Journal of the Formosan Medical Association (2016) xx, 1—7

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Chemotherapeutic effects of luteolin on radio-sensitivity enhancement and interleukin-6/signal transducer and activator of transcription 3 signaling repression of oral cancer stem cells

Dom-Gene Tu [a,b,c,i], Wei-Ting Lin [d,i], Cheng-Chia Yu [e,f,g,i], Shiuan-Shinn Lee [h], Chih-Yu Peng [e,f], Taichen Lin [e,f,*], Chuan-Hang Yu [e,f,*]

[a] Department of Nuclear Medicine, Ditmanson Medical Foundation, Chia-Yi Christian Hospital, Chia-Yi, Taiwan
[b] Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
[c] College of Health Sciences, Chang Jung Christian University, Tainan, Taiwan
[d] Department of Oral and Maxillofacial Surgery, Ditmanson Medical Foundation Chia-Yi Christian Hospital, Chia-Yi, Taiwan
[e] School of Dentistry, Chung Shan Medical University, Taichung, Taiwan
[f] Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan
[g] Institute of Oral Sciences, Chung Shan Medical University, Taichung, Taiwan
[h] School of Public Health, Chung Shan Medical University, Taichung, Taiwan

Received 19 July 2016; received in revised form 18 August 2016; accepted 22 August 2016

KEYWORDS
cancer stem cells;
luteolin;
oral carcinomas;
radio-sensitivity

Background/Purpose: Previously, we successfully identified oral cancer stem cells (OCSC) displaying enhanced stemness and tumorigenic potentials. In the study, we investigated the chemotherapeutic effect of the flavonoid luteolin, commonly found in fruits and vegetables, on targeting OCSC.
Methods: Oralspheres was applied to isolate OCSC. aldehyde dehydrogenase 1 activity and CD44 positivity of OCSC with luteolin treatment were assessed by flow cytometry analysis. Radio-sensitivity of OCSC treated with luteolin was examined. Invasion and colony-forming

Conflicts of interest: The authors have no conflicts of interest relevant to this article.
* Corresponding authors. School of Dentistry, Chung Shan Medical University, Number 110, Section 1, Jianguo North Road, Taichung 40201, Taiwan.
E-mail addresses: linperi@tmd.ac.jp (T. Lin), tao2008@csmu.edu.tw (C.-H. Yu).
[i] These authors contributed equally to the results of this study.

http://dx.doi.org/10.1016/j.jfma.2016.08.009
0929-6646/Copyright © 2016, Formosan Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Tu D-G, et al., Chemotherapeutic effects of luteolin on radio-sensitivity enhancement and interleukin-6/signal transducer and activator of transcription 3 signaling repression of oral cancer stem cells, Journal of the Formosan Medical Association (2016), http://dx.doi.org/10.1016/j.jfma.2016.08.009

assays were performed to assess oncogenicity in OCSC. The expression of interleukin-6 (IL-6)/ signal transducer and activator of transcription 3 (STAT3) was examined by enzyme-linked immunosorbent assay and western blot analysis.
Results: We showed that luteolin effectively inhibited the proliferation rate, self-renewal, aldehyde dehydrogenase 1 activity, and CD44 positivity of OCSC but did not cause significant cytotoxicity of normal epithelial cells. Moreover, luteolin restored radio-sensitivity in OCSC. Combined treatment with luteolin and radiation displayed synergistic effect on invasiveness and clonogenicity of OCSC. Mechanistically, treatment of luteolin resulted in inactivation of IL-6/STAT3 signaling.
Conclusion: These results suggest that combined treatment of luteolin and radiation therapy can attenuate tumorigenicity of OCSC through IL-6/STAT3 signaling inactivation.
Copyright © 2016, Formosan Medical Association. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

The cancer stem cells theory postulates that a subpopulation of cells termed cancer stem cells (CSC) or tumor-initiating cells, drive tumor initiation, radio-resistance, progression, and high rate of relapse and metastasis; thus development of a novel approach targeting CSC is imperative.[1–5] Our group has previously identified a functional subset of oral CSC (OCSC) in oral squamous cell carcinomas (OSCC) marked by oralspheres,[6] CD133,[7] aldehyde dehydrogenase (ALDH),[3] membrane 78 kDa glucose-regulated protein (GRP78),[8] or side population.[9] We also demonstrated that these OCSC display enhanced tumorigenic potential *in vitro* and *in vivo*. Screening novel compounds for drug candidates that targets the CSC of OSCC specifically will be instrumental for future advanced OSCC therapy.

Luteolin (3′,4′,5′,7′-tetrahydroxyflavone), belonging to the flavone subclass, is found in plants such as chamomile tea, celery, perilla leaf, and green peppers.[10] Through a variety of experimental cancer models, luteolin has been found to possess pleiotropic antineoplastic activity including stimulation of cancer cell apoptosis, cell cycle arrest, repression of cancer cell proliferation, suppressing angiogenesis, and metastasis capacity.[11–13] Evidence suggests that luteolin inhibits proliferation and induces apoptosis of cancer cells via protein kinase B/Akt, p38, c-Jun N-terminal kinases, or nuclear factor-κB signaling.[14–17] Luteolin can reverse multidrug resistance in a variety of cancer cells.[16] Luteolin chemosensitizes ovarian cancer cells to paclitaxel through repression of epithelial–mesenchymal transition markers and traits.[18] Luteolin inhibits the metastasis of cancer cells *in vitro* and *in vivo* through inhibiting epithelial–mesenchymal transition, integrin β1, or focal adhesion kinase.[19] However, the efficacy of luteolin on the specific subset of OCSC has not been addressed. In this study, we investigated whether luteolin possesses anti-CSC effect and whether interleukin-6 (IL-6)/ signal transducer and activator of transcription 3 (STAT3) signaling is involved in luteolin-targeted CSCs in OSCC cells.

Materials and methods

Cell culture and reagents

Normal human gingival epithelioid S-G cells and OSCC cells lines (SAS and GNM) were cultivated as previously described.[20] Luteolin was purchased from Sigma–Aldrich (St Louis, MO, USA).

Cell proliferation determination by MTT assay

Cells were plated in wells of 96-well-plate as 1×10^4 cells/well in 0.1% dimethyl sulfoxide (DMSO) or different concentrations of luteolin-containing medium and cultured at 37°C for 24 hours. Cell proliferation/survival was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The 570 nm absorbance of the DMSO-treated group was set as 100% and data are presented as percentage of DMSO control.[21]

Oralsphere-forming assay

OSCC cells will be dissociated and cultured as oralspheres in modified Dulbecco's modified Eagle medium/F-12 supplemented with N-2 (R&D, Minneapolis, MN, USA), 10 ng/mL epidermal growth factor (EGF; Invitrogen, Carlsbad, CA, USA), 10 ng/mL basic fibroblast growth factor (bFGF; Invitrogen), and penicillin/streptomycin at 10^3 live cells/low-attachment six-well plate (Corning Inc., Corning, NY, USA), with the medium changed every other day until oralsphere formation was observed in about 2 weeks. For serial passage of oralsphere cells, single cells were obtained from Accutase (Sigma–Aldrich)-treated spheroids at a cell density of passage of 1000 cells/mL in the serum-free medium described above.[2]

Flow cytometry analysis for cancer stemness marker

The ALDEFLUOR kit (Stem Cell Technologies, Durham, NC, USA) was used to examine the ALDH enzymatic activity

Please cite this article in press as: Tu D-G, et al., Chemotherapeutic effects of luteolin on radio-sensitivity enhancement and interleukin-6/signal transducer and activator of transcription 3 signaling repression of oral cancer stem cells, Journal of the Formosan Medical Association (2016), http://dx.doi.org/10.1016/j.jfma.2016.08.009

Download English Version:

https://daneshyari.com/en/article/5680083

Download Persian Version:

https://daneshyari.com/article/5680083

Daneshyari.com