Cellular resistance to vincristine suppresses NF-κB activation and apoptosis but enhances c-Jun-NH₂-terminal protein kinase activation by tumor necrosis factor

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Tumor necrosis factor (TNF) is a pleiotropic cytokine that potentiates the cytotoxic effects of chemotherapeutic drugs. Although emergence of resistance to chemotherapeutic drugs is a major problem in cancer therapy, its mechanism is incompletely understood. Recently, activation of a nuclear transcription factor NF-κB has been reported to be a signal for anti-apoptosis. In this report, we investigated the effect of TNF on activation of NF-κB, c-Jun N-terminal kinase (JNK), and apoptosis in vincristine-resistant human histiocytic lymphoma U937-VR cells. Unlike the parent clone (U937-VS), no activation of caspase-3, known to be required for apoptosis, was found in vincristine-resistant cells on exposure to vincristine. These cells were also more resistant than U-937-VS cells to doxorubicin, daunomycin, and taxol. TNF-induced NF-κB activation, IκB degradation, and nuclear translocation of p65 were all found to be highly suppressed in the U-937-VR cells. NF-κB activation by LPS, H₂O₂, and okadaic acid was also suppressed. However, vincristine resistance enhanced TNF-induced JNK activation. When examined for apoptosis, vincristine resistance suppressed the cytotoxic effects and caspase-3 activation by TNF. The resistant phenotype in U937-VR cells was independent of the expression of the apoptosis-suppressor, Bcl-2. Thus, overall these results indicate that vincristine resistance correlates with suppression of NF-κB activation, cytotoxicity, and caspase-3 activation but enhancement of JNK activation by TNF.

Keywords: Apoptosis; Bcl-2; chemoresistance; NF-κB; TNF; vincristine.

Introduction

Acquisition of resistance to chemotherapeutic drugs and cytokines poses a major obstacle to the successful therapy of cancer. The emergence of resistance is commonly encountered during the course of initial therapy. Furthermore, tumors that are initially sensitive to an antineoplastic agent and then acquire resistance to this agent often concurrently become resistant to a broad spectrum of drugs, known as multidrug resistance. How the tumor cells develop resistance is incompletely understood. Multiple factors such as, overexpression of P-glycoprotein, multidrug resistance proteins (MRP), Bcl-2; mutations in topoisomerase and p53, and glutathione-S transferase, protein kinase C, transglutaminase, and heat shock proteins (viz., hsp 27) have been implicated in the resistance phenotype.¹⁻¹⁸

Attempts to identify the molecular mechanisms of resistance have suggested a role for the activated form of NF-κB, in particular in cellular resistance to apoptosis.⁹⁻¹¹ Nuclear factor-κB (NF-κB) is a dimeric ubiquituous transcription factor, whose activity is tightly regulated by cytokines and other external stimuli.¹²¹³ The subunit members of this transcription factor belong to the Rel family and participate in various biological processes including immunological responsiveness, inflammation, cell proliferation, and apoptosis.¹⁴⁻¹⁷ The genes regulated by this transcription factor encode proteins involved in rapid response to stress and pathogens, including the acute-phase proteins, cytokines, and cellular adhesion molecules.¹⁸ The activation of NF-κB requires sequential phosphorylation, multubiquitination, and degradation of IκBα, which exposes of the nuclear localization signal in the NF-κB molecule.¹⁹

In parallel with the attempts to understand the molecular mechanisms, various approaches to circumventing the resistance phenotype have been investigated. These approaches have included use of calcium channel antagonists, steroids, antisense oligonucleotides, anti-Pgp antibodies, and liposomal encapsulation of cytotoxic agents.²⁰⁻²² However, these approaches have met with
mixed results. Among the more promising ideas, cytokines have been shown to improve the therapeutic efficacy of the anticancer agents.23 One of them, TNF is a pleiotropic cytokine with cytotoxic activity against a variety of tumor cells. It was found to be capable of inducing tumor regression comparable to that of chemotherapeutic drugs. Although the issue of systemic toxicity was resolved by locoregional administration of TNF, emergence of resistance of some tumor cells to TNF poses the main drawback to its successful clinical application.24 Transfection of the human TNF gene into tumor cells resulted in expression and secretion of biologically active TNF that reduced the cytotoxicity of vincristine and Adriamycin.25

In the present study we examined the effect of vincristine resistance on TNF-induced apoptosis, and activation of transcription factor-κB and c-Jun kinase. We found that development of resistance to vincristine induces suppression of NF-κB activation by TNF and various other agents and decreases cytotoxicity and caspase-3 activation by TNF and other chemotherapeutic agents, but increases JNK activation.

Experimental procedures

Cell lines. Vincristine-resistant cells, U937-VR, were generated by culturing U937 cells, a human histiocytic lymphoma cell line obtained from American Type Culture Collection (Rockville, MD), in successively increasing concentrations of vincristine. 2 × 10^4 cells were grown for 4–5 days in fresh drug-containing medium and remained exposed to each drug-concentration increment for 3 weeks. Continuous exposure generated cells that can grow in the presence of 200 nM vincristine. The U937-VR cells were further grown in the presence and absence of vincristine for 12 months. Details of the development of the vincristine resistant cells have been described.26 The U937-VR cells were subcloned by limiting dilution wherein isolated cells were grown in 96-well tissue culture plate. The colonies thus formed from single cells were expanded.

Human myeloid leukemia cells HL-60 transfected with Bcl-2 and Bcl-xl and control cells HL-60/neo have been described.27 These cells were a generous gift of D. K. Giri, P. Pantazis and B. B. Aggarwal. Okadaic acid was purchased from L. C. Laboratories, Woburn, MA, [γ-32P] ATP from ICN Pharmaceutical, Inc. Costa Mesa, CA, polynucleotide kinase from New England Biolabs, Beverly, MA, polycl.βC from Pharmacia Biotech, Almeda, CA, double-stranded oligonucleotide for AP-1 from Santa Cruz Biotechnology, Santa Cruz, CA. The polyclonal antibodies used in this study were: anti-p65, against the epitope corresponding to amino acids mapping within the amino terminal domain of human NF-κB; anti-IκB-α, against amino acids 297–317 mapping at the carboxy terminus of IκB-α/MAD-3 of human origin and anti-c-Jun antibodies; were all purchased from Santa Cruz Biotechnology. Anti-poly ADP ribose polymerase (PARP) monoclonal antibody that recognizes PARP and its degradation product (PharMingen, San Diego, CA) and anti-Bcl-2 antibody (Dako corp., Carpinteria, CA) were purchased.

Electrophoretic mobility shift assays (EMSA). The preparation of nuclear extracts and the assay procedure for NF-κB have been described elsewhere.28 Nuclear extracts were either used immediately or stored at −70°C. Typically, 4–6 μg protein was used per assay. The protein content of the extract was measured by the method of Bradford.29

EMSA were performed by incubating nuclear extract with 32P-end-labeled 45 mer double-stranded NF-κB oligonucleotide from the HIV terminal repeat, 5′TTGTT ACAAGGGAATTCGGCGGAGCTTTCCAGGGGA GGGTGGG-3′. A double-stranded mutated oligonucleotide, 5′TTGTTCACAACTACTTTCCGGTGCTGACTCTT CCCAGGGAGCGGTG-3′, was used to examine the specificity of binding of NF-κB to the DNA. The specificity of binding was also examined by competition with the unlabeled oligonucleotide. Visualization and quantitation of radioactive bands were carried out by PhosphorImager (Molecular Dynamics) using IMAGEQUANT software (National Institutes of Health, Bethesda).

Immunoblot analysis. Immunoblot analysis was generally carried out with 30–40 μg of extract. Following fractionation by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), the proteins were electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with phosphate buffered saline with 0.5% Tween 20 (PBST) containing 5% fat free milk followed by exposure to primary antibodies. The membranes were washed with PBST and treated with secondary antibody conjugated to horse radish peroxidase (HRPO). The antigen-antibody reaction was visualized by an enhanced chemiluminescence (ECL, Amersham) followed by exposure to film.