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Aging and NFkB in Androgen Receptor Gene Regulation

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The androgen receptor(AR) serves as a ligand-activated transcription factor for a large number of genes that are directly and indirectly involved in the process of reproduction. Tissue-specific regulation of androgen sensitivity during development, maturation, and aging is primarily mediated by selective expression of this receptor in target cells. Sequence-specific DNA-protein interactions mediate these regulatory functions. In this study, nuclear factor-kB(NFkB) regulates the activity of AR promoter in prostate cells (LNCaP). DNase I footprinting analysis showed that binding of the p50 NFkB subunit protected two regions on the rat AR promoter. Electrophoretic mobility shift and antibody supershift assays with nuclear extracts from tissue and cells determined two binding sites, termed kB1 (-491/-482 bp) and kB2 (-574/565), upstream of the rat AR promoter. Analysis of AR promoter-luciferase reporter gene activity after transfection of LNCaP cells showed that p65 kB subunit serves as an activator and conversely p50 subunit serves as a repressor. The balance of two subunit is important for AR gene expression during aging and oxidative stress, namely, p50/p50 homodimer prefer to bind to kB1-sequence and p65/p50 heterodimer prefer to bind to kB2-sequence as demonstrated by electromobility shift assay (EMSA). During aging process p50/p50 homodimer is the major binding complex, on the other hand, oxidative stress increases the p65/p50 heterodimer complex as shown by EMSA.

Taken together, kB1 site has a role in aging and kB2 site is important for oxidative stress in the rat AR gene.

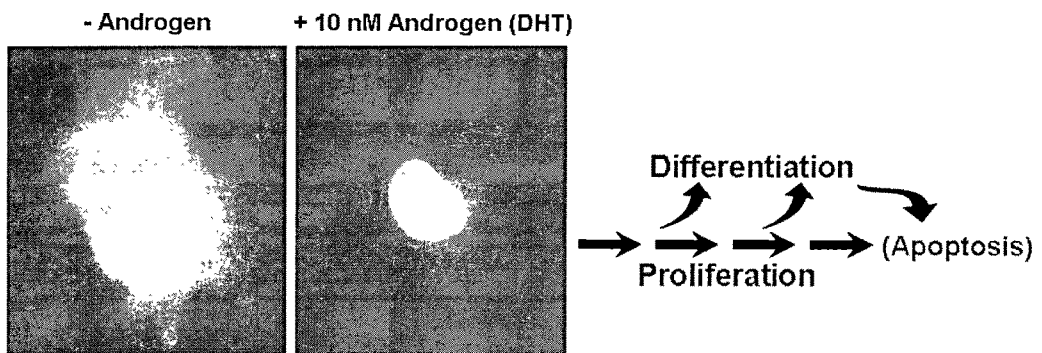
Androgen Receptor and Prostate Cancer

1. Prostate cancer is a leading cause of cancer deaths among men in the 50+ age group.

2. Androgen, acting via androgen receptor, plays an essential role in the development and function of the normal prostate.
3. Prostate cancer cells are initially androgen-sensitive. However, the tumor cells become androgen insensitive later on.

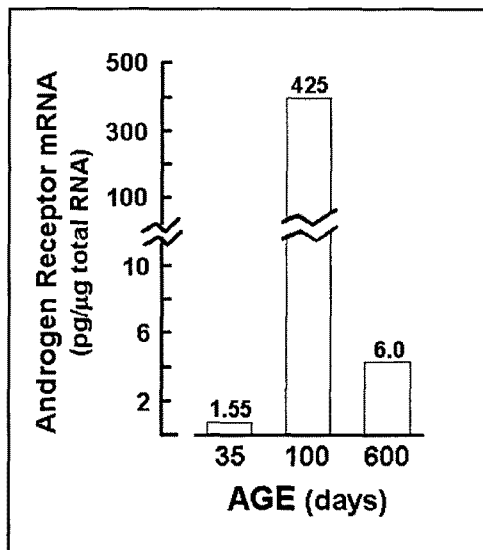
Therefore, regulation of androgen receptor expression in androgen-sensitive and androgen-insensitive prostate cancer cells may hold clues to halting cancer progression to the hormone refractory stage, and also to successful treatment strategies for therapy resistant cancer.

Androgen Receptor (AR) is a Ligand-Driven Transcription Factor and Promotes Proliferation and Differentiation of Target Cells



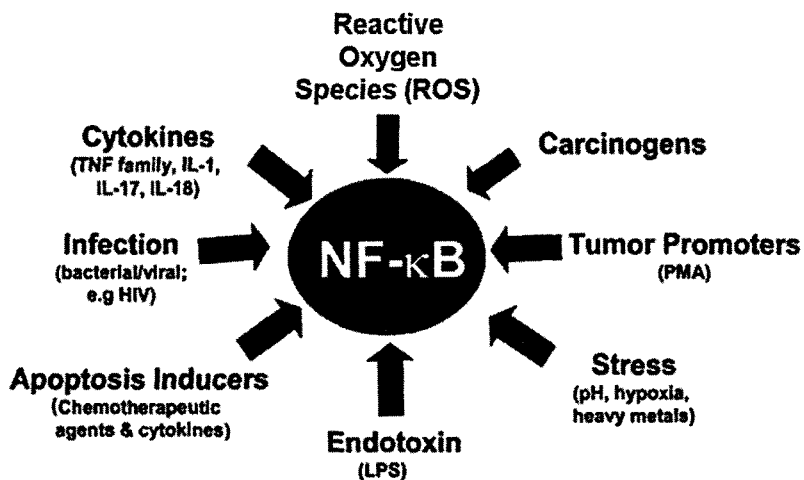
Transfected GFP-AR in COS-1 cell

Changes in Hepatic AR mRNA Levels with Age



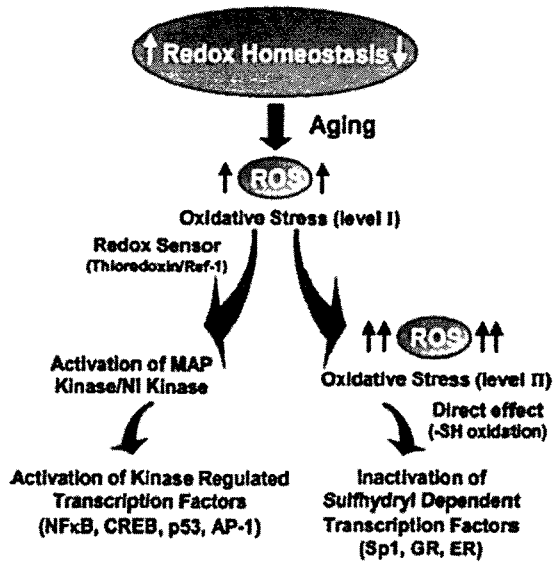
Roy et al. Exp. Geront 1996

Factors that Induce NFκB



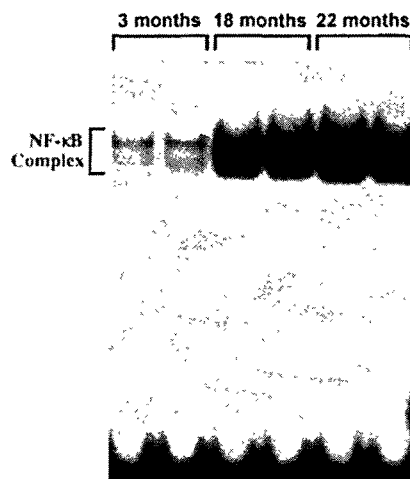
Leukemia 16:1053-1068, 2002

Redox Regulation of Aging



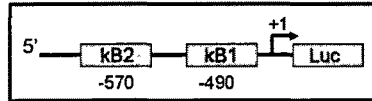
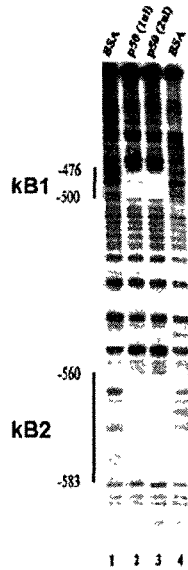
Roy et al Ageing Research Review 2002

Age-dependent Activation of NFκB in Rat Liver



Roy et al. Exp. Geront 1996

Rat AR Gene Promoter Contains an NFkB Binding Site



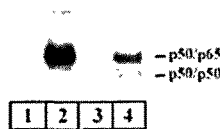
DNase I Footprinting of the rat Androgen Receptor Promoter by the p50 subunit of NFkB

Zhang et al Endocrinology 2004

Differential Increase in p50/p50 and p50/p65 Forms of NFkB During Aging and after Activation with Phobol Ester and H2O2

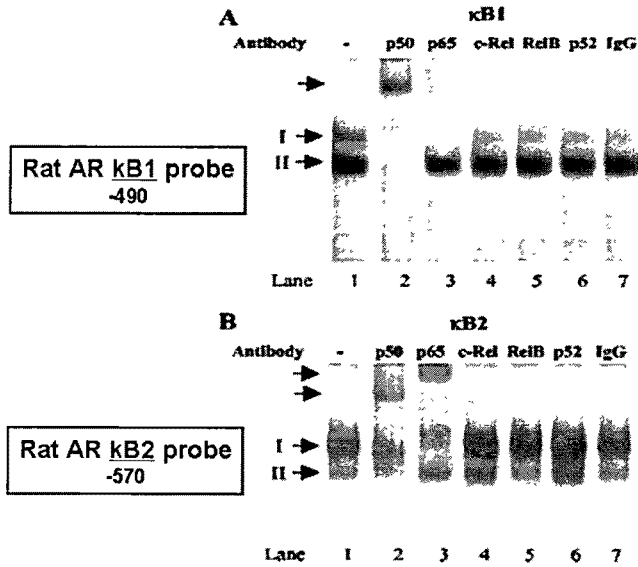


Rat AR kB2 probe used for EMSA



Roy et al JBC 1994

Supershift Assay for kB1 and kB2 Site with NFkB Antibodies

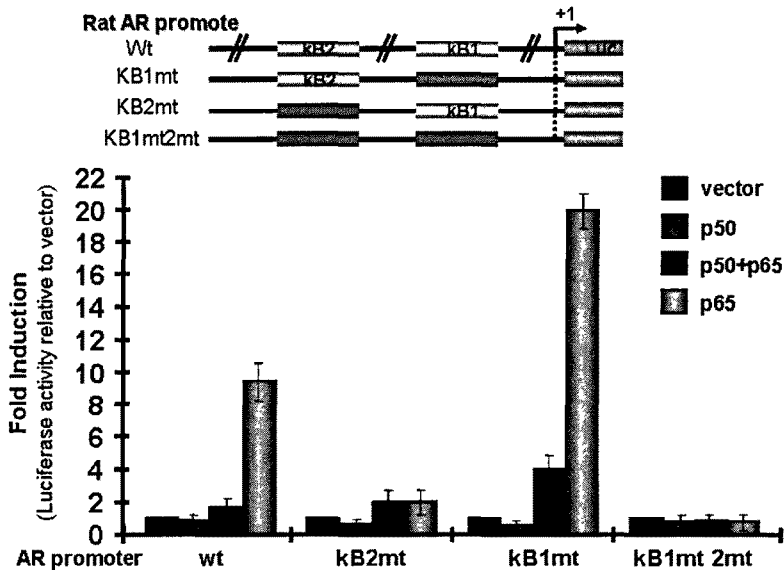


kB1 sequence-prefer to p50 Homodimer, kB2 prefer to p65-p50 complex Zhang et al Endocrinology 2004

Differential Preference of p50 and p65 Binding Sequence in AR promoter

Consensus NFkB	5' GGGRNNYYCC	
kB1 site	5' GGGACTCTCC	p50
kB2 site	5' GGGAATTCCC	p65
Il6-promoter site	5' GGGATTTTCC	p65
Mouse IgG promoter	5' GGGACTTTCC	p65

Biological Activity of the Rat AR Promoter with Mutations at the NFkB Binding Site in LNCaP Cells



Summary

1. Two NFkB binding sites were identified in rat AR promoter.
2. kb1 binding site in AR promoter prefers to p50 subunit homodimer complex
3. kb2 binding site in AR promoter prefers to p50-p65 subunit heterodimer complex
4. p65 activity increases with environmental stresses
5. p50 activity increases with aging process
6. Balance between p65 and p50 activity controls the AR expression level
7. In rodent prostate, AR expression level goes down with aging, however environmental stresses increase p65 activity (reference).