Phytomedicine 23 (2016) 566–577

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Resveratrol inhibits STAT3 signaling pathway through the induction of SOCS-1: Role in apoptosis induction and radiosensitization in head and neck tumor cells

Seung Ho Baek[a,1], Jeong-Hyeon Ko[a,1], Hanwool Lee[a], Jinhong Jung[b], Moonkyoo Kong[b], Jung-woo Lee[c], Junhee Lee[a], Arunachalam Chinnathambi[d], ME Zayed[d], Sulaiman Ali Alharbi[d], Seok-Geun Lee[a], Bum Sang Shim[a], Gautam Sethi[d,e], Sung-Hoon Kim[a], Woong Mo Yang[a], Jae-Young Um[a], Kwang Seok Ahn[a,*]

[a] College of Korean Medicine, Kyung Hee University, 24 Kyungheedae-ro, Dongdaemun-gu, Seoul 130-701, Republic of Korea
[b] Department of Radiation Oncology, Kyung Hee University Medical Center, Kyung Hee University School of Medicine, 23 Kyungheedae-ro, Dongdaemoon-gu, Seoul 130-872, Republic of Korea
[c] Department of Oral & Maxillofacial Surgery, Kyung Hee University Dental Hospital, Kyung Hee University School of Dentistry, 23 Kyungheedae-ro, Dongdaemun-gu, Seoul 130-872, Republic of Korea
[d] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[e] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 117600, Singapore

ARTICLE INFO

Article history:
Received 8 December 2015
Revised 12 February 2016
Accepted 15 February 2016

Keywords:
Resveratrol
STAT3
Squamous cell carcinoma of the head and neck
SOCS-1

ABSTRACT

Background: Signal transducer and activator of transcription 3 (STAT3) is persistently activated in squamous cell carcinoma of the head and neck (SCCHN) and can cause uncontrolled cellular proliferation and division.
Hypothesis: Thus, its targeted abrogation could be an effective strategy to reduce the risk of SCCHN. Resveratrol is known for its anti-cancer efficacy in a variety of cancer models.
Study design: The effect resveratrol on STAT3 activation, associated protein kinases, phosphatases, cellular proliferation and apoptosis was investigated.
Methods: We evaluated the effect of resveratrol on STAT3 signaling cascade and its regulated functional responses in SCCHN cells.
Results: We found that HN3 and FaDu cells expressed strongly phosphorylated STAT3 on both tyrosine 705 and serine 727 residues as compared to other SCCHN cells. *The phosphorylation was completely suppressed by resveratrol in FaDu cells, but not substantially in HN3 cells.* STAT3 suppression was mediated through the inhibition of activation of upstream JAK2, but not of JAK1 and Src kinases. Treatment with the protein tyrosine phosphatase (PTP) inhibitor pervanadate reversed the resveratrol-induced down-regulation of STAT3, thereby indicating a critical role for a PTP. We also found that resveratrol induced the expression of the SOCS-1 protein and mRNA. Further, deletion of *SOCS-1* gene by siRNA suppressed the induction of SOCS-1, and reversed the inhibition of STAT3 activation. Resveratrol down-regulated various STAT3-regulated gene products, inhibited proliferation, invasion, as well as induced the cell accumulation in the sub-G1 phase and caused apoptosis. Beside, this phytoalexin also exhibited the enhancement of apoptosis when combined with ionizing radiation treatment.
Conclusion: Our results suggest that resveratrol blocks STAT3 signaling pathway through induction of SOCS-1, thus attenuating STAT3 phosphorylation and proliferation in SCCHN cells.

Introduction

Head and neck cancer is a malignancy that starts in the lip, oral cavity (mouth), nasal cavity (inside the nose), paranasal sinuses, pharynx, and larynx. 90% of head and neck cancers are squamous cell carcinomas, so they are often referred to as head and neck

Abbreviations: ECL, enhanced chemiluminescence; HRP, horseradish peroxidase; ICC, immunocytochemistry; JAK, Janus tyrosine kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PTPs, protein tyrosine phosphatases; RTCA, real-time cell analysis; SCCHN, squamous cell carcinoma of the head and neck; SDS-PAGE, sodium dodecyl-polyacrylamide gel electrophoresis; SOCS, suppressor of cytokine signaling; STAT3, signal transducer and activator of transcription 3.

* Corresponding author. Tel.: +82 2 961 2316.
E-mail address: ksahn@khu.ac.kr (K.S. Ahn).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.phymed.2016.02.011
0944-7113/

squamous cell carcinomas (HNSCC). It is the sixth most common cancer worldwide and eighth leading cause of mortality (Parfenov et al. 2014), with approximately 600,000 new cases diagnosed each year (Jemal et al. 2009). Although cancer treatment regimens have been advanced, up to 50% of HNSCC patients will experience recurrence or residual *disease* even after *therapy* and the median survival rate is less than 1 year (Cooper et al. 2004). Approximately 80% of HNSCC also exhibit up-regulation of persistent STAT3 expression, which can mediate radioresistance and chemoresistance. The hyperactivation of STAT3 in response to the aberrant activation of upstream receptor signals is frequently observed in a variety of human cancers, including head and neck cancer (Leong et al. 2003; Yin et al. 2010).

Signal transducer and activator of transcription 3 (STAT3) is an oncogenic transcription factor that transmits signals from cytokines and growth-factor receptors to the nucleus (Yu and Jove 2004). The aberrant STAT3 activation promotes the growth and survival of tumor cells through the modulation of cell cycle regulators (e.g., cyclin D1/D2 and c-Myc), upregulation of anti-apoptotic proteins (e.g., Mcl-1, Bcl-xL, and Survivin), downregulation of the tumor suppressor p53, and induction of angiogenesis by vascular endothelial growth factor (VEGF); these oncogenic overexpression eventually lead to tumor progression and resistance to chemotherapy and radiotherapy (Song and Grandis 2000; Yu and Jove 2004).

The suppressor of cytokine signaling (SOCS) proteins make up a family of intracellular proteins (Kubo et al. 2003; Yoshimura et al. 2007). There are seven SOCS family proteins: SOCS-1, SOCS-2, SOCS-3, SOCS-4, SOCS-5, SOCS-6, and SOCS-7, each of which has a central SH2 domain, an amino-terminal domain of variable length and sequence, and a carboxy-terminal 40-amino-acid module known as the SOCS box (Tamiya et al. 2011). SOCS has been reported to inhibit STAT phosphorylation by binding and inhibiting JAKs or competing with STATs for phosphotyrosine binding sites on cytokine receptors (Krebs and Hilton 2001). Specifically, the SH2 domain of SOCS-1 directly binds to the activation loop of JAKs (Yasukawa et al. 1999). The SH2 domains of SOCS-2 and SOCS-3 bind to phosphorylated tyrosine residues on activated cytokine receptors (Kubo et al. 2003). SOCS-3 binds to gp130-related cytokine receptors, including the phosphorylated tyrosine 757 (Tyr757) residue of gp130, the Tyr800 residue of IL-12 receptor β2, and Tyr985 of the leptin receptor (Lehmann et al. 2003; Sasaki et al. 2000). Additionally, both SOCS-1 and SOCS-3 can block JAK tyrosine kinase activity directly via their kinase inhibitory regions (Yasukawa et al. 1999).

Resveratrol(3,5,4′-trihydroxy-*trans*-stilbene), a stilbenoid, is a phytoalexin produced naturally by several plants in response to injury or when the plant is constantly under attack by pathogens such as bacteria or fungi (Fremont 2000). Resveratrol is found in the skin of grapes, blueberries, raspberries, and mulberries. Resveratrol has been reported to exhibit a variety of biological activities including related to the inhibition of lipid peroxidation (Berrougui et al. 2009), free radical scavenging (Leonard et al. 2003), and the suppression of platelet aggregation (Stef et al. 2006). It can also exert anti-inflammatory effects (Bognar et al. 2013), vasorelaxing activity (Fitzpatrick et al. 1993), estrogenic activity (Klinge et al. 2003), and antineoplastic activity (Aggarwal et al. 2004) against a wide variety of disease states. Wung et al. first showed that resveratrol suppressed STAT3 phosphorylated at Tyr705 residue in IL-6-treated endothelial cells (Wung et al. 2005). Also, resveratrol exerted pro-apoptotic effects via suppression of STAT3 signaling against human prostate cancer DU145 cells and v-Src-transformed mouse fibroblasts (NIH3T3/v-Src) (Kotha et al. 2006), human multiple myeloma U266 cells (Bhardwaj et al. 2007), medulloblastoma cells (Yu et al. 2008), leukemia cells (Li et al. 2010a), malignant natural killer cells (Quoc Trung et al. 2013), human and murine melanoma cells (Habibie et al. 2014), and ovarian cancer cells (Zhong et al. 2015). Moreover, the effect of resveratrol in combination with irradiation and chemotherapy in Merkel cell carcinoma has been reported (Heiduschka et al. 2014). In addition, the chemopreventive potential of resveratrol in head and neck squamous cell carcinoma has also been observed (Shrotriya et al. 2015). Although resveratrol has been found to mitigate aberrant activation of STAT3 in various cell lines, our study is the first one to explore the effects of resveratrol both on STAT3 signaling cascades and on the negative regulators of phosphorylated STAT3 (SOCS-1) in HNSCC cells.

Along with standard treatments (such as surgery and chemotherapy), *radiation therapy is one of the most important modalities* for the *treatment* of head and neck *cancer because* the treatment modality generally consists of surgery with postoperative radiation therapy in patients (Vokes 1997). Unfortunately, the use of radiotherapy is largely limited by intrinsic or acquired resistance to ionizing radiation (IR). In an effort to overcome the radioresistance of cancer cells to improve the therapeutic efficacy of radiotherapy, a variety of phytochemicals have been examined for their potential radiosensitizing effects. For instance, curcumin (Chendil et al. 2004; Qian et al. 2015), resveratrol (Fang et al. 2012; Zoberi et al. 2002), genistein (Liu et al. 2013), luteolin (Cho et al. 2015), shikonin (Kwak et al. 2014), and rosmarinic acid (Alcaraz et al. 2014) have been reported to possess radiosensitizing effects on a variety of tumor cells. A significant amount of scientific evidence indicates that the blockage of STAT3 enhances radiation sensitivity in various tumor cells (Choe et al. 2015; Gao et al. 2010; Li et al. 2010b). To the best of our knowledge, the effect of resveratrol on radiosensitization of head and neck cancer cells has not yet been reported. We found that resveratrol suppressed constitutive and STAT3 activation through induction of SOCS-1; down-regulated STAT3-regulated gene products; and potentiated IR-induced apoptotic effects in the SCCHN cell line FaDu.

Materials and methods

Reagents

Resveratrol, with a purity greater than 99%, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Tris base, glycine, NaCl, sodium dodecylsulfate (SDS), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). RPMI 1640 and fetal bovine serum (FBS) were obtained from Thermo Fisher Scientific Inc. (Waltham, MA). Annexin V was purchased from BD Biosciences (Palo Alto, CA). Anti-p-STAT3 (Tyr705 and Ser727), anti-p-JAK2, anti-p-JAK1, anti-JAK2, anti-JAK1, anti-p-Src, anti-Src, anti-Cyclin D1, anti-cleaved caspase-3, anti-caspase9, and anti-cleaved caspase-9 were purchased from Cell Signaling Technology (Beverly, MA). Anti-STAT3, anti-Lamin B, anti-SOCS-1, anti-Bcl-2, anti-Bcl-xL, anti-Survivin, anti-IAP-1, anti-VEGF, anti-MMP-9, anti-MMP-2, anti-caspase-3, anti-PARP, anti-β-actin, and horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell lines

The human head and neck squamous cell carcinoma (HNSCC) SCC4 and FaDu cells were obtained from the American Type Culture Collection (Manassas, VA), YD-8 and YD-10B cells were obtained from Korean Cell Line Bank (Seoul, Korea), HN3, HN9, LN686, and J17AR cells were provided by Dr. Sang-wook Lee (ASAN Medical Center, Korea), immortal human keratinocyte (HaCaT) cells were provided by Cell Lines Service (Eppelheim, Germany). SCC4 cells were cultured in DMEM containing 10% FBS, vitamin solution,