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ORIGINAL INVESTIGATION

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Apoptosis regulatory gene NEDD2 maps to human chromosome segment 7q34–35, a region frequently affected in haematological neoplasms

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Abstract Developmentally regulated mouse gene Nedd2 encodes a protein similar to the product of the nematode *Caenorhabditis elegans* cell death gene ced-3 and the mammalian interleukin-1β-converting enzyme. Overexpression of Nedd2 in cultured mammalian cells induces apoptosis that can be blocked by proto-oncogene BCL2. We have isolated cDNA clones for the human homologue of the mouse gene and, by using these as probes, mapped the human NEDD2 gene to 7q34–35 by fluorescence in situ hybridisation. The potential tumour suppressor function of NEDD2 is discussed.

Introduction

By subtraction cloning, we have previously identified a set of mouse genes (named Nedd1 to Nedd10) with developmentally down-regulated expression in the brain (Kumar et al. 1992). We have recently shown that one such gene, Nedd2, encodes a protein similar to the mammalian interleukin-1β-converting enzyme (ICE) and the product of the cell death gene, ced-3, of *Caenorhabditis elegans* (Kumar et al. 1994). Both ICE and ced-3 encode putative cysteine proteases and induce apoptosis when overexpressed in cultured cells (Miura et al. 1993). Overexpression of Nedd2 in cultured cells also results in cell death by apoptosis; this is suppressed by the expression of the human BCL2 proto-oncogene indicating that Nedd2 is functionally similar to the ced-3 gene in *C. elegans* (Kumar et al. 1994). Recently, a human homologue (NEDD2) of the mouse Nedd2 gene (the mouse Nedd2 and human NEDD2 symbols used in this paper have been approved by the International Committee on Mouse Genetic Nomenclature and the HUGO Nomenclature Committee, respectively), which the authors call Ich-1, has also been shown to induce apoptosis in various cell lines (Wang et al. 1994). An alternately spliced form of NEDD2 mRNA, which encodes for a truncated protein (Ich-1s), protects Rat-1 cells from serum-deprivation-induced cell death (Wang et al. 1994). During embryonic development, Nedd2 is highly expressed in several mouse tissues undergoing high rates of apoptosis, such as the central nervous system and kidney (Kumar et al. 1994). These data suggest that Nedd2 is an important component of the mammalian programmed cell death machinery.

Oncogenes, such as BCL2, act by enhancing cell viability (reviewed in Korsmeyer 1992), whereas tumour suppressor genes, such as p53, may be required for normal physiological cell death to occur (Yonish-Rouach et al. 1992: Shaw et al. 1992). Therefore, genes such as Nedd2 and ICE, which regulate cell death in a positive fashion, probably function as tumour suppressor genes and may be important in multistep carcinogenesis. We have previously localised the mouse Nedd2 gene to the proximal region of chromosome 6 (Kumar et al. 1994). This region of mouse chromosome 6 shares a region of homology with human chromosome arm 7q. Structural abnormalities involving 7q frequently occur in a number of human haematological neoplasms and the presence of a candidate tumour suppressor gene on 7q has been suggested (Neuman et al. 1992; Johansson et al. 1993). Therefore, it is of interest to determine the chromosomal localisation of the human NEDD2 gene. In the present study, we have isolated human NEDD2 cDNA clones and, using these as probes, have mapped the gene to human chromosome region 7q34–35 by fluorescence in situ hybridisation (FISH).

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Materials and methods

DNA probes and labelling

Using an *Eco*RI fragment of mouse Nedd2 cDNA as a probe (Kumar et al. 1994), we screened approximately 500000 clones from a human fetal brain cDNA library (Strategene) by standard protocols (Sambrook et al. 1989) and isolated two NEDD2 cDNA clones (Hb N2.1 and Hb N2.3). These clones contained approximately 1.0 kb and 0.8 kb cDNA inserts, respectively, covering a part of the 5′ region of the human NEDD2 mRNA. Probes were prepared by labelling the plasmids either with biotin-14-dATP using the BioNick Labelling System (Life Technologies) according to manufacturer's instructions, or with biotin-16-dUTP (Boehringer Mannheim) as described previously (Takai et al. 1993, 1994, 1995). The probe for the human centromeric region of chromosome 7 (D7Z1/D7Z2) was purchased from Oncor directly labelled with digoxigenin.

FISH and detection

Metaphase spreads were prepared using the method of Webber and Garson (1983). R-banded human chromosomes were prepared following the method of Viegas-Pequignot and Dutrillaux (1978) with modifications by Takahashi et al. (1990). FISH techniques were as developed by Lawrence et al. (1988) and Lichter et al. (1990) with some modifications (Takahashi et al. 1991; Takai et al. 1993, 1994). Signal amplification was performed according to Pinkel et al. (1986) and Takai et al. (1994). The slides were finally stained with propidium iodide. The stained chromosomes were observed by an Olympus BH2 or a Nikon OPTIPHOT-2-EFD2 microscope with appropriate filters.

Results and discussion

We have isolated two human NEDD2 cDNA clones (Hb N2.1 and Hb N2.3) from a human fetal brain cDNA library. Sequence analysis of these clones indicates that Hb N2.1 contains the entire coding region of the small, alternately spliced NEDD2 protein, also termed Ich-1s (Wang et al. 1994). Hb N2.3 contains a 0.8-kb fragment that corresponds to the nucleotide residues 464-1269 of the mouse Nedd2 cDNA sequence encoding 268 amino acids corresponding to residue 153-421 of the mouse protein (Kumar et al. 1994); it represents the Ich-1L form (Wang et al. 1994). The nucleotide sequence of both forms of NEDD2 cDNA has been published by Wang et al. (1994). The only significant variation in our sequence is that the amino terminus of the predicted human NEDD2 protein is similar to that of mouse Nedd2 protein (Kumar et al. 1994), whereas sequences reported by Wang et al. (1994) for the Ich-1L and Ich-1s forms appear to lack 17 and 31 amino acids, respectively, from the amino terminus (data not shown). A sequence alignment of mouse and human proteins indicates that they are approximately 90% identical to each other (data not shown).

DNA from both clones was labelled with biotin and hybridised to the metaphase spreads of healthy individuals. Initial FISH studies were carried out using unbanded human chromosomes and chromosome-specific centromeric probes. Among the 30 metaphases analysed, 9 (30%) had symmetrical double spots on 7q, 18 (60%) showed a single spot on 7q, whereas 3 (10%) had no signals in this region. From these analyses, NEDD2 was placed on the 7q distal region (Fig. 1 A). For further analysis, we used FISH in conjunction with R-banded chromosomes. Fifty (pro)metaphase chromosome spreads with R-bands were analysed. Among these, 18 (36%) of the cells revealed symmetrical double fluorescence signals on chromosome 7, 30 (60%) cells showed a single spot on one chromatid at the same location, and two (4%) had no signal on this chromosome. The signals on chromosome 7 could be assigned to bands q34–35 (Fig. 1B). No other symmetrical double spots were detected on any of the

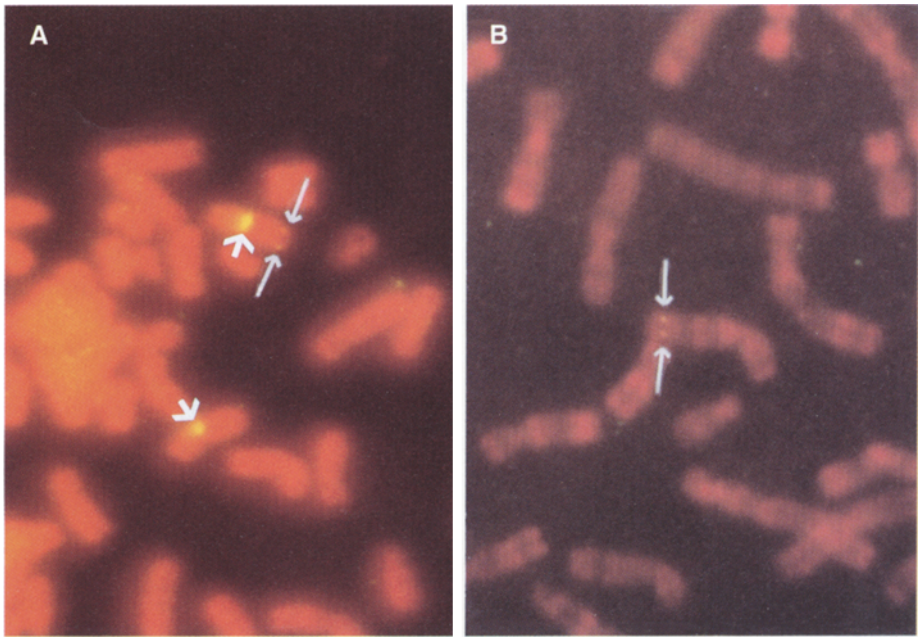

Fig. 1 Partial metaphases of human unbanded (**A**) and R-banded (**B**) chromosomes. *Short thick arrows* in **A** indicate the fluorescence signals from the chromosome-7-specific centromeric probe. *Long thin arrows* in **A** and **B** indicate symmetrical double fluorescent spots for the human NEDD2 gene on 7q as detected by FISH

other chromosomes, indicating that the human NEDD2 gene is located at 7q34–35.

By interspecific backcross mapping, we have previously localised the mouse Nedd2 gene to the proximal region of chromosome 6, within 2.1 cM of the T-cell receptor β-chain gene (TCRB) (Kumar et al. 1994). The human localisation of NEDD2 to 7q34–35 is in good agreement with the mouse mapping, as the proximal region of mouse chromosome 6 shows a region of similarity with human 7q. Moreover, the human TCRB gene is known to be localised to 7q35 (Le Beau et al. 1985; Isobe et al. 1985; Morton et al. 1985).

Loss of the whole or part of chromosome 7 is one of the most common cytogenetic features in haematological neoplasms (reviewed in Mitelman et al. 1991). Monosomy 7 or partial deletions of the long arm of chromosome 7 are frequent in acute myeloid leukaemia (AML), myelodysplastic syndrome and chronic myeloproliferative disorders (Johansson et al. 1993). These cytogenetic changes are especially common in secondary disorders in patients who have undergone radio- and chemo-therapy (e.g. Heim 1992). Loss of heterozygosity has been found for several markers on 7q (e.g. Kere et al. 1989). Non-random deletions of the 7q22–36 region are common in AML (Johansson et al. 1993). Additionally, translocations involving the 7q34–35 region have also been reported in several cases of leukaemia, although these are sometimes associated with the TCRB locus on 7q35 (reviewed in Mitelman et al. 1991; Rabbitts and Boehm 1991). Based on these cytogenetic observations, the presence of a candidate tumour suppressor gene on chromosome arm 7q has been suggested (e.g. Neuman et al. 1992; Johansson et al. 1993). The biological activity of NEDD2 in the regulation of programmed cell death clearly makes it a potential candidate for tumour suppressor function on 7q; it will be of much interest to analyse the structure and expression of NEDD2 in haematological neoplasms with aberrations in the 7q region. Work along these lines is currently in progress.

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