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MDM2/CDK4 gene amplification in large/deep-seated 'lipomas': incidence, predictors and clinical significance

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Summary

This study of 140 cases assessed the incidence of MDM2/CDK4 gene amplification in lipomatous neoplasms with histological features of a lipoma but which were of clinical concern due to large size (≥50 mm) and/or deep-seated (subfascial) location. Univariate and multivariate statistical analyses were used to identify clinical, radiological and pathological predictors of gene amplification. Differences in local recurrence rates between amplified and non-amplified cases were assessed using survival analysis. The findings indicate that the incidence of MDM2/CDK4 amplification in this setting is low at 5% (95%CI 1.4–8.6%). Variables associated with amplification on univariate analysis were tumour site (thigh, $p = 0.004$), size (>100 mm, $p = 0.033$) and presence of equivocal atypia ($p = 0.001$). Independent predictors on multivariate analysis were size (OR 3.9, 95%CI 1.4–11.3, $p = 0.012$) and presence of equivocal atypia (OR 12.5, 95%CI 1.9–80.3, $p = 0.008$). There was no significant difference in local recurrence rates between amplified and non-amplified cases ($p = 0.461$) based on a median follow-up time of 31 months. Assessment for MDM2/CDK4 amplification, therefore, should be considered in 'lipomas' which are >100 mm in size, show equivocal atypia and arise in the thigh. However, the clinical significance of gene amplification in this setting is unclear and requires confirmation in larger studies.

Key words: MDM2; CDK4; amplification; lipoma; liposarcoma.

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INTRODUCTION

Lipomas are among the most common soft tissue neoplasms encountered by surgical pathologists. Separating lipomas from well differentiated liposarcomas is usually not difficult in routine practice based on their microscopic appearances. In more problematic cases, assessment of genetic alterations may be useful to help aid in this distinction. Specifically, well differentiated liposarcomas are cytogenetically characterised by giant marker and ring chromosomes involving the chromosomal region 12q13-15.[1,2] MDM2 (murine double minute 2) and CDK4 (cyclin dependent kinase 4) are two of several genes known to be amplified in this region and detection of these genetic changes has demonstrated high sensitivity and specificity for the histological diagnosis of well differentiated liposarcoma.[3–12]

What is less certain, however, is whether testing for MDM2/CDK4 gene amplification is indicated in bland lipomatous neoplasms with histological features of a lipoma but which are concerning due to large size and/or a deep-seated (subfascial) location. Whilst it would seem that this issue has been previously addressed, most studies which have examined these alterations in lipomatous neoplasms have only included small numbers of lipomas in their validation series.[3–12] Equally uncertain is the clinical significance of demonstrating MDM2/CDK4 amplification in this setting. Although it is commonly assumed, extrapolating from early studies, that these molecular changes warrant a diagnosis of liposarcoma irrespective of the microscopic findings,[6,13] there are presently few published empirical data to support this assumption.

The aim of this study, therefore, was to address some of the issues related to this subject: first, to determine the incidence of MDM2/CDK4 amplification in a series of apparent 'lipomas' of large size and/or deep-seated (subfascial) location; second, to identify clinical, radiological and pathological factors which might predict for these alterations and help avoid unnecessary costly and time consuming molecular tests; and finally, to investigate whether the finding of gene amplification in this setting is associated with a more aggressive clinical course, warranting classification separate from simple lipomas.

MATERIALS AND METHODS

Well differentiated lipomatous neoplasms submitted for assessment of MDM2/CDK4 amplification by fluorescence *in situ* hybridisation (FISH) between January 2009 and August 2015 were retrospectively identified from our laboratory database. Inclusion criteria for this study were: (1) largest macroscopic dimension ≥50 mm and/or deep-seated (subfascial) location; (2) absence of diagnostic atypical morphological features which would lead to a

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histological diagnosis of well-differentiated liposarcoma (namely, overtly atypical stromal cells showing enlarged and hyperchromatic nuclei with or without atypical lipoblasts).

Retroperitoneal and pelvic lipomatous neoplasms, which have a marked predisposition for local recurrence even in the absence of atypical morphological features,[14,15] were excluded. Cases with a prior diagnosis of liposarcoma and presenting with a recurrent mass were also excluded.

Most tumours (n = 134, 95.7%) were reported by subspecialty soft tissue pathologists in our department (DDW, ICL, PDR and DVS). Haematoxylin and eosin (H&E) slides from all remaining cases were reviewed to confirm that the histological features satisfied the inclusion criteria for the study.

Clinical and radiological data were obtained from pathology requisition forms and radiology reports. Variables considered included patient age, gender, tumour location, tissue plane involved (superficial, subfascial or intramuscular), tumour size (largest dimension) on imaging studies, imaging modality used [ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI)] and the presence of atypical imaging features (presence of thick septa, abnormal signal patterns and lesional enhancement following administration of contrast). The imaging diagnosis favoured by the reporting radiologist was noted, retrieved from the conclusion of radiology reports and in many cases, confirmed at our soft tissue multidisciplinary team meetings in which an experienced musculoskeletal radiologist was present. Follow-up information including time to first local recurrence as confirmed on surveillance imaging studies was obtained by interrogating the databases of radiological centres known to service our soft tissue surgeons who were involved in the management of the majority of the cases included in this study (n = 106, 75.7%).

Pathological data considered included tumour size (largest dimension) on macroscopic examination, number of blocks submitted, the presence of unusual histological features (fat necrosis, inflammation, equivocal atypia in stromal cells falling short of that allowing a definite morphological diagnosis of liposarcoma) and the final histological diagnosis (including classification as a lipoma variant). For specimens which were received in multiple pieces, the maximum dimension of the largest piece was accepted as the best approximation for the overall lesion size (compared to aggregate dimensions which, in our experience, are less reproducible). To assess the adequacy of sampling, a block:size ratio was determined by dividing the number of blocks submitted for microscopic examination by the largest dimension of the tumour size in cm.

Interphase FISH analysis to assess for the presence of MDM2/CDK4 amplification was performed on representative formalin fixed, paraffin embedded sections using in-house DNA probes specific for the MDM2 gene at 12q15, CDK4 gene at 12q15 and chromosome 12 centromere (CEP12). Areas of interest were targeted by correlating with serial H&E stained sections. Two hundred successive nuclei were examined by two independent analysts (including JP) using the Applied Imaging CytoVision image capture system (Leica Biosystems, Germany). MDM2:CEP12 and CDK4:CEP12 signal ratios $\geq$2 in more than 15% of tumour cells were considered amplified. Signal ratios between 2 and 10 were classified as low level amplification and ratios >10 as high level amplification.

Statistical comparisons of proportions and means were performed using Pearson's chi-square test and Student's independent samples t-test, respectively. Pearson's correlation was used to assess the strength of the correlation between the largest dimension of lesions measured macroscopically compared to those measured on imaging studies. Multivariate logistic regression analysis was then performed to identify any independent predictors of MDM2/CDK4 amplification. The forward stepwise conditional method was used to introduce selected variables of clinical interest and those found to be significant on univariate analysis (including age, gender, tumour site, tissue plane involved, tumour size, presence of equivocal atypia and final radiological diagnosis) into the multivariate equation. Kaplan–Meier survival curves were used to analyse the time to first local recurrence according to MDM2/CDK4 amplification status and the difference between the curves was examined using the log-rank test. Statistical analyses were performed using SPSS for Windows Version 20.0 (IBM Corporation, USA) with the two-sided statistical significance level set at 5%.

This study was approved by the Institutional Review Board (QA# 9748). Informed consent was waived as there was no direct patient involvement and the results of this retrospective study were not anticipated to alter clinical management.

RESULTS

A total of 140 cases were identified from our database meeting the inclusion criteria for the study, including 83 (59.3%) males and 57 (40.7%) females. The mean and median ages were 56.5 years and 57 years, respectively (range 21–88 years). Specific sites of the tumours are detailed in Table 1. The lower limbs were involved in 50 (35.7%) cases, upper limbs in 47 (33.6%), trunk (chest wall, abdominal wall and back) in 41 (29.3%) and head/neck region in two (1.4%). Of those involving the extremities, 65 (50.4%) involved the left side and 64 (49.6%) involved the right. Up to 23 (16.4%) were situated within the subcutaneous fat, 54 (38.6%) intramuscular and the remaining 62 (44.3%) were deep/subfascial but extramuscular.

Imaging data were available for 100 (71.4%) cases, of which 82 (82%) were assessed with MRI, 10 (10%) with CT and 8 (8%) with ultrasound. Of these, the preferred radiological diagnosis by the reporting radiologist was that of a simple lipoma in 78 (78%), whereas a diagnosis of a well-differentiated liposarcoma was either favoured or could not be confidently excluded in the remaining 22 (22%), based purely on the imaging characteristics, irrespective of size and location. The frequency of atypical imaging features (the presence of thick septa, abnormal signal and abnormal enhancement following contrast administration) in the latter group is detailed in Table 2.

Up to 124 (88.6%) specimens were received intact. In the remaining 16 (11.4%) which were received in multiple pieces, tumour size was approximated by taking the maximum dimension of the largest piece. Mean and median tumour size on macroscopic examination was 95 mm and 85 mm, respectively (range 20–275 mm). Tumour dimensions as measured on imaging studies were available in 96 (68.6%) cases with a mean and median size of 102 mm and 90 mm, respectively (range 18–390 mm). Correlation between the macroscopic and radiologically determined dimensions for this subset was strong [r = 0.74, 95% confidence interval (CI) 0.64–0.82, $p < 0.001$; Fig. 1]. The average number of paraffin blocks submitted per centimetre of the maximum tumour dimension was 1.2 ± 0.4 (range 0.4–3.0).

Microscopic examination of all 140 cases revealed features consistent with a simple lipoma in 119 (85%). Specifically, the lesions comprised solid sheets and large lobules of mature adipose tissue often forming circumscribed but encapsulated masses. Occasional thin, intervening, paucicellular fibrous septa containing isolated bland spindled fibroblastic cells were seen in most tumours (Fig. 2). Adipocytes displayed no more than mild variability in size and shape without the presence of well-developed univacuolated and multivacuolated lipoblasts. Fat necrosis featured in five (4.2%) cases whilst a patchy lymphocytic infiltrate was noted in two (1.7%). Ten (7.1%) showed more frequent and thicker paucicellular bands of fibrosis and were classified as fibrolipomas. Spindle cell lipomas accounted for eight (5.7%) cases showing the well-recognised features of a lipomatous neoplasm with plump, uniform CD34+ spindle cells set in a variably fibrous and myxoid stroma, with coarse collagen fibrils and mast cells. Other variants included osteolipomas [lipoma with metaplastic ossification, n = 2 (1.4%)] and a