The human homologue of the retroviral oncogene *qin* maps to chromosome 14q13

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ABSTRACT Chromosomal mapping of the human *QIN* gene (renamed *FKH2* by the Human Genome Organization Nomenclature Committee) was initially accomplished by correlation of the presence of the *QIN* locus with specific chromosome regions in a rodent–human hybrid panel. This analysis revealed that the human *QIN* gene maps to chromosome region 14q11.2→14q32, between the *TCR* and *IGH* loci. Further analysis by fluorescence *in situ* hybridization techniques with a human *QIN* genomic clone refined the human *QIN* gene localization to 14q13.

The *qin* gene has been identified recently as the cell-derived insert in avian sarcoma virus 31 (ASV31) (1). It is the putative oncogene of that virus. The *qin* product belongs to a large family of transcription factors that includes the product of the homeotic gene fork head (*fkh*) of *Drosophila* and the hepatocyte nuclear factor 3 (HNF3) and brain factor 1 (BF-1) of mammals (2). The family is referred to here as the *fkh*/HNF3 family. Members of the *fkh*/HNF3 family share a unique DNA-binding domain, the *fkh* box. The three-dimensional structure of this domain has been determined on HNF-3–DNA cocrystals by x-ray diffraction at a resolution of 2.5 Å and shows a compact arrangement of β -sheets, loops, and three α -helices, one of which makes contact with the major groove of the DNA double helix, binding to DNA as a monomer (3). This DNA recognition domain has been called a “winged helix” motif; it is structurally related to histone 5.

The proteins of the *fkh*/HNF3 family are expressed in exquisitely tissue-specific manner and play a major role in determining embryonal development and tissue differentiation. The *qin* gene of the avian retrovirus ASV31 is most likely of chicken origin and is very closely related to the mammalian BF-1 protein, possibly representing the avian homolog of BF-1 (1, 4). Using a viral *qin* probe, we have isolated human clones homologous to *qin* and here report the chromosomal localization of the human *QIN* gene (renamed *FKH2* by the Human Genome Organization Nomenclature Committee).

MATERIALS AND METHODS

Probes. *QIN* belongs to a multigene family. In order to avoid cross-hybridization, *QIN*-specific probes outside the DNA-binding domain were selected that showed minimal sequence relatedness to other *fkh*/HNF3 family members. First, human *QIN* cDNA clones were isolated under stringent hybridization conditions by using a probe that represents nucleotides 768–1397 of the viral *qin* sequence (1). With this probe, clones were obtained from a human fetal cDNA library (Stratagene) and from a human placental genomic library (Stratagene). For Southern blot hybridization in the

current study, we used a cDNA fragment of human *QIN* corresponding to nucleotides 399–1352 of the viral *qin* sequence. The probe was labeled by random hexamer priming using [³²P]dCTP. For chromosomal fluorescence *in situ* hybridization (FISH), a human genomic clone obtained as an insert of 9 kb in λ phage was labeled by nick-translation with biotinylated 11-dUTP.

Rodent–Human Hybrids. Most of the hybrids used are available through the Human Genetic Mutant Cell Repository (HGMCR, Coriell Institute, Camden, NJ). Other hybrid DNAs were from previously described rodent–human hybrid cell lines (5, 6). Hybrid DNAs were tested for the presence of restriction enzyme fragments detected by a radiolabeled *QIN* probe by standard Southern hybridization methods.

FISH. The procedure used in this study is a modification (7) of previously described methods (8, 9). Probes were prepared by nick-translation using biotin-labeled 11-dUTP (Bionick kit; BRL). Hybridization of biotin-labeled probes was detected with fluorescein isothiocyanate-conjugated avidin. Metaphase chromosomes were identified by Hoechst-33528 staining and UV irradiation (365 nm), followed by 4',6-diamidino-2-phenylindole (DAPI) staining to produce the banding pattern. The fluorescent signal was observed with filter block I3 (BP450-490/LP515; Leitz Orthoplan) on the background of red chromosomes stained with propidium iodide. Q-banding was observed with filter block A (BP340-380/LP430). Micrographs were taken with Kodak Ektachrome 400 film and printed in color and black and white.

RESULTS

The Human *QIN* cDNA Probe Detects a Single-Copy *QIN* Gene in the Human Genome. DNA was extracted from mouse, Chinese hamster, and human cells as well as from several mouse–human hybrids carrying all or part of the human chromosome 14. The DNA was digested with the restriction endonuclease *Eco*RI, electrophoresed, transferred to nylon filter, and hybridized with the radiolabeled human *QIN* cDNA probe (Fig. 1). This probe detects a single restriction fragment in the mouse, hamster, and human cells, suggesting that it is specific for a single-copy gene in these mammalian genomes. Similar results were obtained with DNA cleaved by the *Hind*III endonuclease.

Detection of *QIN* Sequences in Human–Rodent Hybrids Is Correlated with the Presence of Chromosome 14q11.2→14q32.3. The radiolabeled human *QIN* cDNA probe, which detects a single *Hind*III and *Eco*RI restriction fragment in human, mouse, and hamster, was hybridized to a panel of restriction enzyme-cleaved DNAs from 17 rodent–human hybrids that together represent most human chromosome regions. Hybrids carrying human chromosome 14 in common

FIG. 1. Southern blot analysis for the presence of the *QIN* gene in a chromosome 14 regional mapping panel. *Eco*RI-cleaved DNAs ($\approx 10 \mu\text{g}$ per lane) from mouse (lane 1), Chinese hamster (lane 2), human (lane 3), mouse-human hybrid GM100479 ("479" in Figs. 2 and 3) (lane 4), mouse-human hybrid GM10660A carrying human chromosome 14 and a der 1 (1qter $\rightarrow$ 1p34.3::17q11.2 $\rightarrow$ 17qter) chromosome (lane 5), mouse-human hybrid AB3 (lane 6), and mouse-human hybrid M44 (lane 7) were electrophoresed, transferred to nylon filter, and hybridized to the radiolabeled human *QIN* cDNA probe. Each hybrid contains all or a portion of human chromosome 14, allowing a regional localization as illustrated in Fig. 2. Chromosomes present in hybrids GM100479 AB3, and M44 are illustrated in Fig. 3. Sizes are shown in kbp.

contained the *QIN* gene; all hybrids without chromosome 14 did not contain the *QIN* gene (Fig. 2). A chromosome 14 hybrid mapping panel, in which each hybrid DNA retained a specific portion of chromosome 14, was similarly tested. Hybrids carrying 14q11.2 $\rightarrow$ 14q32.3 in common retained the *QIN* gene as illustrated in Fig. 3.

Human *QIN* Gene Maps to Chromosome Band 14q13 by FISH. λ phage DNA containing a 9-kb human genomic *QIN* clone was biotinylated and hybridized to metaphase chromosomes to determine the chromosomal band position of the *QIN* gene by FISH analysis. The hybridization signal was amplified and detected with fluorescein-conjugated avidin; staining of metaphase chromosomes was with propidium iodide, and 4',6-diamidino-2-phenylindole was used to visualize the hybridization signal on banded chromosomes. A total of 14 metaphases from peripheral blood of a normal human male was analyzed; 46 fluorescent spots were ob-

FIG. 3. Regional localization of the *QIN* gene in rodent-human hybrids carrying partial chromosome 14. Hybrids carrying chromosome 14 or a region of 14 are illustrated to the right of the chromosome 14 ideogram, with results of filter hybridization to the *QIN*-specific probe indicated below the lines representing specific hybrids. The *QIN* locus is present in hybrids which retain the region 14q11.2 $\rightarrow$ 14q32.3 in common. The chromosome break in 14q11.2 is most likely within the *TCRA/TCRD* locus in hybrid AB3, and the chromosome break in 14q32.3 is within the *IGH* locus in hybrid M44. 479, Hybrid GM100479. Chromosome drawings were made by using IDIOGRAM ALBUMS software with permission (copyright 1993, David Adler, University of Washington, Seattle).

served on band 14q13. An example of a metaphase with signal at 14q13 is shown in Fig. 4.

DISCUSSION

The *fkx/HNF3* family of transcription factors attracted initial interest because of the tissue-specific expression of these genes and their involvement in developmental regulation (2). The isolation of *qin* from a retrovirus that induces sarcomas suggested that genes encoding *fkx/HNF3* also function in the control of cell growth and have oncogenic potential (1). A

FIG. 2. Presence of the *QIN* locus in a panel of 17 rodent-human hybrids. $\square$ or $\blacksquare$, Absence or presence, respectively, of the chromosome listed above the columns in the hybrid named to the left; $\blacksquare$, presence of the long arm of the chromosome (or part of the long arm represented by a smaller fraction of stippling); $\blacksquare$, presence of the short arm (or partial short arm) of the chromosome. The column for chromosome 14 is boldly outlined and stippled to highlight correlation of the presence of this chromosome (or region of the chromosome) with the presence of the *QIN* gene. The pattern of retention of the gene in the panel is shown to the right of the figure, where presence of the locus in a hybrid is indicated by a stippled box with a plus sign and absence of the locus is indicated by an open box enclosing a minus sign. 479, hybrid GM100479.

FIG. 4. FISH of human metaphase chromosomes with the *QIN* probe. (A) Complete metaphase with hybridization of the *QIN* probe to two chromosomes 14 (arrows). (B) Partial metaphase with one chromosome 14 labeled on both chromatids (arrow).

striking example of this oncogenicity was discovered recently in an analysis of the t(2;13) translocation that is found regularly in human alveolar rhabdomyosarcoma, a relatively rare pediatric tumor (10–12). This translocation generates two chimeric transcripts, one of which is seen in all cases of the tumor. It codes for a fusion protein containing the DNA-binding region of the product of *PAX3* gene of chromosome 2 and the presumptive transcriptional activator domain of a *fkf/HNF3* family member whose gene is located on chromosome 13. The *PAX3-fkf* fusion product could function as an aberrant transcription factor; it probably plays an important role in the pathogenesis of alveolar rhabdomyosarcoma.

The chromosomal localization of *QIN* is of interest in relation to a possible involvement in human disease. A loss of 14q has been observed in human neuroblastoma (13). The critical region has not yet been defined but may be distal to 14q32 (14). There are several pediatric acute T-cell leukemias in which 14q is rearranged. In a t(10;14) translocation, enhanced expression of *HOX11* has been found; in a t(11;14) translocation, a T-cell receptor gene appears to be involved; and in a t(8;14) translocation, the genes at the breakpoint have

not yet been identified (15–17). A translocation involving 14q13 has also been observed in non-Hodgkin lymphoma (18).

Few genes or loci have thus far been assigned to chromosome band 14q13 (19). A cluster of myosin heavy chain polypeptide genes has been mapped to 14q11.2→14q13 and to mouse chromosome 14 near the murine *Tcra* locus (20). The 14q11→14q12 portion of chromosome 14 is homologous to a portion of mouse chromosome 14, and this homology may extend to and possibly beyond chromosome band 14q13, which may locate the corresponding murine *QIN* in the mouse genome of chromosome 14. The 14q23→14q23 portion of human chromosome 14 is homologous to regions of mouse chromosome 12. The border of the region on human chromosome 14 that is homologous to mouse chromosome 14 has not been determined.

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