**Preliminary Communication**

Mapping of Human DNA-binding Nuclear Protein (NP220) to Chromosome Band 2p13.1-p13.2 and Its Relation to Matrin 3

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Human NP220 (hNP220) is a novel DNA-binding nuclear protein, which has an arginine/serine-rich motif and polypyrimeidine tract-binding motif, and NP220s and matrin 3 are thought to form a novel family of nuclear proteins. We have determined a chromosomal localization of the cDNA encoding human NP220 to 2p13.1-p13.2 by using fluorescence in situ hybridization. Human matrin 3 cDNA was mapped to chromosomes 1p13.1-p21.1 and 5q31.3, demonstrating that these novel nuclear proteins with similar functions are on different chromosomes.

Key words: gene mapping; nuclear protein; FISH

The functional expression of eukaryotic genes is accomplished through a series of intranuclear events including packaging, transferring, and processing of transcripts. These processes are mediated by various RNA-binding proteins and small nuclear RNAs to form complexes with ribonucleoproteins. Human NP220 is a novel DNA-binding nuclear protein that was isolated by Inagaki et al.7) from human cDNA libraries. This protein has an arginine/serine-rich (RS) motif found in non-small nuclear RNP splicing factors, a polypyrimeidine tract-binding RNA recognition motif (RRM) found in heterogeneous nuclear RNP1s 1/L, and a Cys2-His2 zinc finger-like motif. Human NP220 shares three types of unique domains with matrin 3,2,6) MH1 (matrin-homologous domain 1), and MH3, with unknown function at N- and C-termini, respectively, MH2 with high sequence similarity to RRM, and together with matrin 3 forms a novel family of nuclear proteins. Based on the structural aspects of NP220s, these proteins are expected to be mammalian factors regulating RNA splicing.

We have now located the human NP220 and matrin 3 genes on chromosomes by fluorescence in situ hybridization (FISH) analysis using human cDNAs as probes. Clone K1 (2.6 kb), isolated from a λgt11 library of Hela cells (Clontech), and its overlapping clone N9 (2.8 kb), selected from a λZapII library of Namalwa cells, were used for the mapping of NP220. As for human matrin 3, the cDNA clone used as a probe was isolated as follows: The partial sequence data of matrin 3 from rat (Accession No. M63485) and human (Accession No. M63483) from GenBank were used to develop PCR primers. A set of primers were designed to amplify almost the whole ORF of matrin 3 cDNA by RT-PCR to the RNA fraction from human keratinocyte cultured cells; upper primer (5′-ATTCGCCGATCTCAGTCCACGCCGTC-3′, BamHI site underlined) and lower primer (5′-TTGGTCTGAGTCTGATTAAAGCCTTGTTCTCCTTGGT−3′, SalI site underlined). Following digestion with BamHI and SalI, the PCR product was cloned into the BamHI- SalI site of pBluescript KS(-). The 2.7 kb cloned fragment (hMAT3) was confirmed to be human matrin 3 by sequencing (data not shown).

Chromosome spreads were obtained from phytohemagglutinin-stimulated blood lymphocytes of a healthy donor after thymidine synchronization and bromodeoxyuridine incorporation by the method of Takahashi et al.8) The cDNA clones were labeled with biotin-16-dUTP (Boehringer Mannheim) by nick-translation. These biotinylated cDNA probes (K1 and N9 mixed, or hMAT3) were hybridized to R-banded chromosome spreads and the hybridized probes were detected by fluorescein-conjugated avidin (Boehringer Mannheim) without further signal amplification as described previously.9) Chromosomes were counterstained with 200 ng/ml propidium iodide for R-banding. In this method, R-banded chromosomes also show the counterpart G-banding pattern in the Hoechst 33258 staining. Fluorescence signals were examined with an oil x 63 Plan-APochromat objective on a Zeiss Axiplan 2 MOT epifluorescence microscope with a cooled charge coupled device (CCD) camera (Princeton Instruments, Inc., PentaMax-1317-K1). Digital images that passed through each fluorescence filter were captured using the software program IPLab (Signal Analytics Co.) and were pseudocolored and merged using an Adobe Photoshop 4.01 (Adobe Systems Inc.) on a Power Macintosh 9600/ 200MP computer to assign the chromosomal localization of the genes. Each fluorescence image or a merged image was printed in a gray scale by Fuji Pictography 3000. A and B in Fig. are the typical examples of the images for human NP220 cDNA; fluorescein (A) and G-banding (Hoechst) (B) from the same whole spread. Twenty metaphase spreads were observed in a experi-

Abbreviations: FISH, fluorescence in situ hybridization; CCD, charged coupled device.

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Fig. Chromosome Localizations of Human NP220 and Matrin 3 Genes by FISH.

Fluorescence in situ hybridization was done using cDNA fragments as probes. Typical images of fluorescence signals (A and D) and the G-banded chromosomes (B and E) are shown for NP220 and matrin 3, respectively. The positions of each fluorescence signal are indicated by arrowheads (Open arrowheads of D and E point to the signal positions of chromosome 1). The merged image of prophase-like R-banded chromosome 2 and fluorescence signal of NP220 is shown in (C) for its precise assignment and the R-banding ideogram of chromosome 2 with the assigned position is also shown on the right side. The chromosomal localizations are assigned to 2p13.1-p13.2 for NP220 and to 1p13.3-p21.1 and 5q31.2-q31.3 for matrin 3, respectively.

We have identified two chromosomal positions for human matrin 3 gene using cDNA clone as a probe. Although we cannot tell which is the functional gene at the moment, the active gene was suggested to be on chromosome 5 judging from its synteny with the mouse matrin 3 gene.\cite{10} It will be necessary to isolate genomic clones that have exon/intron structures to identify the position of functionally active matrin 3 gene(s).

The domain structure of NP220 is very attractive because these proteins have a zinc finger-like motif which has been found in many transcription factors in addition to the DNA binding domain, acidic repeat, RS motif, and polypyrrimidine tract-binding motif. Moreover, MH1 and MH3 domain of both NP220 and matrin 3 have unique motifs that have not been found in any other nuclear proteins.\cite{2,5,7} Thus, this major protein family of the nuclear matrix may have important functions for intranuclear events. Our results show that these proteins have different chromosomal locations, suggesting that they are expressed by individual mechanisms. Further studies are necessary to clarify their functions and the mechanisms regulating their expression.

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