Original Article

Radiation Response of Human Leukemia/Lymphoma Cells was Improved by 7-Geranyloxycoumarin

Dose-Response:
An International Journal
July-September 2022:1–10

DOI: 10.1177/15593258221124479
journals.sagepub.com/home/dos
SAGE

Ramin Bagheri[1], Fatemeh B. Rassouli[2], Hamid Gholamhosseinian[3], Keyhan Ebrahimi[1], Shakiba Mahdavi[4], Sajad Goudarzi[1], Mehrdad Iranshahi[5], Houshang Rafatpanah[6], and Mohammad Reza Keramati[1]

Abstract

Objectives: Adult T-cell leukemia/lymphoma (ATLL) is a blood neoplasm with specific geographic distribution. Although radiotherapy is a palliative treatment that provides long-term local control, single use of radiation leads to complications for patients. To introduce a novel multimodal approach against ATLL, we investigated combinatorial effects of 7-geranyloxycoumarin and radiation in vitro.

Methods: Viability of MT-2 cells was determined by resazurin assay upon administration of 7-geranyloxycoumarin alone and followed by radiation. Then, apoptosis was detected by annexin V and propidium iodide, and the expression of candidate genes was analyzed by qPCR.

Results: Findings revealed significant (P<.0001) improvement in radiation effects upon 7-geranyloxycoumarin pretreatment, most notably when cells were pretreated with 5 μg/ml 7-geranyloxycoumarin for 96 h, exposed to 6 Gy radiation and recovered for 48 h. These results were confirmed by flow cytometry, as the percentage of early and late apoptotic cells was increased after combinatorial treatment. In addition, significant (P< .0001) changes in *CD44*, *c-MYC*, *cFLIPL*, *BMI-1*, *NF-κB* (*Rel A*), and *P53* expression was induced by 7-geranyloxycoumarin and radiation.

Conclusions: Current research indicated, for the first time, that combinatorial use of 7-geranyloxycoumarin and ionizing radiation could be considered as an effective therapeutic modality for ATLL.

Keywords

radiation response, 7-geranyloxycoumarin, combinatorial treatment, adult T-cell leukemia/lymphoma

[1] Cancer Molecular Pathology Research Center, Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
[3] Department of Medical Physics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
[5] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[6] Immunology Research Center, Inflammation and inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Authors:
Houshang Rafatpanah, Immunology Research Center, Inflammation and Inflammatory Diseases Division, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: RafatPanahH@mums.ac.ir

Mohammad Reza Keramati, Cancer Molecular Pathology Research Center, Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: KeramatiMR@mums.ac.ir

Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a rare lymphoproliferative disorder of mature $CD4^+$ T cells that is caused by human T-lymphotropic virus type 1 (HTLV-1).[1] ATLL requires a long latent period before onset, demonstrating that viral genes, as well as modifications of the host genome (genetic and epigenetic), are crucial in leukemogenesis.[2] HTLV-1 has specific geographical distribution, as Japan, South America, West Africa, the Caribbean and Melanesian islands, and the Middle East are main endemic regions for this virus.[3-5] Aside from these areas, HTLV-1 is known to be widespread in Northeast Iran, particularly in Mashhad and Neyshabour.[6,7] HTLV-1 incidence varies amongst different populations and increases with age, and the virus is known to be more prevalent amongst females.[8,9] Approximately 3–5% of HTLV-1 infected patients develop ATLL, which can be classified as acute (60%), lymphomatous (20%), chronic (15%), and smoldering (5%).[10,11] Despite advancements in chemotherapy and supportive care, the median survival time for ATLL is still less than a year.[12] The introduction of antiviral agents, monoclonal antibodies, and allogeneic hematopoietic stem cell transplantation seems to be promising; however, these methods are insufficient to ensure long-term survival for most ATLL patients.[13] Radiotherapy is an excellent palliative treatment option that provides long-term local control and clinical benefit, even in advanced-stage lymphomas. Nevertheless, the use of radiotherapy as a single modality requires wide extension of the radiation field, as well as raising the dose to normal tissue tolerance levels, which lead to the late development of complications for patients.[14]

7-geranyloxycoumarin, also known as auraptene, is a natural monoterpene coumarin found in Rutaceae and Umbelliferae plants. This simple prenylcoumarin owns various pharmaceutical properties, such as antibacterial, antigenotoxic, antioxidative, and anti-inflammatory activities.[15] Studies have also reported cancer chemopreventive effects of 7-geranyloxycoumarin, in addition to its combinatorial effects with anticancer drugs and ionizing radiation.[16-22]

Numerous laboratory investigations are currently focused on the administration of agents that could boost the sensitivity of cancer cells to conventional therapies, while having minimal negative effects on non-neoplastic cells. Since radiotherapy is used for palliation of ATLL patients, improving the radio response of cancer cells would result in better clinical outcomes. Our research team has demonstrated radiosensitizing effects of 7-geranyloxycoumarin on colon carcinoma, gastric adenocarcinoma and esophageal carcinoma cells.[19-22] Herein, we applied a combined therapeutic strategy against ATLL based on 7-geranyloxycoumarin and radiation. To do so, the viability of ATLL cells was determined by resazurin assay upon administration of 7-geranyloxycoumarin alone and followed by radiation exposure. Then, apoptosis was detected using fluorescein isothiocyanate (FITC)-annexin V and propidium iodide (PI), and the expression of *CD44*, *c-MYC*, *cFLIPL*, *BMI-1*, *NF-κB* (*Rel A*), and *P53* was analyzed by quantitative polymerase chain reaction (qPCR).

Materials and Methods

Synthesis of 7-Geranyloxycoumarin

Under standard laboratory conditions, 7-geranyloxycoumarin ($C_{19}H_{22}O_3$, MW: 298.4 g/mol) was sensitized through a reaction between 7- hydroxycoumarin (1 M) and transgeranyl bromide (1.5 M) in the presence of 1,8-diazabicyclo [5.4.0] undec-7-ene (2 M) as previously described.[23] Once the mixture was concentrated at low pressure, column chromatography (petroleum ether/ethyl acetate 9:1 v/v) was carried out to purify 7-geranyloxycoumarin (mp = 62.7°C–63.4°C). Then, ^1H and ^{13}C NMR were utilized to validate the structure of 7-geranyloxycoumarin. To prepare various concentration of 7-geranyloxycoumarin, dimethyl sulfoxide (DMSO) was used as solvent, and thus, equal amount of DMSO in all 7-geranyloxycoumarin concentrations (.4% v/v) was considered as control.

Treatment and Viability Assessment of Cells

MT-2 cells, lymphoma cells infected with HTLV-1, were derived from normal human cord leukocytes of a healthy donor by co-cultivation with leukemic cells from an adult T-cell leukemia (ATL) patient.[24] In the present study, MT-2 cells (donated by Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences) were cultured in Roswell Park Memorial Institute-1640 supplemented with 10% fetal bovine serum, 1% (W/V) penicillin/streptomycin and 1% L-glutamine, and incubated at 37°C with 5% CO_2 in air.

To determine the toxicity of 7-geranyloxycoumarin, cells were seeded (5×10^4 cells/well in 96-well plates) and treated with 5, 10, 20, and 40 µg/ml 7-geranyloxycoumarin for 24, 48, 72, 96, 120, and 144 h. For combinational treatment, cells were pretreated with 5 and 10 µg/ml 7-geranyloxycoumarin for 24, 48, 72, and 96 h, followed by radiation exposure using Elekta Compact™ linear accelerator (Crawley) at three different doses (2, 4, and 6 Gy). Finally, cells were recovered for 48 and 72 h and assessed for viability.

Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) was used to assess cell viability upon treatment with 7-geranyloxycoumarin, alone or followed by radiotherapy. In this regard, 20 µl resazurin (.1 mg/ml, Sigma) was added to each well, and cells were incubated for 2 h at 37°C. Finally, the absorbance of wells was measured at 600 nm using a microplate reader (Epoch), and cell viability (%) was calculated using the following formula: (100-(AT-AU/AB-AU))×100, in which AT and AU represent the absorbance of treated and untreated cells, respectively, and AB was the absorbance of blank control.

Detection of Apoptosis

Cell apoptosis was measured by FITC-annexin V and PI. In summary, MT-2 cells (5×10^5 cells/well in 6-well plates) were incubated with 7-geranyloxycoumarin (5 µg/ml) for 96°h and irradiated at 6 Gy. After 48°h recovery, cells were collected and stained with FITC-annexin V and PI (MabTag) according to the manufacturer's protocol. Finally, the degree of apoptosis was analyzed by flow cytometry (BD FACS) using FL1 and FL2 filters.

Gene Expression Analysis

The expression pattern of candidate genes was studied by qPCR. Briefly, the total RNA was extracted from treated cells (96 h pretreatment with 5 µg/ml 7-geranyloxycoumarin alone or followed by 6 Gy radiation and 48°h recovery) and their relevant controls (96 h pretreatment with .4% DMSO alone or followed by 6 Gy radiation and 48 h recovery) using Tripure (Roche). Then, synthesis of cDNAs was carried out by random hexamer, dNTPs, and M-MuLV reverse transcriptase (Thermo Fisher Scientific) according to the manufacturer's protocol. qPCR was conducted in Rotor-Gene 6000 detection system (Qiagen) using SYBR green mix and primers listed in Table 1 for *c-MYC*, *cFLIPL*, *BMI-1*, and *CD44*, while TaqMan probes and specific primers were used for *P53* and *NF-κB* (*REL-A*). β_2M transcripts were used as the internal control in all analyses. PCR cycling conditions were 95°C for 2 min, [95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec] (40 cycles) for *CD44*, *BMI-1*, *c-MYC*, and *cFLIPL*, and 95°C for 2 min, [95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec] (45 cycles) for *NF-κB* (*REL-A*) and *P53*.

Statistical Analyses

All results were plotted in the form of bar graphs using GraphPad Prism. Dunnet and Bonfferroni statistical analyses were used to determine the significant difference between groups. Results were expressed as mean ± standard deviation (SD), and *P* values less than .05, .01, .001, and .0001 (shown by *, **, ***, and ****, respectively) were considered to be statistically significant.

Results

Resazurin assay demonstrated that 7-geranyloxycoumarin reduced the viability of MT-2 cells in a dose- and time-dependent manner. As presented in Figure 1, upon 24, 48, 72, 96, 120, and 144 h treatment with 5 µg/ml 7-geranyloxycoumarin, viability was calculated as 98%, 96%, 84%, 79%, 83%, and 81%, respectively. However, upon treatment with the highest concentration (40 µg/ml) during the same consecutive time periods, cell viability was determined as 92%, 65%, 52%, 42%, 37%, and 30%, respectively. Accordingly, 5 and 10 µg/ml 7-geranyloxycoumarin were used as optimum concentrations of this agent for combinatorial experiments.

To investigate radiosensitizing effects of 7-geranyloxycoumarin, MT-2 cells were pretreated with 5 and 10 µg/ml 7-geranyloxycoumarin for 24, 48, 72, and 96°h, then exposed to 2, 4, and 6 Gy X-radiation and recovered for 48 and 72°h. As shown in Figure 2, assessment of cell viability revealed significant ($P < .0001$) increase in the toxicity of applied radiation, almost in all combinatorial treatments, in comparison with relevant controls (.4% DMSO +2, 4, and

Table 1. List of primers and probes used for qPCR analysis in current study.

Name of Gene	Length (Bp)	5′→3′
β₂M	127	Forward: AATTGAAAAAGTGGAGCATTCAGA Reverse: GGCTGTGACAAAGTCACATGGTT
c-MYC	159	Forward: ACTCTGAGGAGGAGGAACAAGAA Reverse: TGGAGACGTGGCACCTCTT
CFLIPL	126	Forward: ATTGGCAATGAGACAGAGCTTC Reverse: CTCGGGCATACAGGCAAA
BMI-1	192	Forward: CTGCAGCTCGCTTCAAGATG Reverse: CACACACATCAGGTGGGGAT
CD44	176	Forward: CGGACACCATGGACAAGTTT Reverse: GAAAGCCTTGCAGAGGTCAG
P53	132	Forward: CAGCATCTTATCCGAGTGGAAGG Reverse: GTTGTAGTGGATGGTGGTACAGTC Probe: CTCAGGCGGCTCATAGGGCACCAC
NF-κB (REL-A)	145	Forward: ACCCCTTCCAAGTTCCTATAGAAGAG Reverse: CGATTGTCAAAGATGGGATGAGAAAG Probe: ACTACGACCTGAATGCTGTGCGGCTCT
β₂M	127	Forward: TTGTCTTTCAGCAAGGACTGG Reverse: CCACTTAACTATCTTGGGCTGTG Probe: TCACATGGTTCACACGGCAGGCAT

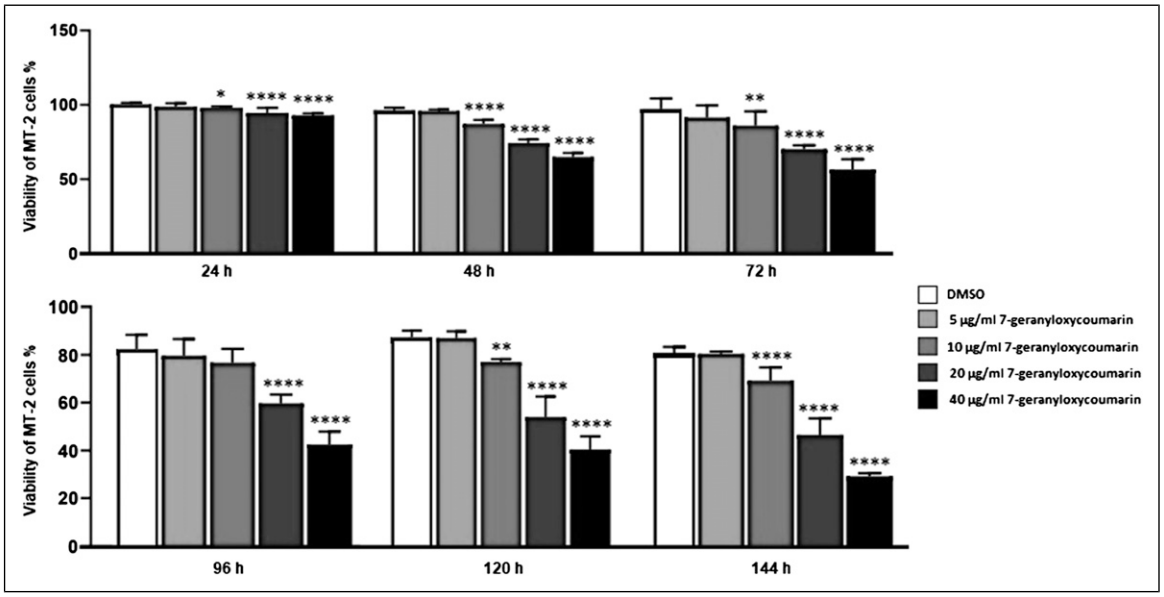

Figure 1. Time-based dose response analysis of MT-2 cells after treatment with 5, 10, 20 and 40 µg/ml 7-geranyloxycoumarin for 24, 48, 72 h, 96, 120, and 144°h. Resazurin assay was carried out for at least three times and results were presented as mean ± SD. *P < .05, **P < .01 and ****P < .0001 indicate significant difference with DMSO control.

6 Gy). With regard to 2 Gy radiation, the lowest viability was 60.8% when cells were recovered for 48°h, and 49.5% when cells were recovered for 72°h (Figure 2-A and B). In addition, when 4 Gy radiation was applied, viability decreased down to 47.6% and 26.9% upon 48 and 72°h recovery, respectively (Figure 2-C and D). More interestingly, after radiation exposure at 6 Gy, viability was determined as .5% and 1.8% when cells were recovered for 48 and 72°h, respectively (Figure 2-E and F).

Figure 3 demonstrates flow cytometry analysis after FITC-annexin V and PI staining. As shown, upon 144 h treatment with 5 µg/ml 7-geranyloxycoumarin, the percentage of alive cells was 64.2%, lower than that for untreated and DMSO treated cells (97.4% and 70.4%, respectively). When 6 Gy radiation was applied and cells were recovered for 48°h, 64.1%, 18.8%, and 16.9% of cells were alive, early apoptotic and late apoptotic, respectively, which was similar to the percentage of cells detected after DMSO and radiation treatment. Interestingly, 96 h pretreatment of cells with 5 µg/ml 7-geranyloxycoumarin followed by 6 Gy radiation and 48 h recovery induced considerable changes in the cell population, as 45.1%, 24%, and 30.9% of cells were alive, early apoptotic and late apoptotic, respectively.

In order to evaluate alterations induced by our combined approach in the expression of *CD44*, *c-MYC*, *cFLIPL*, *BMI-1*, *NF-κB* (*Rel A*), and *P53*, qPCR was carried out and results were presented in Figure 4. As illustrated, 5 µg/ml 7-geranyloxycoumarin alone and followed by 6 Gy radiation significantly (P< .0001) reduced the expression of *CD44*, *c-MYC*, and *cFLIPL* (Figure 4-A-C). With regard to *BMI-1* expression, significant (P< .0001) downregulation was detected after single and combinatorial administration of 7-geranyloxycoumarin in comparison with their relevant DMSO controls (Figure 4-D). On the other hand, 7-geranyloxycoumarin alone and in combination with radiation significantly (P< .0001) upregulated the expression of *NF-κB* (*REL-A*), while significant (P< .0001) induction in *P53* expression was only detected upon 7-geranyloxycoumarin and radiation treatment (Figure 4E and F).

Discussion

ATLL is a public health concern in specific geographic areas including our country Iran.[6,7] Although radiotherapy is an effective modality for the treatment of most lymphomas, single-use of radiation requires raising the dose to normal tissue tolerance levels, which is associated with late development of complications for patients.[14] Hence, introduction of novel multimodal therapies that improve clinical outcomes is a critical demand. In the present study, we designed a new approach against ATLL cells and investigated combinatorial effects of 7-geranyloxycoumarin, a valuable pharmaceutical agent, and radiation in vitro. Our findings indicated improvement in radiation effects upon pretreatment with 7-geranyloxycoumarin, most notably when MT-2 cells were pretreated with 5 µg/ml 7-geranyloxycoumarin for 96°h, then exposed to 6 Gy radiation and recovered for 48°h. Results of cell viability assay were confirmed by flow cytometry, since the percentage of early and late apoptotic cells was increased after 7-geranyloxycoumarin and radiation treatment.

Studying the mechanisms involved in our combinatorial approach revealed significant downregulation in *CD44*, *c-MYC*,

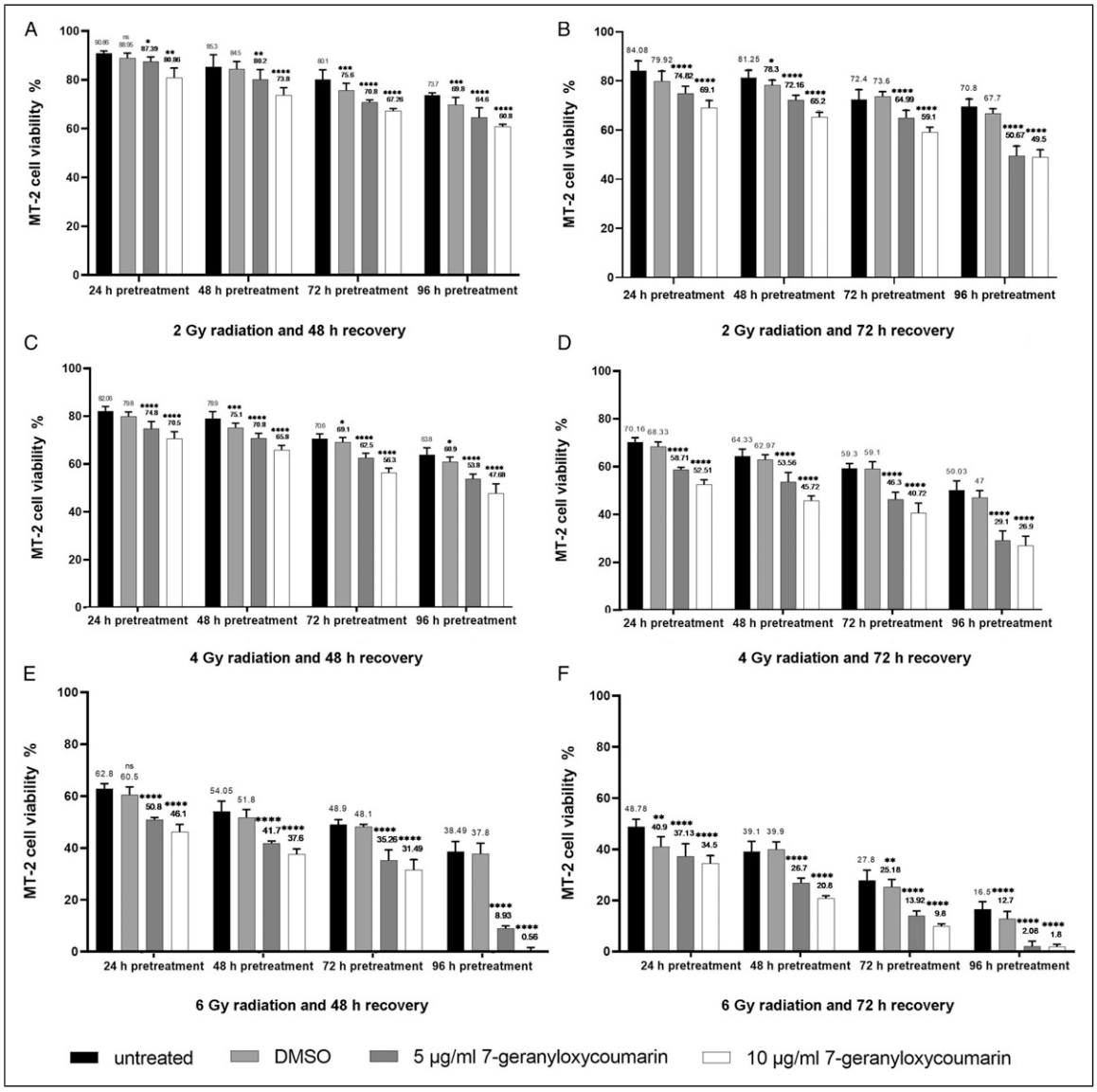

Figure 2. Viability of MT-2 cells after combinatorial treatment with 7-geranyloxycoumarin and radiation. After pretreatment of cells with 5 and 10 µg/ml 7-geranyloxycoumarin for 24, 48, 72, and 96°h, radiation was applied at 2 Gy (A and B), 4 Gy (C and D), and 6 Gy (E and F), and cells were recovered for 48 h (A, C, and E) and 72 h (B, D, and F). Resazurin assay was carried out for at least three times and results were presented as mean ± SD. *P < .05, **P < .01, ***P < .001, and ****P < .0001 indicate significant difference with radiation alone.

and *cFLIPL* expression, as well as significant overexpression of *NF-κB* (*REL-A*) and *P53*. CD44 is a transmembrane glycoprotein involved in drug resistance and anti-apoptosis mechanisms.[25] Upregulation of *CD44* after high-dose irradiation contributes to longer-term cell survival through maintaining extracellular signal-regulated kinase (Erk) phosphorylation.[26] Induction of *CD44* by radiation has been reported in glioblastoma, pancreas, bladder, colon, and prostate carcinoma cells,[26-30] similar to our results that revealed upregulation of *CD44* expression in ATLL cells upon single use of 6 Gy radiation. Interestingly, 7-geranyloxycoumarin downregulated *CD44* expression, and when used in combination with radiation, expression of this gene was reduced down to lower levels. In line with this finding, negative regulatory effect of 7-geranyloxycoumarin on *CD44* expression has been previously reported in esophageal carcinoma, colorectal carcinoma, and ATLL cells.[20,21,31] *c-MYC* is an oncogene that controls cell proliferation, differentiation, and apoptosis. The radioresistant phenotype of embryonal rhabdomyosarcoma, glioblastoma, osteosarcoma, and lung adenocarcinoma cells has been linked to *c-MYC* expression, and radiosensitizing effects of *c-MYC* knockdown has been reported recently.[32-35] cFLIPL prevents caspase-8 activation and cell apoptosis mediated by death receptors. It has been reported that cFLIPL negatively modulated radiosensitivity of lung cancer cells, and knockdown of this gene sensitizes cervical adenocarcinoma cells to

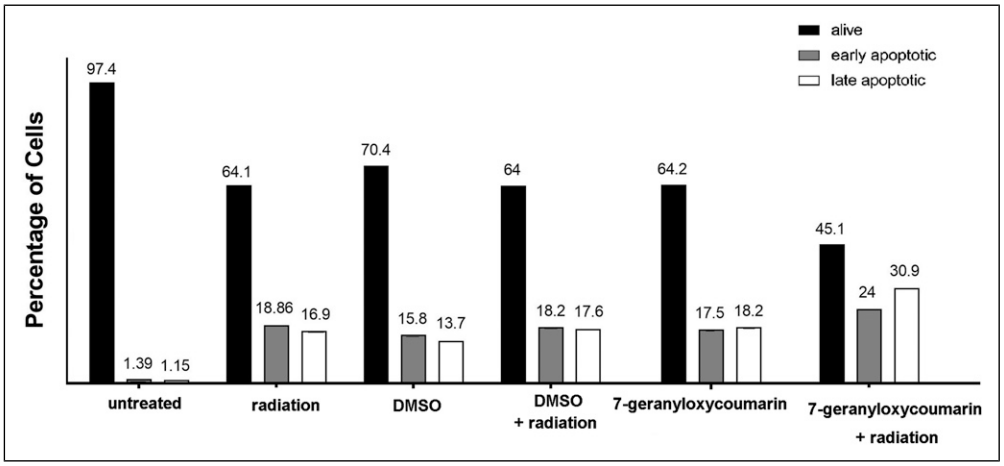

Figure 3. Flow cytometry analysis after single and combinatorial treatments to differentiate alive cells from early and late apoptotic cells.

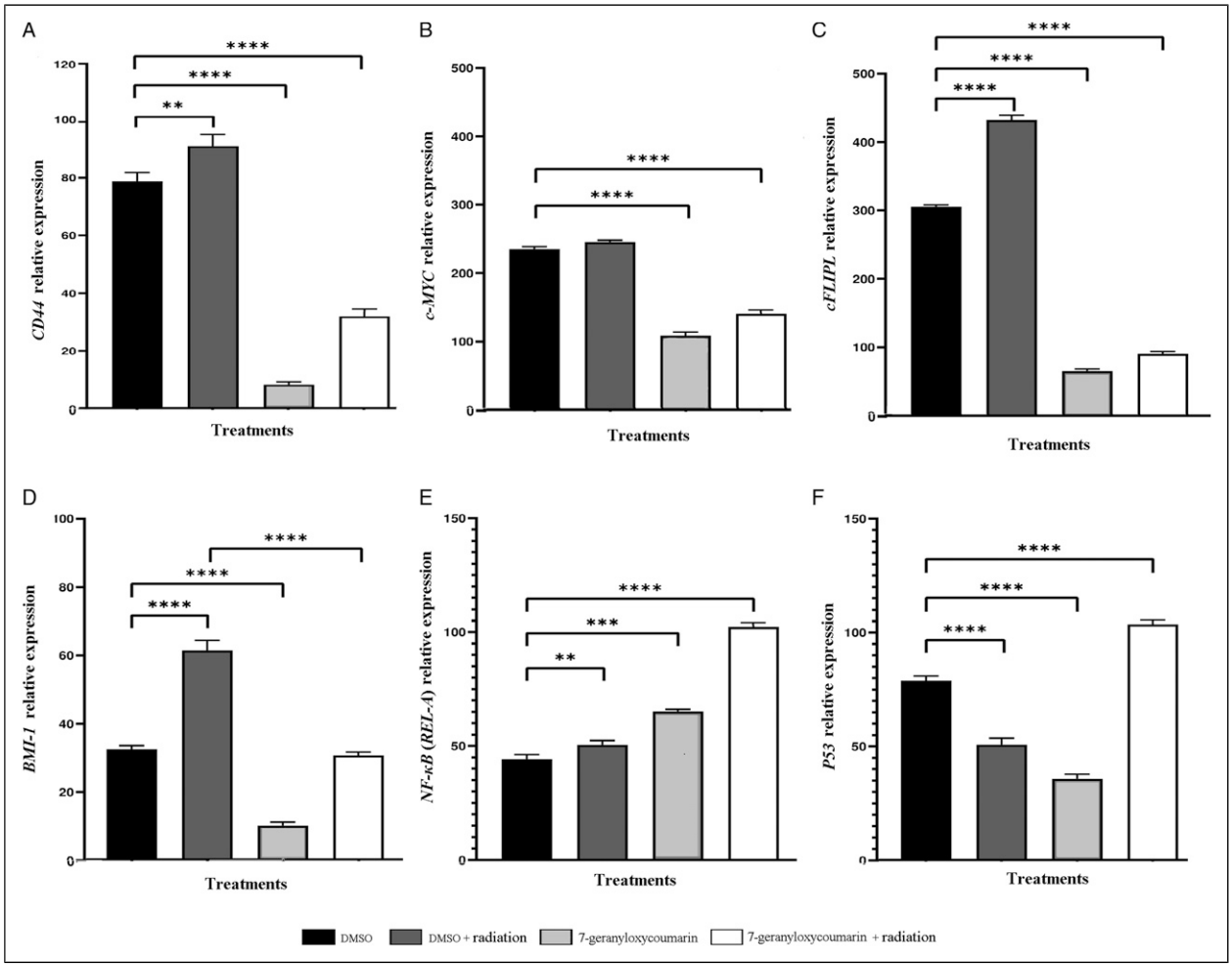

Figure 4. Analysis of gene expression by qPCR. After 96 h pretreatment of cells with 7-geranyloxycoumarin (5 μg/ml), radiation (6 Gy) was applied, cells were recovered for 48 h and the relative expression of *CD44*, *c-MYC*, *cFLIPL*, *BMI-1*, *NF-κB* (*Rel A*) and *P53* was evaluated. Normalized values were plotted over untreated cells. **P < .01, ***P < .001 and ****P < .0001 indicate significant difference with control.

radiotherapy.[36,37] Present results demonstrated significant decrease in *c-MYC* and *cFLIPL* expression upon single and combinatorial use of 7-geranyloxycoumarin, which are in consistence with other studies.[31,38] Accordingly, negative regulation of *CD44*, *c-MYC*, and *cFLIPL* explains, to some extent, the observed effects of our novel combinatorial approach.

The overexpression of BMI-1, an oncogene connected to cell cycle progression and immortalization, has been linked to disease progression and poor clinical outcomes in various human cancers.[39-41] BMI-1 induced radioprotective effects via reducing the genotoxicity of radiation, and therefore, reduction of BMI-1 protein in cancer cells elevates susceptibility to radiotherapy.[42] Previous reports on radiation-induced *BMI-1* expression in glioblastoma, breast, and esophageal carcinoma cells[43-45] confirm our results on significant up-regulation of this gene in ATLL cells by 6 Gy radiation. Intriguingly, 7-geranyloxycoumarin counteracts radiation effects on *BMI-1* expression, as significant downregulation of this gene was detected upon treatment with 7-geranyloxycoumarin alone and in combination with radiation. In agreement with current findings, negative regulatory effects of 7-geranyloxycoumarin on *BMI-1* expression has been reported on other cancer cell types.[20,31] *NF-kB* controls radioresistance through the regulation of DNA double-strand break repair, cell cycle arrest and apoptosis. It has been shown that activating *NF-kB* confers resistance of hepatocellular, oropharyngeal and esophageal carcinoma cells to radiation.[46-48] In addition, it has been demonstrated that *NF-kB* stimulates the expression of death receptors (DR) 4 and 5, Fas and Fas ligand (FasL), which are associated with induction of apoptosis.[49] Results of present study are supported by above mentioned reports, as single and combinatorial use of 7-geranyloxycoumarin and radiation induced the expression of *NF-kB* in ATLL cells. *P53* is a tumor suppressor gene with low expression level in non-transformed cells. Several studies have defined *P53* as a key molecule involved in radioresponse of cells, since it is linked to radiotherapy efficacy,[50-52] and is considered as a biomarker for clinical radiosensitivity.[53] Radiation-induced expression of *P53* has been demonstrated in glioblastoma and hepatocellular carcinoma cells,[54,55] similar to our results that indicated up-regulation of *P53* by 7-geranyloxycoumarin and radiation. Hence, observed combinatorial effects in present study could also be explained by changes induced in the expression of *BMI-1*, *NF-κB* (*REL-A*), and *P53*.

Beside modulation of *CD44*, *c-MYC*, *cFLIPL*, *BMI-1*, *NF-κB* (*Rel A*), and *P53* expression, other possible mechanisms (Figure 5) could be associated with observed effects of 7-geranyloxycoumarin and radiation, although more research is required to confirm their involvement. For instance, aldehyde dehydrogenase 1 (ALDH1), which oxidizes intracellular aldehydes to carboxylic acids, reduces the level of reactive oxygen species (ROS) and oxidative stress induced by ionizing radiation.[56,57] In this regard, negative regulatory effect of 7-geranyloxycoumarin on *ALDH1* expression has been demonstrated previously.[19] Moreover, inhibition of poly (ADP-ribose) polymerase1 (*PARP1*), an essential enzyme involved in the repair of radiation-induced DNA damage, has been reported upon 7-geranyloxycoumarin treatment.[38,58] Accordingly, it is possible that combinatorial effects of 7-geranyloxycoumarin and radiation were somehow due to the inhibition of *ALDH1* and *PARP1* expression.

Proliferating cell nuclear antigen (PCNA) is an important protein involved in DNA replication and repair, as well as cell

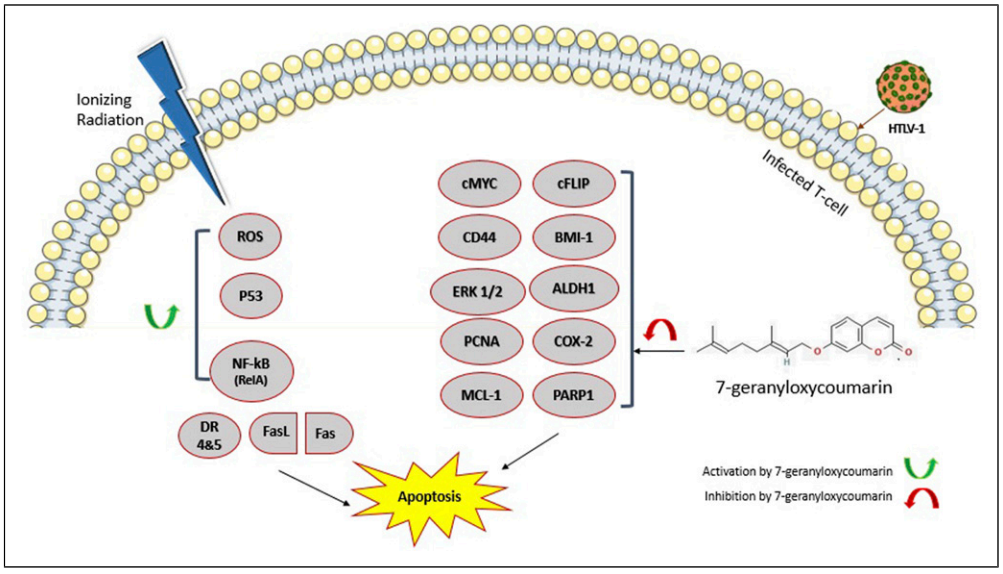

Figure 5. Schematic representation of changes induced by 7-geranyloxycoumarin and radiation on the expression of *CD44*, *c-MYC*, *cFLIPL*, *BMI-1*, *NF-κB* (*Rel A*), and *P53* in ATLL cells, and possible involvement of *ALDH1*, *COX-2*, *PARP1*, *ERK 1/2*, *MCL-1*, *CD44*, and *PCNA* in observed effects of our combinatorial approach.

cycle regulation.[59] Cyclooxygenase-2 (COX-2) promotes proliferation, angiogenesis, inflammation, invasion, and apoptotic resistance in cancer cells.[60] As previously reported, 7-geranyloxycoumarin downregulated *PCNA* and *COX-2* expression,[17] therefore, it is presumable that 7-geranyloxycoumarin improved radioresponse of ATLL cells via negative regulation of *PCNA* and *COX-2*.

As a mitogen-activated protein kinase family member, Ras-dependent extracellular signal-regulated kinase (*ERK*) 1/2 regulates cell cycle and proliferation, and inactivates apoptotic genes such as *BAX*.[61] Myeloid leukemia 1 (MCL-1) is a member of BCL-2 family involved in apoptosis resistance of cancer cells.[62,63] Since previous studies indicated inhibitory effects of 7-geranyloxycoumarin on *ERK* 1/2 and *MCL-1*,[64,65] downregulation of both genes might also be involved in observed effects of 7-geranyloxycoumarin and radiation.

In conclusion, we reported for the first time, that 7-geranyloxycoumarin improved radioresponse of human ATLL cells. Complementary studies on other ATLL cell lines, such as Hut-102, SP, and MT-4, are recommended for comprehensive evaluation of 7-geranyloxycoumarin and radiation effects. In addition, future in vivo studies and clinical trials are necessary before introducing 7-geranyloxycoumarin as a safe and effective radiosensitizer agent for ATLL treatment.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This study was financially supported by a grant from Mashhad University of Medical Sciences (No. 970298).

Ethical Approval

This study does not involve human participants and/or animals.

Data Availability

The data that support the findings of this study are included in the manuscript and available from the corresponding author upon reasonable request.

ORCID iD

Ramin Bagheri https://orcid.org/0000-0003-1889-0964

References

1. Qayyum S, Choi JK. Adult T-cell leukemia/lymphoma. *Arch Pathol Lab Med*. 2014;138(2):282-286.
2. Nosaka K, Matsuoka M. Adult T-cell leukemia-lymphoma as a viral disease: Subtypes based on viral aspects. *Cancer Sci*. 2021; 112(5):1688.
3. Tabei S. Human T Lympmphotrophic Virus (HTLV1) related diseases. *Iran Red Crescent Med J*. 2011;13:374.
4. Naderi M, Paryan M, Azadmanesh K, Rafatpanah H, Rezvan H, Mirab Samiee S. Design and development of a quantitative real time PCR assay for monitoring of HTLV-1 provirus in whole blood. *J Clin Virol*. 2012;53:302-307.
5. Yamashiro T, Kamiya H, Miyara T, et al. CT scans of the chest in carriers of human T-cell lymphotropic virus type 1: Presence of interstitial pneumonia. *Acad Radiol*. 2012;19:952-957.
6. Rafatpanah H, Hedayati-Moghaddam MR, Fathimoghadam F, Bidkhori HR, Shamsian SK, Ahmadi S. High prevalence of HTLV-I infection in Mashhad, Northeast Iran: A population-based seroepidemiology survey. *J Clin Virol*. 2011;52:172-176.
7. Hedayati-Moghaddam MR, Fathimoghadam F, Eftekharzadeh Mashhadi I, Soghandi L, Bidkhori HR. Epidemiology of HTLV-1 in Neyshabour, Northeast of Iran. Iran. *Red Crescent Med J*. 2011;13:424-427.
8. Van Tienen C, van der Loeff MFS, Peterson I, et al. Research HTLV-1 in rural Guinea-Bissau: Prevalence, incidence and a continued association with HIV between 1990 and 2007. *Retrovirology*. 2010;8:12.
9. Souza VG, Martins ML, Carneiro-Proietti ABF, et al. High prevalence of HTLV-1 and 2 viruses in pregnant women in Sao Luis, state of Maranh ao, Brazil. *Rev Soc Bras Med Trop*. 2012; 45:159-162.
10. Yared JA, Kimball AS. Optimizing management of patients with adult T cell leukemia-lymphoma. *Cancers*. 2015;7:2318-2329.
11. Bittencourt AL, Vieira MG, Brites CR, Farre L, Barbosa HS. Adult T-cell leukemia/lymphoma in Bahia, Brazil: analysis of prognostic factors in a group of 70 patients. *Am J Clin Pathol*. 2007;128:875-882.
12. Mesnard JM, Barbeau B, Césaire R, Péloponèse JM. Roles of HTLV-1 basic zip factor (HBZ) in viral chronicity and leukemic transformation. Potential new therapeutic approaches to prevent and treat HTLV-1-related diseases. *Viruses*. 2015;7(12): 6490-6505.
13. Ishikawa T. Current status of therapeutic approaches to adult T-cell leukemia. *Int J Hematol*. 2003;78(4):304-311.
14. Yahalom J. Radiotherapy of follicular lymphoma: updated role and new rules. *Curr Treat Options Oncol*. 2014;15(2):262-268.
15. Genovese S, Epifano F. Auraptene: A natural biologically active compound with multiple targets. *Curr Drug Targets*. 2011;12: 381-386.
16. Tanaka T, de Azevedo MB, Durán N, et al. Colorectal cancer chemoprevention by 2 beta-cyclodextrin inclusion compounds of auraptene and 4'-geranyloxyferulic acid. *Int J Cancer*. 2010; 126:830-840.
17. Kohno H, Suzuki R, Curini M, et al. Dietary administration with prenyloxycoumarins, auraptene and collinin, inhibits colitis-related colon carcinogenesis in mice. *Int J Cancer*. 2006;118: 2936-2942.
18. Krishnan P, Yan KJ, Windler D, et al. Citrus auraptene suppresses cyclin D1 and significantly delays N-methyl nitrosourea induced mammary carcinogenesis in female Sprague-Dawley rats. *BMC Cancer*. 2009;9:259.
19. Moussavi M, Haddad F, Rassouli FB, Iranshahi M, Soleymanifard S. Synergy between auraptene, ionizing

radiation, and anticancer drugs in colon adenocarcinoma cells. *Phytother Res*. 2017;31:1369-1375.
20. Saboor-Maleki S, Rassouli FB, Matin MM, Iranshahi M. Auraptene attenuates malignant properties of esophageal stem-like cancer cells. *Technol Cancer Res Treat*. 2017;16:519-527.
21. Salari H, Afkhami-Poostchi A, Soleymanifard S, et al. coad-ministration of auraptene and radiotherapy; a novel modality against colon carcinoma cells in vitro and in vivo. *Int J Radiat Biol*. 2020;96:1051-1059.
22. Movaffagh J, Salari H, Merajifar E, et al. 7-geranyloxycoumarin enhanced radiotherapy effects on human gastric adenocarcinoma cells. *J Cancer Res Ther*. 2021;17(4):1-6.
23. Askari M, Sahebkar A, Iranshahi M. Synthesis and purification of 7-prenyloxycoumarins and herniarin as bioactive natural coumarins. *Iran J Basic Med Sci*. 2009;12:63-69.
24. Miyoshi I, Kubonishi I, Yoshimoto S, et al. Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. *Nature*. 1981;294:770-771.
25. Chen C, Zhao S, Karnad A, Freeman JW. The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol*. 2018;11(1):1-23.
26. Tsubouchi K, Minami K, Hayashi N, et al. The CD44 standard isoform contributes to radioresistance of pancreatic cancer cells. *J Radiat Res*. 2017;58(6):816-826.
27. Wu CT, Lin WY, Chang YH, Chen WC, Chen MF. Impact of CD44 expression on radiation response for bladder cancer. *J Cancer*. 2017;8(7):1137.
28. Sahlberg SH, Spiegelberg D, Glimelius B, Stenerlöw B, Nestor M. Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells. *PLoS One*. 2014;9(4):e94621.
29. Xiao W, Graham PH, Power CA, Hao J, Kearsley JH, Li Y. CD44 is a biomarker associated with human prostate cancer radiation sensitivity. *Clin Exp Metastasis*. 2012;29(1):1-9.
30. Liu WH, Lin JC, Chou YC, Li MH, Tsai JT. CD44-associated radioresistance of glioblastoma in irradiated brain areas with optimal tumor coverage. *Cancer Med*. 2020;9(1):350-360.
31. Kazemi M, Kouhpeikar H, Delbari Z, et al. Combination of auraptene and arsenic trioxide induces apoptosis and cellular accumulation in the subG1 phase in adult T-cell leukemia cells. *J Basic Med Sci*. 2021;24(12):1643-1649.
32. Gravina GL, Festuccia C, Popov VM, et al. c-Myc sustains transformed phenotype and promotes radioresistance of embryonal rhabdomyosarcoma cell lines. *Radiat Res*. 2016;185(4): 411-422.
33. Rodrigues PMG, De Souza RS, Borges HL, Martins RAP. Regulation of c-Myc and NBS1 by ionizing radiation in glioblastoma cells. *Oncology Signaling*. 2018;1(1):1-5.
34. Fan Y, Jia X, Xie T, Zhu L, He F. Radiosensitizing effects of c-myc gene knockdown-induced G2/M phase arrest by intrinsic stimuli via the mitochondrial signaling pathway. *Oncol Rep*. 2020;44(6):2669-2677.
35. Wang R, Chen DQ, Huang JY, et al. Acquisition of radioresistance in docetaxel-resistant human lung adenocarcinoma cells is linked with dysregulation of miR-451/c-Myc-survivin/rad-51 signaling. *Oncotarget*. 2014;5(15):6113.
36. Luo A, Wang W, Sima N, et al. Short hairpin RNA targeting c-FLIP sensitizes human cervical adenocarcinoma Hela cells to chemotherapy and radiotherapy. *Cancer letters*. 2008; 271(2):323-332.
37. McLaughlin KA, Nemeth Z, Bradley CA, et al. FLIP: A targetable mediator of resistance to radiation in non-small cell lung cancer. *Mol Cancer Ther*. 2016;15:2432-2441.
38. Jun DY, Kim JS, Park HS, et al. Apoptogenic activity of auraptene of Zanthoxylum schinifolium toward human acute leukemia Jurkat T cells is associated with ER stress-mediated caspase-8 activation that stimulates mitochondria-dependent or -independent caspase cascade. *Carcinogenesis*. 2007;28(6): 1303-1313.
39. Vonlanthen S, Heighway J, Altermatt HJ, et al. The bmi-1 oncoprotein is differentially expressed in non-small cell lung cancer and correlates with INK4A-ARF locus expression. *Br J Cancer*. 2001;84:1372-1376.
40. Kim JH, Yoon SY, Kim CN, et al. The Bmi-1 oncoprotein is overexpressed in human colorectal cancer and correlates with the reduced p16INK4a/p14ARF proteins. *Cancer Lett*. 2004; 203:217-224.
41. Silva J, García V, García JM, et al. Circulating Bmi-1 mRNA as a possible prognostic factor for advanced breast cancer patients. *Breast Cancer Res*. 2007;9:R55.
42. Dong Q, Oh JE, Chen W, et al. Radioprotective effects of Bmi-1 involve epigenetic silencing of oxidase genes and enhanced DNA repair in normal human keratinocytes. *J Invest Dermatol*. 2011;131:1216-1225.
43. Griffith J, Andrade D, Mehta M, et al. Silencing BMI1 radiosensitizes human breast cancer cells by inducing DNA damage and autophagy. *Oncol Rep*. 2017;37(4):2382-2390.
44. Ye L, Wang C, Yu G, et al. Bmi-1 induces radioresistance by suppressing senescence in human U87 glioma cells. *Oncol Lett*. 2014;8(6):2601-2606.
45. Wang G, Liu L, Sharma S, et al. Bmi-1 confers adaptive radioresistance to KYSE-150R esophageal carcinoma cells. *Biochemical and biophysical research communications*. 2012; 425(2):309-314.
46. Shen ZT, Chen Y, Huang GC, Zhu XX, Wang R, Chen LB. Aurora-a confers radioresistance in human hepatocellular carcinoma by activating NF-κB signaling pathway. *BMC Cancer*. 2019;19(1):1-14.
47. Qiao Q, Sun C, Han C, Han N, Zhang M, Li G. Endoplasmic reticulum stress pathway PERK-eIF 2α confers radioresistance in oropharyngeal carcinoma by activating NF-κB. *Cancer Sci*. 2017;108(7):1421-1431.
48. Park M, Yoon HJ, Kang MC, Kwon J, Lee HW. PTK7 regulates radioresistance through nuclear factor-kappa B in esophageal squamous cell carcinoma. *Tumour Biol*. 2016;37(10): 14217-14224.
49. Kucharczak J, Simmons MJ, Fan Y, Gélinas C. To be, or not to be: NF-kappaB is the answer–role of Rel/NF-kappaB in the regulation of apoptosis. *Oncogene*. 2003;22(56):8961-8982.

50. Gudkov AV, Komarova EA. The role of P53 in determining sensitivity to radiotherapy. *Nat Rev Cancer*. 2003;3:117-129.
51. Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer*. 2009;9:749-758.
52. Cuddihy AR, Bristow RG. The p53 protein family and radiation sensitivity: Yes or no? *Cancer Metastasis Rev*. 2004;23: 237-257.
53. Kong X, Yu D, Wang Z, Li S. Relationship between p53 status and the bioeffect of ionizing radiation. *Oncol Lett*. 2021;22(3): 1-8.
54. Sasaki A, Udaka Y, Tsunoda Y, et al. Analysis of p53 and miRNA expression after irradiation of glioblastoma cell lines. *Anticancer research*. 2012;32(11):4709-4713.
55. Gomes AR, Abrantes AM, Brito AF, et al. Influence of P53 on the radiotherapy response of hepatocellular carcinoma. *Clin Mol Hepatol*. 2015;21(3):257.
56. Huang EH, Hynes MJ, Zhang T, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res*. 2009;69(8):3382-3389.
57. Singh S, Brocker C, Koppaka V, et al. Aldehyde dehydrogenases in cellular responses to oxidative/electrophilicstress. *Free Rad Biol Med*. 2013;56:89-101.
58. Wiman KG. p53 talks to PARP: the increasing complexity of p53-induced cell death. *Cell Death Dif*. 2013;20(11):1438-1439.
59. Lv Q, Zhang J, Yi Y, et al. Proliferating cell nuclear antigen has an association with prognosis and risks factors of cancer patients: a systematic review. *Mol Neurobiol*. 2016;53(9): 6209-6217.
60. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: A review. *J Cell Physiol*. 2019; 234(5):5683-5699.
61. Meloche S, Pouysségur J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene*. 2007;26(22):3227-3239.
62. Fogarty LC, Flemmer RT, Geizer BA, et al. Mcl-1 and Bcl-xL are essential for survival of the developing nervous system. *Cell Death Dif*. 2019;26(8):1501-1515.
63. Gores GJ, Kaufmann SH. Selectively targeting Mcl-1 for the treatment of acute myelogenous leukemia and solid tumors. *Genes Dev*. 2012;26(4):305-311.
64. Lee JC, Shin EA, Kim B, et al. Auraptene induces apoptosis via myeloid cell leukemia 1-mediated activation of caspases in PC3 and DU145 prostate cancer cells. *Phytother Res*. 2017;31(6): 891-898.
65. Sekiguchi H, Irie K, Murakami A. Suppression of CD74 expression and Helicobacter pylori adhesion by auraptene targeting serum starvation-activated ERK1/2 in NCI-N87 gastric carcinoma cells. *Biosci Biotech Biochem*. 2010; 74(5):1018-1024.