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Microarray analysis reveals influence of the sesquiterpene lactone parthenolide on gene transcription profiles in human epithelial cells

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Abstract

Sesquiterpene lactones are known for their anti-inflammatory activity which has been proven in various assays on DNA, mRNA and protein level. Here we report on the change in the gene expression profile in TNF-α stimulated human 293 cells after treatment with parthenolide using a cDNA microarray analysis. Twenty-one of 7028 genes were found to be up- and 18 down-regulated. They encode for chemoattractants, immune system proteins, glycoproteins, metabolism, serine proteinases, and transcription factors. Confirmatory analyses were carried out using quantitative real-time RT-PCR (TaqMan®). Additional studies with selected genes revealed the concentration-dependent influence of parthenolide on the expression of these genes.

Keywords: Sesquiterpene lactones; Parthenolide; Gene transcription; cDNA microarray; Real-time quantitative PCR

Introduction

Sesquiterpene lactones (SLs) are the active constituents of many medicinal plants from the Asteraceae family and possess a variety of biological effects (Schmidt, 1999). In particular, their potent anti-inflammatory and cytotoxic properties have received considerable attention. Their anti-inflammatory activity has been corroborated using various assays and it has been established that one of the main targets inhibited by SLs is the transcription factor NF-κB (Bork et al., 1997; Garcia-Pineres et al., 2001; Hehner et al., 1999; Lyss et al., 1997). This protein plays a pivotal role in controlling the expression of multiple proinflammatory, inflammatory and immune genes involved in diseases, such as asthma, rheumatoid arthritis or cancer (Aggarwal et al., 2004; Bacher and Schmitz, 2004). NF-κB is a dimeric transcription factor formed by the hetero- or homodimerization of proteins of the rel-family (Bonizzi and Karin, 2004). In most cell types, NF-κB dimers are cytoplasmic and retained in an inactive complex by binding to IκB, its inhibitory subunit (Cramer and Muller, 1999; Hoffmann et al., 2002a). Translocation to the nucleus takes place in response to a variety of proinflammatory stimuli. Two major pathways account for translocation of NF-κB dimers from the cytoplasm to the nucleus where they activate target genes by binding with high affinity to κB elements in their promotors (Bonizzi and Karin, 2004; Hoffmann and Baltimore, 2006).

It has been shown that SLs exert most of their biological effects, e.g., cytotoxicity and anti-inflammatory activity, by alkylating biological nucleophiles. The α,β-unsaturated carbonyl structures of SLs, such as α-methylene-γ-lactone and α,β-en-one groups, react with thiol groups of enzymes and proteins via a Michael type addition thus interfering with their functions (Picman et al., 1979; Schmidt, 1999). Recently, we could demonstrate that these structural elements are also involved in inhibiting NF-κB DNA binding, probably by alkylating the p65 subunit (Garcia-Pineres et al., 2001).

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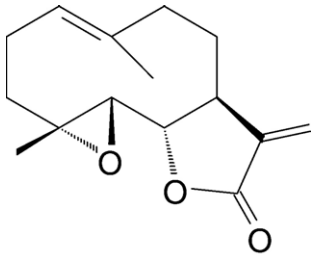

Fig. 1. Structure of parthenolide.

Inhibition of IκB kinase also occurs (Hehner et al., 1999), but this might not be the decisive step in NF-κB inhibition (Garcia-Pineres et al., 2001).

SLs do not only inhibit the DNA binding activity of NF-κB but also influence the NF-κB dependent gene expression (Bork et al., 1997; Li-Weber et al., 2002a; Mazor et al., 2000) and consequently mRNA levels of NF-κB regulated genes, (Li-Weber et al., 2002a; Mazor et al., 2000; Lindenmeyer et al., 2006; Gertsch et al., 2003) as well as the respective protein (Li et al., 2006). Recently, a comprehensive analysis of the effects of the SL parthenolide on gene expression in keratinocytes has been carried out using cDNA microarray technology (Banno et al., 2005). This technique allows the monitoring of the expression of thousands of individual genes including those regulated by NF-κB (Zhou et al., 2003).

Using a 7 K cDNA microarray, we here analyzed gene expression changes in cultures of human 293 cells, a permanent line of primary human embryonal kidney cells, after treatment with the SL parthenolide (for structure see Fig. 1) and stimulation with TNF-α. Some of the results were confirmed using quantitative real-time RT-PCR (TaqMan® analysis). We found distinct alterations in the expression pattern of genes associated with the immune response and with a variety of cellular pathways. This study represents the second global analysis of the transcriptional response of cells to SLs using parthenolide as an example. As SLs may serve as lead structures for the development of therapeutically and cytokine supressing remedies valuable for the treatment of various inflammatory diseases (Wagner et al., 2006), knowledge concerning their effects on genes stimulated by TNF-α and regulated by NF-κB and other transcription factors is important for estimating possible side effects.

Materials and methods

Reagents

Parthenolide was purchased from Sigma-Aldrich, TNF-α from Roche Molecular Biochemicals.

Cell culture

Human 293 cells were maintained in Dulbecco's modified Eagle Medium and supplemented with 10% fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin (all Gibco-BRL).

Microarrays

cDNA microarrays were produced and processed essentially according to the Standford protocol described recently (Eisen and Brown, 2004). In short: approximately 7000 annotated genes from the RZPD (Resource Center and Primary Database, Berlin, Germany) were obtained as bacterial stocks. Plasmids were purified using the Qiagen 96-well Turbo Kit (Qiagen, Hilden, Germany). Inserts were purified by polymerase chain reaction (PCR) using vector primers flanking the individual inserts (5′-CTG CAA GGC GAT TAA GTT GGG TAA C-3′ and 5′-GTG AGC GGA TAA CAA TTT CAC ACA GGA AAC AGC-3′). PCR products were purified by ethanol precipitation and dissolved in H_2O. Aliquots were transferred into 384-well plates, dried and dissolved in 3× standard saline citrate to a final concentration of approximately 40 ng/μl. Printing was performed on aminosilane-coated slides (CMT-GAP II slides, Corning, NY), using an arrayer that was assembled according to specifications by the Stanford group with software provided by J. de Risi (http://cmgm.stanford.edu/pbrown).

Cell treatment and RNA preparation for microarray analysis

293 cells were maintained in Dulbecco's modified eagle medium (Gibco), supplemented with 10% fetal bovine serum (Sigma), 100 IU/ml penicillin and 100 μg/ml streptomycin (Roche Diagnostics). TNF-α was purchased from Roche Diagnostics.

293 cells were plated 48 h prior to treatment with parthenolide (20 μM) at a density of 1.5×10^6 cells per 10 cm-dish in 10 ml medium. Cells were preincubated with parthenolide for 1 h and subsequently stimulated with TNF-α (2 ng/ml) for 1 h. Untreated cells were used as negative control; cells stimulated only with TNF-α as positive control. Cells from four 10 cm-dishes were pooled and homogenized in 4 M guanidinium isothiocyanate with 0.72% β-mercaptoethanol. Total RNA was purified on a caesium chloride gradient. After ethanol precipitation, RNA was dissolved in water and stored at −80 °C.

Hybridization

All 12 hybridizations were performed in the presence of an equal amount of human reference RNA (Stratagene, La Jolla, CA) as recently described (Boldrick et al., 2002). Twelve micrograms of total RNA from 293 cells or reference RNA were transcribed into cDNA in the presence of Cy3-and Cy5-labeled dUTP, respectively, using Superscript II reverse transcriptase (RT) (Invitrogen, Carlsbad, CA). All other steps were performed according to the protocol published by P. Brown et al. (http://cmgm.stanford.edu/pbrown). A cDNA purification kit (Qiagen, Hilden, Germany) was used for cDNA purification after dye labeling.

Data analysis

Signal intensities were measured by an Axon 4000A scanner using GenePix 4.1 software (Axon Instruments Inc., Union City, CA). The experimental design included a color-reversal experiment for every sample to correct for dye-specific effects. The log