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Progress of fundamental research in Wilms' tumor

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Abstract The progress of fundamental research on the histopathological and molecular genetic properties, model systems, growth factor involvement, and tumor markers of clinical nephroblastoma (Wilms' tumor) are reviewed. Histologically, Wilms' tumor (WT) has been found to reveal a disorganized renal developmental process in which blastema and epithelia are randomly interspersed in varying amounts of stroma. Anaplasia is the only criterion for assigning a WT as having an "unfavorable histology." Cytogenetic analysis identified WT genes at chromosome 11p13 (WT1), 11p15 region (WT2), and 16q (WT3). Permanent in vitro WT cell lines and in vivo WT models, such as human xenografts, have been established which provide indefinite sources of tumor material for fundamental, as well as therapy-directed, research. Abnormalities of growth factor (GF) expression in WT indicate that GF may play an important role in WT pathogenesis. A series of monoclonal antibodies was tested in WT by immunohistochemical techniques to identify specific diagnostic and prognostic markers. p53 expression in anaplastic WT is significantly higher than in differentiated WTs, indicating p53 may be a prognostic marker. Although significant progress has been made in the fundamental research, our basic knowledge of this malignancy is still limited. The availability of suitable experimental models, particularly the human xenograft system, offers the opportunity for further study of the cell biological and molecular aspects of WT and its clinical progression.

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Wilms' tumor (WT) has been a tumor of frequent occurrence in children since its first report by Wilms in 1889[92], occuring in approximately 1/10 000 children between 1 and 6 years of age and accounting for 85% of all childhood kidney cancers [6, 50]. The treatment outcome of children with a WT has considerably improved during the last 2 decades, in terms of both quantity of survivors and quality of long-term survival [6, 47, 50]. Despite this remarkable improvement, recurrence occurs in approximately 20% of patients with stage II and III disease and in over 50% of those with stage IV disease or with tumors with unfavorable histologic characteristics [16, 35, 91]. Nevertheless, there is a clear need for more fundamental research into the cell-biological properties and the mechanisms underlying the process of the progression of WTs. This paper reviews the current status and progress in fundamental research of WT.

Histopathological properties and molecular genetics

Histologically WT is composed of three major elements in variable proportions: undifferentiated condensed embryonal cells (blastema), differentiated epithelial cells (tubular), and variably differentiated mesenchymal cells (stroma). Typically, the histology of WT reveals a disorganized renal developmental process showing blastema and epithelium randomly interspersed in varying amounts of stroma [63]. The classical form is composed of embryonic blastemal cells, while the differentiated form may contain a variety of cell types, including striated muscle cells, squamous epithelial cells, and cartilaginous cells. The coexistence of blastemal, epithelial, and stromal cells has led to the term "triphasic" to characterize the histologic composition of "classic" WT, while other WTs present only biphasic or even monomorphous histologic patterns [9].

The blastemal part of WT is the most malignant component, with a relatively high proliferative activity and with the potency to metastasize, whereas epithelial and stromal components are hardly, if at all, represented in metastases [49, 91].

Each of the three cellular components of WT can exhibit focal or diffuse anaplasia which has been defined by the National Wilms' Tumors Study (NWTS) as having enlarged hyperchromatic nuclei and abnormal mitotic figures [8, 11]. Anaplasia is the only criterion for assigning a WT as having "unfavorable histology". The adverse prognostic significance of anaplasia mandates careful sampling of the whole tumor. Experience suggests that anaplasia is a marker of increased resistance to chemotherapy but does not denote increased tumor aggressiveness. Therefore, stage I WT with anaplasia did clinically as well as classic WT of the same stage when treated similarly. This result is apparent because in stage I tumors all malignant cells are usually removed at the time of surgery and therefore increased resistance to therapy has had no adverse significance [9].

Nodular renal blastema representing metanephric blastema persists after 36 weeks of gestation. When these focal remnants become confluent, invade the kidney, and cause massive enlargement, the condition is called nephroblastomatosis [57]. Hou was the first to describe a unique specimen of symmetric, diffuse nephromegaly in a neonate, in whom the entire renal parenchyma microscopically resembled WT [42]. This condition was termed "nephroblastomatosis," which subsequently has been used more generally to describe a variety of lesions thought to represent precursors of WT or their derivatives. In other literature the term "nephrogenic rest" (found in about 1% of routine infant postmortem examinations) is used to denote any precursor lesion of WT. This term implies the abnormal persistence of incompletely differentiated cells from the stage of renal embryogenesis, and the presence of nephrogenic rests in patients with WT seems to coincide with a worse prognosis [7, 9]. Unilateral and bilateral kidneys that give rise to WT frequently contain foci with nephrogenic rests which are located either around the periphery of the renal lobule, or within the lobule [65].

Remarkable advances have been made in elucidating specific molecular events involved in WT pathogenesis. Cytogenetic analysis of cells from WT patients has identified deletions encompassing the p13 band of chromosome [11, 54]. Furthermore, restriction fragment length polymorphism (RFLP) analyses have determined that a reduction to homozygosity of DNA markers has occurred at two independent loci; one at 11p13, designated WT1, and one at 11p15 (or 11p15.5 region), designated WT2 [23, 67]. These observations correlate the loss of a genetic locus in the p13 and 15p regions of chromosome 11 with malignant expression in WT and therefore these regions have been postulated to contain tumor suppressor loci [19, 53, 76]. The possibility of a third WT locus was suggested by lack of linkage to either 11p13 or 11p15 markers in familial WT [37, 43]. Allotyping of over 100 WT cases has demonstrated that the long arm of chromosome 16 is also a location for loss of heterozygosity, suggesting the presence of another putative WT gene at 16q [55].

WT1 is defined as a tumor suppressor gene which was tested by cell fusion experiments and by introducing fragments of chromosome 11 in the tumor-derived cell line G401, leading to suppression of tumorigenicity in nude mice [90]. WT2 has been implicated in Beckwith-Wiedemann pathogenesis, and is suggested to play an important role in pathogenesis of WT [6].

Transcriptional repression by WT1 of appropriate receptor constructs in transfection assays has identified the in vitro target genes for the WT1 product. These include epidermal growth factor receptor (EGFR)-1, Pax-2, insulin-like growth factor (IGF)-2, platelet-derived growth factor (PDGF)-A, and IGF-1 receptor. Based on sequence analysis, insulin receptor, *EGFR*, c-*raf*, k-*ras*, and c-*myc* genes could also be candidates. WT1 expression was shown to be a good marker for tumor differentiation and reveals how the normal pattern of differentiation is disrupted in WT histogenesis [31, 58, 64].

Experimental Wilms' tumors

Many aspects related to the properties of clinical WT, and to the treatment of this malignancy, can only be investigated by the use of suitable in vivo and vitro model systems. Such models include tumors that have been developed in laboratory animals, transplantable human tumors in athymic nude mice, and in vitro cell lines derived from either human or nonhuman tumor tissue [29, 39, 60, 80, 74, 93]. Generally such models provide indefinite sources of cell material or tumor tissue and a tool to study response to drug treatment and molecular genetic manipulations.

Models of nonhuman origin

Spontaneous WTs occur with relative high frequency in swine and have also been reported in numerous other mammals. Experimental WTs have been developed in laboratory animals including rats, rabbits, and hamsters. Rat renal tumors induced transplacentally with *N*-ethyl-nitrosourea (ENU) are a suitable animal model of human WT, because the histologic features have several similarities to their human counterpart [80]. One of the unique morphological features of rat WT is recapitulation of normal renal development within the tumor, which consists of blastemal, organoid, and mature tubular elements. An animal model for heritable WT was also established in the Upjohn Sprague-Dawley rat, with an incidence of WT of 33–58.3% [56]. This tumor has been consistently transplanted to syngenetic rats through ten successive passages. However, these animal WTs have certain disadvantages, such as nonhuman origin, slow growth rate, and difficulties in analysis at the

cellular level in vivo. Reports on in vitro cell lines derived from animal WTs are scarce. Sumino [80] succeeded in establishing a cell line from a xenotransplanted ENU-induced rat nephroblastoma. This cell line possessed characteristics of early nephrogenic epithelial cells.

Heterotransplantable human tumor in athymic nude mice

Far the best animal model for WT is the heterotransplanted human WT in athymic nude mice. In general, WT appears to grow in athymic nude mice with greater frequency than do many other surgically excised tumors [74]. Successful tumor formation has been reported in over 30% of heterotransplanted WT specimens [29, 60]. A couple of years ago, at the Institute of Urology, Rotterdam, a program was started to transplant specimens of resected WTs of chemotherapeutically pretreated patients directly into athymic nude mice. The first series of 20 tumor transplants resulted in six permanent tumor cell lines which were shown to be serially transplantable.

Both classic and anaplastic WTs have been reported to be capable of at least 20 serial passages in nude mice, with some tumors completing 100 passages over a period of 5 years [65]. Heterotransplants derived from classic (triphasic) WTs show a tendency to shift towards a predominately blastemal makeup beyond the sixth serial passage [65]. The blastemal cells are morphologically and histochemically identical to those of the primary tumor, whereas tubular elements become more rare in each advancing passage. Primary tumors containing anaplastic tubular elements result in heterotransplants that maintain a constant proportion of tubules despite advancing passage number. Anaplastic heterotransplants retain all of the morphological and growth characteristics associated with anaplasia throughout extensive serial passages. Classic WT heterotransplants have failed to show spontaneous progression to anaplasia. Neither classic nor anaplastic xenografts showed any spontaneous formation of metastatic lesions in athymic mice [29], a phenomenon that is observed in other heterotransplanted tumors in nude mice. We transplanted WT tissue into NMRI nude mice [78], a strain that we demonstrated to be a suitable host for permanent transplantation of human prostatic carcinomas [89], which resulted in six positive takes, yielding permanent tumor lines, i.e., tumors which were shown to be serially transplantable. Staining with bisbenzimide for all models revealed a pattern consistent with human tumor tissue. All six permanent WT lines contained blastemal tissue, and some of the tumors also included (immature) mesenchymal tissue with variable degrees of epithelial (tubular) differentiation. More specifically, these two components were identified by immunohistochemical staining of tissue sections with vimentin and cytokeratin antibodies, respectively [78].

Garvin [29] inoculated tumor cells of permanent in vitro cell lines into the flank subcutaneously or into the peritoneal cavity of mice. The site of transplantation (subcutaneous, peritoneal, or renal capsule) had no effect on the differentiation of the tumors. Yeger [93] found that the tumor originating from subcutaneously injected tumor cells consisted almost entirely of blastema, while tumors injected peritoneally consisted of blastema with large areas of tubular epithelium.

In vitro cell lines of Wilms' tumor

Considerable effort has been made to isolate and propagate homogeneous cultures of the various WT components to study their biological and molecular properties. Two experimental approaches have been used in obtaining homogeneous populations of distinct elements of WT including: (1) enzymatic digestion combined with Percoll gradient fractionation and (2) the use of specific and selective growth-promoting tissue culture media and substrates. So far only a limited number of human WT cell lines in vitro have been established permanently.

Long-term adherent cultures of blastemal cells have been developed using selective growth-promoting tissue culture formulations [28]. Only 10% of triphasic WTs yield epithelial cultures by using growth medium formulations and culture matrices identical to those used to generate successful normal kidney epithelial cell cultures [39]. Long-term cultures of the blastemal component, and skeletal muscle component of WTs, have been established by using serum-free medium or conditioned media from cultures of human kidney [27, 28]. Both in vitro systems provided potential models for testing factors influencing growth and differentiation of different tissue components of WT. Recently we attempted to bring xenograft-derived tumor tissue into culture, which resulted in two permanent cell lines. After ten passages, both cell lines (designated WT-15C and WT-15LNC), which originated from a collagenase digest of WT-15 and WT-15LN tumor tissues, were demonstrated to consist of human cells. Both cell lines could be permanently propagated in serum-free (Ultra-MDCK) medium.

Growth factors and Wilms' tumor

Synthesis and actions of growth factors, i.e., insulin-like growth factor (IGF), epidermal growth factor(EGF), etc., have been characterized in the developing metanephric kidney. Studies have defined not only the localization of growth factor mRNAs, receptors, and peptides, but also delineated patterns of growth factor synthesis, established the growth factor dependency of embryonic kidney development, and identified abnormalities of growth factor expression as potentially causative of aberrancies in metanephrogenesis [25, 34, 38]. Growth factors abnormally expressed in WT have been reported in the literature [1, 10, 18, 20, 22, 24, 25, 30, 34, 41, 61, 66–69, 71, 73, 84–87, 94].

Insulin-like growth factors

At least five members of a genetic superfamily of IGFs are known including insulin, IGF-I, IGF-II, relaxin, and the 7S β-subunit of nerve growth factor. In humans the growth of many tissues during embryogenesis and prenatal development is associated with abundant IGF-II levels [65]. Postnatally, tissue expression of IGF-II appears to decrease, while growth hormone and IGF-I levels begin to increase at about 6 months of age, reaching a peak during early to mild adolescence. In the developing kidney, IGF-II was primarily expressed in blastemal cells and lost with their differentiation [41, 61, 66].

In relation to WT, IGFs are the most extensively studied growth factors. As early as 1971, tumor extracts were shown to have a growth-promoting effect on human embryonic kidney cells in culture [10]. In later years, two studies showed that IGF-II messenger RNA was elevated in tumor cells, with levels similar to those of fetal kidney [67, 73]. Subsequently, the IGF-II peptide was shown to be produced in tumor tissue [68]. Furthermore WT samples were found to have type 1 IGF receptor, which is thought to be the mitogenic receptor for IGF-II. These data suggest that IGF-II may be a marker for organogenic cells, responsible for their proliferation via autocrine-stimulating pathways [30]. The whole loop of elevated synthesis, secretion, receptor binding, and autocrine growth stimulation of IGF-II through type I IGF receptors in WT cell cultures has been demonstrated [69]. In serum-free culture medium, tumor cell growth is reversibly inhibited by suramin through interference with IGF-II binding and is also arrested by IGF-binding protein-3, one of the six known IGF-binding proteins [15, 20], capturing continuously produced IGF-II, and by αIR-3, a type I IGF receptor-blocking antibody [26, 69].

Epidermal growth factor

EGF is a polypeptide growth factor which stimulates the growth of epithelial and stromal cells. In testing of WT xenograft for EGF, there seemed to be no definite correlation between EGF expression in tumor cells and tumorigenicity in nude mice because EGF-positive and EGF-negative cells were arranged alternately in the neoplastic tissues. The epithelial cells were positive for EGF and mesenchymal cells were negative in WT [94].

Activins

Activins are glycoproteins that are important not only as regulators of FSH secretion but also as mediators of growth control and cell differentiation in several normal and malignant tissues. The expression of activin receptor-mRNA was studied in the six permanent WT xenografts by an RNAse protection assay. Activin receptor-II (A and B)-mRNA was differentially expressed in all tumor models. Expression of ActRIIA appeared to correlate positively with the size of the mesenchymal component of the tumor, whereas in the models which were largely composed of blastemal tissue the expression of ActRIIB mRNA was relatively high [78].

Midkine expression

Midkine (MK) and heparin-binding growth-associated molecule/trophin form a new family of heparin-binding growth/differentiation factors with a molecular weight of 13 000 [86]. All surgically removed specimens of WT highly expressed MK [87]. This suggests an important role in regulating the development and/or biological behavior of tumors and raised the possibility of using MK as a diagnostic marker. The growth inhibition of WT cells by the anti-MK antibody implies that MK is involved in the growth of these cells [87].

Neurotrophin receptors

In earlier studies, Thomson [85] found WT cells in cultures expressing the low-affinity nerve growth factor receptor(NGF), p75, and that these cells were capable of responding to the neurotrophin (NT) NGF. Donovan [18] examined a group of WTs immunohistochemically with antibodies recognizing known tyrosine kinase (trk) receptor proteins, such as the p75 receptor. The trk receptor is an NT receptor with high affinity which has been identified at distinct sites during the development of the rodent kidney [22]. The *trk* gene family encodes three receptor tyrosine kinases, trk A, B, and C, which selectively interact with the NTs, NGF, etc. [84]. The p75 receptor was demonstrated to be predominantly expressed in the epithelial and blastemal components. The trk A and B receptors were primarily found within stromal components, whereas the trk C and C' receptors were present within epithelial structures [18]. The selective presence of NT receptors and growth factors in WT implies that NT may be involved in WT pathogenesis.

Platelet-derived growth factor A-chain

Platelet-derived growth factor (PDGF) A-chain in fetal and normal adult kidneys is uniformly expressed by visceral glomerular epithelial cells and the epithelial cells of the distal nephron, including collecting ducts and contiguous urothelium lining the renal pelvis [1]. Fetal kidneys also demonstrate expression of PDGF A-chain at the earliest stages of vesicle formation from the metanephric blastema [1, 24]. This expression is only intermittently detectable in developing glomeruli until differentiation of visceral epithelial cells occurs. PDGF A-chain expression has been identified in epithelial elements of WT [1]. The results suggest the sites of activity for PDGF A-chain are involved in the pathogenesis of WT.

Markers for clinical Wilms' tumor

The development of immunoenzymatic techniques for identification of specific cell components or products has revolutionized tumor diagnosis. The most widely used method employs antibodies linked to various chromogens that are rendered visible by the action of peroxidase. Different markers for WT differentiation are being developed [3, 21, 40, 52, 70].

Intermediate filaments

Desmin is a 53-kDa protein that belongs to the intermediate filament class of cytoskeletal proteins. Expression of desmin is limited to muscle tissue and is especially highly expressed in immature muscle fibers, both during fetal life and regeneration as well as in certain congenital myopathies [33, 62]. WT stromal cells with rhabdomyomatous differentiation exhibited cytoplasmic staining for desmin [52].

Cytokeratins are a multigene family of intracellular fibrous polypeptides that are expressed in virtually every true epithelial cell. In WT, immunohistochemical stains of the primary tumor demonstrated intense reactivity of the tubular or epithelial component with cytokeratin and EMG, but the blastemal component was negative for both antigens [28]. The epithelial component consists of discrete islands of blastemal cells that are partially or fully differentiated toward tubular, tubulopapillary, or papillary structures. Embryonal epithelium immunostains for cytokeratin but not EMG [29, 52].

Vimentin is a specific marker for mesenchyme and stroma. This also applies to WT, whereas in this tumor blastema was occasionally found positive or minimally reactive for vimentin [28, 39, 94]. Some laboratories reported blastemal elements within classic WT, and endothelial cells of the tumor vasculature were characteristically immunoreactive for vimentin [21, 28, 39]. The mesenchymal component showed fascicular proliferation of tightly interlaced, uniform, benign-appearing spindle cells that expressed vimentin and fibronectin, but not desmin or actin [40]. Garvin [29] showed in histologic sections of heterotransplants of a WT small aggregates of vimentin-positive cells in a configuration resembling avascular "glomeruloid bodies", indicating that vimentin may be a marker for glomerular cells.

In addition, the consistent vimentin positivity of clear cell sarcoma of the kidney (CCSK) cells is a useful feature in differentiating it from "blastemal-predominant" WT [52, 62]. Negative reactivity for desmin and vimentin was the major immunohistochemical distinction between neuroblastomas and rhabdomyosarcomas [70]. However, combined evaluation of morphology and the pattern of immunoreactivity using multiple markers is of importance for differential diagnosis. This is due to limited reactivity of blastematous parts of WT with neuroblastoma-specific antibodies [70].

p53

In 1988, Baker [3] showed that deletions of the short arm of chromosome 17 were associated with point mutations in the p53 allele on the homologous chromosome. Since then, much evidence has supported that p53 is a tumor suppressor gene involved in a wide range of human malignancies. This gene encodes a 375-amino-acid nuclear phosphoprotein, which is involved in the regulation of cell proliferation. The wild-type p53 acts as a tumor suppressor gene in normal cells and is lost or inactivated during the development and/or progression of many neoplasms [12, 75].

Intrinsic to the function of p53 is its ability to induce apoptotic cell death and to cause cell cycle arrest. Moreover, p53 plays an important role in controlling the cellular response to DNA-damaging agents such as ionizing radiation and cancer chemotherapeutic drugs. Mutations of the p53 gene lead to the accumulation of mutant proteins in the cell nucleus, which can be detected immunohistochemically [2, 36, 48, 75].

The prevalence of p53 alterations in pediatric tumors is, however, less well established in comparison to other malignancies. Conflicting reports regarding the possible existence of p53 mutations in WT have emerged from various studies [14, 46, 51, 81]. One study suggested that WT samples very frequently express high levels of p53 [51], whereas another report showed that the frequency of p53 mutations was low [81]. Waber [88] demonstrated that p53 is not involved in the pathogenesis of WT by examining a group of 38 WTs with single-stand conformational polymorphism (SSCP) analysis.

The anaplastic histologic variant of WT is frequently associated with p53 gene mutations. Bardeesy [4] found p53 mutations in 8 of 11 anaplastic WTs after screening 140 WT specimens. Seven WTs for which they had paired samples from non-anaplastic and anaplastic regions were analyzed. Five WTs were demonstrated to have mutations restricted to anaplastic regions. Non-anaplastic cells of the sixth example were heterozygous for a p53 mutation, whereas the anaplastic area of this tumor showed reduction to homozygosity [5]. These results indicate that progression to anaplasia is associated with clonal expansion of cells which have acquired a p53 mutation and which show attenuated apoptosis. It suggests that such lesions may provide a selective advantage in vivo by decreasing cell death [5]. There is a good correlation between the immunohistochemical expression of p53 protein and recurrence/metastasis of WT [13, 46]. These data indicate that p53 may be a valuable diagnostic and prognostic marker, but there is still a need to further study in detail the frequency of p53 alterations and its clinical significance in WT.

Proliferation markers

Proliferating cell nuclear antigen (PCNA) is a 36-kDa nuclear protein expressed in association with the cell cycle, and is now known to represent a component of

DNA polymerase-δ [45]. It is expressed in the nucleus of cells in all but the G0 phase of proliferation. PCNA immunolocalization can be used as an index of cell proliferation and has, as such, been proposed as an alternative to flow-cytometric DNA analysis to detect proliferative activity of cells. Ki-67, a marker of proliferation probably more closely related to the DNA replication phase of the cell cycle [32, 45, 79].

Nagoshi [59] performed immunohistochemical studies for PCNA on formalin-fixed paraffin-embedded specimens of WT. Image analyses determined the percentage of positive areas for PCNA, which were then represented in the PCNA score. The result showed the PCNA scores of tumors correlated with neither the clinical outcome of the patients nor the histologic groups [59]. However, Delahunt [17] assessed the proliferative activity in post-chemotherapy WT, showing the PCNA may be a useful indicator of prognosis. Enumeration of silver-staining nucleolar organizer regions (AgNORs) and PCNA staining of the tumors showed the PCNA and AgNOR scores derived from the blastemal and epithelial components of the tumors were significantly higher than those of the stromal component. There was a significant difference in the survival of the two groups for tumors treated with preoperative chemotherapy [17].

Tenascin

Tenascin is an extracellular matrix (ECM) protein prominently expressed in the stroma of several epithelial malignancies [82]. Interactions between tumor cells and the ECM are of importance for tumor invasion and metastasis. The role of tenascin is unclear, but it may facilitate epithelial cell spread during carcinogenesis and embryogenesis, by reducing rather than increasing cell-matrix interactions. A human WT cell line in nude mice that did not produce tenascin but induced synthesis of this in the adjacent normal cells of the host animal has been established [82].

Other possible markers

Other potential markers studied immunohistochemically in WT are carbohydrate antigen 125 (CA 125), tissue polypeptide antigen (TPA), blastema-associated antigen (BLA-1), P-glycoprotein (Pgp), and PAL-E.

CA 125 and TPA were detected in the conditioned culture media of a WT cell line, and the levels of CA 125 in the serum and the total tumor load of human heterotransplants into nude mice appeared to correlate well [44]. BLA-1 antibody usually stained cell surfaces of all WT-containing blastemal components [83]. The multidrug resistant-associated Pgp was found in both "favorable histology"(FH) and anaplastic (ANA) WT, especially with higher levels of expression in FH patients who had a relapse. However, there was no statistically significant difference between relapsed ANA and FH tumor relapse or FH tumor-negative relapse [77]. PAL-E is an anti-endothelial cell antibody which was found not only restricted to the endothelium, but also specifically stained blastemal cells in WT, whereas mesenchymal and tubular components of WT were negative for this antigen [72].

Conclusion

Significant progress has been made during the last 2 decades with respect to the chemotherapeutic treatment of WT, and in addition some insight was obtained into the mechanisms underlying the process of WT progression. Remarkable advances have particularly been made in elucidating molecular events involved in WT pathogenesis. By studying series of clinical WT specimens factors were recognized with possible prognostic value, such as the tumor suppressor gene p53 and proliferation-associated markers. An independent prognosticator has not been identified as yet. Thus, histologic typing is the only criterion predicting the clinical outcome of the tumor. In spite of our current knowledge, mechanisms of WT aggression are still unclear and, therefore, there is a need to continue and further intensify fundamental WT research. Studies to further improve therapeutic intervention of WT as well as investigations into cell biological and molecular aspects of the incompletely understood mechanism of tumor progression can be performed at best using available human xenograft models. Continuous research efforts will lead to better classification of individual WTs making use of recognized prognostic markers, provide insight into the mechanism of WT recurrence, and ultimately lead to more and better options for the treatment of this malignancy.

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