

GENETICS OF SMALL ROUND CELL TUMORS OF CHILDREN

Mohammed Akhtar, MD, FCAP, FRCPA; M. Anwar Iqbal, PhD, FACMG

Small round cell malignant tumors (SRCT) of children represent a heterogeneous group of neoplasms which are histologically characterized by relatively undifferentiated small round cells. SRCT usually include malignant lymphoma, Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, Wilm's tumor and desmoplastic small round cell tumor.¹⁻⁴ Since many SRCT are predominantly composed of undifferentiated elements, morphologic distinction between the various subtypes may be difficult. A variety of ancillary studies may be employed for making these distinctions and arriving at a correct diagnosis. These include electron microscopy, immunohistochemistry, flow cytometry, image analysis, cytogenetics and molecular genetics, among others.⁵⁻⁹

In recent years, important strides have been made in the area of the genetics of cancer. It is now understood that many cancers arise as a result of genetic abnormalities developing in a multistep fashion, although in SRCT, only a small number of genetic mutations may be sufficient for development and progression of these neoplasms.⁶⁻⁸ Knowledge and understanding of these genetic abnormalities may provide valuable insight into the biology of these tumors, which may be extremely useful not only for establishing a definitive diagnosis, but also for predicting their clinical behavior. A definitive pathologic diagnosis may be crucial in view of ongoing development of tumor-specific therapeutic modalities and concomitant improvement in prognostic outlook. Precise information regarding specific genetic abnormalities may be used for developing specific therapeutic regimens in which intensity of treatment may be tailored to the perceived aggressiveness of the neoplasm.

Routine cytogenetic studies have provided valuable information regarding the cytogenetic abnormalities in solid tumors, although technical difficulties may limit the usefulness of this technique. The drawbacks inherent in routine karyotyping include the requirement for fresh tumor tissue, frequent overgrowth of normal stromal cells during culture, and the difficulty in accurate karyotyping

of poor quality metaphases. Furthermore, increased utilization of primary chemotherapy and the increasing popularity of minimally invasive biopsy techniques such as fine-needle aspiration biopsy, which provide an extremely small tissue sample, has compounded these difficulties.¹⁻⁵ Successful cytogenetic analysis is usually possible in about 50% of Ewing's sarcoma, and 30% of neuroblastomas.⁷ Recently, additional, more effective and easily applicable modalities for studying the various genetic mutations and translocations have been developed. These include fluorescent in-situ hybridization (FISH), polymerase chain reaction (PCR), and reverse transcriptase polymerase chain reaction (RT-PCR). These techniques have an advantage over the conventional karyotyping, since the success rate is high and fresh unfixed material as well as formalin-fixed, paraffin-embedded tissue may be used.⁹

In this review, a multitude of genetic abnormalities encountered in various subtypes of SRCT are discussed and their significance in the diagnosis and management of these tumors is briefly reviewed.

Malignant Lymphoma

An overwhelming majority of malignant lymphomas encountered in infancy and childhood belong to the category of Burkitt's and non-Burkitt's lymphoma (BL) and lymphoblastic lymphoma (LBL).¹¹ Occasionally, large cell lymphoma (LCL) and anaplastic large cell lymphoma (ALCL) may be seen. In most human lymphomas, translocations of genetic material between chromosomes are responsible for activation of oncogenes, which lead to initiation and progression of these neoplasms. The oncogene involved in BL is C-myc, which is transcriptionally silent at its normal location at chromosome 8q24 region. However, translocation of C-myc to loci of immunoglobulin heavy chain (14q32) or immunoglobulin light chain lambda (22q11) or immunoglobulin light chain kappa (2p12) results in the deregulation of the C-myc gene, which becomes expressed at higher levels. Thus the translocations encountered in BL are: t(8;14)(q24;q32) (Figure 1), t(8;22)(q24;q11) and t(2;8)(p12;q24), respectively. BL usually arises in a background of excessive proliferative stimulus provided by Epstein-Barr virus infection, or an antigenic stimulation resulting in expansion of rearranged immunoglobulin genes. It is generally believed that due to excessive cellular proliferation, the possibility of chromosomal translocation

From the Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Address reprint requests and correspondence to Dr. Akhtar: Department of Pathology and Laboratory Medicine, MBC-10, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.

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FIGURE 1A. Typical chromosome translocation $t(8;14)(q24;q32)$ seen in Burkitt's lymphoma. Partial karyotype showing $t(8;14)$, arrows pointing to the breakpoint.

also increases with time. Translocation of C-myc appears to be crucial but not necessarily the only genetic event in the initiation and irreversible progression of BL.^{11,12}

Gene rearrangement studies in lymphoblastic lymphoma have revealed rearrangement of T-cell receptor β -chain gene as the most frequent abnormality. Unlike in BL, there is no single recurrent translocation or chromosomal abnormality that is observed in most LBL. Up to 30%, however, contain one of several possible translocations involving chromosome band 14q11 or 7q35, which are now known to be the site of the T-cell receptor gene complex.^{11,12}

Neuroblastoma

Neuroblastoma is the most common SRCT among infants and children. Cytogenetic and molecular genetic studies have identified characteristic chromosomal changes which not only support the diagnosis but also correlate with disease stage and help to predict tumor behavior.^{6-11,13} The most frequent and consistent cytogenetic abnormality identified in neuroblastoma is the deletion of the short arm of chromosome 1, resulting in loss of heterozygosity (LOH). This deletion has been mapped to the distal end of the short arm of chromosome 1 at p36.2-3. The loss of one or more suppressor genes located at this locus may be partly responsible for the development of neuroblastoma, although no specific genes have yet been identified.

Another important cytogenetic abnormality in neuroblastoma is amplification of N-myc, which is present in approximately 25%-30% of the tumors.^{6-11,12} It is seen more frequently in advanced stages (30%) of neuroblastoma as compared to those in early stages (5%-10%). Amplification of N-myc may be recognized in routine cytogenetic preparations as well as by FISH analysis as extrachromosomal double minutes or homogeneously stained regions in various chromosomes. FISH analysis on touch imprints or aspiration smears has been shown to be an effective way to identify N-myc



FIGURE 1B. Ideogram of chromosome 8 and 14 shows the genes involved.

amplification (Figure 2). Several studies have now established N-myc amplification as an important indicator of prognosis independent of all other determinants of clinical behavior. Neuroblastomas with N-myc amplification are associated with aggressive behavior and extremely poor clinical outcome.

Neuroblastoma may undergo regression or differentiation, either spontaneously or in response to therapy. Since nerve growth factors are crucial for the development and differentiation of sympathetic neurons, many authors have studied the expression of nerve growth factor receptors in neuroblastoma and the response of neuroblastoma cells to nerve growth factors. The principal component of nerve growth factor receptor is encoded by the TRKA proto-oncogene. NB with high expression of TRKA proto-oncogene in neuroblastoma have been shown to behave in a less aggressive fashion, while those tumors which lack such expression tend to have poor prognosis.^{6-11,13} TRKA proto-oncogene expression may be studied by RT-PCR or by immunohistochemistry using monoclonal or polyclonal antibodies. These antibodies may be applied to fresh unfixed tissue as well as formalin-fixed, paraffin-embedded material. It is not yet known how nerve growth factor and its receptors affect the development, progression and regression of NB.

Analysis of DNA content of NB may also provide valuable information regarding the clinical behavior and response to therapy.^{6,13} Tumors with hyperdiploid, especially triploid, DNA content tend to have a good response to chemotherapy and a favorable outcome. Diploid and tetraploid tumors, on the other hand, are associated with poor prognosis. Evaluation of DNA may be particularly useful in infants less than 12 months old, since the predictive value of DNA ploidy is especially pronounced in this group of patients.

Recently, Brodeur et al. have suggested a clinical genetic classification of patients with neuroblastoma based on a variety of known prognostic markers.¹⁵ These patients

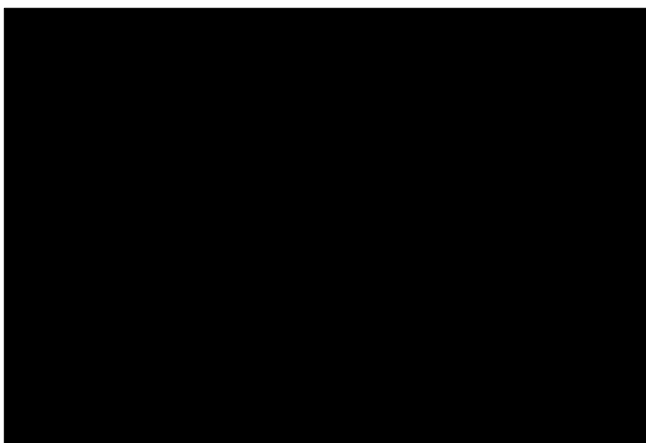


FIGURE 2A. Amplified copies of N-myc gene in neuroblastoma. Diagram showing the mechanism of N-myc gene amplification either in the form of double minute chromosomes or homogeneously staining region (HSR).

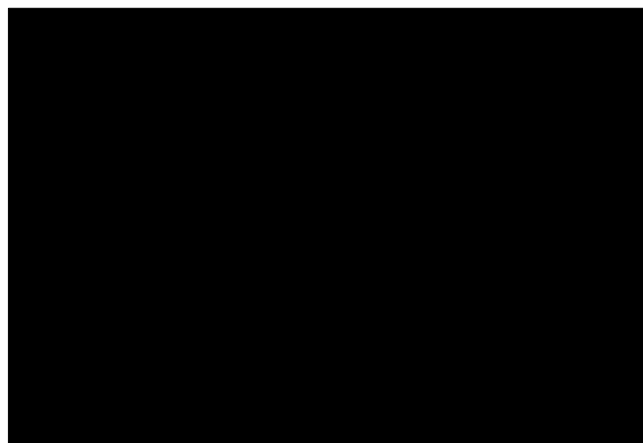


FIGURE 2B. Detection of N-myc amplification by FISH analysis. Touch prep cells from an advanced stage NB tumor hybridized by a digoxigenin-labelled N-myc DNA probe (Cat #P5115, Oncor, Inc, USA). Tumor cells show 10 to 50 fold increases in N-myc signals.

may be divided into three distinct risk groups in order to predict prognosis and devise specific therapeutic regimens tailored to the aggressiveness of the tumor. The first group consists of those tumors with hyperdiploid DNA, high TRKA expression but no 1p deletion or N-myc amplification. Patients with these tumors are usually infants with a low stage of disease. The second group consists of tumors that have a near-diploid DNA content, usually with 1p deletion, high TRKA expression but without N-myc amplification. The patients are generally older with advanced stages of the disease. The third group is characterized by tumors with N-myc amplification, 1p loss and low or absent TRKA expression. The patients are one to five years of age, and have advanced stages of the disease. The prognosis in group I patients is excellent, while patients in group III have an extremely poor outcome. The clinical outcome of group II is intermediate between the other two groups. These groups appear to be genetically distinct and one type seldom evolves into another. Recognition of these clinical/genetic subsets may permit an accurate prediction of outcome and may help evolve therapeutic modalities which are tailored to the perceived aggressiveness of the tumor, thus optimizing the chances of success with minimum side effects.

Several additional markers have also been studied for their potential prognostic significance in NB.^{6,12} These include expression of CD44, multiple drug resistance, BCL2 and NB84. However, the available data is conflicting regarding the predictive value of these markers in terms of the clinical behavior and ultimate outcome.

Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in infants and children. The light microscopic diagnosis of RMS is dependent on demonstration of myogenic differentiation, which may on occasion be difficult, especially when the tumor is poorly

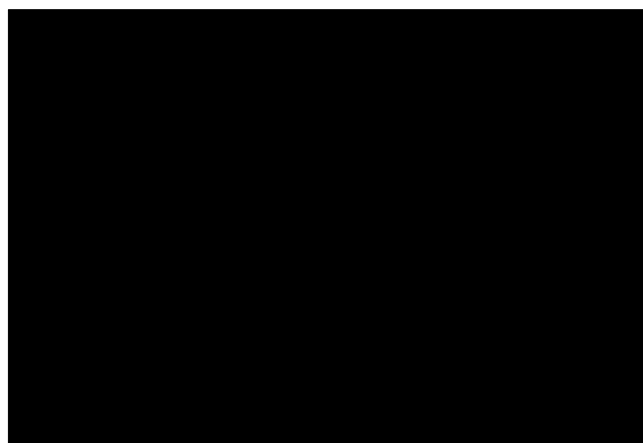


FIGURE 3. An ideogram representing the typical translocation t(2;13)(q35;q14) characteristic of alveolar rhabdomyosarcoma with the respective genes involved.

differentiated. Classification of RMS in children generally includes three subtypes, namely, embryonal, alveolar, and a solid variant of alveolar.⁶ Pleomorphic RMS, which may occasionally be encountered in adults, is extremely rare in children. This classification, although based on morphology alone, has excellent correlation with clinical outcome and prognosis. Thus embryonal RMS has a much better survival rate compared to the alveolar and the solid variant of alveolar RMS. In many of the cases, the distinction between the various subtypes of RMS may be possible on morphology alone, but in some cases there may be considerable morphologic overlap, so that additional studies may be required for arriving at a precise classification. Genetic studies may be extremely helpful, not only for distinguishing RMS from other subsets of SRCT, but also for assigning RMS into subtypes.⁶⁻⁸

Several genes involved in development and differentiation of normal muscle may also be expressed in RMS. The commitment to myogenic lineage is under the



FIGURE 4A. The typical translocation $t(11;22)(q24;q12)$ observed in Ewing's sarcoma. This shows a partial karyotype of $t(11;22)(q24;q12)$, arrows indicating the breakpoint.

control of a small set of regulatory proteins, including Myo D1, a nuclear phosphoprotein which is expressed during normal myogenesis and in normal skeletal muscle, but which has not been detected on other normal or neoplastic tissues. Detection of Myo D1 gene expression both at the RNA and at the protein level has been shown to be an extremely valuable aid to diagnosis of RMS.^{6,16} Diagnostic potential of Myo D1 expression has been expanded by the development of polyclonal and monoclonal antibodies to the Myo D1 protein. Their use in the differential diagnosis of RMS has been demonstrated in a large series of pediatric solid tumors.

Embryonal subtype is the most common RMS in children. Genetic studies of RMS have revealed that embryonal subtype is consistently associated with LOH at 11p15.5.⁶⁻⁸ The genes involved in this LOH appear to be the insulin-like growth factor II (IGFII) gene, which is a potential oncogene, and H19, which is probably a tumor suppressor gene. These two genes are regulated by parental gene imprinting, i.e., the activity of their alleles is dependent upon parental origin. The paternal allele controls expression of IGFII gene, while the maternal allele controls H19. In embryonal RMS, there is usually increased expression of IGFII, suggesting that both alleles may be expressing IGFII due to gene duplication or to loss of imprinting. Since H19 has been shown to have a growth suppressor function, loss of its expression on the maternal allele may contribute towards development of RMS.

Alveolar RMS, including the solid variant, is considerably less common than the embryonal RMS, but is associated with aggressive behavior and adverse clinical outcome. These tumors are mostly characterized by $t(2;13)(q35;q14)$ translocation (Figure 3).⁶⁻⁸ This translocation involves two genes, namely PAX3, located at 2q35, and the FKHR gene at 13q14. In this translocation, the 5' region of PAX3 gene fuses with the 3' region of FKHR gene, resulting in a PAX3-FKHR fusion gene on chromosome 13. The chimeric protein is a powerful



FIGURE 4B. Ideogram representing $t(11;22)$ and the genes involved.

transcriber and thus functions as an oncogene, which may be an important factor in the development of alveolar RMS. PAX3 is an evolutionary conserved transcription factor which inhibits myogenic differentiation of the primitive myoblasts until their migration to the developing limbs is complete. Naturally occurring mutations in mice result in mutant *Spotch* type, which develops without limb musculature. In humans, very rare homozygous mutations of PAX3 are associated with major limb defects and complete absence of muscles.⁸ PAX3-FKHR chimeric protein has been shown to have a more pronounced inhibitory effect on myogenic differentiation of cultured myoblastic cells, compared to wild type PAX3. It is interesting to note that most of the alveolar rhabdomyosarcomas occur in the extremities, and are generally composed of undifferentiated cells in which myogenic differentiation may be difficult to demonstrate.

In addition to PAX3-FKHR gene translocation, a variant $t(1;13)(p36;q14)$ translocation has also been reported to occur in approximately 10% of alveolar RMS.⁶⁻⁸ This translocation involves fusion of PAX7 gene on chromosome 1 to FKHR on chromosome 13, and the protein encoded by this fusion gene is also a powerful transcriber. PAX3-FKHR and PAX7-FKHR overexpression occurs by separate mechanisms. Transcription of PAX3-FKHR gene is increased relative to wild type PAX3 by a copy number independent process. In contrast to PAX7-FKHR, overexpression results from fusion gene amplification.²¹ Both these mechanisms ensure a critical level of gene product for oncogenic effect of these tumors. Recently, it has been suggested that PAX3-FKHR and the variant PAX7-FKHR fusion gene may be associated with distinct clinical phenotypes.²² The group with PAX7-FKHR is young and presents more often with extremity lesions.

The metastatic disease in this group was generally limited to bone. In patients with PAX3-FKHR fusion, the metastases were more widespread, involving lung, bone,

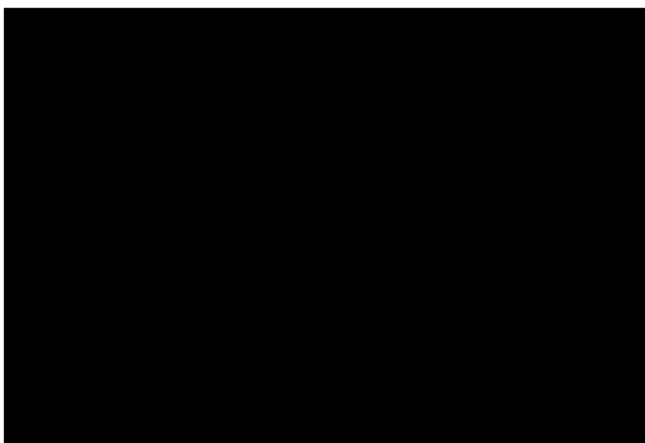


FIGURE 5A. Partial karyotype of chromosome 11 showing the deletion of WT1 gene located at 11p13 region, which is commonly seen in Wilm's tumor.

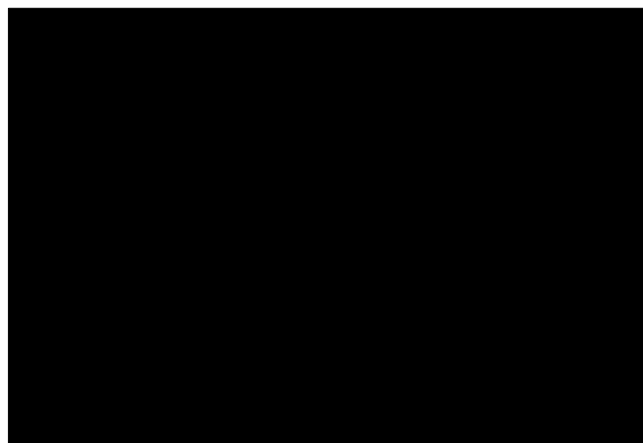


FIGURE 5B. Ideogram of chromosome 11 showing the deletion of WT1 gene located at 11p13 region.

lymph nodes and brain. Event-free survival for the PAX7-FKHR group was significantly longer. Thus identification of fusion gene status may be a useful prognostic tool in alveolar RMS.

DNA ploidy of status of RMS may have a correlation with morphologic subtypes and clinical outcome. Ploidy may be determined by flow cytometry, image analysis or cytogenetic and FISH analysis. Diploid or near tetraploid tumors usually have alveolar morphology and a poor survival rate. Hyperdiploid tumors, on the other hand, are embryonal type and have a considerably better prognosis.^{7,23}

Ewing's Sarcoma

Ewing's sarcoma (ES) and peripheral primitive neuroectodermal tumor (PNET) belong to a family of tumors which manifest considerable morphologic overlap and have a similar histogenesis.²⁴ While Ewing's sarcoma occurs primarily in bone, PNET is most often seen in the soft tissue. A close relationship between these tumors is further emphasized by the fact that both have identical genetic abnormalities. The most frequently encountered genetic anomaly in ES/PNET is t(11;22) (q24;q12), which is present in up to 90% of these neoplasms (Figure 4).^{6-11, 24,25} This translocation involves FLI1 gene at chromosome 11 and ES gene at chromosome 22. Ewing's sarcoma gene is a ubiquitously expressed RNA-binding gene, while FLI1 is a member of the ETS family of genes which encode DNA-binding transcription factors. The translocation results in an ES-FLI1 fusion gene on chromosome 22 encoding DNA-binding protein, which is a powerful transcriptional activator with transforming activity. In a small minority of cases, variant translocations involving fusion of ES gene with other members of the ETS family of genes may be seen.^{6-11,23,25,26} These include the ERG gene on chromosome 21, and ETV-1 gene on chromosome 7. The resulting translocations are t(21;22) (q22;q12) and t(7;22) (q22;q12), respectively. Both these translocations

result in fusion genes with ES gene on chromosome 22 and encode DNA-binding proteins which are powerful transcription factors. There appear to be no significant clinicopathologic differences associated with these variant translocations.

Staining for MIC2 gene product has recently provided surgical pathologists with an extremely useful tool for recognition of ES/PNET. MIC2 appears to be a ubiquitous gene located in the pseudoautosomal regions of X and Y chromosomes. The gene product is normally found in virtually all human tissues and is thought to play a role in cellular adhesion processes.²⁴ It is a transmembrane glycoprotein, a 30/32 kd cell surface antigen which has recently been clustered as CD99. A 29 kd intracellular protein has also been recognized. MIC2 gene product also appears to be similar to E2 antigen on T-lymphocytes. Three commercially available antibodies, namely 12E7, 013 and HBA 71, have been used for recognizing CD99 in formalin-fixed, paraffin-embedded material. More than 90% of ES show positivity for these markers, although reactivity may also be seen in other SRCT, such as RMS and WT. Lymphoblastic lymphoma also shows consistent CD99 positivity. Therefore, it is essential that diagnosis of SRCT be based on a panel of antibodies rather than CD99 alone.

Wilms' Tumor

Wilms' tumor (WT) is the most common malignant tumor of the kidney in infants and children. At least two genes are associated with the development of WT. WT-1, a suppressor gene located at 11p13, was isolated in 1990 through the study of syndromes associated with Wilms' tumor.^{6-11,26,27} WAGR syndrome (Wilms' tumor, aniridia, genito-urinary anomalies and mental retardation) is associated with constitutional deletion of one allele of WT-1 gene (Figure 5). On the other hand, a constitutional mutation of WT-1 is encountered in Denys-Drash syndrome, and in patients with sporadic bilateral WT and

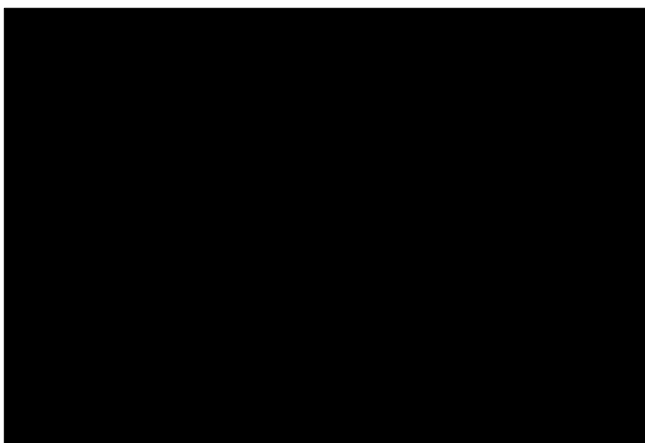


FIGURE 6. An ideogram representing the common translocation t(11;22)(p13;q12) seen in DRCT tumors. The fusion genes involved are shown on the der (22) chromosome.

about 10% of sporadic unilateral WT. In these patients, the mutation affects only one allele, but in the tumor cells the mutation may be homozygous. The mutation may result in a truncated protein or may give rise to abnormal proteins with new properties.

WT-1 gene encodes a zinc finger protein which can function as a transcription factor.^{6-11,27} The WT-1 messenger RNA is subjected to alternative splicing, giving rise to up to 4 isoforms which differ in their DNA-binding specificity and in their effect on transcription. Expression of WT-1 is restricted to only a few tissues, namely, developing kidney, gonad, spleen, intestine, muscle, central nervous system and mesothelium. Within the developing kidney, the WT-1 expression is primarily seen in blastemal tissue and in the differentiated kidney in podocytes. WT-1 appears to be essential for the development of normal kidneys and gonads, since mice homozygous for WT-1 null mutations fail to form kidneys and gonads.

A second WT gene (WT-2) maps to chromosome 11p15.5. Beckwith-Weidmann syndrome (BWS) carries a propensity to develop embryonal tumors, including WT and is characterized by loss of heterozygosity restricted to this region.^{6-11,27} However, it is not clear whether single or adjacent genes are involved in both the tumor and the syndrome. In tumors with loss of heterozygosity, only the maternal copy of 11p15.5 is lost, suggesting that the two copies of WT-2 gene are functionally different. This phenomenon is due to gene imprinting, where one allele is imprinted in a parental-specific manner to be inactive. Studies have shown that paternal allele is imprinted to control the expression of insulin growth factor II, a potential oncogene, while the maternal gene controls H19, which is a tumor suppressor gene. Loss of the maternal allele or loss of imprinting usually results in overexpression of IGFII and loss of H19. A similar preferential loss of maternal allele may be seen in approximately one-third of sporadic WT, suggesting a

similar mechanism may be operating. Linkage studies of rare but large pedigrees of familial WT have excluded WT-1 and WT-2, suggesting the existence of yet another unidentified locus responsible for these tumors.

In addition to the above-mentioned three genetic loci which are implicated in predisposition or genesis of WT, there is evidence for genetic loci which may be involved in the progression to more malignant or aggressive tumors.⁸ LOH at chromosome 16q has been observed in 15%-20% of WT and at 1p in about 8%. Constitutional loss of these loci has not been associated with a predisposition to develop WT, however, tumor-specific loss of either region is associated with an adverse outcome.

The tumor suppressor gene P53 appears to play a role in a subset of tumors.⁸ Tumor-specific mutations have been shown to be specifically associated with the so-called anaplastic histology, a subgroup with a particularly adverse prognosis. The mutation appears to be limited to the anaplastic component of the tumor.

Desmoplastic Round Cell Tumor

Desmoplastic round cell tumor (DRCT) is a rare tumor generally encountered in young adult male patients. The tumor usually presents as a hard multinodular mass, which is histologically characterized by a nesting arrangement of undifferentiated round cells associated with marked desmoplasia.^{6,9} Most of the tumors are located in the abdomen and pelvis, although an increasing number of additional sites have been involved, including ovary, scrotum, pleura and central nervous system. Only a few karyotypic studies have been performed which have consistently shown a translocation involving Ewing's sarcoma gene and WT-1 gene t(11;22)(p13;q12) (Figure 6).^{6,9} Similar findings have been noted in studies using RT-PCR.^{30,31} This translocation results in an EWS-WT-1 fusion gene on chromosome 22. These abnormalities may be extremely useful in arriving at a correct diagnosis, but have no prognostic value since this tumor has a uniformly poor prognosis.

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