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Review article: chromosomal diagnosis in rhabdomyosarcoma

Artigo de revisão: diagnóstico cromosômico em rabdomiossarcoma

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Resumo

Os rabdomiossarcomas (RMS) são considerados tumores clinicamente agressivos com origem a partir de células mesenquimais imaturas e que se caracterizam pela presença de células com diferenciação pouco definida. O emprego das técnicas citogenéticas convencionais em RMS vem contribuindo consideravelmente para a diferenciação entre os rabdomiossarcomas alveolares e os outros tumores de células pequenas e redondas, além de fornecer informações prognósticas importantes referente ao rabdomiossarcoma do tipo alveolar. Assim, este trabalho visa a realizar uma revisão das alterações citogenéticas observadas nos diferentes subtipos histológicos de RMS, enfocando não só os trabalhos de citogenética convencional, mas também novas abordagens utilizadas para o estudo de neoplasias tais como FISH, CGH, SKY e M-FISH. Tais metodologias vêm contribuindo de maneira significativa para a melhor compreensão da heterogeneidade cariotípica observada nos RMS.
Palavras-chave: Cromossomos; Citogenética; Rabidomiossarcoma.

Abstract

Rhabdomyosarcomas (RMS) are considered clinically aggressive tumors, originated from immature mesenchymal cells and characterized by the presence of cells with an ill-defined differentiation. The use of conventional cytogenetic techniques has contributed considerably to distinguish the alveolar RMSs from the other types of solid tumors in children and adolescents. Besides that, it provides important prognostic informations about alveolar RMSs. Thus, the present work was aimed at reviewing the cytogenetic alterations observed in the different histological subtypes of RMS, focusing not only on the studies performed with conventional cytogenetics, but also on new approaches used in the study of neoplasms, such as FISH, CGH, SKY and M-FISH. These methodologies have contributed significantly to a better understanding of the karyotype heterogeneity observed in RMS.
Key words: Chromosomes; Cytogenetics; Rhabdomyosarcomas.

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INTRODUCTION, EPIDEMIOLOGY AND ETIOLOGY

Rhabdomyosarcomas (RMS) are considered clinically aggressive tumors originated from primitive and immature mesenchymal cells, which are located in a skeletal muscle line and can be formed within a variety of organs and tissues, including those without striated muscles[1]. This tumor presents immunohistochemical expression of myosin, actin, desmin, myoglobin and Z-band protein[2] and expresses a DNA binding protein, MYOD1, which may be a lineage marker for rhabdomyosarcomas[3].

This pathology was originally described by Weber[4] in 1854 and is characterized mainly by the presence of cells with an ill-defined differentiation, which significantly increases the difficulty of making a histopathological diagnosis and distinguish the alveolar RMSs from the other types of solid tumors in children and adolescents (e.g. neuroblastomas, non-Hodgkin lymphomas and the tumors of the Ewing's family)[5]. In the United States of America a collaborative Intergroup Rhabdomyosarcoma Study Group (IRS) was established in order to investigate the biology and treatment of this tumor[6]. IRS-V is currently ongoing and four prior studies have been concluded[6,7].

RMS is the most common soft tissue sarcoma in the first two decades of life[3]. It accounts for 10-15% of solid malignant tumors and 6% of all malignancies in infants under 15 years of age[2]. In children, RMS has an annual incidence of 4.3 cases per million individuals and some studies show a significant predominance of this type of tumor in males (11.8 per million) as compared to females (10.3 per million)[8,9]. In the United States of America the proportion between male to female is 1.5:1, the tumor is twice as common in Caucasians as in African-Americans and approximately 250 new cases are diagnosed every year[2].

Recently published studies on the possible etiologic factors related to the development of this disease demonstrate that the great majority of RMS cases occur sporadically. It is believed, however, that the development of this pathology may be related to certain kinds of syndromes, such as neurofibromatosis type 1[10], Li-Fraumeni Syndrome (LFS)[11], Beckwith-Wiedemann Syndrome[12] and Costello Syndrome[13]. Besides, other known risk factors include marijuana and cocaine use, maternal exposure to radiation, and female health care workers may be contributing to the development of this disease[14].

So far, only a small number of RMS cases have been characterized cytogenetically, although these tumors are considered relatively common in childhood and adolescence. This is probably due to the difficulty in obtaining metaphases from primary tumors, which contributes to the small number of studies available in the literature[15].

CLASSIFICATION

Generally, RMSs are classified into three groups: (a) pleomorphic, (b) embryonal (ERMS) and it´s relative variants (botryoid and spindle-cell RMS) and (c) alveolar (ARMS) (including the solid alveolar variant)[16].

Pleomorphic tumors usually arise on the limbs and trunks of adults over 45 years old, but isolated cases in pediatric age group have been reported[17,18]. It comprises only 1% of childhood rhabdomyosarcomas and, microscopically, this tumor presents large pleomorphic cells with multinucleated giant cells[19]. Immunohistochemistry technics are usually required to distinguish it from liposarcoma or malignant fibrous histiocytoma[2].

ERMS is the most common variety and comprises over half of all RMS cases diagnosed[20]. This subtype usually occurs before 8 years of age and frequently arises (60% of the cases) in the head and neck region (particularly the orbit, nasopharynx, oral cavity and middle ear)[21,2]. ERMS tumors may occur also in retroperitoneum, bile ducts and urogenital tract[21].

It shows a mixture of spindle and undifferentiated round cells and immature striated muscle-like cells (called rhabdomyoblasts) with abundant eosinophilic cytoplasm either tightly or loosely packed in a myxoid background. The botryoid variant is a morphologic subtype of the embryonal variety and its name derives from its gross appearence which resembles a "cluster of grapes"[22]. This subtype accounts for 5% of all RMS cases and usually arises under mucosal surfaces such as nasopharynx, oral cavity, vagina and bladder[23,20]. Botryoid tumors have the best prognosis and are typically observed in infants under 5 years of age[14].

Another rare variant of ERMS (spindle-cell) accounts for 3% of all RMS[24] and the higher survival rate supports that this group has a favorable histologic subtype[14]. Histologically, it is characterized by fascicles of spindle cells, reminiscent of a leiomyosarcoma and tends to appear in an unproportional manner in the paratesticular region. It can be also seen in the extremities, cavities, head and neck[25].

ARMS generally occurs in 10-30 year old patients[2]. This subtype is more frequent in tumors arising in adolescents[26] and comprises about 25% of all RMS[20]. ARMS tumors are often more firm, less myxoid and occur more commonly on the limbs and trunk[2]. Under the microscope, small, round or oval tumor cells are observed in nest by connective tissue septa[16]. Eosinophilic cytoplasm growing in thin strands of fibrovascular stroma with "free floating" tumor cells are observed[2]. The acidophilia of the cytolplasm and the presence of occasional multinucleated giants cells are important diagnostic features[27]. A variant form (solid ARMS), with small and round cells, has been identified[28].

CONVENTIONAL CYTOGENETICS AND RMS

The use of conventional cytogenetic techniques in RMS has contributed considerably to make important prognostic information available. Numerical and structural abnormalities thus observed can help classifying the histological subtypes when tumor characterization by microscopy is difficult[29].

ARMS

In approximately 70% of the ARMS cases, a characteristic translocation is observed involving chromosomes 2 and 13 t(2;13)(q35;q14)[30], affecting the PAX3 gene at band 2q35 and the FKHR gene at band 13q14[31]. On the other hand, in about 15-20% of cases, a variant translocation t(1;13)(p36;q14)[32] can be observed, juxtaposing gene PAX7 at band 1p36 and gene FKHR at band 13q14 on the chromosome[33,34].

Structural alterations involving chromosome 1 were described, such as del(1)(p11)[29], del(1)(p21-pter)[35], the i(1q)[36] and the der(1) observed by Magnani et al. (1991)[37] in a RMS cell line. Other chromosome 1 alterations, such as t(1,22)[38], t(1;5) [39] and t(1;11)[40] have also been described in the literature. These reports emphasize the crucial role of chromosome 1 in the development and/ or progression of this kind of tumor[29].

Abnormalities related to chromosome 17 were observed as well, such as add(17)(q25)[29] and t(17;?)(q25;?)[27]. Other alterations of chromosome 17 were identified as t(14;17)(q24;q11), add (17)(q?) and t(17;22)(q21;q13) in different cases studied by Kullendorf et al. (1998)[40]. Gains of 17q21[41] and the presence of an i(17q) were also described[39]. A variety of different genes have been found to be amplified in the ARMS subtype, which leads to frequent observations of double-minute chromosomes double minutes (dmins)[42].

Botryoid RMS and Spindel-Cell Variant

Even though the number of papers describing cytogenetic findings in botryoid RMS is not significant, some sporadic cases have been reported, as one inv(9)(p11q13),+del(1)(p12),+13,+18[43];one add(11)(q21),t(8;11)(q12 approximately 13;q21)[44]; one i(17)(q10)[45], and one psu dic (1;10)(p13;p15), der(16)t(16;17)(p13.3;q21)[41]. In addition to these alterations, gains involving chromosomes 2, 4, 8, 19 and 20 were also described in the literature. Due to this karyotype heterogeneity, further cytogenetic studies are necessary, in order to determine: (1) whether these cytogenetic findings are consistently associated with this malignant lesion; and (2) whether their presence has any prognostic significance[41].

The cytogenetic analysis of one spindle-cell variant revealed the abnormal karyotype 46,XX,der(2)t(2;7)(q36 approximately q37;q3?), del(14)(q24), der(16)t(1;16) (q21;q13)[46].

ERMS

Although no consistent alteration has been found so far, a number of studies have been performed on ERMS from the cytogenetic point of view[41]. Gil-Benso et al. (2003)[47] reported a case with a der(11)t(3;11)(p21;p15). In addition to this abnormality, these tumors are often hyperploid, presenting extra chromosomes 2[40], 8[48], 9, 11, 12, 13, 17, 18, and 20, besides loss of chromosomes 10, 14, 15 and 16[49]. Scrable et al. (1989)[50] and Koufos et al. (1985)[51] demonstrated that there was a loss of heterozygosity (LOH) at 11p15.5 in 13 out of 14 ERMS tumors analyzed. Trisomy of chromosomes 2 and 13, structural abnormalities involving 1q and/or 1p and regions 3p14-21 have also been reported in ERMS and undifferentiated sarcomas[35]. Recently, Ho et al. (2004)[52] described a novel chromosomal t(2;20)(q35;p12) occurring in a case of childhood RMS with embryonal histology.

RMS AND FLUORESCENCE IN SITU HIBRIDIZATION (FISH)

The success of cytogenetic studies from solid tumors has been limited by the difficulties faced in obtaining an adequate number of metaphase cells and by the poor quality of the spread and banded chromosomes[53,54]. However, lately these limitations of the conventional method have been bypassed using the fluorescence in situ hybridization (FISH) method[55]. The use of this technique on metaphases and interphase nuclei, associated with the classical cytogenetic studies, has played a major role in the detection of specific chromosome rearrangements[56].

Using FISH, McManus et al. (1996)[57] detected the translocation t(2;13)(q35;q14) in four ARMS cases, but this alteration was not detected in the ERMS type. The amplification of the gene HER-2/neu (which encodes a protein that takes part in the structure of the epidermal growth factor receptor) was clearly demonstrated from paraffin-embedded histological sections of ERMS[58]. FISH was also used to detect amplifications of the oncogene MYCN in 15 ARMSs and 14 ERMSs. This amplification was observed in 9 out of the 15 ARMSs, but in none of the 14 ERMSs[59].

The results obtained by Afify & Mark, (1999)[48] showed that 6 out of the 12 ERMSs studied by them presented trisomy of chromosome 8. Other trisomies, such as trisomy 2, were detected in nine out of ten ARMS cases studied by Biegel et al. (1995)[60].

Using probes for 6 different chromosomes, Lee et al. (1993)[61] found multiple copies of chromosomes 8 and 12 and one clone with trisomy 11, but no numerical aberration whatsoever involving chromosomes 6, 17 or 18 in RMS. Trisomy of chromosome 11 was described in three ERMS cases by Scrable et al. (1989)[50] and in five cases by Wang-Wuu et al. (1988)[62].

SPECTRAL KARYOTYPING (SKY), MULTI-FLUOROPHORE FLUORESCENCE IN SITU HYBRIDIZATION (M-FISH), COMPARATIVE GENOMIC HYBRIDIZATION (CGH) AND RMS

The use of CGH, SKY and M-FISH techniques has proven to be a more efficient approach for defining complex structural and numerical abnormalities in the study of solid tumors and in the distinction of patients with different biological types of RMS[63]. These techniques are alternative methods which require specific digital analysis programs and the use of expensive probes. They were developed, nevertheless, because of the limitations of the analysis and the poor quality of the slides obtained by the traditional banding methods from chromosome preparations of RMS cells[5,64].

The use of CGH in RMS has shown that genomic amplifications observed as dmins and homogeneously stained regions are present in a higher proportion in ARMS and only in a few embryonal cases[65].

Using several molecular cytogenetic techniques such as SKY, M-FISH and CGH, Roberts et al. (2001)[5] observed a number of recurrent cytogenetic abnormalities in 5 ERMS lines and in one ARMS line that was negative for the PAX-FKHR fusion. These abnormalities included translocations involving chromosomes 1 and 15 (4 of the 6 lines) and chromosomes 2 and 15 (2 of the 6 lines). All 6 lines displayed chromosome 15 abnormalities[5].

In some ERMS cases, the cytogenetic studies have shown gains of entire chromosomes or chromosome regions, especially of chromosomes 2, 7, 8, 11, 12, 13, 17, 18, 19, 13q21, and 20 (in 33-67% of these tumors), and these findings have been confirmed by CGH[66]. Losses of 1p35~p36, 16, 6, 9q22, 10, 14, 15, 14q21~q32, and 17 (in 20-42% of these tumors) that had been detected by conventional cytogenetics were also confirmed by CGH[67, 66].

Finally, Meddeb et al. (1996)[65] observed the presence of a single point in region 12q13q14 that contained amplified copies of the gene MDM2 by using CGH. This observation had already been made in RMS by conventional cytogenetics through the presence of dmins.

CONCLUSION

Cytogenetic analysis of RMSs have shown to be important for the clinical diagnosis, besides being useful in identifying the genes involved in the tumorigenesis process. Its use has been helpful in clarifying inconclusive histological findings, making the differential diagnosis easier. It is however worth pointing out that the molecular cytogenetic methods, although useful in detecting chromosome alterations, should not replace the conventional cytogenetic method, because this one provides information based on a complete karyotype.

The cytogenetic techniques described in this review have been used at the Pediatrics Laboratory of the FMRP-USP Hospital das Clínicas and at the Cytogenetic Laboratory of the EPM/UNIFESP, where our research team carries out chromosome studies aimed at diagnosing such neoplasias. With this review, we intend to contribute to the publication of the molecular technological advances developed starting from the classical methodology that allowed identifying chromosome markers currently used for the study of proliferative processes in pediatrics.

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