European Journal of Cancer, Vol. 33, No. 12, p. 2138, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

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0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00414-0

Human *trk*-B Promotor: Isolation and Characterisation Reveals Novel Retinoic Acid Inducible Transcription Unit Involving INR and 3′ Direct Repeat Element

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Poor prognosis neuroblastoma tumours, especially those with amplified *MYC*N express full-length *trk*-B and expression of *trk*-B and its ligand, BDNF, stimulate tumour cell survival, invasion and differentiation. The ability of retinoic acid (RA) to transcriptionally regulate the human *trk*-B receptor tyrosine kinase gene, facilitated the isolation of the human *trk*-B gene promoter. The *trk*-B promoter lacks a TATA motif and the major transcription initiation site has a strong homology with an initiator (INR) element. Transient transfection analysis using a series of deletion mutants in 5′-flanking region of human *trk*-B gene linked to a reporter gene indicates that a 57 bp minimal unit is sufficient for basal as well as RA inducible transcription. RA inducible expression occurs only in neuroblastoma cells and not in several cell lines tested, indicating cell type specific restriction of *trk*-B expression. The 57 bp minimal *trk*-B promotor contains an initiator (INR) element (−3 to +5) as well as the direct repeat (DR) element (+12 to +24) and both are required for RA inducible as well as basal transcription. Gel mobility shift using INR or DR oligos indicated the presence of factors binding to the INR or DR in nuclear extracts from RA treated NGP cells. Both the INR and DR cis-element are critical for *trk*-B transactivation in NGP cells. RA induced INR binding protein(s) (IBPs) and DR binding protein(s) (DRBPs) cooperatively mediate transcription of *trk*-B gene. Proteins regulating transcription via binding to the INR have been identified. An understanding of the molecular mechanisms mediating transcription of *trk* genes may enable the manipulation of *trk* gene levels *in vivo*.

PII: S0959-8049(97)00415-2

Identification of a Novel Thyroid Hormone Receptor Responsive Element in the Human *trk*-B Promoter

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The tyrosine kinase active form of *trk*-B is expressed in poor prognosis neuroblastomas tumours with *MYC*N amplification, and studies in cell lines indicate that activation of *trk*-B stimulates tumour cell survival, differentiation and invasiveness. To study the mechanisms regulating *trk*-B expression, the human *trk*-B promoter was isolated and characterised. A 30 bp region from nucleotides −3 to +27, spanning an initiator of transcription (INR) site and a putative novel direct repeat (DR) element, was found to contain full promoter activity and responsiveness to RA in transient transfection analyses. Gel shift analysis, using nuclear extracts of RA treated neuroblastoma cells, was used to explore DNA/protein interactions on a ^{32}P-labelled DR oligo probe. Four complexes with distinct electrophoretic mobilities were detected. Sequence comparison of the *trk*-B promoter revealed the presence of several putative cis-acting elements partially related to known retinoic acid receptors (RAR and RXR) and thyroid hormone receptor (THR) binding sites. Specific anti-RARs, anti-RXRs and anti-THRs antibodies were added to gel shift mixtures prior to electrophoresis. Anti-THR-β1 and anti-RXR-β but not anti-RAR or anti-THR-a antibodies supershift some of the DR complexes. Preliminary functional analysis indicates that *trk*-B promoter activity increases (9-fold) in the presence of both T3 and RA. This is the first evidence that a neurotrophin receptor gene may be transcriptionally regulated by specific nuclear hormone receptors and indicates that these trans-acting factors regulate neurotrophin receptor levels in normal and transformed neuroblasts.