Assignment¹ of the chicken *NOV* gene (alias CCN3) to chromosome 2q34→q36: Conserved and compared synteny between avian, mouse and human

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¹ To our knowledge this is the first time this gene has been mapped in chicken.

Rationale and significance

The nephroblastomas induced by the integration of MAV-1(N) (avian myeloblastosis-associated virus type 1 N) (Perbal et al., 1985; Soret et al., 1989) in the chicken genome was found to be an attractive model for the study of genes responsible for initiation and establishment of the human pediatric Wilms tumor (Perbal, 1995). Screening of lambda libraries of tumor DNAs allowed us to discover the *NOV* (nephroblastoma over-expressed) gene (Joliot et al., 1992) whose expression was found to be altered both in avian and human nephroblastomas (Chevalier et al., 1998). The NOV protein belongs to the CCN family of multimodular secreted regulators of cell growth (for a recent review see Perbal, 2001). In normal conditions its expression is associated with polarized differentiation of several different tissues. In human tumors, increased expression of NOV is either a marker of heterotypic differentiation (Chevalier et al., 1998) or associated with increased tumorigenic potential (Manara et al., 2002). Recently, NOV was reported to act as a tumor suppressor in glioblastoma cells (Gupta et al., 2001). The human NOV homolog was previously assigned to human chromosome 8q24 (Martinerie et al., 1992). In the framework of our continued study of the MAV1-(N) integration sites, we report here the localization of the chicken *NOV* gene on chicken chromosome 2q by fluorescent in situ hybridization.

Materials and methods

The isolation of MAV1-(N) junction fragments in tumor DNA (Joliot et al., 1992) allowed us to derive 23 probes representing MAV integration sites. Screening of chicken BAC (Bacterial Artificial Chromosomes) libraries with these probes made it possible to isolate several BACs containing the normal chicken sequences in which MAV was integrated (Li et al., manuscript in preparation). The NOV sequences previously reported to be a target for MAV integration were localized on BAC15. The DNA of this BAC was labeled with digoxigenin dUTP by nick translation and used as a probe for fluorescent in situ hybridization (FISH) on chicken chromosomes. FISH experiments were realized as adapted from Goguel et al. (1996), on chicken metaphases (*Gallus gallus*) obtained from embryonic cell cultures. The digoxigenin was detected using an anti-digoxigenin-rhodamine-labeled antibody and DAPI was used as counterstaining. Pictures were acquired on a Zeiss epifluorescent microscope using a tri-CCD camera and Vysis computer software. Chromosome assignment was made by reference to the DAPI banded ideogram of the chicken.

Results and discussion

Probe names: P1 (12) D10 (BAC 15)

Insert size: 110 kb

Proof of authenticity: Southern blot

Gene reference: gi|63702|emb|X59284.1|GGNOVMRNA

Probe type: BAC clone

Vector: pbleloBACII

Mapping data

Most precise location: chicken chromosome 2q3.4→q3.6

Number of cells examined: 50

Number of cells with specific signals: 1(0), 2(0), 3(2), 4(45), 7(1), 8(2) chromatids per cell. (A percentage of tetraploid cells, present in the embryonic chicken fibroblast culture, was taken into account.)

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Fig. 1. (a) Chromosomal localization of the *NOV* gene to chicken metaphase chromosomes by fluorescent in situ hybridization of digoxigenin labeled BAC 15 clone. Staining was performed with anti-Dig-Rhodamine and counterstained with DAPI. (b) Reverse DAPI banding; (c) GTG-banded ideogram of chicken chromosome 2.

The *NOV* gene was localized next to a MAV integration site in a nephroblastoma generated after its inoculation in a chicken. The physical mapping performed (Li et al., manuscript in preparation) indicates that it is not a general feature; in other tumors, MAV integration sites are not in the vicinity of *NOV*. Recent studies suggest that the synteny of the markers is relatively well conserved between chicken and man at the level of the cytogenetic band or chromosome segments (Nanda et al., 1999, Schmid et al., 2000). To date, a series of nine genes have been assigned on chicken chromosome 2q: *PRKDC* (q24→q25), *PENK*, *MOS*, *LYN* (q26), *CALB1* (q26), *CA2*, *TRHR*, *MYC* and *HSF1* of which the human homologs lie on chromosome segment 8q11→q24.3 (Schmid et al., 2000). The respective localizations of *NOV* in human 8q24 and *NOV* in chicken 2q34→q36 reinforce this chromosomal homology and suggest that the syntenic segment between the two species could be extended up to avian 2q3. Furthermore, the mouse *Nov* gene maps to chromosome 15 (Snaith et al., 1996) in a region of conserved synteny with man including *TRHR*, *MYC* and *HSF1* but not the six proximal markers of the previous man-avian shared segment: *PRKDC*, *PENK*, *MOS*, *LYN*, *CALB1* and *CA2* which are distributed on four mouse chromosomes (Schmid et al., 2000). The “*NOV* region” illustrates one of the frequent examples where synteny is better conserved between chicken and man than between man and mouse.

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