Á, Flores AI. Cell-Based Nanoparticles Delivery Systems for Targeted Cancer Therapy: Lessons from Anti-Angiogenesis Treatments. Molecules. 2020;25(3).
10. Azimifar MA, Salmasi Z, Doosti A, Babaei N, Hashemi M. Evaluation of the efficiency of modified PAMAM dendrimer with low molecular weight protamine peptide to deliver IL-12 plasmid into stem cells as cancer therapy vehicles. Biotechnol Prog. 2021;37(4):e3175.
11. Stein A, Folprecht G. Immunotherapy of colon cancer. Oncology research and treatment. 2018;41(5):282-5.
12. A. Engel M, F. Neurath M. Anticancer Properties of the IL-12 Family - Focus on Colorectal Cancer. Current Medicinal Chemistry. 2010;17(29):3303-8.
13. Floros T, Tarhini AA. Anticancer Cytokines: Biology and Clinical Effects of Interferon-α2, Interleukin (IL)-2, IL-15, IL-21, and IL-12. Seminars in Oncology. 2015;42(4):539-48.
14. Tugues S, Burkhard SH, Ohs I, Vrohlings M, Nussbaum K, vom Berg J, et al. New insights into IL-12-mediated tumor suppression. Cell Death & Differentiation. 2015;22(2):237-46.
15. Weiss JM, Subleski JJ, Wigginton JM, Wiltrout RH. Immunotherapy of cancer by IL-12-based cytokine combinations. Expert opinion on biological therapy. 2007;7(11):1705-21.

[Downloaded from humangeneticsgenomics.ir on 2026-11-24]

[DOI: 10.52547/jhgg.74]