significantly increase breast cancer risk. (2) There are many deleterious mutations, and each mutation is individually rare. That is, for none of these genes (individually or in combination) does increased risk of breast cancer result from additive effects of multiple common alleles, each of small influence. Inherited breast cancer is highly genetically heterogeneous with respect to both loci and alleles involved. All evidence to date is that the model that best reflects this heterogeneity is not a "common disease-common allele" model, but instead a "common disease-multiple rare alleles" model.

The ten known genes for inherited breast cancer function in a pathway whose role is to preserve genomic integrity. Roughly 50% of familial breast cancer remains unresolved by any of these genes. Clearly other genes in this pathway are worthy of in-depth genomic analysis in unresolved families. Furthermore, in thus far unrecognized members of this pathway, mutations may also be associated with inherited breast cancer.

REFERENCES

Erkko, H., Xia, B., Nikkila, J., Schleutker, J., Syrjakoski, K., Mannermaa, A., Kallioniemi, A, Pylkas, K., Karppinen, S-M., Rapakko, K., et al. (2007). Nature. Published online February 7, 2007. 10.1038/nature05609.

Heikkinen, K., Rapakko, K., Karppinen, S.M., Erkko, H., Knuutila, S., Lundan, T., Mannermaa, A., Borresen-Dale, A.L., Borg, A., Barkardottir, R.B., et al. (2006). Carcinogenesis *27*, 1593–1599.

Howlett, N.G., Taniguchi, T., Olson, S., Cox, B., Waisfisz, Q., De Die-Smulders, C., Persky, N., Grompe, M., Joenje, H., Pals, G., et al. (2002). Science *297*, 606–609.

Levy-Lahad, E., Lahad, A., Eisenberg, S., Dagan, E., Paperna, T., Kasinetz, L., Catane, R., Kaufman, B., Beller, U., Renbaum, P., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 3232–3236.

Litman, R., Peng, M., Jin, Z., Zhang, F., Zhang, J., Powell, S., Andreassen, P.R., and Cantor, S.B. (2005). Cancer Cell *8*, 255–265.

Meijers-Heijboer, H., van den Ouweland, A., Klijn, J., Wasielewski, M., de Snoo, A., Oldenburg, R., Hollestelle, A., Houben, M., Crepin, E., van Veghel-Plandsoen, M., et al. (2002). Nat. Genet. *31*, 55–59.

Rahman, N., Seal, S., Thompson, D., Kelly, P., Renwick, A., Elliott, A., Reid, S., Spanova, K., Barfoot, R., Chagtai, T., et al. (2006). Nat. Genet. Published online December 31, 2006. 10.1038/ng1959.

Reid, S., Schindler, D., Hanenberg, H., Barker, K., Hanks, S., Kalb, R., Neveling, K., Kelly, P., Seal, S., Freund, M., et al. (2006). Nat. Genet. Published online December 31, 2006. 10.1038/ng1947.

Renwick, A., Thompson, D., Seal, S., Kelly, P., Chagtai, T., Ahmed, M., North, B., Jayatilake, H., Barfoot, R., Spanova, K., et al. (2006). Nat. Genet. *38*, 873–875.

Seal, S., Thompson, D., Renwick, A., Elliott, A., Kelly, P., Barfoot, R., Chagtai, T., Jayatilake, H., Ahmed, M., Spanova, K., et al. (2006). Nat. Genet. *38*, 1239–1241.

Shen, W.H., Balajee, A.S., Wang, J., Wu, H., Eng, C., Pandolfi, P.P., and Yin, Y. (2007). Cell *128*, 157–170.

Taniguchi, T., and D'Andrea, A.D. (2006). Blood *107*, 4223–4233.

Walsh, T., Casadei, S., Coats, K.H., Swisher, E., Stray, S.M., Higgins, J., Roach, K.C., Mandell, J., Lee, M.K., Ciernikova, S., et al. (2006). JAMA *295*, 1379–1388.

Xia, B., Dorsman, J.C., Ameziane, N., de Vries, Y., Rooimans, M.A., Sheng, Q., Pals, G., Errami, A., Gluckman, E., Llera, J., et al. (2006a). Nat. Genet. Published online December 31, 2006. 10.1038/ng1942.

Xia, B., Sheng, Q., Nakanishi, K., Ohashi, A., Wu, J., Christ, N., Liu, X., Jasin, M., Couch, F.J., and Livingston, D.M. (2006b). Mol. Cell *22*, 719–729.

Wilms Tumor Genetics: A New, UnX-pected Twist to the Story

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The study of the genetics of Wilms tumor has led to several highly unexpected and precedent-establishing discoveries. Ironically, however, the identification of "WT genes" has been painfully slow, and gene mutations have been identified in only ~25% of tumors. The discovery of an X chromosome gene, *WTX*, that is mutated somatically in ~30% of Wilms tumors is notable both for helping to explain the genetic etiology of a substantial proportion of tumors and also for underscoring the role that X chromosome genes can play in cancer genetics.

Wilms tumor (WT) is a childhood embryonal cancer of the kidney. Unlike most tumors, Wilms tumors generally exhibit few, if any, chromosomal abnormalities, and therefore WT was originally thought to represent a simple model for studying the genetic etiology of cancer. WT genetics, however, has turned out to be anything but simple; the road to identifying and understanding "WT genes" has been littered with false leads and dashed

hopes, but also marked with highly unexpected and precedent-establishing discoveries. The recent report by Rivera et al. (2007) of the mutation of the novel X chromosome gene, *WTX*, in ~30% of Wilms tumor adds a significant milestone to this road—and has provided yet another unexpected twist to the ever-evolving story.

Mathematical modeling of the age of onset and the frequency of bilateral tumors in both familial and sporadic WT patients suggested that two genetic "hits" are critical, rate-limiting steps in tumor development (Knudson and Strong, 1972). Subsequently, a WT gene, *WT1*, was localized to chromosomal band 11p13, and assessment of DNA polymorphisms in tumor and matched normal tissue from patients demonstrated that ~40% of tumors displayed loss of heterozygosity (LOH) at 11p loci. These data were consistent with a tumor suppressor gene model in which inactivation of both copies of the gene was required for tumorigenesis. Gene inactivation often occurred by mutation of one allele followed by loss of the remaining wild-type allele by chromosome deletion, chromosome loss/reduplication, or somatic recombination. Once the *WT1* gene was cloned, this model was confirmed at the molecular level.

But things weren't so simple. Even before *WT1* was isolated, unexpected data with—at the time—heretical implications began accruing. First was the observation that, in those tumors displaying 11p LOH, it was invariably the 11p alleles inherited from the father that were retained in the tumor (Schroeder et al., 1987). With the subsequent identification of a cluster of syntenic imprinted genes at 11p15 (telomeric of the *WT1* locus), this novel observation in Wilms tumors brought the issue of gene imprinting out of the arena of mouse development and into the arena of human disease and cancer genetics. It is now thought that the retention of paternal alleles in tumors is likely due to selection for the expression of the fetal mitogen, *IGF2*, that is located in the 11p15 imprinted region and is expressed during embryonic development only from the paternally derived allele. In addition to those tumors displaying LOH, ~70% of Wilms tumors lose the normal 11p15 imprint and express both copies of the *IGF2* gene (Ogawa et al., 1993; Rainier et al., 1993). This loss of imprinting (LOI) in WT was another "first" in cancer genetics and added to the complexity of molecular alterations that occur in WT.

Another startling finding came from studies of several large WT families. Familial predisposition to WT was *not* genetically linked to the *WT1* region. These data implied that familial predisposition was due to mutation of another, unknown gene and that WT was a genetically heterogeneous disease. This notion was reinforced once *WT1* was cloned and mutational analyses were carried out on panels of tumors. In one large study in which the entire *WT1* coding region was assessed for both point mutations and intragenic deletions, only 18% of Wilms tumors carried a *WT1* mutation (Huff, 1998). Additionally, in tumors with wild-type *WT1* the gene was almost invariably expressed, and *WT1* transcripts were normally spliced and of wild-type sequence. Clearly there was another "WT" gene(s) whose alteration was critical for tumor development in those non-*WT1*-mutated tumors. But where was it and what was it?

Progress in gene identification was slow. Identification of genes aberrantly expressed or silenced in tumors was difficult due to the fact that tumors, which are thought to arise from undifferentiated renal mesenchyme, can be very histologically variable and are composed of cells that are reminiscent of the differentiated cell types that normally develop from renal mesenchyme. Were alterations in expression of a given gene etiologically important or simply a reflection of variable tumor histology?

p53 mutations were identified in ~5% of Wilms tumors, but only in those with an anaplastic histology (Bardeesy et al., 1994). β-catenin mutations were identified in ~15% of tumors (Koesters et al., 1999), but these mutations were highly significantly associated with *WT1* mutations (Maiti et al., 2000) and so would not be responsible for the genetic etiology of non-*WT1* mutant tumors. Extensive cytogenetic, LOH, CGH, and genetic linkage studies revealed several regions of the genome thought to harbor a WT gene. However, until now, these studies have not resulted in the clear identification of a WT gene. Thus, the report of a gene (*WTX*) that is mutated in almost 30% of a series of 51 Wilms tumors is a major breakthrough.

However, as is usual with any such discovery, a multitude of questions arise. Why haven't deletions of the X chromosome been observed before? The answer is, deletion of the entire X chromosome was noted occasionally, but intrachromosomal deletions were not. Presumably only with higher-resolution arrays utilizing long oligonucleotides could the relatively small (~1 Mb or less) interstitial X chromosome deletions reported by Rivera et al. (2007) be detected. And, luckily, the commonly deleted region mapped within a single gene. Even more fortunately, *WTX* sequence variants observed in tumors were somatic, and almost all were truncating mutations and so were likely of functional significance.

What does WTX do? The sequence of the predicted encoded protein provides few clues. Although primate, mouse, rat, dog, and cow orthologs have been identified or predicted, *WTX* shares no significant homology with genes of known function. Ectopic expression of *WTX* in two cell lines resulted in a reduction in colony formation and an increase in apoptosis in one line. However, these were not WT cell lines, nor were the observed *WTX* truncation mutations tested in these assays, so these observations may or may not relate to the actual function of *WTX*.

WTX mutations and *WT1* mutations were mutually exclusive, suggesting that *WT1* and *WTX* may function in the same cellular pathway. Does *WT1*, a known transcription factor, regulate *WTX*? *WTX*, unlike *WT1*, was expressed more robustly in neonatal and adult lung than in neonatal and adult kidney. However, in kidney the expression pattern for the two genes was similar. Although this similar pat-

tern may simply be due to both genes being expressed in the same precursor and adult cell types, the presence of two putative *WT1* binding sites in the promoter region of *WTX* (E.C. Ruteshouser, personal communication) hints that *WT1* may regulate *WTX*, at least in kidney. If this is the case, mutation of either gene could serve to abrogate the function of the same pathway critical for normal kidney development.

Do *WTX* mutant tumors represent a distinct subset of tumors with respect to tumor histology, presence of preneoplastic lesions, age of onset, family history, disease progression, prognosis, etc? This is currently unknown, but this type of data may help provide clues to the function of *WTX*. The *WTX* mutations observed to date are somatic mutations and therefore cannot account for the familial predisposition to WT. Additionally, in most WT families predisposition is not inherited as an X-linked disease. Do tumors from familial cases, however, sustain somatic *WTX* mutations, suggestive of the importance of mutating at least two distinct loci? Does the fact that a single alteration can inactivate a gene residing on the X chromosome factor into the relatively high (for Wilms tumors) frequency of tumors with *WTX* mutations?

Interestingly, the frequency of *WTX* mutations in tumors from male and female WT patients is the same. This is unexpected, since a *WTX* somatic mutation, if randomly occurring on both the active and inactive X chromosomes, would always mutate the functional gene in males, but would mutate the functional gene on the active X chromosome only half the time in females. Along the same lines, one would also expect that the Wilms patient population would be skewed toward males, but this is not the case. In fact, in North America, there is a small, but statistically significant predominance of females (Breslow et al., 1988). Is the lack of male predominance for a disease that often (30% of the time) involves an X chromosome gene mutation telling us something about the mutability of the *WTX* gene on the active versus the inactive X chromosome?

So yet again, a discovery in the field of WT genetics is at once exciting, unexpected, novel, and puzzling. For such a simple model, WT keeps challenging us.

REFERENCES

Bardeesy, N., Falkoff, D., Petruzzi, M.J., Nowak, N., Zabel, B., Adam, M., Aguiar, M.C., Grundy, P., Shows, T., and Pelletier, J. (1994). Nat. Genet. *7*, 91–97.

Breslow, N., Beckwith, J.B., Ciol, M., and Sharples, K. (1988). Cancer Res. *48*, 1653–1657.

Huff, V. (1998). Am. J. Med. Genet. *79*, 260–267.

Koesters, R., Ridder, R., Kopp-Schneider, A., Betts, D., Adams, V., Niggli, F., Briner, J., and von Knebel Doeberitz, M. (1999). Cancer Res. *59*, 3880–3882.

Knudson, A.G., Jr., and Strong, L.C. (1972). J. Natl. Cancer Inst. *48*, 313–324.

Maiti, S., Alam, R., Amos, C.I., and Huff, V. (2000). Cancer Res. *60*, 6288–6292.

Ogawa, O., Eccles, M.R., Szeto, J., McNoe, L.A., Yun, K., Maw, M.A., Smith, P.J., and Reeve, A.E. (1993). Nature *362*, 749–751.

Rainier, S., Johnson, L.A., Dobry, C.J., Ping, A.J., Grundy, P.E., and Feinberg, A.P. (1993). Nature *362*, 747–749.

Rivera, M.N., Kim, W.J., Wells, J., Driscoll, D.R., Brannigan, B.W., Kim, J.C., Feinberg, A.P., Gerald, W.L., Vargas, S.O., Chin, L., et al. (2007). Science Published online January 4, 2007. 10.1126/science.1137509.

Schroeder, W.T., Chao, L.Y., Dao, D.D., Strong, L.C., Pathak, S., Riccardi, V., Lewis, W.H., and Saunders, G.F. (1987). Am. J. Hum. Genet. *40*, 413–420.