Antiproliferation Effects of Curcumin on the STAT5 Signaling Pathway in K562 Cells

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OBJECTIVE Curcumin is the major component of the spice turmeric and the yellow pigment in curry powder. Many studies have shown that curcumin (diferuloylmethane) has significant antiproliferative and apoptotic effects in cancer cells by several mechanisms. Signal transducers and activators of transcription (STAT) proteins are critical in mediating a response in hematopoietic cells. This study was designed to investigate whether curcumin is associated with proteins involved in signal transduction and activation of transcription (STAT) and to investigate the expression of signal transducers and activators of transcription and the significance of the STAT5 signaling pathway by treating K562 cells and cells from CML patients with curcumin.

METHODS The study was divided into the following groups: normal control cells (human bone marrow cells), untreated K562 cells, curcumin treated K562 cells, IFN-γ treated K562 cells, curcumin plus IFN-γ treated K562 cells, and CML patient cells with and without curcumin treatment. Cell proliferation was measured by the MTT assay. The expression of STAT5 mRNA was determined by RT–PCR. The expression of the STAT5 protein was assayed by Western-blotting and the expression of STAT5 in K562 cells was examined under confocal laser–scanning microscopy. The expression of STAT5 mRNA of K562 cells was determined with in situ hybridization. EMSA was used to assess the change in binding of STAT5 with DNA in CML patient cells.

RESULTS The proliferation of the K562 cells and CML primary cells was decreased in the curcumin–treated group and/or IFN-γ group. The expression of STAT5 mRNA and protein were decreased the curcumin–treated group as compared with the K562 untreated group (P<0.01). STAT5 mRNA and protein expression was decreased in the IFN-γ group compared to the untreated K562 group (P<0.01). Combined use of curcumin with IFN-γ inhibited the proliferation of K562 cells and decreased the expression of STAT5 mRNA and protein of the K562 cells. For the CML patient cells, the OD value of STAT5–DNA binding in the curcumin treated cells was less than that compared to untreated cells.

CONCLUSION The antiproliferation effects of curcumin may partly be mediated through signal transduction and activation of transcription and may involve the STAT5 signaling pathway.

KEYWORDS: curcumin, STAT5 signaling pathway, K562 cells.

Curcumin, the major component of the spice turmeric and the yellow pigment in curry powder, has been widely used in India oth-
er parts of Southeast Asia as a spice and a coloring agent in cooking. Many studies have been conducted indicating that curcumin (diferuloylmethane) has significant antiproliferative and apoptotic effects in cancer cells by several mechanisms. Curcumin is under clinical evaluation as a potential cancer chemopreventive agent. Proteins involved in signal transduction and activation of transcription (STAT) are critical in mediating the response to curcumin in hematopoietic cells.

The development of more effective prevention and treatment strategies for solid tumors is limited by an incomplete understanding of the critical growth pathways that are activated in carcinogenesis. Proteins which are signal transducers and activators of transcription have been linked to transformation and tumor progression. Studies to date have not elucidated clear and distinct roles for Stat5 genes in human cancers. The constitutive activation of signal transducers and activators of transcription proteins has been demonstrated in many diverse human cancer cell lines and clinical tumors including acute and chronic leukemias. While STAT activation is a common characteristic of leukemia, the specific pattern of activated STAT and the manner by which STAT activation occurs varies with each disease. In the K562 cell line, BCR/ABL can activate the STAT5 signaling pathway. The effects of curcumin in combination with IFN-γ on STAT5 signaling pathway was studied in K562 cells and cells from CML patients.

### MATERIALS AND METHODS

#### Reagents

Phosphated-STAT5 rabbit polyclonal anti-human antibodies and goat polyclonal anti-rabbit antibodies were purchased from the BD Co. (USA). Tris-Hcl, SDS, DTT, and EDTA etc. were purchased from Sigma (USA). An EMSA kit and Trizol were obtained from Promega. Reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were acquired from Bio-Rad. Curcumin and IFN-γ were purchased from Gibco (USA). Curcumin was dissolved in dimethyl sulfoxide (DMSO), stored at -20°C and thawed only before use. The working curcumin concentration was 25 μmol/L. IFN-γ was dissolved in 0.9% sodium chloride. Its working concentration was 500 U/ml.

#### Primers used for amplification

The primer sequences used for PCR were as follows: the forward primers were 5'-GGAGATCATCTGGCA-GAAC-3' and the reverse primers were 5'-CAGCT-CATTCCACCAAC-3', the product size was 499 bp. β-Actin: the forward primers were 5'-CAGAGCAGAGAGGATCCT-3' and the reverse primers were 5'-GCATAGCACAGCCTGGATAG-3', the product size was 250 bp.

#### Cell culture and grouping

The K562 cell line was obtained from the China Center for Typical Culture Collection (Wuhan, China). Normal human bone marrow cells (NHBMC) were obtained from normal adults and isolated by centrifugation on a Ficoll-Hypaque density gradient. The following groups were formed: a normal control group (human bone marrow cells), a K562 cell group, a curcumin group (K562 cells were treated with curcumin for 24 h), a IFN-γ group (K562 cells were treated with IFN-γ for 24 h), a curcumin plus IFN-γ group (K562 cells were treated with curcumin and IFN-γ for 24 h) and a CML patient group. The cells in groups were grown in RPMI-1640 culture medium containing 10% FCS, 2 mmol/L L-glutamine, at 37°C in a 5% CO₂ incubator.

#### MTT assay

The K562 and NHBMC were adjusted to 4×10⁵/ml and cultured in a 96-well plate at 37°C under 5% CO₂ for 24 h. Depending on the requirements of the research, the assay was divided into the following groups: the normal control group, K562 cell group, curcumin group, IFN-γ group, curcumin plus IFN-γ group and the CML group. The curcumin working concentration was 25 μmol/L and IFN-γ concentration was 500 U/ml. OD values were measured by CliniBio.

#### Reverse transcription–polymerase chain reaction (RT–PCR)