Radiation Hybrid Mapping and mRNA Expression of Chicken N-myc downregulated gene 4

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ABSTRACT

N-myc downregulated gene 4 (NDRG4) is a member of the N-myc downregulated gene family which belongs to the alpha/beta hydrolase superfamily. The protein encoded by this gene is a cytoplasmic protein that may be involved in the regulation of mitogenic signalling in vascular smooth muscle cells. To map NDRG4 gene in chicken chromosome, a 6,000 rads chicken-hamster radiation hybrid panel was used. Primers were designed according to the published human sequence for amplification of chicken NDRG4. We compared the corresponding human mRNA sequence with the predicted coding sequence of chicken NDRG4, and found that the assembled contig shared a high percentage of similarity with that of human gene. PCR of samples from ChickRH6 revealed the locations of NDRG4 to be linked to the maker PARD6G (5 cR away) with a LOD score 20.46. In addition, we detected the mRNA expression and distribution of chicken NDRG4 in various tissues by RT-PCR, and found that NDRG4 was highly expressed in chicken brain and heart, whereas lowly but detectable in thymus. The mRNA expression of this gene in chicken liver, spleen, lung and muscle was rarely detectable under present experimental conditions.

Key words: chicken, RH panel, NDRG4, mapping, mRNA expression, genetic marker.

INTRODUCTION

Radiation hybrid (RH) mapping is a somatic cell genetic mapping technique, and it has become a general way to construct high resolution, contiguous physical map of human and animal chromosomes (Walter and Goodfellow, 1993). Based on earlier studies of Goss and Harris (1975) and modification later by Cox et al. (1990), high dose of X-rays was used to break the chicken candidate chromosome into several fragments. These randomly broken chromosomal fragments were recovered in hamster cells, and stable hamster-chicken hybrid clones were analyzed for the presence or absence of specific chicken DNA markers by Southern blot hybridization or by PCR-based assays. The further apart
two markers are on the chromosome, the greater the probability that a given dose of X-rays will break the chromosome between them, placing the markers on two separate chromosomal fragments. After estimating the frequency of breakage and thus the distance, between markers by statistical analysis (Boehnke et al., 1991), it is possible to determine their order on the chromosome. RH was initially developed to make a single chromosomal map, but now it has been used to construct a high resolution map of the whole genome (Walter et al., 1994).

The human *NDRG4* is located at chromosome 16q21–22.1, and specifically expressed in brain and heart (Zhou et al., 2001; Qu et al., 2002). The protein encoded by this gene is a cytoplasmic protein. It plays a role in neurite outgrowth and has an influence on an NGF-stimulated AP-1 activation by an undefined mechanism in PC12 cells (Ohki et al., 2002), and may play a role in supporting the activation of ERK and its target proteins needed for neuronal differentiation and in reducing the activation of Elk-1 implicated in cell growth (Hongo et al., 2006). In spite of our knowledge of this gene, little is known about it in chicken. In this study, the chromosome localization of chicken *NDRG4* was firstly performed by a radiation hybrid panel, and the mRNA expression of this gene was investigated in various tissues by RT-PCR.

**MATERIALS AND METHODS**

**Primer design, PCR conditions and sequencing**

The PCR primers for the chicken *NDRG4* sequence were designed using Oligo 6.0 software (MBI Cascade, Co, USA) and supplied from Integrated Technologies Inc. (Coralville, IA, USA). Based on consensus sequences from human (NC_000016) and mouse (NC_000074), primers were designed to amplify chicken *NDRG4* genomic sequence, and this procedure has been described previously (Chen et al., 2006).

PCR amplification was performed in 25 µl reaction mixture containing 0.8 U Taq DNA polymerase (Takara, Dalian, China), 1 × PCR buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 0.5 µM of each primer and 50 ng chicken genomic DNA. The PCR conditions were as follows: 95°C for 4 min and 30 cycles of 94°C for 45 s, 62°C for 45 s, and 72°C for 1 min, followed by a 10 min extension at 72°C for 10 min. The amplified products were then analyzed by electrophoresis on 1.5% agarose gels. The PCR products were purified with Wizard Prep PCR purification system (Promega) and sequenced. Identity of the PCR product was confirmed by sequence analysis. Using this sequence, new primers of the *NDRG4* were designed for radiation hybrid (RH) mapping (Table 1). In all cases, the primers designed for RH mapping only amplified chicken genomic DNA, but not hamster controls.

**Radiation hybrid mapping and analysis**

The *NDRG4* was mapped using the INRA Chicken Radiation Hybrid Panel (ChickRH6) consisting of 90 hamster-chicken hybrid cell lines (Morisson et al., 2002). PCR amplification was performed in 12.5 µl volumes containing 30 ng of RH template, 0.3 U Taq DNA polymerase (Takara, Dalian, China), 1 × PCR buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, and 0.5 µM of each primer. The PCR conditions were 95°C for 4 min, 33 cycles of 94°C for 45 s, 56°C for 45 s and 72°C for 1 min, followed by a 10 min extension at 72°C.

The gene was typed in duplicate with the chicken ChickRH6 panel, and the results were scored as present

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**Table 1.** Primers used for isolating and mapping *NDRG4* in chicken.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chicken/Human</th>
<th>Identity (%)</th>
<th>Primer sequence (5'→3')</th>
<th>Size (bp)</th>
</tr>
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<tr>
<td>NDRG4</td>
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<td>82</td>
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<td>462</td>
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<tr>
<td></td>
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<td>PR₁: CACTCCACCAACCCCTCTTC</td>
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<td></td>
<td></td>
<td>PF₂: CAACATCGCCATCTTCACCTG</td>
<td>355</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PR₂: GCAACTGGAATGAGAGGAGG</td>
<td></td>
</tr>
</tbody>
</table>

*GenBank accession number.

Identity scores after comparison of predicted coding regions in the human sequence.

Primers used for sequencing.

Primers used for mapping.