

Mapping of the gene for Nischarin, a Novel Integrin Binding Protein, to Chromosome 3 by Fluorescence In Situ Hybridization

Suresh K. Alahari

Department of Pharmacology, University of North Carolina School of Medicine, CB#7365, Chapel Hill, NC 27599-7365, USA

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ABSTRACT Recently, we have reported the cloning and characterization of a novel protein, Nischarin that associates with $\alpha 5$ integrin *in vitro* and *in vivo*. Nischarin appears to have a negative regulatory function in cell migration by antagonizing the actions of Rho GTPases on cytoskeletal organization and cell movement. I now report chromosomal localization of the gene for mouse Nischarin. Fluorescence In Situ Hybridization (FISH) analysis of metaphase chromosomes derived from mouse embryo fibroblast cells revealed that Nischarin is localized to chromosome 3. Also further labeling of the telomere and middle portion of chromosome 3 indicated that the gene position is 53% of the distance from the heterochromatic boundary to the telomere of chromosome 3, corresponding to 3F1-F2-1. Mouse chromosome 3 seems to share a region of homology with the human chromosome 3, however the homologous genes mapped so far are all on human 3q. Interestingly, the human homolog of Nischarin has been mapped to chromosome 3p. The possibilities for this conundrum between human and mouse genomes are also discussed.

INTRODUCTION

Integrins are a major family of cell surface receptors that mediate cell to cell or cell to extracellular interactions (Aplin et al. 1998). They play a role in diverse cellular processes including cell migration, signal transduction, tumor growth and apoptosis (Giancotti and Ruoslahti, 1999; Aplin et al. 1999; Parise et al. 2000). The $\alpha 5 \beta 1$ integrin is a fibronectin receptor, that has been shown to be involved in suppressing tumor cell growth (Varner et al. 1995), and protecting cells against apoptosis (Lee and Juliano, 2000). Nischarin is a novel protein that was discovered by us and shown to be involved in $\alpha 5$ integrin mediated events. Murine Nischarin (Genbank AF315344) interacts with $\alpha 5$ integrin *in vitro* and *in vivo*. Nischarin is ubiquitously expressed, and encodes a novel cytoplasmic protein that inhibits

cell migration and alters actin filament organization (Alahari et al. 2000). The predicted 1354 amino acid protein has a cytochrome p450 motif and two consensus leucine zippers that may be involved in protein-protein interactions (Alahari et al. 2000). Here I report the localization of mouse Nischarin to chromosome 3, specifically band 3F1-F2.1.

METHODS

Probe: To isolate a Nischarin genomic clone, a BAC ES mouse library was screened with Nischarin cDNA. The BAC ES mouse library is derived from RW4 cells of male 129/SVJ mice. For hybridization screens, a full length cDNA probe (4.0 kb) was random primed using Amersham's rediprime DNA labeling systems, and hybridized to the double spotted filters.

Sequence Analysis: DNA was isolated from the positive clones by standard procedures (Alahari et al. 1993). For sequencing analysis, the following primers were used. (primer 1: GACTACCTCTAGACCTGAGCCAC; primer 2: GAGGCTTCAGGAGCAAGCAC). Sequence analysis was performed at the UNC Sequencing core facility.

Fluorescent in Situ hybridization (FISH) Analysis: Dual color fluorescence in situ hybridization was performed on metaphase chromosomes as described (Shi et al. 1997). DNA from mouse Bac clone F788 was labeled with digoxigenin dUTP by nick translation. Metaphase chromosomes of mouse were prepared from mouse embryonic fibroblasts. Molecular cytogenetic procedures were performed exactly as described (Shi et al. 1997). A labeled probe was combined with sheared mouse DNA and hybridized to metaphase chromosomes derived from a mouse embryo fibroblast cell line, in a solution containing 50% formamide, 10% dextran sulfate and 2X SSC. The positive signals were detected by antidigoxigenin antibodies.

Address for correspondence: Dr. S. K. Alahari, Department of Pharmacology, CB#7365 University of North Carolina, School of Medicine Chapel Hill, NC 27599-7365, USA
Telephone: 919-966-4343, Fax: 919-966-5640
E-Mail: alahari@med.unc.edu

RESULTS AND DISCUSSION:

Screening of BAC ES cell library with Nischarin cDNA yielded three positive colonies. By performing sequence analysis using primers derived from Nischarin cDNA one of these clones was identified to be a true positive. In addition, the sequence analysis revealed the presence of introns, indicating that the genomic clone identified was not a pseudogene. FISH analysis was performed to map the chromosomal location of Nischarin.

Specific hybridization signals (hybridization between the probe and metaphase chromosomes) were detected by incubating the hybridized slides in fluoresceinated antidigoxigenin antibodies followed by counterstaining with DAPI. This stained chromosome 3. Chromosome identification was further confirmed by cohybridizing clone F788 with a probe specific for the telomeric region of chromosome 3. Ten hybridized chromosomes were measured revealing that Nischarin is located at a position 53% of the distance from

the heterochromatic euchromatic boundary to the telomere of mouse chromosome 3. Out of 80 analyzed metaphase cells, 71 exhibited specific labeling. Based on these data, Nischarin is assigned to chromosome band 3F1-F2.1.

The human homolog of Nischarin has been identified and suggested to be a imidazoline receptor, and localized to chromosome 3p [(Ivanov et al. 1998); and www.ncbi.nlm.nih.gov/genmap]. Interestingly, this region is not syntenic to mouse chromosome 3, and as a result there are no mouse orthologs on human 3p except human latexin which is simply localized to chromosome 3 without any sublocalization. It is possible this part of the mouse genome is scrambled relative to the human genome. It has been shown by various investigators that many parts of the mouse genome have suffered multiple rearrangements involving multiple chromosomes (Burt et al. 1999; Ehrlich et al. 1997). This is clearly depicted in the mouse and human homology map created by The Jackson Laboratory, Bar Harbor, Maine

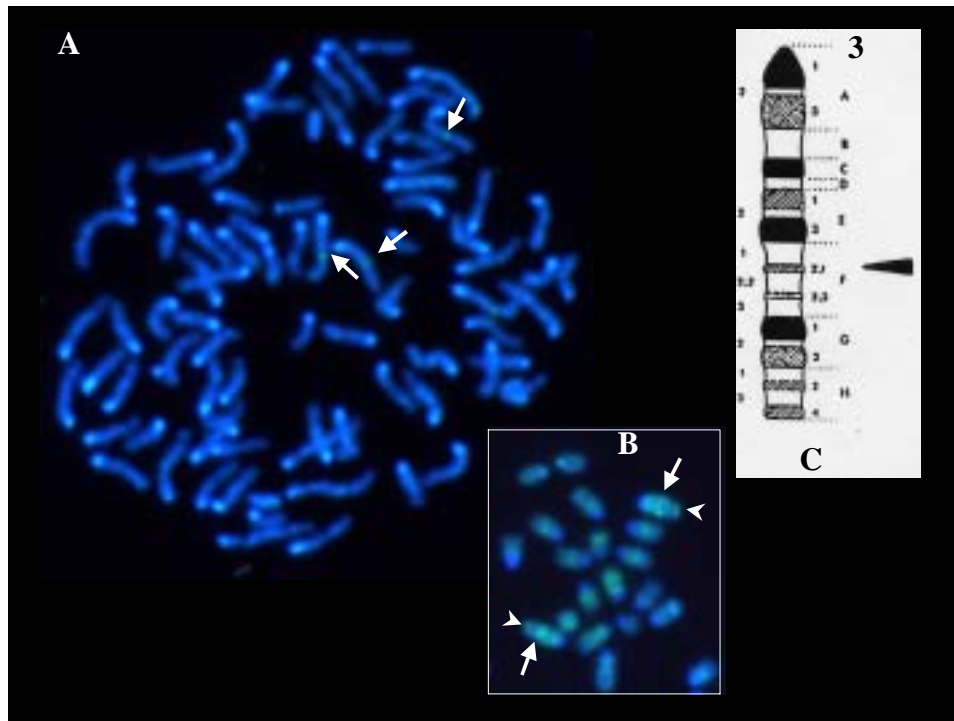


Fig. 1. Fish Localization of the mouse Nischarin: The specific signals detected on chromosome 3 are shown with arrows (A). The specificity of the mapping was confirmed by co-hybridization with chromosome 3 telomeric region (arrowheads) (B). An ideogram of mouse chromosome 3 is included showing the location of Nischarin (C). The measurements of these hybridized chromosomes indicated that Nischarin is located at band 3F1-F2.1.

(www.informatics.jax.org), where it was shown that human genes of chromosome 3 aligned against mouse chromosomes were in clusters (around 10-15 cM and 30-35 cM). This indicates that human chromosome 3 is not represented on mouse chromosome 3 by a single block of conserved synteny. Despite this, there are big blocks of conserved synteny, but Nischarin is not in one of these, as per the data. Some conserved syntenic genes previously described between mouse and human chromosome 3 were depicted in the table.

Mouse genes located close to the Nischarin region, map to different regions on different human chromosomes or do not have human orthologs (Table 1); this further supports that the mouse genome has undergone various rearrangements. Although some mouse and human genes exist in conserved syntenic groups, considerable evolutionary changes prevail in the two species.

Interestingly, the human 3p21 region is of special interest. As discussed above, human Nischarin has been localized to human chromosome 3p21, and this region has been implicated in a role in cancer, because this region is deleted or rearranged in various cancers (Timmer et al. 1999). Some of the genes that are localized to this chromosome include RASSF1A, a tumor suppressor involved in kidney tumorigenesis (Dreijerink et al. 2001), Semaphorin III which is involved in lung cancer (Xiang et al. 1996), and other tumor suppressor genes involved in small cell lung cancer, cervical carcinoma, colorectal carcinoma and breast carcinoma (Herzog et al. 2001; Maestro et al., 2000; Agathangelou et al. 2001; Fullwood et al. 1999; Uzawa et al. 1998; Sekido et al. 1998). The

importance of this region in tumor suppression nicely correlates with our data (Alahari et al. 2000; Alahari et al, 2001 unpublished) showing that Nischarin's role in inhibiting cell migration and invasion, which are essential events in metastasis and tumor progression.

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Table 1: Genes that are adjacent to Nischarin on chromosome 3 (compiled from www.informatics.jax.org)

<i>Gene on Mouse Chromosome 3</i>	<i>Human Chromosome</i>	<i>Human Gene</i>
Adar	1q21.1-1q21.2	ADAR
Rorc	Not Localized	THOR
Mif-ps3	-----	Ortholog Unknown
Glrb	4q31.3	GLRB
Ugt8	4q26	UGT8
Shox2	3q25-3q26	SHOX2
Mab2111	13q13	MAB21L1
Inac	4q31.2-2q-28	INAC
Terc	3q21-q28	TERC
Thbs3	-----	Ortholog Unknown

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