
Re: Loss of Imprinting of Insulin-Like Growth Factor-II (IGF2) Gene in Distinguishing Specific Biologic Subtypes of Wilms Tumor

In a recent issue of the Journal, Ravenel et al. (1) claim that loss of imprinting (LOI) of the insulin-like growth factor-II (IGF2) gene defines a molecular subgroup of Wilms tumors that have a different pathologic subtype, a later age of onset, and greater IGF2 expression than those without LOI. On the basis of their findings, they propose a model for Wilms tumorigenesis. We question their conclusions and model for several reasons.

First, their conclusions rely on the pathologic classification of Wilms tumors into perilobar nephrogenic rest (PLNR)-like or intralobar nephrogenic rest (ILNR)-like tumors proposed by Beckwith et al. (2). However, Ravenel et al. (1) used only two of the four original criteria proposed by Beckwith et al.—the presence or absence of PLNR or ILNRs and the presence or absence of heterologous elements—to classify the Wilms tumors in their study. Although the use of only two criteria may simplify the classification of Wilms tumors according to whether they are ILNR-like or PLNR-like, Ravenel et al. found, as we have (see below), that a substantial proportion of the tumors in their study were unclassifiable.

Second, Ravenel et al. found strong associations between chromosome 11p loss of heterozygosity (LOH) and ILNR-like tumors and between IGF2 LOI and PLNR-like tumors. We previously reported a detailed study of 24 Wilms tumors and did not find similarly strong associations (3). In our study, all

five tumors associated with PLNRs displayed LOI, whereas heterologous elements (always rhabdomyoblastic) were found in both LOI tumors (5 of 9) and non-LOI tumors (6 of 15). In addition, our data illustrate the difficulties in classifying Wilms tumors, because only three of our nine tumors that displayed LOI would have been classified as PLNR-like (presence of PLNR, absence of heterologous elements) according to the criteria used by Ravenel et al.

Third, the model of Ravenel et al. (1) proposes that increased IGF2 expression is restricted to tumors with LOI. However, LOH of chromosome 11p15, which is associated with reduplication of the paternal chromosome (4,5), also doubles the number of active copies of IGF2. Furthermore, in Wilms tumors, the major determinants of IGF2 expression are independent of heterozygosity or imprinting status (5). In addition, because Ravenel et al. did not distinguish between the different types of LOH (e.g., those that affect chromosome 11p15 only, those that affect chromosome 11p13 only, and those that affect both regions), the model they propose cannot separate the effects of increased expression of IGF2 from loss of expression of the tumor suppressor gene WT1, which resides at 11p13.

Fourth, we found no difference in age at diagnosis for patients with Wilms tumors that did and did not display LOI—the median age of nine children with LOI tumors was 30 months and the median age of 15 children with non-LOI tumors was 41 months (Becroft DM, Reeve AE, Morison IM: unpublished data).

The model proposed by Ravenel et al. may oversimplify the complex interplay of multiple genetic events that are associated with Wilms tumorigenesis, because it is based on findings from a selected group of Wilms tumors. More work is needed to derive models that address such complexity.

IAN M. MORISON
DAVID M.O. BECROFT
ANTHONY E. REEVE

REFERENCES

- (1) Ravenel JD, Broman KW, Perlman EJ, Niemitz EL, Jayawardena TM, Bell DW, et al. Loss of imprinting of insulin-like growth factor-II (IGF2) gene in distinguishing specific biologic subtypes of Wilms tumor. *J Natl Cancer Inst* 2001;93:1698–703.

- (2) Beckwith JB, Kiviat NB, Bonadio JF. Nephrogenic rests, nephroblastomatosis, and the pathogenesis of Wilms' tumor. *Pediatr Pathol* 1990;10:1–36.
- (3) Becroft DM, Okamoto K, Morison IM, Reeve AE. Perilobar nephrogenic rests are associated with epigenetic changes at the IGF2/H19 locus in developing kidney [abstract]. *Pediatr Dev Pathol* 1998;1:251.
- (4) Fearon ER, Vogelstein B, Feinberg AP. Somatic deletion and duplication of genes on chromosome 11 in Wilms' tumours. *Nature* 1984;309:176–8.
- (5) Wang WH, Duan JX, Vu TH, Hoffman AR. Increased expression of the insulin-like growth factor-II gene in Wilms' tumor is not dependent on loss of genomic imprinting or loss of heterozygosity. *J Biol Chem* 1996;271:27863–70.

NOTES

Affiliations of authors: I. M. Morison, A. E. Reeve, Cancer Genetics Laboratory, Department of Biochemistry, University of Otago, Dunedin, New Zealand; D. M. O. Becroft, Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand.

Corresponding author: Ian M. Morison, M.B., Ch.B., Ph.D., Cancer Genetics Laboratory, Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand (e-mail: ian.morison@otago.ac.nz).

RESPONSE

Morison et al. raise several specific reasons for doubting the results and conclusions of our work. We believe our methodology and interpretation are correct. They first criticize our classification of Wilms tumors as intralobar nephrogenic rest (ILNR)-like or perilobar nephrogenic rest (PLNR)-like, because we did not classify the Wilms tumors according to four characteristics described by Beckwith et al. (1). However, what Beckwith et al. (1) described are the features commonly seen in tumors derived from PLNRs and ILNRs, not the criteria for making the distinction between PLNR-like and ILNR-like tumors. Only two features unambiguously distinguish ILNR-like tumors from PLNR-like tumors: identification of the rest on the periphery of the tumor and the presence of heterologous elements. Failure to use these stringent histopathologic criteria might cause one to miss this important molecular difference. In our study, tumor classification was performed in a blinded fashion by the pathologist for the National Wilms Tumor Study, and many of the slides were also examined by Beckwith himself. That only 60% of the tumors in our study were classifiable by these methods

serves to emphasize the point of the study, because it shows the power of molecular analysis in augmenting histopathologic examination.

Second, Morison et al. challenge our results showing relationships between loss of heterozygosity (LOH) and ILNR-like tumors and between loss of imprinting (LOI) and PLNR-like tumors. They cite their own data on 24 tumors (2). However, those data were presented in a meeting abstract rather than in a peer-reviewed study and, thus, cannot be analyzed critically. Nevertheless, in that abstract, Morison et al. make no reference to examining LOH, and they report that tumors with PLNRs show LOI (in support of our observations of more than 100 tumors). None of the Wilms tumors we examined was rhabdomyomatous and, thus, we could not determine whether this subcategory is different.

Third, Morison et al. challenge our finding of a statistically significant association of LOI with an increase in insulin-like growth factor-II (IGF2) expression levels. Their argument is based on their hypothesis that LOH would also be associated with a doubling of IGF2 expression and on work by Wang et al. (3). Our data do not support their hypothesis. We found that LOH is not associated with an increase in IGF2 expression when examined by real-time quantitative polymerase chain reaction (RTQ-PCR; Ravenel JD, Broman KW, Perlman EJ, Niemitz EL, Jayawardena TM, Bell DW, et al.: unpublished results). Furthermore, the study by Wang et al. (3) has several important limitations that our study has addressed. For example, Wang et al. used multiplex, semiquantitative reverse transcription-PCR, which is less sensitive and specific than the RTQ-PCR technique we used. Wang et al. compared IGF2 expression levels by examining the ratio of expression in tumor to that in normal postnatal kidney. We question postnatal kidney as the most appropriate control, as Wilms tumors are most closely related to fetal kidney, not postnatal kidney. By contrast, we compared tumors with and without LOI, or those with and without LOH. Wang et al. used expression of 18S ribosomal RNA (rRNA) as their control for normalization, whereas we used the expression of 12 different housekeeping genes to validate normalization. Expression levels of 18S rRNA

are an order of magnitude greater than those of IGF2 RNA, which is likely to greatly reduce the sensitivity of the experiments that used 18S rRNA as a normalization control. Morison et al. further propose that we did not consider the effect of loss of WT1 expression due to LOH. However, we examined WT1 expression directly and found that it did not correlate with IGF2 levels in Wilms tumor, normal kidney, or fetal kidney.

Fourth, Morison et al. question our findings that patients' ages at diagnosis differ for PLNR-like and ILNR-like tumors. However, our data, which were obtained from a large set of tumors, were statistically significant ($P < .001$) and are consistent with the findings of Breslow et al. (4), who also found an age difference associated with the two types of nephrogenic rests.

Finally, Morison et al. comment that our model is an oversimplification based on a limited number of samples. However, we analyzed four times more samples than the number reported in their abstract; our tumors were not, as they suggest, selected; our molecular and pathologic analyses were performed by separate investigators who were blinded to the others' results; and our data were subjected to rigorous statistical analysis. Our work shows, for the first time, that the different pathologic classifications of Wilms tumors are indeed associated with differing genetic and epigenetic alterations.

JASON D. RAVENEL
ELIZABETH J. PERLMAN
KARL W. BROMAN
ANDREW P. FEINBERG

REFERENCES

- (1) Beckwith JB, Kiviat NB, Bonadio JF. Nephrogenic rests, nephroblastomatosis, and the pathogenesis of Wilms' tumor. *Pediatr Pathol* 1990;10:1-36.
- (2) Becroft DM, Okamoto K, Morison IM, Reeve AE. Perilobar nephrogenic rests are associated with epigenetic changes at the IGF2/H19 locus in developing kidney. *Pediatr Dev Pathol* 1998;1:251.
- (3) Wang WH, Duan JX, Vu TH, Hoffman AR. Increased expression of the insulin-like growth factor-II gene in Wilms' tumor is not dependent on loss of genomic imprinting or loss of heterozygosity. *J Biol Chem* 1996;271:27863-70.
- (4) Breslow N, Beckwith JB, Ciol M, Sharples K. Age distribution of Wilms' tumor: report from the National Wilms' Tumor Study. *Cancer Res* 1988;48:1653-7.

NOTES

Affiliations of authors: J. D. Ravenel, A. P. Feinberg, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; E. J. Perlman, Department of Medicine, Children's Memorial Hospital, Chicago, IL; K. W. Broman, Department of Biostatistics, Bloomberg School of Public Health, Baltimore.

Corresponding author: Andrew P. Feinberg, M.D., M.P.H., Johns Hopkins University School of Medicine, Ross 1064, 720 Rutland Ave., Baltimore, MD 21205 (e-mail: afeinberg@jhu.edu).