Cytogenetics of hepatoblastoma

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1. ABSTRACT

The cytogenetics of hepatoblastoma demonstrate recurring events which include whole chromosome trisomies, most commonly trisomy of chromosome 2, 8, or 10. In addition, unbalanced translocations involving a breakpoint on the proximal short arm of chromosome 1 are observed which result in a duplication of the long arm of chromosome 1q. The most commonly involved reciprocal chromosomal arm is 4q, although the reciprocal chromosome is highly variable and always results in a loss of chromosomal material. The full significance of these chromosomal changes has yet to be confirmed in large studies, however a suggestion of an association of duplication of regions of 2q with a poor prognosis. A rare sub-type of hepatoblastoma, known as the small cell undifferentiated variant, is associated with deletion or translocation of 22q, the locus of the rhabdoid tumor gene, *SMARCB1*.

2. TEXT

The cytogenetics of many childhood malignancies has led the way to identification of crucial genes which contribute to the oncogenic process, either through the creation of chimeric proteins, or by transposing an existing gene into a different regulatory setting. In many instances of childhood cancer, selection of treatment options and prediction of outcome is influenced by cytogenetics, either by the absolute number of chromosomes as in childhood leukemia, in which the presence of chromosomal trisomies, particularly trisomy of chromosome 4 or 10, is associated with a favorable prognoses, or alternatively the presence of the Philadelphia chromosome associated with a poor prognosis (1, 2).

Hepatoblastoma is a tumor characterized by recurring chromosomal abnormalities, however the full significance of these abnormalities is not yet fully
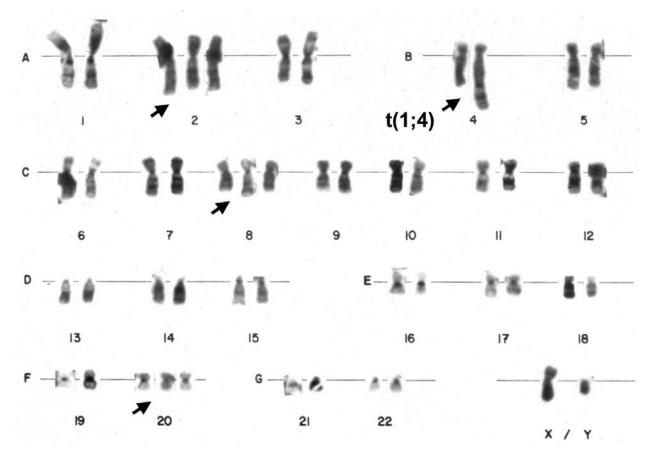
Figure 1. Standard karyotype of a hepatoblastoma of mixed fetal and embryonal histology. This tumor displays the most common chromosomal trisomies of chromosomes 2, 8, and 20 as well as the recurring translocation in which an extra copy of the long arm of chromosome 1 is translocated to the long arm of chromosome 4. Trisomies as well as the t(1;4) translocation are indicated by arrows.

understood. Some of the earliest reported cases karyotyped from young children with hepatoblastoma carried a single extra chromosome (3, 4). Other early studies focused on single cases or small series which revealed chromosome trisomes, most often chromosome 2, 8, or 20, often seen together with structural alterations (5-13).

In 1997, Schneider *et al* described a recurring translocation in which the entire long arm of chromosome 1 is translocated to the distal region of chromosome 4, with net loss of material at the distal region of chromosome 4 and a net gain of chromosome 1q (14). This translocation in most cases is seen in conjunction with the observed chromosomal trisomies. A perhaps "classic" hepatoblastoma karyotype is seen in Figure 1, demonstrating trisomy 2, 8, and 20 as well as the t(1;4) translocation.

It soon became apparent that the cytogenetics of hepatoblastoma are complex, but recurring themes have emerged. Karyotypic changes in hepatoblastomas can clearly be classified into two broad categories: numerical changes and structural changes of individual chromosomes. Numerical aberrations in hepatoblastomas are not random, but are characterized cytogenetically by distinct patterns.

Most result in addition of whole chromosomes, but occasionally result in loss of chromosomes. Trisomies of chromosome 2, 8 and 20 are the most common recurring numerical aberrations, with trisomy of chromosome 20 observed most frequently. The distribution of trisomies among the different chromosomes in hepatoblastoma karyotypes previously published is shown in Figure 2. (15). As indicated above, such non-random chromosomal trisomies are not unique to hepatoblastoma, but are seen in other pediatric tumors including acute lymphoblastic leukemia which demonstrate extra copies of chromosomes 4 and 10 and the solid renal mesoblastic nephroma which demonstrate extra copies of chromosomes chromosome 11 (1, 16).

The most common structural cytogenetic abnormality in hepatoblastoma involves an unbalanced translocation involving the long arm of chromosome 1. The initial recurring translocation described was the translocation involving chromosomes 1 and 4, t(1;4)(q12;q34), which was reported by several groups (17-19). It is notable that the reciprocal breakpoints on chromosome 4 are not identical in all tumors and have been observed to occur at both 4q32 and 4q34. Likewise the breakpoints on chromosome 1 have been observed both at chromosome

Table 1. Derivative chromosomes resulting from unbalanced translocations in which the long arm of chromosome 1 is translocated to a reciprocal chromosome with loss of distal material from the reciprocal chromosome
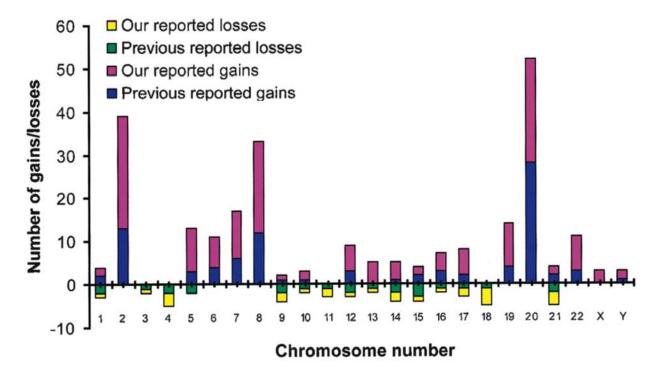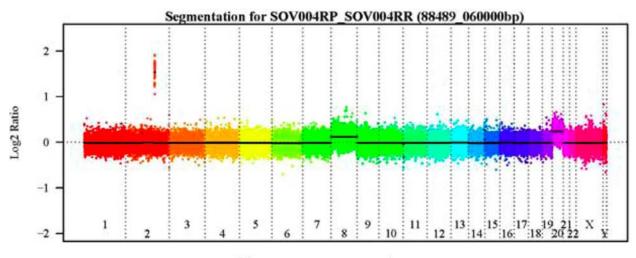
der(1)t(1;1)(p36;q12)	der(5)t(1;5)(q21.3;q31.3)
der(2)t(1;2)(q12;q35)	der(6)t(1;6)(q12;q22.2)
der(2)t(1;2)(q12;q37) (n=2)	der(6)t(1;6)(q12;q23)
der(3)t(1;3)(q12;q27)	der(6)t(1;6)(q21;q25)
der(4)t(1;4)(q12;q27)	der(11)t(1;11)(q21;q25)
der(4)t(1;4)(q12;q33)	der(12)t(1;12)(q12;q24.1)
der(4)t(1;4)(q12;q34) (n=3)	der(12)t(1;12)(q12;q24.3)
der(4)t(1;4)(q12;q35)	der(12)t(1;12)(q12;q24.3)
der(4)t(1;4)(21;q32)	der(14)t(1;14)(q12;p12)

Figure 2. Distribution of chromosome gains and losses in previously reported cases of abnormal karyotypes. Columns above the x axis represent total number of instances of chromosome gain; below the x-axis, total number of chromosome losses. Reproduced with permission from (15).

1q12 and 1q21. It had since been reported in a large series that hepatoblastomas that there exists a family of chromosome translocations with similar breakpoints on either chromosome 1q12 or 1q21 and multiple different reciprocal chromosome arms (15). The different resulting derivative chromosomes are shown in Table 1. Each such translocation observed is unbalanced, resulting in a gain of the long arm of chromosome 1 and loss of material on the distal part of the reciprocal chromosome. These translocations are frequently associated with numerous whole chromosomal gains as well.

The clinical significance of these translocations and numerical abnormalities is just beginning to be explored. An interesting case of partial duplication of chromosome 2 revealed by fluorescence *in situ* hybridization in which a segment on the long arm of chromosome 2 is inserted into chromosome 9, suggested that the critical region for trisomy 2 is on the long arm

between 2q21 and 2qter (20). A region of amplification of chromosome 2q localized to 2q24 has been noted to be associated with a poor prognosis (21).

Although these whole chromosome changes have described previously by classical karyotype analysis, they can also be visualized by whole genome comparative genomic hybridization (CGH) as shown in Figure 3. CGH determines copy number such that whole chromosome additions or deletions are easily detected. The recurring translocations involving breakpoints on chromosome 1q can also be detected as an increase in copy number along the length of the q arm of chromosome 1. CGH is used increasingly in analyzing tumors for genetic changes at the chromosome level, as it does not rely on dividing tumor cells.   There are limitations in CGH, however, in that it is not possible to ascertain with certainty the reciprocal arms of unbalanced translocations, particularly in complex karyotypes. In addition any balanced chromosomal translocations, although infrequent in hepatoblastoma, would   not   be   detected   by   CGH.

Segmentation for SOV004RP_SOV004RR (88489_060000bp)

Log2 Ratio

Chromosome number

Figure 3. Oligonucleotide array Comparative Genomic Hybridization (oaCGH) plot of data comparing DNA copy number in tumors compared to DNA from non-malignant tissue (blood). The 22 chromosomes, X and Y chromosomes are plotted on the x-axis. On the y-axis is plotted the log of the difference in copy number in tumor tissue compared to non-malignant tissue from the same individual. A value of 0 is equal to no change in copy number in tumor compared to normal. Note that there is an excess in all of chromosome 8 and chromosome 20, indicating that this tumor has trisomy 8 and 20, although the extent of increase is different in the two chromosomes. The difference could be due to clonal evolution, in which some clones had only and extra chromosome 8 and some clones had both and extra 8 and extra 20. Alternatively, this observation could represent tetrasomy 20 in some cells, although this is not frequently seen. Also seen in this oaCGH plot is amplification of a narrow region on chromosome 2. This corresponds to the 2q24 region previously described as associated with a poor prognosis (21).

Approximately 5% of cases of hepatoblastoma are of the small cell undifferentiated (SCU) variant. Several cases of SCU hepatoblastomas have been reported in the literature with karyotypes showing deletion or translocation of 22q. (22, 23) We recently reported that SCU variant of hepatoblastoma was characterized by absence of the SMARCB1 gene (also known as hSNF5/INI1, or the rhabdoid tumor gene), which maps to chromosome 22q11. Using oaCGH a small interstitial homoyzgous deletion on chromosome 22q11 encompassing *SMARCB1* was detected in tumor tissue from a young infant with a hepatoblastoma with SCU histologic features. (24) These molecular cytogenetic findings, together with demographic and clinical characteristics, suggest that this variant of hepatoblastoma has similarity to rhabdoid tumors of other sites.

Hepatocellular carcinoma is less common in children, but is also characterized by chromosomal gains and losses, with loss of the Y chromosome notable. (25). Duplication of chromosome 1q is also seen in hepatocellular carcinoma with increased expression of numerous chromosome 1 genes (26, 27). Cytogenetic data on the fibrolamellar variant of HCC is sparse, but one childhood fibrolamellar carcinoma has been characterized by a hypertriploid karyotype with clonal evolution with multiple additional chromosomal gains as well as loss of the Y chromosome. (28).

The field of cytogenetics of hepatoblastoma continues to move forward. Current efforts are being applied both in Europe and in the United States, to understand the impact on prognosis of both the numerical aberrations as well as the structural abnormalities. The precise breakpoint on chromosome 1 has remained elusive to date because of the difficulty in mapping this highly repetitive region of the genome. (29).

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