The expression of MDM2/CDK4 gene product in the differential diagnosis of well differentiated liposarcoma and large deep-seated lipoma

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Summary Ordinary lipomas are cytogenetically characterized by a variety of balanced rearrangements involving chromosome segment 12q13–15, whereas well differentiated liposarcomas (WDL) show supernumerary ring and giant marker chromosomes, known to contain amplified 12q sequences. The tight correlation between the presence of ring chromosomes and both amplification and overexpression of MDM2 and CDK4 genes suggests the exploration of the possibility that immunocytochemistry (ICC) might assist in the differential diagnosis of lipoma-like well differentiated liposarcomas (LL-WDL) and large deep-seated lipomas (LDSL). For this purpose, 21 cases of the former and 19 cases of the latter tumours were analysed by ICC and, according to the availability of material, by molecular and cytogenetic approaches. All lipomas displayed a null MDM2/CDK4 phenotype, whereas all LL-WDL showed MDM2/CDK4 or CDK4 phenotypes. Southern blot analysis performed on 16 suitable cases, complemented by fluorescence in situ hybridization and classical cytogenetic analysis in 11 cases, was consistent with, and further supported the immunophenotyping data. In conclusion, MDM2/CDK4 product-based immunophenotyping appears to represent a valuable method for the categorization of arguable LDSL.

Keywords: MDM2; CDK4; lipoma; liposarcoma lipoma-like; differential diagnosis

Non-subcutaneous ordinary lipomas, including subfascial and intramuscular lipomas, account for 40% of ordinary lipomas (Fletcher et al, 1996). These tumours, also called deep-seated ordinary lipomas, may achieve a large size, particularly the intramuscular ones. In these cases the differential diagnosis between large deep-seated ordinary lipoma (LDSL) and the lipoma-like subtype of well differentiated liposarcoma (LL-WDL) may be difficult due to their similar histologic characteristics and focality of nuclear atypia (Rosai et al, 1996).

Furthermore, although lipomatous tumours in the retroperitoneum are mainly represented by well differentiated liposarcomas (WDL), lipomas may also appear at this site (DeWeerd et al, 1952). Thus, it would be valuable to support histologic classification with a simple immunophenotypic procedure in arguable cases. The 12q13–15 chromosome region is complex and contains several genes that are amplified or rearranged in lipomatous tumours such as the MDM2, CDK4, SAS or CHOP and HMG1-C (High Mobility Group 1-C protein) genes respectively (Smith et al, 1992; Forus et al, 1993; Khatib et al, 1993; Leach et al, 1993; Schoenmakers et al, 1995). HMG1-C gene translocations have been suggested to facilitate the development of lipomas (Schoenmakers et al, 1995; Tkachenko et al, 1997). On the other hand, MDM2 and CDK4 amplification and overexpression, which in turn correlate with the presence of chromosomes containing material from 12q, have been indicated as frequent in WDL (Dal Cin et al, 1993; Khatib et al, 1993; Pedeutour et al, 1993; Hunter and Pines, 1994; Nilbert et al, 1994; Pilotti et al, 1997, 1998).

More recently it has also been shown that overexpression of 12q sequences is a recurrent finding in WDL but not in lipoma (Mandahl et al, 1996; Szymanska et al, 1997).

Here we applied a MDM2/CDK4 gene-product-based immunophenotypic analysis, complemented in suitable cases by molecular and cytogenetic approaches, to a series of LL-WDLs and of LDSLs for comparison purposes.

The results show that MDM2/CDK4 immunophenotyping may assist in the differential diagnosis of the two entities.

MATERIALS AND METHODS

Materials

The case material consisted of 21 cases of LL-WDL including 12 primary and nine recurrent tumours and 19 primary LDSL from patients treated at our institution between 1988 and 1997. Eleven cases had been included in a previously reported series (cases 5, 9–13, 17–21) (Pilotti et al, 1997). All cases have been reviewed in order to verify the diagnosis and select representative tumoural samples for immunophenotypic analysis. The morphologic diagnoses were confirmed in all cases following the criteria of the pathologists of the CHAMP study group (Willen et al, 1998) and the criteria of Evans (1988) updated by Mentzel and Fletcher (1995). The cases were consecutive according to the criteria of selection: histologic subtype (LL-WDL) for liposarcoma and size (> 7 cm in largest diameter) for lipoma.

The age and sex of the patients as well as the location, type of specimens analysed (primary, recurrence) and size of the tumours are presented in Tables 2 (LL-WDL) and 3 (LDSL).
**Histologically, the lipoma-like component constituted 100% of the tumour in 16 out of 21 LL-WDLs including primaries and recurrences. In three retroperitoneal cases a sclerosing subtype component constituted from 20% (case no. 3) to 30% (cases 4 and 5) of the total of sampled tumours respectively, and in two non-retroperitoneal tumours (cases 1 and 7) this component corresponded to less than 20%. In two cases radiotherapy (case no. 6) and chemotherapy (case no. 11) were performed before surgical resection of tumour samples for study.**

**Table 1** Lipoma-like well differentiated liposarcomas: clinical features

| Case no. | Age/Gender | Site | Primary treatment | Number/Time of local recurrence | Treatment of recurrence | Follow-up |
|----------|------------|------|-------------------|---------------------------------|-------------------------|-----------|
| 1        | 64/F       | Thorax wall | Resection | 2, at 7 and 9 years | Resection + CT | NED, 1 year |
| 2        | 84/M       | Thigh | Resection | – | – | NED, 1 year |
| 3        | 42/M       | Retroperitoneum | Resection | – | – | Lost |
| 4        | 36/F       | Retroperitoneum | Resection² | 1, at 1 year | Resection + RT | AWD, 2 years |
| 5        | 49/M       | Retroperitoneum | Resection² | 3, at 4, 6 and 9 years | Resection | AWD, 9 years |
| 6        | 50/M       | Retroperitoneum | RT³-resection² | 1, at 1 year | RT + resection | NED, 2 years |
| 7        | 59/M       | Thigh | Resection² | 1, at 4 years | Resection + CT | NED, 1.5 years |
| 8        | 67/M       | Retroperitoneum | Resection² | 1, at 3 years | Resection | NED, 5 years |
| 9        | 56/F       | Retroperitoneum | Resection² | – | – | NED, 4 years |
| 10       | 57/F       | Retroperitoneum | Resection² | 1, at 3 years | Resection | NED, 1 year |
| 11       | 64/M       | Retroperitoneum | CT³-resection² | 2, at 2 and 5 years | Resection + RT | AWD, 5 years |
| 12       | 46/F       | Retroperitoneum | Resection² | 2, at 3 and 6 years | Resection + CT + RT | AWD, 9 years |
| 13       | 51/F       | Retroperitoneum | Resection² | 1, at 3 years | Resection | NED, 6 years |
| 14       | 62/M       | Thigh | Resection | – | – | NED, 2 years |
| 15       | 72/M       | Thigh | Resection | 2, at 4 and 7 years | Resection | NED, 9 years |
| 16       | 52/M       | Shoulder | Resection | – | – | Lost |
| 17       | 66/M       | Thigh | Resection | 3, at 5, 12 and 13 years | Resection + CT | AWD, 2, 5 years |
| 18       | 45/F       | Arm | Resection | – | – | NED, 1, 5 years |
| 19       | 28/M       | Thigh | Resection | – | – | NED, 9 years |
| 20       | 26/F       | Buttock | Resection | – | – | NED, 9 years |
| 21       | 44/F       | Pelvis | Resection | 1, at 1 years | CT | Lost |

**RT = radiotherapy; CT = chemotherapy; NED = no evidence of disease; AWD = alive with disease.²For details see text.³Primary tumour resection performed elsewhere.⁴Synchronous renal carcinoma.**

**Fluorescence in situ hybridization**

Interphase fluorescence in situ hybridization (FISH) analysis was carried out on cryopreserved cytopathic scrapings of the surgical specimen. Each slide was hybridized with the biotinylated probe Amplification unit probe 12q13 (Li Biomedical Corporation) corresponding to the ampiclon MDM2, SAS and GLI genes. Hybridization was performed according to Lichter et al (1990), Mezzelani et al (1996) and the manufacturer’s recommendations. The sample was scored as amplified when the single nuclei showed more than two signals. Metaphase FISH analysis was performed using whole chromosome 12 and 6 paintings (CAMBIO, Cambridge, UK) using standard procedures (Pilotti et al, 1997, 1998). Overall, cytogenetic analysis and/or interphase and metaphase FISH were performed in eight out of 21 cases of LL-WDL and in six out of 19 cases of LDSL.

**RESULTS**

Table 1 summarizes the main clinical data, treatment and outcome on LL-WDLs. Thirteen out of 21 (61.9%) patients experienced from one to three recurrences, whereas only one case of recurrence was observed among the LDSLs (case no. 33). Given the uneventful follow-up of the remaining 18 cases a dedicated table has not been produced.

Immunophenotyping (Table 2) shows MDM2/CDK4 expression in 18 of the 21 cases (85.7%) and CDK4 immunoreactivity in 100% of the cases. The analysis of two samples was sufficient to achieve information about the immunophenotypic profile in the majority of the cases. In the three MDM2-CDK4+ cases (Table 2, cases 9, 14 and 16) all available samples were immunostained in order to confirm the null MDM2 immunophenotype.

All LDSL samples analysed turned out to be MDM2-CDK4- (Table 3). Tables 2 and 3 and Figure 1A–F summarize
immunophenotypic, genotypic and cytogenetic results. In keeping with immunophenotypic findings amplification of both MDM2 and CDK4 genes was observed in the nine LL-WDLs and no abnormality of the two genes was observed in the seven LDSLs analysed by Southern blot. Interestingly, case no. 9, categorized routinely on morphologic grounds as lipoma and reported as MDM2-negative in a previous investigation (Pilotti et al, 1997), showed CDK4 immunoreactivity (Figure 1C) and CDK4 as well

Table 2 Lipoma-like well differentiated liposarcomas: immunophenotyping, molecular and cytogenetic features (21 cases)

| Case no. | Specimens analysed: primary (P), recurrences (R) | Immunocytochemistry | Southern blot analysis | Interphase FISH | Cytogenetics |
|----------|-------------------------------------------------|----------------------|-----------------------|----------------|-------------|
|          | Specimens analysed: primary (P), recurrences (R) | MDM2 CDK4 | MDM2 CDK4 | A | 48,XY,+r(12)|
| 1        | P (2/7) | + + + + | + + | A | 48,XY,+r(12)|
| 2        | P (2/6) | + + + + | + + | A | 48,XY,+r(12)|
| 3        | P (2/9) | + + + + | + + | A | 48,XY,+r(12)|
| 4        | R (2/17) | + + + + | + + | A | 48,XY,+r(12)|
| 5        | R (2/13) | + + + + | + + | A | 48,XY,+r(12)|
| 6        | R (2/26) | + + + + | + + | A | 48,XY,+r(12)|
| 7        | P (2/11) | + + + + | + + | A | 48,XY,+r(12)|
| 8        | R (2/23) | + + + + | + + | A | 48,XY,+r(12)|
| 9        | P (7/7) | - + + + | + + | A | 48,XY,+r(12)|
| 10       | R (2/7) | + + + + | + + | A | 48,XY,+r(12)|
| 11       | R (2/6) | + + + + | + + | A | 48,XY,+r(12)|
| 12       | R (2/6) | + + + + | + + | A | 48,XY,+r(12)|
| 13       | R (2/6) | + + + + | + + | A | 48,XY,+r(2r)|
| 14       | P (7/7) | + + + + | + + | A | 48,XY,+r(2r)|
| 15       | P (2/26) | + + + | + + | A | 48,XY,+r(2r)|
| 16       | P (2/26) | + + + | + + | A | 48,XY,+r(2r)|
| 17       | P (2/26) | + + + | + + | A | 48,XY,+r(2r)|
| 18       | P (2/26) | + + + | + + | A | 48,XY,+r(2r)|
| 19       | P (2/26) | + + + | + + | A | 48,XY,+r(2r)|
| 20       | P (2/26) | + + + | + + | A | 48,XY,+r(2r)|
| 21       | R (2/26) | + + + | + + | A | 48,XY,+r(2r)|

*In brackets: number of samples immunostained for MDM2 and CDK4 by the total of specimens sampled for each tumour. A: features consistent with gene amplification.

Table 3 Lipomas: immunophenotyping, molecular and cytogenetic features (19 cases)

| Case no. | Age gender | Site | size (cm) | Immunocytochemistry | Southern blot analysis | Interphase FISH | Cytogenetics |
|----------|------------|------|-----------|----------------------|-----------------------|----------------|-------------|
| 22       | 54/F       | Thigh | 18        | - - - -               | - - - -               | - - - -         | - - - -     |
| 23       | 79/M       | Neck  | 7         | - - - -               | - - - -               | - - - -         | - - - -     |
| 24       | 56/M       | Lower back | 10 | - - - -               | - - - -               | - - - -         | - - - -     |
| 25       | 40/M       | Thigh | 11        | - - - -               | - - - -               | - - - -         | - - - -     |
| 26       | 69/M       | Arm   | 7         | - - - -               | - - - -               | - - - -         | - - - -     |
| 27       | 67/M       | Thorax region | 10 | - - - -               | - - - -               | - - - -         | - - - -     |
| 28       | 66/M       | Abdominal wall | 30 | - - - -               | - - - -               | - - - -         | - - - -     |
| 29       | 39/M       | Thorax region | 17 | - - - -               | - - - -               | - - - -         | - - - -     |
| 30       | 52/M       | Thigh | 7         | - - - -               | - - - -               | - - - -         | - - - -     |
| 31       | 57/F       | Abdominal wall | 13 | - - - -               | - - - -               | - - - -         | - - - -     |
| 32       | 66/M       | Lower back | 13  | - - - -               | - - - -               | - - - -         | - - - -     |
| 33       | 76/M       | Abdominal wall | 10 | - - - -               | - - - -               | - - - -         | - - - -     |
| 34       | 31/M       | Thorax wall | 7   | - - - -               | - - - -               | - - - -         | - - - -     |
| 35       | 64/F       | Shoulder | 9   | - - - -               | - - - -               | - - - -         | - - - -     |
| 36       | 59/M       | Lower back | 11  | - - - -               | - - - -               | - - - -         | - - - -     |
| 37       | 68/M       | Shoulder | 12  | - - - -               | - - - -               | - - - -         | - - - -     |
| 38       | 56/F       | Thorax region | 7   | - - - -               | - - - -               | - - - -         | - - - -     |
| 39       | 53/F       | Shoulder | 10  | - - - -               | - - - -               | - - - -         | - - - -     |
| 40       | 59/M       | Thigh | 21        | - - - -               | - - - -               | - - - -         | - - - -     |

NE = not evaluable; TS = two signals.
as MDM2 gene amplification (Figure 1F) despite MDM2 null immunophenotype. In light of these findings the case, which since the time of the first categorization showed no completely convincing benign features, was reconsidered as possible LL-WDL and included in Table 1.

FISH was successfully applied in the five cases of LL-WDL and in three out of four cases of LDSL. Nuclei obtained from the LL-WDL cases showed an amplification level ranging from eight to 15 signals (Figure 1B). Although FISH cannot demonstrate that the amplified gene is MDM2 (since the probe covered MDM2, SAS and GLI sequences), this assumption is supported by the immunostaining (Figure 1A) and Southern blot results obtained with the MDM2-specific probe (Figure 1E). By contrast, nuclei from LDSL showed two normal signals corresponding to the two copies of the region (1D). Karyotypic analysis showed the presence of ring chromosomes in three LL-WDL cases analysed: two of them (case no. 2 and no. 14) also showed the presence of a minor clone (10% of the total metaphases), that contained ring chromosomes, giant markers and double minutes. In these two cases FISH experiments with a whole chromosome 12 probe completely painted the ring chromosomes of the major clone, and the ring chromosomes, the double minutes and part of the giant markers of the minor clone. A translocation with a breakpoint at band q13 of chromosome 12 was found in case no. 26 of LDSL, and a novel translocation t(6;18)(q13–14; q21) was found in case no. 40 of LDSL.

**DISCUSSION**

On cytogenetic grounds, about two-thirds of ordinary lipomas and most WDLs share aberrations in the chromosome 12q13–15 segment (Mandahl et al, 1996). However, in lipomas the majority of these aberrations are consistent with the creation of chimaeric genes made up of a fusion of the HMG1-C gene in 12q15 with multiple partners, although the LPP (lipoma preferred partner) gene, at 3q27–28, is preferentially involved (Petit et al, 1996). On the other hand, in WDLs the aberrations of chromosome 12q are represented by the amplification of genes spanning the 13q–15q region (Berner et al, 1997; Dei Tos and Dal Cin, 1997). Assuming that these findings represent two different transformation pathways, and taking into account that MDM2 and CDK4 gene amplification parallels the presence of ring chromosomes as well as MDM2 and CDK4 protein overexpression (Pilotti et al, 1997, 1998), it is reasonable to assume that immunophenotyping will assist in the differential diagnosis between LL-WDL and LDSL. The present data confirm this assumption that, in turn, makes immunophenotype more consistent with biology than morphology. Similar results have recently been observed using different, less easily handled and not always applicable methodologic approaches, such as classical cytogenetic methods and comparative genomic hybridization (CGH) (Suijkerbuijk et al, 1994; Mandahl et al, 1996; Szymbanska et al, 1997).
Support to our immunophenotypic results is provided by the molecular and cytogenetic data which fitted with the immunophenotypic information in all the examined cases. For the MDM2 gene, the Southern blot data were further confirmed by FISH which showed amplification in the 5 LL-WDL analysed. By contrast, FISH revealed only two signals in the three LDSLs successfully analysed. Moreover, in one of these three cases of lipoma (case no. 26) the diagnosis was further supported by the presence of a translocation with a breakpoint at q13 of chromosome 12, a cytogenetic feature characteristic of lipoma (Heim and Mitelman, 1995), and a novel translocation t(6;18)(q13–14;q21) was found in a second case. Even though rearrangement of 12q13–14 and 6p are the most frequently found in lipoma, other observations which do not involve these chromosome arms have been reported (Willen et al, 1998). The finding that the MDM2 antibody failed to decorate tumour cells in two cases of LL-WDL (cases 14 and 16 respectively), points out the value of CDK4 gene assessment in differential diagnosis, and reconfirms that even amplification excluding MDM2 may contribute to transformation (Pilotti et al, 1998). It is rather unlikely, in fact, that immunostaining failure be ascribed to cellular variation as immunostaining was performed in all samples. However, this possibility cannot be completely excluded in case no. 9 because of the discrepancy between immunochemical and molecular analyses regarding the MDM2 amplification which, on the other hand, could suggest that an MDM2 gene amplification can occur without the expression of the relative product.

In conclusion our results support the notion that MDM2 and CDK4 immunophenotyping may represent a valuable ancillary procedure in the assessment of arguable LDSL cases. This diagnostic approach will also clarify if retroperitoneal lipoma represents an anecdotal occurrence or a reality (DeWeerd and Dockerty, 1952).

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