

Biochemistry **38(45)**, 14763-14774 (1999)

Activation of Nuclear Factor κ B by Polyamines In Breast Cancer Cells

Neha Shah, Thresia Thomas, Akira shirahata (白幡 晶), Leonard H. Sigal, and T. J. Thomas

Polyamines- putrescine, spermidine, and spermine-are Involved In the growth of breast cancer cells. A possible target of polyamine action is at the site of interaction of transcription factors with their response elements. NF- κ B is a member of the rel family of transcription factors that regulate transcription of genes In the proliferative/anti-apoptotic pathways. We performed electrophoretic mobility shift assays to study the role of polyamines In NF- κ B binding to NF- κ B response elements (NREs), the consensus sequence of which is GGGGAATTCCCC. Using cellular extract from MCF-7 breast cancer cells,we found very little binding of NF- κ B to NRE In the absence of polyamines. Addition of 1 mM spermidine or spermine caused a 4- and 6-fold Increase In NF- κ B-NRE binding, respectively. Putrescine induced a 2-fold Increase In the binding at 2 mM concentration. Using antibody supershift assays. We identified the p50 subunit of NF- κ B to be a major component in NF- κ B-NRE complex formation In the presence of polyamines. However, the decreased intensity of the band corresponding to NF- κ B-NRE complex In the presence of anti-p65, c-rel, relB and p52 antibodies suggested the participation of these subunits also. Spermine also stimulated NF- κ B-NRE binding using cellular extracts from other breast cancer cell lines and a normal breast epithelial cell line. A differential effect of spermine analogues on NF- κ B-NRE binding was observed, with spermine exerting the maximal effect. CD spectra of NRE containing oligonucleotides was asymmetric and distinct from that of a typical B-DNA CD spectrum. A concentration-dependent increase In T_m of the duplex NRE was seen in the presence of polyamines.

In transient transfection experiments using an NF- κ B driven secreted alkaline phosphatase (SEAP) reporter, spermine induced NF- κ B activity by ~2-fold as compared to controls. Spermine induced activation of NF- κ B was also confirmed using an NF- κ B-EGFP (enhanced green fluorescent protein) vector. In transient transfections in which expression of the green fluorescent protein was visualized by fluorescence microscopy. These data show a gene regulatory function of polyamines involving enhanced binding of NF- κ B to NRE and a possible mechanism for the action of polyamines in breast cancer cell proliferation.