Effects of Chemotherapy on the Cytogenetic Constitution of Wilms’ Tumor

Thorsten Schlomm,¹ Bastian Gunawan,² Hans-Jürgen Schulten,² Björn Sander,² Karthinathan Thangavelu,³ Norbert Graf,⁶ Ivo Leuschner,⁶ Rolf-Hermann Ringert,⁴ and László Füzesi²

Abstract

The management of Wilms’ tumors consists of a combination of surgery, chemotherapy, and possibly radiotherapy. To date, chemotherapy is being risk stratified according to histologic subtype and stage. Although the cytogenetic characteristics of Wilms’ tumors are well established, the cytogenetic effects related to chemotherapy are widely unknown. We herein report on comparative genomic hybridization findings in 41 primary Wilms’ tumors of blastemal type, of which 19 had received preoperative chemotherapy (PCT group) and 22 did not (non-PCT group). Overall, imbalances could be detected in 32 tumors, with +1q (17 cases), +7q (10 cases), +7p (6 cases), and −7p (6 cases) as the most common changes. Among these, +7q and −7p were both significantly associated with metastatic disease at the time of surgery (P = 0.002 and 0.007, respectively), and +7q was also associated with higher stage (stages III + IV; P = 0.003). There were significant differences in the cytogenetic constitution of tumors between the two treatment groups. As a trend, tumors in the preoperative chemotherapy group had fewer changes (mean, 2.7) than those in the non-preoperative chemotherapy group (mean, 3.8), and the frequencies of imbalances at 7p or +7q, respectively, were significantly lower compared with tumors in the non-preoperative chemotherapy group (2 of 19 versus 10 of 22, P = 0.019; 1 of 19 versus 9 of 22, P = 0.011). In contrast, +1q was common in both the preop-CT group (10 of 19) and the non-preop-CT group (7 of 22). The results suggest that Wilms’ tumor clones with +1q are not obliterated by preoperative chemotherapy, whereas cytogenetically more complex clones with +7q and/or imbalances at 7p seem more responsive and are more likely to be eliminated by chemotherapeutic treatment.

Wilms’ tumor, or nephroblastoma, is the most common renal neoplasm of childhood with overall long-term survival rates approaching 90% in localized disease and over 70% for metastatic disease using current therapeutic protocols created by the International Society of Pediatric Oncology (SIOP) or the National Wilms’ Tumor Study Group (1). The SIOP protocols advocate preoperative chemotherapy followed by surgery and postoperative treatment which is stratified according to histologic evidence of responsiveness to preoperative therapy, as reflected by post-therapy classification (low-risk, intermediate-risk, and high-risk histology; refs. 2–8). Although the vast majority of children with Wilms’ tumor, particularly of blastemal type, respond well to standardized therapy, a small proportion of patients show nonresponsiveness to chemotherapy. In these cases, extensive residual blastemal tumor cells are frequently found following pretreatment. It seems that in addition to anaplasia as a well-established predictive factor for poor responsiveness to chemotherapy, the persistence of large amounts of viable blastemal cells is also related to low response and reduced prognosis requiring intensified therapy. On the other hand, large amounts of necrosis and/or maturation into differentiated components are considered as evidence for responsiveness and may confer a more favorable prognosis (1, 7, 9). The current SIOP/GPOH 2001 trial is designed to tailor treatment by stratifying patients considering individual clinicopathologic factors to minimize potentially nephrotoxic and cardiotoxic side effects of chemotherapeutic agents. Other biological variables that may eventually allow greater ability to stratify patients may derive from investigations focusing on drug resistance–related proteins (10–13). Recently, cDNA microarray studies suggest that low-stage Wilms’ tumors with a good response to chemotherapy. In these cases, extensive residual blastemal tumor cells are frequently found following pretreatment. It seems that in addition to anaplasia as a well-established predictive factor for poor responsiveness to chemotherapy, the persistence of large amounts of viable blastemal cells is also related to low response and reduced prognosis requiring intensified therapy. On the other hand, large amounts of necrosis and/or maturation into differentiated components are considered as evidence for responsiveness and may confer a more favorable prognosis (1, 7, 9). The current SIOP/GPOH 2001 trial is designed to tailor treatment by stratifying patients considering individual clinicopathologic factors to minimize potentially nephrotoxic and cardiotoxic side effects of chemotherapeutic agents. Other biological variables that may eventually allow greater ability to stratify patients may derive from investigations focusing on drug resistance–related proteins (10–13). Recently, cDNA microarray studies suggest that low-stage Wilms’ tumors with a good response to chemotherapy are characterized by high expression of genes encoding for topoisomerase IIa, stathmin I, and tubulin (14).

Molecular and cytogenetic studies have found recurrent allelic losses and chromosomal imbalances in Wilms’ tumors and some markers were implicated to correlate with clinicopathologic factors (15–18). The closed nonrandomized NWTS-5 trial showed a prognostic value for loss of heterozygosity at
and q-arms of metacentric and submetacentric chromosomes, and q-arms of acrocentric chromosomes, respectively. Differences in the frequencies of individual imbalances between the two treatment groups and associations between tumor stage (SIOP stages I + II versus III + IV) or metastatic disease at the time of surgery and individual imbalances were evaluated using the two-sided Fisher's exact test. Because the analysis was of an exploratory nature, no adjustment for multiple testing was done. The significance level was 5%. All statistical analyses were done using the software system R (http://www.r-project.org/).

Results

Clinicopathologic data. The clinicopathologic data of all patients are summarized in Table 1. The female-to-male ratio was 16:6 in the non-PCT group and 13:6 in the preoperative chemotherapy group. Mean age at diagnosis was 7.1 years in the non-PCT group (range, birth to 32 years) and 5.7 years in the PCT group (range, 9 months to 17 years), and mean follow-up time was 4.3 years in the non-PCT group (range, 9 months to 9 years), and 4.0 years in the PCT group (range, 18 months to 7.3 years). Tumor stage was significantly higher among the 22 cases in the non-PCT group compared with the 19 cases in the PCT group (stages I + II; 11 versus 17; stages III + IV: 11 versus 1; \( P = 0.004 \)). Among 16 patients, where effect of chemotherapy was clinically evaluated, 14 patients responded to chemotherapy with reduction of tumor volume, whereas two patients developed increase of tumor volume (data not shown). In both groups, all patients except one received postoperative chemotherapy combined with radiotherapy in 12 cases. One of six patients in the non-PCT group and all four patients in the PCT group that developed tumor relapse have died of disease during follow-up.

Comparative genomic hybridization analysis. Genomic imbalances were detected in 18 of 22 (82%) tumors of the non-PCT group and 14 of 19 (74%) tumors of the PCT group (Table 1; Fig. 1). Overall, the most frequent individual imbalances were +1q (17 cases), +7q (10 cases), +7p (6 cases), −7p (6 cases), −1p (6 cases), +12p (5 cases), +12q (5 cases), +8q (5 cases), +18p (5 cases), and +18q (5 cases). Among these, +7q and −7p were both significantly associated with metastatic disease at the time of surgery (\( P = 0.002 \) and 0.007, respectively).

In four cases (cases 5, 8, 17, and 20), +7q was associated with a +7q (9 of 22), +1q (7 of 22), +7p (5 of 22), and −7p (5 of 22). In four cases (cases 5, 8, 17, and 20), +7q was associated with −7p, suggesting an isochromosome of the long arm of chromosome 7 as underlying cytogenetic aberration.

In the PCT group, +1q (10 of 19) was by far the most common imbalance, whereas +7q (1 of 19), +7p (1 of 19), and −7p (1 of 19).
Table 1. Clinicopathologic and CGH findings in 41 primary Wilms’ tumors with and without preoperative chemotherapy

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex/age (y)</th>
<th>Therapy</th>
<th>Stage</th>
<th>Follow-up [relapse], OS [EFS] (mo)</th>
<th>CGH findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preop</td>
<td>Adjuv</td>
<td></td>
<td>Gains</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(response)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F/4.2</td>
<td>No CRT</td>
<td>III</td>
<td>NED, 82</td>
<td>7p, 16q</td>
</tr>
<tr>
<td>2</td>
<td>F/3.8</td>
<td>No CRT</td>
<td>I</td>
<td>DOTD [Local], 18 [4]</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M/10</td>
<td>No CT</td>
<td>III</td>
<td>NED, 70</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>F/32</td>
<td>No CRT</td>
<td>III</td>
<td>Alive [LYM], 68 [34]</td>
<td>20q12qter</td>
</tr>
<tr>
<td>5</td>
<td>M/5.3</td>
<td>No No IV</td>
<td>CRT</td>
<td>DOTD [PUL], 9 [0]</td>
<td>1q, 7q</td>
</tr>
<tr>
<td>6</td>
<td>F/17</td>
<td>No CRT</td>
<td>IV</td>
<td>Alive [PUL], 55 [0]</td>
<td>7, 10, 12</td>
</tr>
<tr>
<td>7</td>
<td>F/3.8</td>
<td>No CRT</td>
<td>III</td>
<td>NED, 54</td>
<td>1q31qter, 6, 7, 8, 9, 12, 13q, 20</td>
</tr>
<tr>
<td>8</td>
<td>F/5.5</td>
<td>No CT</td>
<td>IV</td>
<td>Alive [PUL, ADR], 46 [0]</td>
<td>7q</td>
</tr>
<tr>
<td>9</td>
<td>F/3.5</td>
<td>No CT</td>
<td>I</td>
<td>NED, 45</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>F/0.2</td>
<td>No CT</td>
<td>I</td>
<td>NED, 43</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M/6.7</td>
<td>No CRT</td>
<td>III</td>
<td>NED, 34</td>
<td>1q</td>
</tr>
<tr>
<td>12</td>
<td>M/25</td>
<td>No CT</td>
<td>II</td>
<td>NED, 31</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F/0.5</td>
<td>No CT</td>
<td>II</td>
<td>NED, 29</td>
<td>1p11p31, 4, 11, 14q</td>
</tr>
<tr>
<td>14</td>
<td>F/0.0</td>
<td>No CT</td>
<td>I</td>
<td>NED, 42</td>
<td>18</td>
</tr>
<tr>
<td>15</td>
<td>F/3.5</td>
<td>No CT</td>
<td>III</td>
<td>NED, 75</td>
<td>1q</td>
</tr>
<tr>
<td>16</td>
<td>M/3.0</td>
<td>No CT</td>
<td>II</td>
<td>NED, 48</td>
<td>1q25qter</td>
</tr>
<tr>
<td>17</td>
<td>F/6.3</td>
<td>No CT</td>
<td>I</td>
<td>NED, 58</td>
<td>7q</td>
</tr>
<tr>
<td>18</td>
<td>F/16</td>
<td>No CT</td>
<td>II</td>
<td>NED, 54</td>
<td>7, 8, 12</td>
</tr>
<tr>
<td>19</td>
<td>F/2.2</td>
<td>No CRT</td>
<td>III</td>
<td>NED, 39</td>
<td>1q, 2, 3, 6, 7, 12, 13q, 16, 18</td>
</tr>
<tr>
<td>20</td>
<td>M/3.8</td>
<td>No CRT</td>
<td>IV</td>
<td>Alive [HEP], 30 [0]</td>
<td>7q</td>
</tr>
<tr>
<td>21</td>
<td>F/1.6</td>
<td>No CT</td>
<td>I</td>
<td>NED, 108</td>
<td>1q21q23, 7, 13q</td>
</tr>
<tr>
<td>22</td>
<td>F/2.5</td>
<td>No CT</td>
<td>I</td>
<td>NED, 84</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>F/8.7</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 30</td>
<td>1q</td>
</tr>
<tr>
<td>24</td>
<td>F/6.5</td>
<td>CT (R)</td>
<td>CT</td>
<td>II NED, 29</td>
<td>1q</td>
</tr>
<tr>
<td>25</td>
<td>M/17</td>
<td>CT</td>
<td>CT</td>
<td>I NED, 45</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>F/15</td>
<td>CT (I)</td>
<td>CT</td>
<td>II NED, 36</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>F/1.6</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 44</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>M/5.2</td>
<td>CT (R)</td>
<td>CT</td>
<td>II NED, 87</td>
<td>1q</td>
</tr>
<tr>
<td>29</td>
<td>F/5.4</td>
<td>CT (R)</td>
<td>CT</td>
<td>II NED, 84</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>F/3.2</td>
<td>CT (R)</td>
<td>CT</td>
<td>II NED, 82</td>
<td>18</td>
</tr>
<tr>
<td>31</td>
<td>M/5.4</td>
<td>CT CRT</td>
<td>CRT</td>
<td>DOTD [Local, PUL], 32 [12]</td>
<td>1q</td>
</tr>
<tr>
<td>32</td>
<td>F/2.4</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 75</td>
<td>1q21q32, 8, 18</td>
</tr>
<tr>
<td>33</td>
<td>M/9.9</td>
<td>CT (R)</td>
<td>CRT</td>
<td>DOTD [Local, BRA], 18 [10]</td>
<td>1q, 6, 18</td>
</tr>
<tr>
<td>34</td>
<td>F/11</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 64</td>
<td>7p1p14, 7p21p22, 7q22qter</td>
</tr>
<tr>
<td>35</td>
<td>F/3.3</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 57</td>
<td>1q</td>
</tr>
<tr>
<td>36</td>
<td>F/4.4</td>
<td>CT (R)</td>
<td>CRT</td>
<td>III NED, 55</td>
<td>1q, 6, 7p21qter, 8q, 9, 12</td>
</tr>
<tr>
<td>37</td>
<td>F/4.0</td>
<td>CT (I)</td>
<td>CRT</td>
<td>I DOTD [Local], 29 [12]</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>F/1.3</td>
<td>CT</td>
<td>CT</td>
<td>V DOTD [Local, PUL], 45 [21]</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>M/0.8</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 38</td>
<td>1q21q32, 4p</td>
</tr>
<tr>
<td>40</td>
<td>F/1.2</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 26</td>
<td>13q</td>
</tr>
<tr>
<td>41</td>
<td>M/2.5</td>
<td>CT (R)</td>
<td>CT</td>
<td>I NED, 25</td>
<td>1q</td>
</tr>
</tbody>
</table>

Abbreviations: Preop, preoperative; Adjuv, adjuvant; R, reduction of tumor volume; I, increase of tumor volume; OS, overall survival; EFS, event-free survival; CT, chemotherapy; CRT, chemoradiotherapy; NED, no evidence of disease; DOTD, died of tumor disease; LYM, lymph nodes; PUL, pulmonary; ADR, adrenals, HEP, hepatic; BRA, brain.
−7p (1 of 19) were only revealed to be nonrecurrent events. Thus, whereas +1q remained a prevalent imbalance and even was detected at an increased frequency compared with tumors without preoperative chemotherapy, the frequencies of +7q or imbalances at 7p were significantly lower (\( P = 0.011 \) and \( P = 0.019 \), respectively), leading to cytogenetically less complex karyotypes with overall fewer imbalances.

Among relapsed patients, there seemed some differences in event-free survival (EFS) for chromosome 7 imbalances. In the non-PCT group, four patients had synchronous distant metastases (mean EFS, 0 months) and these patients were disclosed to have tumors with +7q. Another two patients without chromosome 7 changes in their tumors only developed relapse during follow-up, either regional lymph node metastasis (EFS, 34 months) or local recurrence (EFS, 4 months). In the PCT group, the four relapsed patients were disclosed to have tumors without chromosome 7 abnormalities, and these patients only developed metastatic disease in the setting of local recurrence. EFS for these patients was 10, 12, 12, and 21 months, respectively. Losses at 16q were observed at lower frequencies (overall 3 of 41). However, both patients in the PCT group with loss of 16q have died of disease.

Discussion

The cytogenetics of Wilms’ tumors has been studied extensively. Chromosomal aberrations that have been identified in these tumors include gains at 1q, 6, 7q, 8, 12, and 17q and losses at 1p, 7p, 11q, 16q, and 22q (17, 24–32). The first study to use comparative genomic hybridization for the analysis of eight familial Wilms’ tumors described common gains on chromosomes 6, 8, and 12 and frequent chromosomal losses at 3q, 4q, 9p, 16q, and 20p (33). Later studies identified gains at 1q as the most prevalent change in Wilms’ tumors, followed by gains at 7q and losses at 7p (34, 35). Hing et al. (16) identified high frequencies of gains at 1q in relapsed tumors. However, the effects of chemotherapy on
the cytogenetic constitution of these tumors are widely unknown.

This study presents the first data on CGH analysis in primary Wilms’ tumors showing differences between tumors with and without preoperative chemotherapy. We could show that high frequencies of +1q seemed maintained in pretreated tumors, whereas imbalances involving chromosome 7, i.e. +7q, +7p, and −7p, were significantly less common after chemotherapeutic treatment, contributing to simpler karyotypes with fewer cytogenetic changes. Steenman et al. (35) investigated 46 Wilms’ tumors and six cases of nephroblastomatosis after preoperative chemotherapy and identified +7q in comparably low frequencies of 9% compared with +1q or +12q in 20% of their tumors. These observations suggest that Wilms’ tumor clones with +1q apparently are not obliterated by chemotherapeutic treatment, in line with a concept that clones with +1q are less responsive to chemotherapeutic treatment and therefore may be detected at a somewhat increased frequency in pretreated tumors. This would also explain previously published data indicating a high incidence of 1q gains in relapsed Wilms’ tumors of otherwise favorable histology (16). It may also be speculated whether chromosomal gains at 1q are related to drug resistance as is being considered in other tumors (e.g. ovarian cancers and neuroblastoma, respectively; ref. 36).

On the other hand, cytogenetically more complex clones with +7q and/or imbalances at 7p seemed obliterated by chemotherapy resulting in low frequencies of clones with chromosome 7 imbalances in pretreated tumors. One explanation for this observation would be that chromosome 7 imbalances rather represent secondary changes associated with increased karyotypic instability and presumably a higher potential for accelerated growth, making these clones more susceptible to chemotherapeutic treatment. This concept is also supported by the present observation that chromosome 7 imbalances seemed associated with higher tumor stage. Considering the imbalanced distribution of stage IV tumors between the two groups in the present series, we cannot rule out that the observed differences in frequencies of +7q may also reflect to a certain extent a covariation with stage. However, previous studies that were done largely on pretreated specimens also reveal lower incidences of +7q compared with those of +1q (24, 26, 35). Additionally, it would seem equally likely that chromosome 7 changes may characterize a distinct cytogenetic subset of Wilms’ tumors and that those tumors per se tend to be more aggressive but are more responsive to chemotherapy.

Whether +7q or +1q may be independent predictive markers for response to chemotherapy has to be clarified ultimately by multivariate analyses in larger studies with data on the cytogenetic constitution of tumors before and after preoperative chemotherapy. Isochromosomes of 7q have been identified as nonrandom aberrations in pediatric and adult Wilms’ tumors (37–41) and it has been suggested that i(7q) is related to loss of a tumor suppressor gene at 7p (42, 43). Recently, the PTH-

B1 (parathyroid hormone–responsive B1 gene) at 7p15 has been identified as a candidate gene for a Wilms’ tumor-related suppressor gene (44). Future studies will have to clarify, whether the cytogenetic constitution may eventually serve as an additional variable to stratify patients in the management of Wilms’ tumors.

Acknowledgments

We thank C. Enders for excellent technical assistance.

References

Clinical Cancer Research

Effects of Chemotherapy on the Cytogenetic Constitution of Wilms' Tumor
Thorsten Schlomm, Bastian Gunawan, Hans-Jürgen Schulten, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/12/4382

Cited articles
This article cites 43 articles, 7 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/12/4382.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/11/12/4382.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC)
Rightslink site.