American Journal of Pathology, Vol. 152, No. 2, February 1998

Detection of the MyoD1 Transcript in Rhabdomyosarcoma Cell Lines and Tumor Samples by Reverse Transcription Polymerase Chain Reaction

Emanuela Frascella and Angelo Rosolen

From the Division of Hemato-Oncology, Department of Pediatrics, University of Padova, Padova, Italy

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of childhood. Diagnosis of RMS can be difficult when it appears as a small round-cell tumor without evidence of differentiation. Recently, a set of regulatory proteins expressed during skeletal muscle development has been described. Among them, MyoD1 has been detected by Northern blot and immunohistochemical analyses in normal skeletal muscle and RMS. Given the relevance of this marker in the diagnosis of RMS, we developed an assay to evaluate the expression of MyoD1 mRNA in small tissue specimens by reverse transcription polymerase chain reaction. Specificity and sensitivity of the assay was determined in a series of 25 tumor cell lines and 39 pediatric tumor samples, including 35 RMSs. Subsequently, we studied the expression of MyoD1 in bone marrow and peripheral blood stem cell specimens. We detected the MyoD1 transcript in normal skeletal muscle and in almost all RMSs, whereas no expression was found in non-RMS samples or in normal hematopoietic tissues. This assay showed high sensitivity and specificity, and it could be a useful molecular tool for the diagnosis of RMS within small round-cell tumors of childhood and for the detection of minimal bone marrow and peripheral blood stem cell involvement in children with RMS, regardless of the histological subtype. *(Am J Pathol 1998, 152:577–583)*

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of childhood, accounting for more than half of this group of tumors.[1] Twenty percent of children with RMS have a disseminated disease at diagnosis, and lungs, lymph nodes, bone marrow (BM), and bones are frequent sites of metastasis.[2] BM involvement is currently evaluated by morphological and immunohistochemical examination of bone marrow aspirates and trephine biopsies.

RMS shows a skeletal muscle phenotype with a wide morphological spectrum ranging from well differentiated rhabdomyoblasts to poorly differentiated cells. In the latter case, distinction of RMS from other small blue round-cell tumors of childhood, such as Ewing's sarcoma, peripheral neuroectodermal tumor (PNET), neuroblastoma, and non-Hodgkin's lymphoma can be difficult.

Different subtypes of RMS have been identified based on morphological features: 1) embryonal rhabdomyosarcoma (ERMS), with its botryoid and spindle cell subtypes, 2) alveolar rhabdomyosarcoma (ARMS), with solid alveolar variant, and 3) pleomorphic subtype.[3] Embryonal and alveolar subtypes show different molecular features; loss of heterozygosity of chromosome 11p15 was observed in ERMs,[4] whereas ARMSs were associated with the specific chromosomal translocations t(2;13)(q35;q14) and t(1;13)(p36;q14).[5]

On the other hand, ERMS and ARMS show an overlapping expression of muscle-specific markers, such as muscle-specific actin, myosin, creatine-kinase, and myogenic regulatory factors of the MyoD protein family.[6] These muscle-specific transcription factors appear to regulate the myogenic program of skeletal muscle,[7,8] and their expression represents a useful marker to identify skeletal muscle precursor cells. Among them, MyoD1 has been well characterized; it is a member of the basic helix-loop-helix protein family with nuclear localization and maps on chromosome 11p15.[9] Human MyoD1 protein shows 90% amino acid sequence homology with mouse MyoD1. Although the human MyoD1 cDNA sequence is known,[10] its genomic sequence has not yet been determined. On the contrary, the mouse gene has been well characterized and was shown to contain two introns, in positions 1443 to 1881 and 1961 to 2288, respectively.[11] MyoD1 is expressed only in skeletal muscle, and its expression can convert a wide range of cell types into muscle cells.[10,12] It has been detected by Northern blot[6,13] and immunohistochemical analyses in

Supported by a fellowship of the Associazione Italiana per la Ricerca sul Cancro (E. Frascella).

Accepted for publication November 6, 1997.

Address reprint requests to Dr. Angelo Rosolen, Division of Hemato-Oncology, Department of Pediatrics, University of Padova, Via Giustiniani 3, Padova 35128, Italy. E-mail: rosolen@child.pedi.unipd.it.

the majority of RMSs,[14,15] and it represents a useful marker in differentiating RMS from other small round-cell tumors.

We developed a reverse transcription polymerase chain reaction (RT-PCR) assay to evaluate the expression of the human MyoD1 and tested its specificity and sensitivity in a series of 13 RMS and 12 non-RMS cell lines and 39 tumor samples, including 35 RMSs. Subsequently, we studied the expression of MyoD1 in bone marrow and peripheral blood stem cell (PBSC) samples to determine whether this assay could represent a useful molecular tool in the diagnostic work-up and in the detection of minimal BM involvement in children with RMS. So far, RT-PCR analysis could be used in the evaluation of BM samples only in the alveolar subtype, which carries the t(2;13)(q35;q14) chromosomal translocation or its variant t(1;13)(p36;q14),[16,17] but there is no specific molecular marker for the identification of BM micrometastasis in RMSs lacking specific translocations.

Materials and Methods

Cell Lines

The human cell lines RH30, RD, IMR32, Namalwa, CA46, MOLT4, CEM, HL60, DAOY, and D283Med were purchased from American Type Culture Collection (Rockville, MD); CHP100 was obtained from the Children's Hospital of Philadelphia (Philadelphia, PA); RH1, RH4, RH18, and RH28 were a gift of D. Shapiro (Memphis, TN); RC2 and CCA were provided by P. L. Lollini (Bologna, Italy); SMS-CTR, RH36, and TC32 were obtained from M. Tsokos (Bethesda, MD); and ACN and 2C2 from M. Ponzoni (Genova, Italy). Short-term cell cultures were established in our laboratory from tumor biopsies. Cells were cultured in RPMI 1640 with 10% fetal bovine serum (Seromed, Berlin, Germany), 100 IU/ml penicillin G and 100 μg/ml streptomycin. All cell lines were mycoplasma free.

Tumor Samples

Tumor specimens were obtained from patients admitted to the Division of Hemato-Oncology, Department of Pediatrics, University Hospital of Padova. Fresh tissue samples were minced into small fragments, quickly frozen in liquid nitrogen, and used, in part, for RNA extraction. A total of 39 tumor samples were studied: 35 RMSs (16 ARMSs, 17 ERMSs, and 1 solid alveolar and 1 spindle cell RMS), 1 leiomyosarcoma, 1 PNET, and 2 Ewing's sarcomas. BMs, PBSCs, and pericardial effusion were obtained from some patients with RMS or PNET/Ewing's tumors. To protect confidentiality, patients were identified by a number and not by name.

Control Tissues

Normal adult skeletal muscle tissue and normal peripheral blood were obtained from the Department of Pathology and the Blood Bank at the University Hospital of Padova, respectively.

PCR and RT-PCR Assays

Total RNA was isolated by using the RNAzol-B reagent (Tel-Test, Friendswood, TX) following the manufacturer's instructions. One microgram of total RNA from each specimen was reverse transcribed by using the M-MLV reverse transcriptase (Life Technologies, Milan, Italy) and random examers. Genomic DNA from total blood was extracted by standard techniques.

PCR amplification was performed by using the Gene Amp PCR kit (Perkin Elmer, Monza, Italy) according to the manufacturer's instructions. The PCR reaction mixture contained 1.5 mmol/L $MgCl_2$, 0.35 μmol/L of each primer, 1.9 μl of 10X PCR buffer, 4 μmol/L of each dNTP, 0.5 U of *Taq* polymerase, and 5% of the RT product in a final 20-μl reaction volume. The 5′ and 3′ primers, specific for the human MyoD1 cDNA sequence, were AGCACTACAGCGGCGACT and GCGACTCAGAAGGCACGTC, respectively. Template cDNA was subjected to 35 PCR cycles consisting of 15 seconds of denaturation at 94°C, 15 seconds of annealing at 60°C, and 15 seconds of extension at 72°C. They were followed by a 10-minute final extension at 72°C. When genomic DNA was used as a template, DNA was subjected to 40 cycles with the same temperature conditions reported above, but the time of each step was increased to 90 seconds. To augment sensitivity in BM and PBSC analyses, PCR was performed with 40 cycles consisting of 30 seconds of denaturation at 94°C, 30 seconds of annealing at 58°C, and 30 seconds of extension at 72°C. They were followed by 10 minutes at 72°C as final extension. Amplification of chimeric products PAX3-FKHR/PAX7-FKHR, derived from t(2;13)(q35;q14) and t(1;13)(p36;q14), respectively, and EWS-FLI1 transcript, derived from t(11;22)(q24;q12), were performed as previously reported.[16–18]

For each sample, the expression of wild-type β-actin was assessed as a control for the presence of amplifiable RNA and the efficacy of reverse transcription. PCR reaction products and the DNA molecular weight marker (marker VI, Boehringer Mannheim, Monza, Italy) were electrophoresed through 1.5% agarose gels and stained with ethidium bromide. Results were confirmed in three different assays for each sample. In each assay, a sample without nucleic acid was included to control for possible cross-contamination, and normal skeletal muscle was used as a positive control for MyoD1 expression.

Southern Blot Analysis of RT-PCR and PCR Products

To ascertain the specificity of the PCR products, 10 μl of the amplified product was electrophoresed through a 2% agarose gel, transferred to a nylon membrane (Sure-blot, Oncor, Genova, Italy), and subsequently hybridized to a ^{32}P-labeled oligonucleotide probe specific for the human MyoD1 cDNA sequence (GCTACGAAGGCGCCTACTACAACGAGGCGC). The membrane was then exposed to x-ray film for 4 hours.

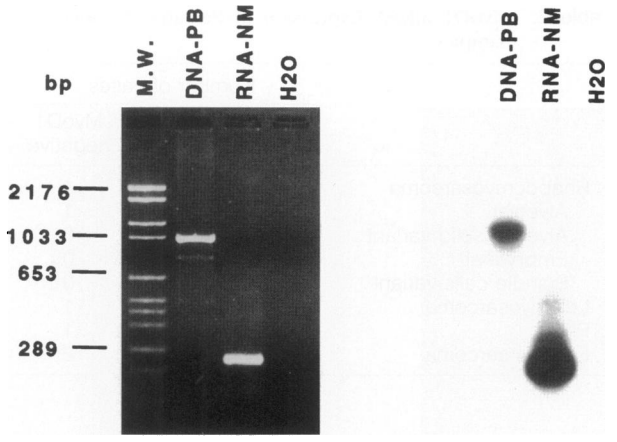

Figure 1. Detection of human MyoD1 mRNA by RT-PCR. To detect MyoD1 transcript we designed primers from the human cDNA regions that showed homology with the first and the third exons of the murine MyoD1 gene. RT-PCR of human adult skeletal muscle showed the predicted product of 266 bp, whereas PCR analysis of genomic DNA obtained from peripheral blood showed a product of approximately 1030 bp including intronic sequences (**left panel**). The specificity of the products was confirmed by hybridization with a MyoD1-specific radiolabeled probe for human mRNA sequence (**right panel**).

Results

Detection of Human MyoD1 mRNA by RT-PCR

We compared the exonic sequence of the murine MyoD1 gene with the human cDNA sequence and identified, by homology, a probable localization of introns in the human gene. To verify our hypothesis we designed sense and antisense primers derived from the regions of human MyoD1 cDNA that showed homology with the first and the third exon of murine MyoD1 and performed RT-PCR and PCR on RNA extracted from normal skeletal muscle and on DNA obtained from normal peripheral blood, respectively. RT-PCR of normal skeletal muscle showed the predicted product of 266 bp, whereas PCR analysis of genomic DNA from total blood, using the same primers, showed a product of approximately 1030 bp, suggesting that it could contain at least one intron. The specificity of both PCR products was confirmed by Southern blot analysis and subsequent hybridization with a human MyoD1-specific radiolabeled probe (Figure 1).

Expression of MyoD1 Transcript in Tumor Cell Lines and Normal Cell Cultures

Specificity and sensitivity of our RT-PCR assay were evaluated on several cell lines and short-term cell cultures obtained from neoplastic and normal human tissues. We studied 13 RMSs (8 ARMSs and 7 ERMSs), 2 PNETs, 3 neuroblastomas, 5 lymphoma/leukemia cell lines, 2 cell lines and 1 short-term culture obtained from pediatric brain tumors, and 1 fibroblast short-term culture.

MyoD1 transcript was detected in 11 of 13 RMS cell lines; the product was absent in the RMS cell lines RH1 and RH18. No expression was found in non-RMS tumor cells and in non-neoplastic cells (Table 1 and Figure 2).

Table 1. MyoD1 Expression in Cell Lines and Short-Term Cultures

Cell line	Histology	MyoD1 mRNA
RH30	ARMS	+
RC2	ARMS	+
RH4	ARMS	+
RH28	ARMS	+
RH18	ARMS	−
CCA	ERMS	+
RD	ERMS	+
SMS-CTR	ERMS	+
RH1	ERMS	−
RH36	ERMS	+
PD-01	ARMS short-term culture	+
PD-02	ERMS short-term culture	+
PD-03	ARMS short-term culture	+
CHP100	PNET	−
TC32	PNET	−
IMR32	Neuroblastoma	−
ACN	Neuroblastoma	−
2C2	Neuroblastoma	−
Namalwa	Burkitt's lymphoma	−
CA46	Burkitt's lymphoma	−
MOLT4	T-cell leukemia	−
CEM	T-cell leukemia	−
HL-60	Acute promyelocytic leukemia	−
D283Med	Medulloblastoma	−
DAOY	Medulloblastoma	−
PD-04	Malignant ependimoma	−
PDF-01	Fibroblast short-term culture	−

The RH1 cell line, derived from an ERMS, had not been previously characterized for expression of myogenic factors. As it was the only ERMS negative for MyoD1 RNA, we studied the expression of chimeric transcripts derived from the specific chromosomal translocations t(2;13)(q35;q14)/t(1;13)(p36;q14) and t(11;22)(q24;q12) by RT-PCR. We found no expression of PAX3-FKHR or PAX7-FKHR transcripts, in accordance with previous reports,[16,19,20] whereas we detected EWS-FLI1 type 1 chimeric transcript, in contrast with previous observations of Dowing et al[21] (Figure 3). Thus, the RH1 cell line might represent a nontypical ERMS.

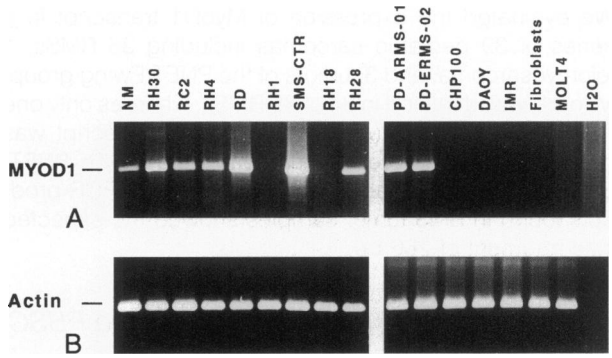

Figure 2. RT-PCR analysis of MyoD1 transcript in normal and tumor cell lines. **A:** Analysis of MyoD1 expression. **B:** Actin RNA was amplified in all samples and served as a control for amplifiable RNA. Normal skeletal muscle (NM; **lane 1**) was used as positive control in each assay. Cell lines and short-term cell cultures are described in Table 1.

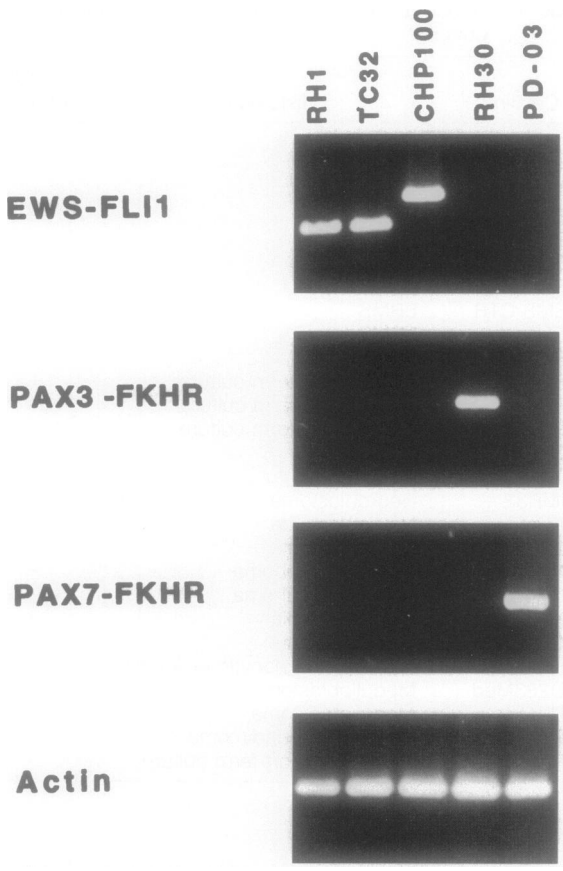

Figure 3. Analysis of the chimeric transcripts originated from the specific chromosomal translocations in the ERMS RH1 cell line. Expression of chimeric transcripts PAX3-FKHR, PAX7-FKHR, and EWS-FLI1 were detected by RT-PCR. TC32 and CHP100 are PNET cell lines carrying the t(11;22)(q24;q12), RH30 is an ARMS cell line used as a positive control for t(2;13)(q35;q14), and PD-03 is a short-term culture derived from an ARMS that carries the t(1;13)(p36;q14). RH1 was positive for the chimeric product derived from the t(11;22)(q24;q12) and showed a type 1 transcript like TC32 cells.

With regard to the RH18 ARMS cell line, the absence of MyoD1 mRNA has been previously described by Tapscott et al[22] using Northern blot analysis.

Expression of MyoD1 in Pediatric Tumors

We evaluated the expression of MyoD1 transcript in a series of 39 pediatric sarcomas including 35 RMSs, 1 leiomyosarcoma, and 3 tumors of the PNET/Ewing group. MyoD1 was identified in 34 of 35 RMSs, whereas only one ARMS was negative (sample 7). MyoD1 transcript was not detected in the leiomyosarcoma and in the PNET/Ewing's sarcomas (Table 2 and Figure 4). All PCR products found in RMS tumor samples showed the expected size fragment of 266 bp.

Analysis of MyoD1 Expression in BM and PBSC Samples

Based on the hypothesis that MyoD1 might represent a specific tumor marker in the evaluation of RMS BM involvement at diagnosis and in the detection of minimal residual disease in RMS patients, we analyzed its expression in BM, PBSC harvests, and pericardial effusion of six cases: three ARMSs, one PNET, and two Ewing's sarcomas. Tumor tissue obtained from the ARMS cases expressed MyoD1 and the chimeric product of the t(2;13)(q35;q14) PAX3-FKHR; the other three tumors did not express MyoD1 but were positive for the chimeric transcript EWS-FLI1 originated from the t(11;22)(q24;q12). In four BM and in all PBSC samples, lack of tumor involvement was confirmed by RT-PCR for PAX3-FKHR (case 115) or EWS-FLI1 (cases 88, 99, and 109) chimeric transcripts. Accordingly, the BM and all PBSC samples were negative for MyoD1 expression. In the other two ARMSs (cases 63 and 46) the BM sample and the pericardial effusion had suspected and proven morphological evidence of tumor cells, respectively. Tumor involvement was confirmed by detection of the RT-PCR PAX3-FKHR product. Accordingly, both samples were positive for MyoD1 expression (Table 3 and Figure 4).

We tested the sensitivity of the assay by diluting ERMS RD cells, which are positive for MyoD1 expression, with CEM T-cell leukemia cells, which do not express MyoD1 mRNA. In our experimental conditions we were able to detect 1 RD cell in 10^3 leukemia cells (Figure 5).

Table 2. MyoD1 mRNA Expression in Pediatric Tumor Samples

	Number of cases		
	Total	MyoD1 positive	MyoD1 negative
Rhabdomyosarcoma	35	34	1
Alveolar	16	15	1
Alveolar solid variant	1	1	0
Embryonal	17	17	0
Spindle cells variant	1	1	0
Leiomyosarcoma	1	0	1
PNET	1	0	1
Ewing's sarcoma	2	0	2

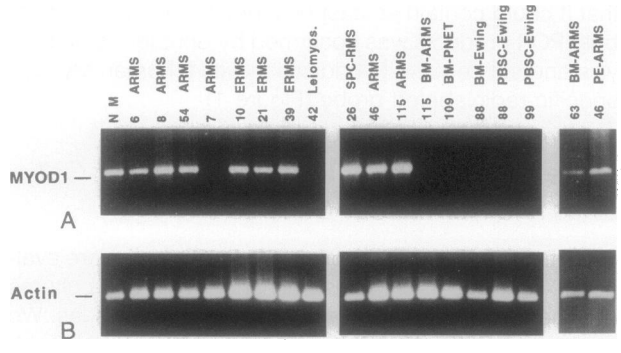

Figure 4. RT-PCR analysis of MyoD1 transcript in pediatric tumor, BM, PBSC, and pericardial effusion (PE) samples. **A:** Analysis of MyoD1 mRNA expression. **B:** Actin RNA was amplified in all of the samples and served as a control for amplifiable RNA whereas normal skeletal muscle (NM; **lane 1**) was used as a positive control in each assay. Samples were identified according to the patient's number. Samples 6, 8, 54, 7, 46, and 115 were ARMS; sample 26 was a spindle cell RMS; samples 10, 21, and 39 were ERMS; and sample 42 was a leiomyosarcoma. A summary of tumor samples analysis is reported in Table 2, whereas BM, PBSC, and pericardial effusion sample analysis is described in Table 3.

Table 3. MyoD1 and Chimeric Transcripts Expression in Primary Tumor, BM, PBSC Samples, or Pericardial Effusion of Six Cases

Case	MyoD1	PAX3-FKHR	EWS-FLI1
115 ARMS			
Tumor	+	+	–
BM	–	–	–
PBSC	–	–	–
63 ARMS			
Tumor	+	+	–
BM	+	+	–
PBSC	NA	NA	NA
46 ARMS (solid variant)			
Tumor	+	+	–
BM	NA	NA	NA
Pericardial effusion	+	+	–
109 PNET			
Tumor	–	–	+
BM	–	–	–
PBSC	NA	NA	NA
88 Ewing's sarcoma			
Tumor	–	–	+
BM	–	–	–
PBSC	–	–	–
99 Ewing's sarcoma			
Tumor	–	–	+
BM	–	–	–
PBSC	–	–	–

NA, not available.

Discussion

MyoD1 is a transcription factor involved in the regulation of the skeletal muscle myogenic program,[7] and it represents a marker for the identification of RMS, regardless of the histological subtype.[3,6,13–15] Its expression has been detected by Northern blot analysis and immunohistochemical staining in normal skeletal muscle and in RMS but not in normal and neoplastic tissues of different histogenesis.

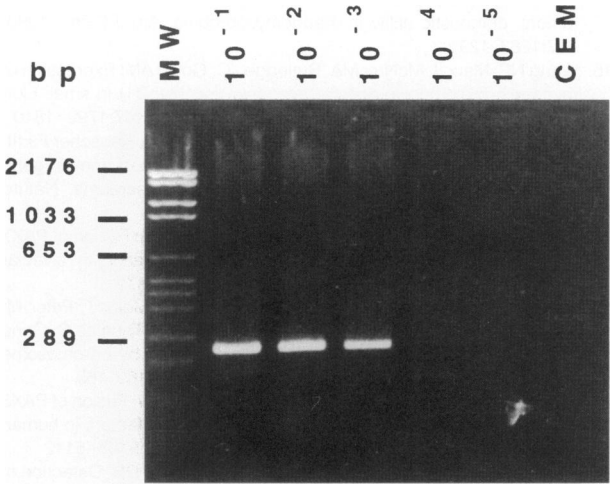

Figure 5. Determination of sensitivity of RT-PCR assay for Myod1 mRNA. Analysis was carried out on RNA extracted from RMS cells (RD) diluted into a T-cell leukemia cell line (CEM). Numbers on the top indicate the RD/CEM cell ratio whereas the last lane (CEM) represents only leukemia cells. The 266-bp band corresponds to the MyoD1 RT-PCR product.

Interpretation of paraffin-wax-embedded tissue immunoreactivity to MyoD1 monoclonal antibodies may be difficult because cytoplasmic staining was observed in some normal tissues and in non-RMS pediatric tumors.[15] In addition, this analysis is not very sensitive as normal adult skeletal muscle was reported as negative and the immunostaining appeared less pronounced in RMS cells with morphological evidence of skeletal muscle differentiation.[15,23,24]

Based on these observations and on the scarce availability of biopsy material from routine diagnostic surgery in chemosensitive pediatric solid tumors, we developed a RT-PCR assay to evaluate the expression of human MyoD1 mRNA from small tumor specimens.

First, we ascertained the presence of one or more introns in the human MyoD1 gene sequence, which allowed us to discriminate between amplification of MyoD1 mRNA and possible DNA contamination, based on the PCR product size. RT-PCR of normal muscle showed a product of 266 bp, whereas, using the same primers, PCR analysis of DNA from total normal blood revealed a product of approximately 1030 bp. The difference of approximately 760 bp between the RT-PCR and PCR products suggests that in the human gene there could be two introns of similar size to the introns of the murine MyoD1 gene.[11]

Specificity and sensitivity of our RT-PCR assay were evaluated in 13 RMSs, in 13 non-RMS cell lines, and in normal fibroblasts. Among the RMS cell lines, MyoD1 transcript was detected in 11 of 13 samples, whereas no expression was found in non-RMS tumor cells or in the short-term fibroblast culture. These results confirm that MyoD1 expression is restricted to tumor cells with skeletal muscle histogenesis. We did not find the MyoD1 product in RH1 and RH18 cell lines, derived from an ERMS and an ARMS, respectively. The absence of MyoD1 mRNA in the RH18 cell line has been previously reported; these cells express Myf5, another member of the MyoD protein family, and they could represent a myogenic precursor.[22]

The absence of MyoD1 transcript and the expression of the chimeric product derived from the t(11;22)(q24;q12) in the RH1 cell line suggest a possible neuroectodermal origin. Alternatively, the RH1 cell line could derive from a biphenotypic sarcoma with concomitant myogenic and neural differentiation and the discordance could depend on technical differences. In fact, Downing et al[21] observed PAX3-FKHR RT-PCR product after hybridization with a radiolabeled probe and used different experimental conditions for RT-PCR to detect EWS-FLI1 chimeric transcript.

To test the sensitivity and the specificity of the assay on tumor samples we evaluated a series of 39 pediatric sarcomas. MyoD1 was identified in 34 of 35 RMSs; only 1 ARMS was negative. In this case, the absence of MyoD1 transcript could depend on absent or negligible expression or molecular analysis of unrepresentative tissue.

The PNET/Ewing's tumors, as well as the leiomyosarcoma, did not express MyoD1, as reported by Braun et al[10] who described the absence of MyoD1 expression in

smooth muscle and in cell cultures of smooth muscle origin by Northern blot analysis.

Detection of MyoD1 by RT-PCR analysis was more sensitive than immunohistochemical staining. In fact, normal skeletal muscle was positive, and we did not observe any difference among the RMSs, regardless of their histological subtype or presence of the t(2;13)(q35;q14) and t(1;13)(p36;q14) specific translocations. Thus, RT-PCR could be used to discriminate RMS from other small round-cell tumors, but an accurate selection of tumor samples and adequate PCR conditions are needed to avoid false positive results due to normal skeletal muscle contamination. In our series, the signal obtained from any RMS was always more intense than the signal from normal skeletal muscle, and all of the non-RMS tumors were negative for MyoD1 transcript, thus supporting the specificity of the assay.

As we detected MyoD1 transcript in almost all RMSs, we studied its expression in BMs, PBSCs, and pericardial effusion to determine whether MyoD1 could represent a useful marker for the evaluation of minimal disease.

We studied six cases carrying specific molecular markers: three ARMSs characterized by the t(2;13)(q35;q14) and one PNET and two Ewing's sarcomas positive for the t(11;22)(q24;q12). In one ARMS and in the PNET/Ewing tumors, lack of involvement of all BM and PBSC samples was confirmed by RT-PCR for PAX3-FKHR and EWS-FLI1 chimeric products. Accordingly, all BM and PBSC samples were negative for the MyoD1 RT-PCR product. In the other two ARMSs, with morphological tumor cell involvement of the pericardial effusion and BM, respectively, RT-PCR for PAX3-FKHR confirmed the presence of tumor cells in both samples. Accordingly, the pericardial effusion and the BM sample were positive for the MyoD1 transcript.

These data suggest that MyoD1 might represent a specific marker in the evaluation of BM and intracavity effusion involvement at diagnosis and in the detection of minimal residual disease in all children with RMS, regardless of the histological subtype. Furthermore, evaluation of minimal disease could also be useful in PBSC harvests for patients who need high-dose chemotherapy followed by stem cell rescue.

Unlike the chimeric products originated from t(2;13)(q35;q14) and t(1;13)(p36;q14) chromosomal translocations, MyoD1 expression did not appear to be restricted to a specific subset of RMS, and it could be detected in virtually all RMSs by RT-PCR. These results and the good sensitivity of the method suggest that this RT-PCR assay for MyoD1 could represent a useful molecular tool in the diagnostic work-up and in the detection of minimal residual disease in children with RMS.

Acknowledgments

We thank Prof. S. Schiaffino and Dr. E. D'Andrea for critical review of the manuscript and Ms. Romina Rossi for excellent technical assistance.

References

1. Wexler LH, Helman LJ: Rhabdomyosarcoma and the undifferentiated sarcomas. Principles and Practice of Pediatric Oncology. Edited by Pizzo PA, Poplack DG. Philadelphia, Lippincot-Raven, 1997, pp 799–829
2. Raney RB, Tefft M, Maurer HM, Ragab AH, Hays DM, Soule EH, Foulkes MA, Gehan EA: Disease patterns and survival rate in children with metastatic soft-tissue sarcoma. Cancer 1988, 62:1257–1266
3. Pappo AS, Shapiro DN, Crist WM, Maurer HM: Biology and therapy of pediatric rhabdomyosarcoma. J Clin Oncol 1995, 13:2123–2139
4. Scrable H, Witte D, Shimada H, Seemayer T, Wang-Wuu S, Soukup S, Koufos A, Houghton P, Lampkin B, Cavenee W: Molecular differential pathology of rhabdomyosarcoma. Genes Chromosomes & Cancer 1989, 1:23–35
5. Sreekantaiah C, Ladanyi M, Rodriguez E, Chaganti RSK: Chromosomal aberrations in soft tissue tumors. Am J Pathol 1994, 144:1121–1134
6. Tonin PN, Scrable H, Shimada H, Cavenee WK: Muscle-specific gene expression in rhabdomyosarcomas and stages of human fetal skeletal muscle development. Cancer Res 1991, 51:5100–5106
7. Weintraub H, Davis R, Tapscott S, Thayer M, Krause M, Benezra R, Blackwell TK, Turner D, Rupp R, Hollenberg S, Zhuang Y, Lassar A: The myoD gene family: nodal point during specification of the muscle cell lineage. Science 1991, 251:761–766
8. Weintraub H: The MyoD family and myogenesis: redundancy, networks, and thresholds. Cell 1993, 75:1241–1244
9. Scrable HJ, Johnson DK, Rinchik EM, Cavenee WK: Rhabdomyosarcoma-associated locus and MYOD1 are syntenic but separate loci on the short arm of human chromosome 11. Proc Natl Acad Sci USA 1990, 87:2182–2186
10. Braun T, Bober E, Buschhausen-Denker G, Kotz S, Grzeschik KH, Arnold HH: Differential expression of myogenic determination genes in muscle cells: possible autoactivation by the Myf gene products. EMBO J 1989, 12:3617–3625
11. Zingg JM, Alva GP, Jost JP: Characterization of a genomic clone covering the structural mouse MyoD1 gene and its promoter region. Nucleic Acids Res 1991, 19:6433–6439
12. Boukamp P, Chen J, Gonzales F, Jones PA, Fusenig NE: Progressive stages of "transdifferentiation" from epidermal to mesenchymal phenotype induced by MyoD1 transfection, 5-aza-2′-deoxycytidine treatment, and selection for reduced cell attachment in the human keratinocyte line HaCaT. J Cell Biol 1992, 116:1257–1271
13. Clark J, Rocques PJ, Braun T, Bober E, Arnold HH, Fisher C, Fletcher C, Brown K, Gusterson BA, Carter RL, Cooper CS: Expression of members of the myf gene family in human rhabdomyosarcomas. Br J Cancer 1991, 64:1039–1042
14. Dias P, Parham DM, Shapiro DN, Webber BL, Houghton PJ: Myogenic regulatory protein (MyoD1) expression in childhood solid tumors: diagnostic utility in rhabdomyosarcoma. Am J Pathol 1990, 137:1283–1291
15. Wang NP, Marx J, McNutt MA, Rutledge JC, Gown AM: Expression of myogenic regulatory proteins (myogenin and MyoD1) in small blue round cell tumors of childhood. Am J Pathol 1995, 147:1799–1810
16. Galili N, Davis RJ, Fredericks WJ, Mukhopadhyay S, Rauscher FJ III, Emanuel BS, Rovera G, Barr FG: Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. Nature Genet 1993, 5:230–235
17. Davis RJ, D'Cruz CM, Lovell MA, Biegel JA, Barr FG: Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. Cancer Res 1994, 54:2869–2872
18. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, Kovar H, Joubert I, De Jong P, Rouleau G, Aurias A, Thomas G: Gene fusion with and ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 1992, 359:162–165
19. Shapiro DN, Sublett JE, Li B, Downing JR, Naeve CW: Fusion of PAX3 to a member of the forkhead family of transcription factors in human alveolar rhabdomyosarcoma. Cancer Res 1993, 53:5108–5112
20. Biegel JA, Nycum LM, Valentine V, Barr FG, Shapiro DN: Detection of the t(2;13)(q35;q14) and PAX3-FKHR fusion in alveolar rhabdomyosarcoma by fluorescence in situ hybridization. Genes Chromosomes & Cancer 1995, 12:186–192
21. Downing JR, Khandekar A, Shurtleff SA, Head DR, Parham, DM, Webber BL, Pappo AS, Hulshof MG, Conn WP, Shapiro DN: Multiplex

RT-PCR assay for the differential diagnosis of alveolar rhabdomyosarcoma and Ewing's sarcoma. Am J Pathol 1995, 146:626–634
22. Tapscott SJ, Thayer MJ, Weintraub H: Deficiency in rhabdomyosarcomas of a factor required for MyoD activity and myogenesis. Science 1993, 259:1450–1453
23. Dias P, Parham DM, Shapiro DN, Tapscott SJ, Houghton PJ: Monoclonal antibodies to the myogenic regulatory protein MyoD1: epitope mapping and diagnostic utility. Cancer Res 1992, 52:6431–6439
24. Engel ME, Mouton SCE, Emms M: Paediatric rhabdomyosarcoma: MyoD1 demonstration in routinely processed tissue sections using wet heat pretreatment (pressure cooking) for antigen retrieval. J Clin Pathol 1997, 50:37–39