Chromosomal Analysis of Sixteen Human Rhabdomyosarcomas

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ABSTRACT

Chromosomal analysis of 16 rhabdomyosarcomas was done from four primary tumors and from 12 tumors after nude mouse passage. Seven tumors were alveolar; four of these had t(2;13)(q37;q14) and in two tumors it was the only structural abnormality. The other three alveolar tumors were near tetraploid with marker chromosomes and double minutes. In the nine embryonal tumors studied, one had a normal karyotype, and eight were abnormal. Although the eight tumors had no common structural abnormality, trisomy 2 was present in all.

INTRODUCTION

Evidence is accumulating to show that specific chromosome rearrangements are associated with specific tumor types and subtypes. Such information is useful not only in diagnosis and classification of tumors, but also in contributing to our understanding of tumor biology, and in providing clues to location and expression of oncogenes (1). At least 29 chromosomal analyses of rhabdomyosarcomas with banding have been published (2-12). Of these, 22 have specified the tumor subtype. This report is an attempt to draw attention to the differences in results between RMS-A and RMS-E subtypes.

MATERIALS AND METHODS

Cytogenetic preparations were made from tumor tissue obtained directly from surgery or following growth in athymic nude mice. Xenografts were established by s.c. injection in NIH II Swiss background 6-8 week-old nude mice. Among the 16 tumors, 14 were obtained from Children's Hospital, Cincinnati, OH; one from Children's Hospital, Columbus, OH; and one from Vanderbilt University Hospital, Nashville, TN. Tumor material, primary tumor in 13 cases and metastasis in 3, was reviewed in our hospital by two pediatric pathologists. Tumors were diagnosed by conventional histological criteria and classified as RMS-A or RMS-E (13). Diagnosis was confirmed by histochemical staining with antidesmin, and with electron microscopy when necessary. Nude mouse samples were also reviewed by a pathologist and histological findings were consistent with original tumor material. Only tumors which could be definitely classified as rhabdomyosarcoma were included in this study. Two tumors, one alveolar (no. 2) and one embryonal (no. 15), were also considered to be undifferentiated.

RESULTS

The clinical history of the 16 rhabdomyosarcomas is summarized in Table 1 and the cytogenetic findings in Table 2. The first seven tumors, found in children 1-12 years old at diagnosis, were classified by pathology as RMS-A. The nine RMS-E tumors were from children 1-15 years of age at diagnosis. Patient 10 had the most abnormal karyotype with at least seven rearrangements. She was treated with radiation and chemotherapy for 15 months before surgery. Patient 15, our oldest patient, was diagnosed at age 6 with RMS-E of nasopharynx and died at age 23 with intradural RMS-E.
### Table 2: Chromosomal analysis of 16 rhabdomyosarcomas

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Source</th>
<th>Number of cells scored (analyzed)</th>
<th>Chromosome number (range)</th>
<th>Karyotype</th>
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<tbody>
<tr>
<td>1</td>
<td>NM°</td>
<td>75 (38)</td>
<td>45–49</td>
<td>48,XX,—8,+20,+20,rcp(2;13)(q37;q14),+der(5)(5;8)(q35;q13)</td>
</tr>
<tr>
<td>2</td>
<td>Primary</td>
<td>16 (7)</td>
<td>46–54</td>
<td>46,XY/53,XY,+8,+10,—13,+20,+dup(1)(p32;q12),der(2)(2;13)(q37;q14), +der(2)(2;13)(q37;q14),del(7)(p13),+17(7)(p11;?),+17(7)(p11;?)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49—54</td>
</tr>
<tr>
<td>3</td>
<td>NM</td>
<td>31 (12)</td>
<td>61–95</td>
<td>90,XXX,-13,-13,der(2)(2;13)(q37;q14),der(2)(2;13)(q37;q14)</td>
</tr>
<tr>
<td>4</td>
<td>NM°</td>
<td>74 (40)</td>
<td>&lt;80–96</td>
<td>92–94,XXXX,—3,+6,+6,—10,+20,+20,rcp(2;13)(q37;q14),rcp(2;13) (q37;q14)</td>
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<tr>
<td>5</td>
<td>NM</td>
<td>30 (20)</td>
<td>&lt;85–93</td>
<td>92,XXYY,—4,der(16)(1;16)(q21;q13),+mar,+dmin</td>
</tr>
<tr>
<td>6</td>
<td>NM°</td>
<td>88 (35)</td>
<td>79–94</td>
<td>92,XXYY,—3,5,5,—9,—12,—18,+2,mar,+dmin</td>
</tr>
<tr>
<td>7</td>
<td>NM°</td>
<td>60 (35)</td>
<td>71–93</td>
<td>93,XXYY,—2,—11,mar,+dmin/19,XXYY,—8,—9,—11,mar2,+dmin</td>
</tr>
<tr>
<td>8</td>
<td>Primary</td>
<td>13 (9)</td>
<td>48–53</td>
<td>51,XX,+8,+11,+12,15,—15,—18,+20,der(14)(14;11)(q11;?),der(17)(17;?),+der(21)(21;10)(p31;10),+dup(12)(p32;q12),+del(18)(q22),+2mar,del(19)(19;7)(11;7),+5mar/related karyotypes</td>
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<tr>
<td>9</td>
<td>NM</td>
<td>111 (35)</td>
<td>47–56</td>
<td>51,XX,+8,+12,+13,der(3)(13;9)(q21;q12),del(9)(q12),rcp(11)(11;21),+2mar,del(18)(q22),+5mar/related karyotypes</td>
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<td>10</td>
<td>NM°</td>
<td>100 (40)</td>
<td>49–61</td>
<td>52,XX,+der(X)(X;11)(q28;q13),—11,13,—17,17,20,+20,rcp(12;12)(q31;31),+der(11;11),+der(13;13),+der(8;18)(q22;q13),t(8;15;q),del(9;9)(q11;p3),dup(12)(q24;q13),del(7;14),del(15;15),del(18;18),+mar,+dmin</td>
</tr>
<tr>
<td>11</td>
<td>NM</td>
<td>60 (38)</td>
<td>50–57</td>
<td>53,XY,+2,+7,+8,+8,+9,11,+20,der(10)(10;15)(p15;q15),+54,XY, +rcp(8;12)(p12;q12),+del(12)(q12),+del(12)(q12)</td>
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<tr>
<td>12</td>
<td>NM°</td>
<td>70 (35)</td>
<td>47–55</td>
<td>55,XX,rcp(X;5)(q28;q22),+1,+2,+2,5,7,11,13,19,19,+1q</td>
</tr>
<tr>
<td>13</td>
<td>Primary</td>
<td>48 (20)</td>
<td>49–59</td>
<td>57,XXY,+2,5,5,8,11,11,13,13,19,20,22</td>
</tr>
<tr>
<td>14</td>
<td>NM</td>
<td>37 (20)</td>
<td>58–62</td>
<td>61,XXXX,+2,2,—4,6,7,8,8,9,11,12,13,15,19,19,rcp(9;14)(p11;q11),del(10)(10;10)(p15;15),+dup(17)(17;17)</td>
</tr>
<tr>
<td>15</td>
<td>NM°</td>
<td>146 (35)</td>
<td>54–67</td>
<td>61,XXYY,+1,1,5,6,6,8,9,12,—15,18,19,20,+der(2)(2;7p25;7), +dup(7;7)(q32;q11),+del(7;7)(q11),+del(7;7)(q11),+del(12;12)(12;12)(q11;q11),+der(2)(2;7p25;7), +der(2)(2;7p25;7),+15;15(15;15),del(15)(15;15), +3mar,61,XXX, del(19)(19;19)(q11;q11)</td>
</tr>
<tr>
<td>16</td>
<td>Primary</td>
<td>30 (20)</td>
<td>43–46</td>
<td>46,XY</td>
</tr>
</tbody>
</table>

* Two to four passages analyzed.

Major clone, i.e., more than 50% cells analyzed.

Box, chromosome aberrations different from those in preceding clone.

Cytogenetic analyses were successful in four primary tumor samples and in 12 other tumors after passage in the nude mouse. A normal karyotype was found in only one, no. 16, a primary tumor, after 4–6-day cultures. Heterogeneity, i.e., evidence of more than one clone, was observed in seven tumors, RMS-A, nos. 2 and 7, and RMS-E, nos. 8, 9, 10, 11, and 15. Analyses from different nude mouse passages were often necessary to demonstrate heterogeneity, e.g., nos. 8, 9, and 15, although an alternative explanation is that the clone seen in the primary tumor or in earlier passages changed after passage in the mouse.

Fifteen tumors showed an abnormal karyotype. Comparison of modal numbers showed near tetraploid in five RMS-A tumors. The remaining two RMS-A tumors and the seven abnormal RMS-E tumors were hyperdiploid with modal numbers of 48–61. All clonal abnormalities were relatively stable after nude mouse passages.

In four of the seven RMS-A tumors, Glemsa banding showed a t(2;13)(q37;q14) readily evident as a 2q+ chromosome (Figs. 1 and 2). In two tumors, nos. 1 and 4, the translocation was balanced and the der(13) was seen (Figs. 1A and 2). The t(2;13) was apparently unbalanced in two other RMS-A, nos. 2 and 3, since the 13q- derivative was not seen in the karyotype (Fig. 2B). In cases 3 and 4, the t(2;13) was the only structural abnormality; cases 1 and 2 had other random structural abnormalities. These four tumors included one which was described as undifferentiated, but suggestive of alveolar. Three other RMS-A, nos. 5, 6, and 7, were tetraploid with marker chromosomes and double minutes (Fig. 3) and did not have t(2;13).

There was no structural abnormality common to the eight RMS-E tumors with abnormal karyotypes, although there was...
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Fig. 1. Partial karyotypes showing t(2;13) from two representative alveolar rhabdomyosarcomas. A, an rcp(2;13)(q37;q14) translocation in case 1; B, an extra der(2) and no der(13) from t(2;13)(q37;q14) in case 2.

Fig. 2. Representative karyotype of an alveolar rhabdomyosarcoma, case 4: 92,XXXX,-3,+6,-10,+20,rcp(2;13)(q37;q14),rcp(2;13)(q37;q14). Arrows, structural chromosome abnormalities; arrowheads, numerical changes.

Fig. 3. Part of a G-banded metaphase from case 6, an alveolar rhabdomyosarcoma, showing many double minutes.

an abnormality involving 12q13 in three tumors. In all eight abnormal RMS-E tumors, there was a +2 or partial trisomy 2 (one case) (Figs. 4 and 5). In nos. 10 and 15, there was also a structural abnormality of chromosome 2 (Fig. 6), but neither of the breakpoints were 2q37. Tumor no. 15 was undifferentiated, but was diagnosed as a rhabdomyosarcoma by positive antidesmin staining and was considered embryonal. Interestingly, tumor no. 7, a RMS-A type, had no t(2;13) and did have +2. In addition, other common numerical abnormalities were seen in eight RMS-E tumors: seven had +20, six had +13, six had +8, five had +11, and five had +19.

Fig. 4. Representative karyotype of an embryonal rhabdomyosarcoma, case 8: 54,XX,+2,+8,+12,+13,+19,der(13)(q21:13.1),del(9)(p23:?),del(9)(q12.1),rcp(11;20)(q23;p13). Arrows, structural abnormalities; arrowheads, numerical abnormalities.

Fig. 5. Representative karyotype of an embryonal rhabdomyosarcoma, case 11: 55,X,rcp(X;5)(q28;q22)+1,+2,+2,+5,+7,+11,+13,+19,+i(1q). Arrows, structural abnormalities; arrowheads, numerical abnormalities.

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DISCUSSION

Eleven published reports of chromosomal analyses of rhabdomyosarcomas with banding include results of 29 tumors from direct preparations, cell lines, or metastasis (2-12). Eleven of the tumors were RMS-A (3, 7, 8-12), eight were RMS-E (2, 5, 12), three were other cell types (8, 12), and the other seven were unclassified. The present report of 16 tumors brings the total to 45 rhabdomyosarcomas with banded analyses, and of these at least 18 are RMS-A.

Four of the seven RMS-A described here had the t(2;13)(q37;q14). The translocation appears to be the same as that first reported by Seidal et al. in one RMS-A (3), and subsequently in another four RMS-A (7, 9, 10, 11). An important case of RMS-A had t(2;11)(q37;q13) (8), indicating that the critical breakpoint in the tumor is probably 2q37. Douglass et al. (12) interpreted the breakpoints as t(2;13)(q37;q14) in five tumors. A review of the Catalog of Chromosome Aberrations in Cancer, 1985 (17) indicates that 2q37 rearrangements, other than RMS-A, have occurred in one lymphoma and in nine other hematological diseases, one of which had t(2;13)(q37;q12). The breakpoint 13q14 has been identified as a critical region in retinoblastomas (18). Constitutive fragile sites on 2q37.3 and on 13q13.2 have also been reported (19), although no oncogenes have been identified in these regions.

In two of our RMS-A tumors, the t(2;13) appeared to be a balanced translocation, as also reported in at least six other RMS-A tumors (7, 9-12). In two of our RMS-A the der(13) was not seen, as noted in two other RMS-A (3, 12). In tumor no. 2, the t(2;13) was seen in a third culture of a primary tumor. Finally, in two of our cases and in two other reported RMS-A (3, 10), the t(2;13) was the only structural abnormality in the karyotype. This information from our first four cases, therefore, appears to confirm the observation by Turc-Carel et al. (7) that the t(2;13)(q37;q14) may be a new specific clonal rearrangement characteristic of RMS-A. Furthermore, RMS-A tumors maintain constitutional heterozygosity with various recombinant DNA probes of 11p13-15, whereas RMS-E tumors show loss of heterozygosity with the same probes (16). Thus, at least some RMS-A appear to be a special subtype and cytogenetic entity of rhabdomyosarcomas with an etiology different from other types.

However, not all RMS-A have t(2;13). Douglass et al. (12) found the t(2;13) in only three of five of their RMS-A and in two other rhabdomyosarcomas, not RMS-A. Furthermore, a constitutive t(2;5)(q37;q31) has been found in a patient with RMS-E (20). Douglass concluded that t(2;13) is not limited to the RMS-A histological type, and may be a secondary abnormality and possibly a marker of disease progression in various types of rhabdomyosarcomas (12). Our three tumors, nos. 5, 6, and 7, which failed to have the t(2;13) were reviewed by the pathologist and confirmed to be alveolar. All three were tetraploid with at least one marker chromosome and double minutes, which have been reported in other rhabdomyosarcomas (6, 9, 12, 21, 22). Thus, it may be possible that there is another undefined cytogenetic entity in alveolar rhabdomyosarcomas.

Tumor no. 7 was unusual in that it had +2, as seen in our RMS-E tumors, and also showed homozygosity with 11p probes (16), suggesting RMS-E. However, it may be reasonable to expect overlap between results of pathology classification and cytogenetic or molecular biology findings (12). The lesson which has been learned from leukemia is that chromosome changes can be used in classifying various subsets, sometimes independent of histology (1). Thus, we have come to regard specific translocations in neoplasia not necessarily as markers of clinically and morphologically defined diseases, but rather markers of a neoplastic cell origin or pathway (23).

Our eight RMS-E tumors were particularly examined for evidence of a common structural abnormality. In three tumors, there was a rearrangement involving breakpoint 12q13. Structural abnormalities of 1q and/or 1p have previously been reported in 14 cases of rhabdomyosarcomas (5, 6, 11, 12). Although three of our tumors had a rearrangement of chromosome 1, there was no common breakpoint. Structural abnormalities of chromosome 3 have also been reviewed in rhabdomyosarcomas (6), but only one of our tumors had a translocation 3q21.

Numerical abnormalities in our eight abnormal RMS-E tumors, plus one RMS-A tumor, no. 7, which did not have t(2;13), were of some interest. In these nine tumors, there was trisomy 2; in six of the nine there was both +2 and +13. A review of embryonal rhabdomyosarcomas in published reports yielded eight informative karyotypes as to numerical abnormalities. In one, an RMS-E with modal number 56, there was +2 and +13 (5). In the other seven RMS-E there was no +2 (2, 12), but at least five of these were cell lines analyzed after 9–40 passages. An examination of four of our RMS-A tumors showed that there was an extra der(2) t(2;13), in one tumor, no. 2, which was also reported in one other RMS-A tumor (3). Two other RMS-A tumors (10, 11) have t(2;13), plus two normal no. 2 chromosomes. Other numerical abnormalities in our RMS-E tumors were: +20 seen in seven tumors, +8 in six, +11 in five, and +19 in five. Of these, +20 and +8 are frequent trisomies in tumors (17).

From these preliminary observations, the nonrandom involvement of +2 and possibly +13 was suspected, either as primary or secondary abnormalities. We reviewed karyotypes...
in 40 other pediatric tumors in our laboratory (11 Ewing's sarcomas, 15 neuroblastomas, and 14 Wilms' tumors) and one case with +2 was found, although +13 was seen twice. The incidence of trisomy 2 and 13 in neoplasms was estimated from the Catalog of Chromosome Aberrations in Cancer (17) and there was no evidence that +2 or +13 is common in any particular tumor or hematological disease. Assigning importance to nonrandom numerical abnormalities in tumors is not as simple as structural abnormalities. We have come to expect such numerical changes to be associated with secondary (subsequent) rather than primary (initial) changes (24), although primary numerical abnormalities are not unknown (23, 25). Certainly, analysis of a larger number of tumors is necessary to decide whether +2 and possibly +13 are significant in rhabdomyosarcomas, and constitute a cytogenetic entity.

ACKNOWLEDGMENTS

We are indebted to Dr. David Witte, Pediatric Pathologist, Cincinnati Children's Hospital, for the critical information on the tumor pathology, and to Marianne Brown who supplied us with nude mouse material. The following individuals have generously contributed information on their patients: Dr. David Swick, Columbus Children's Hospital, Columbus, OH and Dr. Karen Zaboy, Vanderbilt University Hospital, Nashville, TE.

Dr. Edwin Douglass generously allowed us to read his manuscript before publication.

REFERENCES

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