Resveratrol Inhibits IL-6-Induced Transcriptional Activity of AR and STAT3 in Human Prostate Cancer LNCaP-FGC Cells

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Abstract
Prostate cancer is the most frequently diagnosed cancer. Although prostate tumors respond to androgen ablation therapy at an early stage, they often acquire the potential of androgen-independent growth. Elevated transcriptional activity of androgen receptor (AR) and/or signal transducer and activator of transcription-3 (STAT3) contributes to the proliferation of prostate cancer cells. In the present study, we examined the effect of resveratrol, a phytoalexin present in grapes, on the reporter gene activity of AR and STAT3 in human prostate cancer (LNCaP-FGC) cells stimulated with interleukin-6 (IL-6) and/or dihydrotestosterone (DHT). Our study revealed that resveratrol suppressed the growth of LNCaP-FGC cells in a time- and concentration-dependent manner. Whereas the AR transcriptional activity was induced by treatment with either IL-6 or DHT, the STAT3 transcriptional activity was induced only by treatment with IL-6 but not with DHT. Resveratrol significantly attenuated IL-6-induced STAT3 transcriptional activity, and DHT- or IL-6-induced AR transcriptional activity. Treatment of cells with DHT plus IL-6 significantly increased the AR transcriptional activity as compared to DHT or IL-6 treatment alone and resveratrol markedly diminished DHT plus IL-6-induced AR transcriptional activity. Furthermore, the production of prostate-specific antigen (PSA) was decreased by resveratrol in the DHT-, IL-6- or DHT plus IL-6-treated LNCaP-FGC cells. Taken together, the inhibitory effects of resveratrol on IL-6- and/or DHT-induced AR transcriptional activity in LNCaP prostate cancer cells are partly mediated through the suppression of STAT3 reporter gene activity, suggesting that resveratrol may be a promising therapeutic choice for the treatment of prostate cancer.

Key Words: Resveratrol, AR transcriptional activity, STAT3 transcriptional activity, Prostate cancer

INTRODUCTION
Prostate cancer is the most frequently diagnosed cancer among the American men and is the second largest cause of cancer-related mortality in the United States (Siegel et al., 2014). While the radical prostatectomy and radiotherapy are the potential therapies for localized prostate tumors, success of these therapeutic modalities in malignant prostate cancer is poor (Ratan et al., 2002). Although prostate tumors respond to hormone ablation therapy at early stage, they often acquire the potential of hormone-independent growth (Laufer et al., 2000; Walsh et al., 2007; Bommareddy et al., 2013).

One of the most exciting strategies to reduce the burden of prostate cancer is the use of chemopreventive agents (Ratan et al., 2002; Bommareddy et al., 2013; Wang et al., 2013). Resveratrol (3,5,4’-trihydroxy-trans-stilbene), a phytoalexin abundantly present in grapes and other medicinal plants, has been extensively investigated for its chemopreventive and chemotherapeutic potential (Shankar et al., 2007; Kundu and Surh, 2008). Previous studies have shown that resveratrol inhibits the androgen-dependent or -independent growth of prostate cancer cells (Mitchell et al., 1999; Harada et al., 2007). Kotha et al. reported that resveratrol induced apoptosis in human prostate cancer (DU145) cells by blocking STAT3 signaling pathway (Kotha et al., 2006). Others have shown...
that resveratrol attenuates the proliferation of LNCaP prostate cancer cells by downregulating AR protein expression following transcriptional (Mitchell et al., 1999) or post-translational (Harada et al., 2007) mechanisms. However, a molecular link between the inhibitory effect of resveratrol on STAT3 signaling and the downregulation of AR has not been established. Since the cytokine interleukin-6 (IL-6) promotes tumor cell proliferation by activating STAT3 signaling (Guo et al., 2012), we sought to examine whether resveratrol can attenuate IL-6-induced AR transcriptional activity in LNCaP-FGC cells. Moreover, the role of IL-6 in AR activation has not been reported yet. We, therefore, examined the IL-6, in presence or absence of AR ligand dihydrotestosterone (DHT), in inducing the AR transcriptional activity, and its possible modulation by resveratrol in LNCaP-FGC cells.

MATERIALS AND METHODS

Chemicals and reagents
Resveratrol (purity >99%), dimethylsulfoxide and MTT [3-(4, 5-dimethyl-thiazolyl-2)-2, 5-diphenyl-tetrazolium bromide] were procured from Sigma (St. Louis, MO, USA). IL-6 and DHT were purchased from R&D systems (Minneapolis, MN, USA) and TCI (Tokyo, Japan), respectively. RPMI-1640 and fetal bovine serum (FBS) were purchased from Invitrogen (GIBCO, Grand Island, NY, USA).

Cell culture
LNCaP-FGC (human prostate cancer) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and were cytogenetically tested and authenticated before the cells were frozen. Each vial of frozen cells was thawed and maintained in culture for a maximum of 8 weeks. LNCaP-FGC cells were cultured in RPMI 1640 containing penicillin (100 units/mL), streptomycin (100 μg/mL), and 10% FBS or Dextran charcoal coated FBS (DCC-FBS, Clontech). Cells were maintained at 37°C in a 5% CO₂ incubator.

Cell proliferation assay
LNCaP-FGC cells (5,000 cells per well) were plated in 96-well plates and treatment were started after 24 h. Cells were treated with 0, 12.5, 25, 50 or 100 μM resveratrol (dimethylsulfoxide as a vehicle) for 12, 24, 48, or 72 h and cell viability was analyzed using the MTT assay. Values were expressed as the mean ± S.E.M. of at least three independent experiments. Statistical significance was determined by Student’s t-test and a p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Effects of resveratrol on LNCaP-FGC cell growth
Resveratrol has been reported to inhibit the growth of various cancer cells. In the present study, we attempted to examine the effects of resveratrol on the growth of LNCaP-FGC human prostate cancer cells. Treatment of these cells with resveratrol (12.5, 25, 50 or 100 μM) resulted in decreased cell viability in a concentration- and time-dependent fashion (Fig. 1).

Resveratrol inhibited DHT-induced AR transcriptional activity in LNCaP-FGC cells
Since resveratrol suppressed the growth of LNCaP-FGC prostate cancer cells, we sought to examine whether resveratrol can suppress the transcriptional activity of AR in LNCaP cells. We transfected LNCaP-FGC cells with luciferase reporter gene construct containing AR response element and cells were then stimulated with dihydrotestosterone (DHT) (Fig. 2A). We optimized the concentration of DHT to 1 nM. Treatment with DHT (1 nM) induced the AR transcriptional activity, which was attenuated by incubation of cells with resveratrol (Fig. 2B). The transcriptional activity of AR was assessed by corresponding luciferase reporter gene analysis.