Introduction

Chicken nephroblastomas induced by MAV1-N represent a unique animal model of the Wilms tumor, a kidney cancer occurring in young children at a frequency of about 1:6000 births. Early cytogenetic studies have identified multiple chromosomal alterations in Wilms tumors, raising the possibility that several steps in the differentiation pathway of blastemal cells could represent potential targets for tumorigenic events [1].

In an attempt to characterize genes that are altered at various stages of tumor development, we have taken advantage of the histological similarities between Wilms tumor and MAV-1(N) induced nephroblastoma.

MAV is a replication competent retrovirus which can induce nephroblastomas, osteopetrosis and lymphoid leucosis when injected in chicken [2]. Molecular cloning of the MAV1(N) proviral genome permitted us to isolate a pure viral strain inducing specifically nephroblastomas when injected, either intravenously in ovo on embryonic day 18, or intraperitoneally in day-old chickens [3]. The characterization of MAV sequences contained in avian nephroblastomas established that these tumors were polyclonal and that in tumor DNA, MAV was inserted at a limited number of sites, suggesting that either the integration of MAV at other sites was not associated with nephroblastoma induction, or that the selection pressure occuring naturally during tumor development had counterselected cells carrying MAV proviral genomes at other sites [1,4].

The analysis of lambda librairies obtained from these tumors reinforced the idea that in the tumor DNA, the MAV proviral sequences were integrated at a few distinct cellular sites. The MAV genomes present in well-developed tumors were all heavily rearranged whereas in diffuse tumors of smaller size showing a less advanced tumor phenotype, the MAV genomes were full length in size and functional [5].

Use of junction fragments containing viral U3 and adjacent cellular sequences, permitted to establish that in one of the most developed tumor one of the proviral genome was integrated within a gene that is known as ccn3 and that we originally designated "nov" for « nephroblastoma overexpressed » [5].

CCN3 is one of the three founders of the CCN family of proteins which presently consists of six different members. Its expression is associated with cell quiescence [6,7]. In normal conditions, the expression of ccn3 undergoes spatiotemporal regulation in several different tissues originating from the three germ layers, with major sites of expression being adrenal, nervous system, cartilage and bone, muscle, and kidney [7-14]. The production of CCN3 protein can be increased or decreased upon carcinogenesis [7,12,15-20]. In Wilms' tumors, the expression of ccn3 was a marker of differentiation [12] whereas in Ewings' tumors, the expression of ccn3 was associated with a higher risk of developing metastasis [17]. In all cases, the full length CCN3 protein shows antiproliferative activity.

Albeit its expression was elevated in all avian tumors, the ccn3 gene was found to be disrupted in only one case, suggesting that either an unknown viral product, or MAV LTR enhancer was responsible for increased ccn3 expression. Indeed, it is well known that LTR enhancer sequences can activate transcription of genes localized several tens of kilobases away. However, the limited size (20 kb) of the insert DNA that was contained in the lambda recombinants, did not permit to establish whether MAV LTR sequences were present in the vicinity (at a genome scale) of the ccn3 gene in the DNA of all tumor cells.

Since we had isolated and studied tumors representing three increasing developmental stages, we took advantage of this material to ask whether the progression from an initial diffuse tumor to a well developed tumor, was accompanied by the selection of cells carrying particular MAV integration sites. To tackle this problem, we have used the BAC (bacterial artificial chromosome) and FISH (fluorescent in situ hybridization) strategies.

The results we report here confirm that a limited number of MAV-integration sites are detected in the DNA of MAV-induced nephroblastomas, with an over representation of integration sites on chromosome 2. In well-developed tumors, MAV-integration sites are localized in the vicinity of genes encoding proteins involved in matrix remodeling, angiogenesis, and signaling. Our results also indicated that ccn3 is not a common integration site in these tumors.

Materials and methods

Labeling of the BAC DNA fragments

Prior to labelling, the BAC DNA fragments were amplified by PCR using the Expand high fidelity PCR system from BOEHRINGER MANNHEIM. One hundred nanograms of insert was mixed with U and R primers (0.3 μM/L), 8 μl dNTP(10 mM), mix II buffer (10 μl), TaqE (3 U) and water to 50 μl. Amplification was performed for 30 cycles of 94°C for 30 seconds, 50°C for 1 min, 72°C for 1 min. The size of the amplified fragments were checked by electrophoresis in 1% agarose gels prior to purification with the QIAquick PCR Purification Kit (Qiagen). DNA fragments (50 ng of each) were labeled with the Amersham multiprime DNA labelling system (Amersham Pharmacia Biotech RPN161Z LIFE SCIENCE) in the conditions rec-