Cytokeratin immunoreactivity in Ewing sarcoma/primitive neuroectodermal tumour

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Abstract

Ewing sarcoma (ES)/ primitive neuroectodermal tumour (PNET) is an aggressive malignant neoplasm affecting mainly children and young adults. The tumour is included with other primitive neoplasms under the category of small round cell tumour. Cytokeratin expression in ES/PNET has been described in sporadic case reports as well as a few systemic series. We studied this feature in Malaysian patients diagnosed in University Malaya Medical Centre on the basis of typical morphology and immunohistochemical assays. Immunohistochemical staining for AE1/AE3 and MNF116 were performed in 43 cases. Cytokeratin was expressed in 17 cases (39.5%) in focal, intermediate or diffuse patterns. There was no significant association between cytokeratin immunoreactivity and the following parameters: patient age, sex, skeletal and extraskeletal primary location as well as primary, metastastic or recurrent tumours or chemotherapy treatment. A significant association between cytokeratin and neuron specific enolase (NSE) expression was demonstrated. Our study supports evidence of epithelial differentiation in ES/PNET and emphasizes that the expression of cytokeratin does not exclude ES/PNET in the differential diagnosis of small round cell tumours.

Key words: Ewing sarcoma, primitive neuroectodermal tumours, cytokeratin, epithelial differentiation

INTRODUCTION

The Ewing sarcoma (ES)/ primitive neuroectodermal tumour (PNET) family represent malignant undifferentiated “small, blue, round cell tumours” arising in an osseous or extra osseous primary site. These tumours are characterized by the presence of balanced translocations resulting in gene fusion of the EWS gene (Ewing sarcoma gene) and members of the ETS family transcription factors, mainly FLI1 or ERG genes. This family also includes Askin tumour, which is regarded as EW/PNET of the thoracopulmonary region. Skin and viscera are also known primary sites of the tumour. Reaching a definitive diagnosis amongst other small blue round cell tumours is often challenging to the surgical pathologist because of very similar to almost identical morphological and cytological features. It is believed that immunohistochemical stains provide the diagnosis in about 75% of tumours, among which CD99 is the most reliable commonly-used marker for the diagnosis of ES/PNET. Other recently recognized markers include FLi1 and caveolin (CAV1).

The antigenic expression of epithelial markers (especially cytokeratins) of this tumour has been described in several recent studies. This feature is of critical diagnostic importance as pitfalls in the diagnoses has been encountered in many cases, based on strong diffuse or focal immunoreactivity for cytokeratins in these tumours. The evidence for epithelial differentiation was also supported by the demonstration of intermediate filaments and cell junction proteins by several methods including ultrastructural studies. The prognostic significance of epithelial differentiation is not well studied, however, it has been suggested that keratin-expressing tumours may have a more aggressive behaviour. To our best knowledge, all related studies were performed in the Western population and there are no available reports involving the...
Asian populace. We studied this feature in Malaysian patients and analyzed its association with the following parameters: patient age, sex, site of origin (skeletal or extraskeletal), tumour location (primary, metastatic or recurrent), chemotherapy treatment and NSE expression.

MATERIALS AND METHODS

Patients and samples
Archived histopathology reports and slides of EW/PNET cases diagnosed in the Pathology Department, University Malaya Medical Centre between January 1993 and December 2010, including referral and in-house material, were retrieved and reviewed. The selection criteria were confirmed histopathology diagnosis based on histomorphology (Fig. 1) and availability of paraffin-embedded tissue with sufficient tumour for further immunohistochemical studies.

For practical reasons and due to the unavailability of molecular genetic techniques in our centre, CD99 positivity was considered an inclusion criterion. In addition, cases with atypical morphology on H&E sections, which essentially require genetic confirmatory studies, were excluded. Cases with ambiguous histopathology reports pointing to other possible differential diagnoses were also excluded.

For cases without or with faded H&E slides, 5-µm sections were cut from the paraffin blocks and stained with H&E. The staining pattern for vimentin, neuron-specific enolase (NSE) and CD99 was evaluated from available immunohistochemical slides and histopathology reports. The pattern of CD99 expression was graded as: 3+ (diffuse membranous staining), 2+ (focal membranous staining), 1+ (scanty membranous staining of isolated groups of cells). Information regarding patient age, sex, tumour origin and location and treatment history were extracted from histopathology reports and from patient medical records.

Immunohistochemistry
Immunohistochemistry for MNF116 (1:1000, DakoCytomatin, Glostrup, Denmark) and AE1/AE3 (1:100, Dako North America, Carpinteria, CA, USA) were performed on formalin-fixed, paraffin-embedded tissue sections of all cases, using steam heat-induced epitope retrieval and the Dako Envision detection system. Grading of staining results was as follows: 3+ (diffuse staining), >75% of tumour cells staining; 2+ (intermediate staining) 25% to 75% staining; 1+ (focal staining), 1% to < 25% staining; and 0 (negative staining), fewer than 1% staining. Normal or tumour tissue known to express the test antigens (i.e. cytokeratins, CD99) were used as positive controls and included routinely in the immunohistochemical assay.

Statistical analysis
Data was analysed using SPSS version 16. P-value of <0.05 was considered significant.

RESULTS
Of 75 cases of EW/PNET recorded in the pathology archives, 43 were entered into the study on the basis of adequate documentation and available material for further immunohistochemistry.

The 43 tumours arose mainly in children
and young adults (mean age: 26 years; range: 2-67 years) with one patient of unknown age. 27 patients were male and 16 female.

**Tumour location and origin**
There were 37 (86%) primary, 4 (9.3%) recurrent, and 1 (2.3%) metastatic tumours. One was of unknown location. 24 (55.8%) arose in extraskeletal sites: 3 each from the leg, lung/chest wall; 2 each from the thigh, shoulder, arm, uterus and pelvic soft tissue and 1 each involving the abdominal wall, buttock, inguinal region, intrabdominal soft tissue, liver, adrenal gland, pancreas and colon. 18 (41.9%) were primary skeletal tumours, including 6 from the femur, 2 each from the pelvis, fibula, maxilla, scapula and 1 each from the humerus, tarsal bones and vertebra. One tumour was from an unknown bone site. 33 (76.7%) tumours were sampled prior to chemotherapeutic treatment. 8 (18.6%) were post-chemotherapy samples.

**Immunohistochemical profile**
Vimentin was expressed in 96.5% (28/29) whilst NSE was expressed in 78.3% (18/23) of the tumours for which these parameters were tested. The majority (40; 93%) of tumours showed strong membranous staining (3+) for CD99 (Fig. 2). 1 (2.3%) showed focal staining (2+) and 2 (4.7%) showed faint and patchy staining (1+).

**Cytokeratin expression** was demonstrated in 17 (39.5%) of the 43 tumours. Of cytokeratin positive tumours, 11 (64.7%) were solely positive for AE1/AE3 and 6 (35.3%) positive for both AE1/AE3 and MNF116. The expression of AE1/AE3 was diffuse (more than 75%) in 9 (52.9%) tumours (Fig. 3), intermediate (25-75%) in 2 (11.8%) tumours; and focal (1-25%) in 6 tumours.
(35.3%) tumours (Fig. 4). MNF116 positivity was diffuse in 1 (5.9%) tumour, intermediate in 2 (11.8%) (Fig. 5) and focal in 3 (17.6%). None of the tumours showed immunoreactivity for MNF116 alone.

The mean age of the 17 patients with cytokeratin positive tumours was 28 years (range, 2-65 years). 11 were male and 6 were female. 14 (82.4%) samples were from primary tumour sites, 1 (5.9%) from a metastatic site and 2 (11.8%) were recurrent tumours. The primary site was skeletal in 5 (29.4%) cases and extraskeletal in 12 (70.6%). 14 (82.4%) samples were obtained prior to chemotherapy and 3 (17.6%) were obtained post-chemotherapy.

NSE results were available for 9 of the cytokeratin positive cases; all were positive for NSE. Comparison between cytokeratin-positive and negative cases in relation to studied variables is shown in Table 1.

DISCUSSION

Ewing Sarcoma and PNET have in the past been regarded as totally separate clinical and pathological entities. However, in ensuing years, the concept of unity was suggested due to overlapping morphological characteristics, that was subsequently supported by the presence of a balanced translocation (11;22) in over 90% of ES and PNET cases. Thus, now Ewing sarcoma and PNET are regarded respectively as the poorly and well-differentiated ends of the spectrum of a sarcomatous tumour with round-cell morphology.
and partial neuroectodermal phenotype.7 The diagnostic genetic feature is the presence of a reciprocal translocation t(11;22)(q24;q12) or t(21;22)(q2;q12), resulting in fusion of the EWS (Ewing sarcoma) gene with FLI1 or ERG gene, respectively1, 2 or less frequently with other genes such as FEV, ETV1 and E1AF.30,31 Although there has been recent emphasis on utilizing cytogenetic studies for the diagnosis of ES/PNET, these tests are not widely available in the majority of centres and are not routinely performed in many hospitals. Therefore, the combination of morphological and immunophenotypical criteria, especially for typical Ewing sarcoma, remains the main practically-convenient method for its diagnoses.14 A panel of immunohistochemical stains is often required to differentiate this family from other morphologically-similar differentials, among which CD99 is the traditionally-used marker for ES/PNET.

Based on the results obtained, cytokeratin positivity (in focal, intermediate or diffuse patterns) was detected in about 40% of ES/PNET cases, providing an evidence of epithelial differentiation as shown in previous studies.14,20,22 However, this percentage appears high when compared to the results of Gu et al (20%)20 and Collini et al (31.7%)22 who examined 50 and 41 cases respectively. The difference seems meager considering that in the former study, 67% (4 of 6) of cytokeratin-negative cases also showed evidence of epithelial differentiation at the ultra-structural level.20 On the other hand, our result is closer to the overall percentage of cytokeratin immunoreactivity demonstrated by Flope (35.7%).14

Cytokeratin AE1/AE3 is a mixture of two different clones of anticytokeratin antibodies that detects high and low molecular weight keratins, namely high molecular weight cytokeratins 1,2,3,4,5,6,10,14,15 and 16 and low molecular weight cytokeratins 7,8 and 19. MNF116 is another broad spectrum antikeratin with reactivity corresponding to cytokeratin 5,6,8,17 and 19. Among our 17 keratin-positive cases, 11 cases expressed AE1/AE3 only; 6 cases expressed both AE1/AE3 and MNF116; while there was no single case positive for MNF116 alone. This suggests that cytokeratin 17 is not expressed by this group of tumours. If we combine these findings with the immunoreactivity to CAM5.2 (specific marker for cytokeratin 8) that was demonstrated by Gu,20 together with the almost negative staining for 34βE12 (specific for high molecular weight cytokeratin 1,5,10 and 14/15) that was shown by Flope,14 the expressed cytokeratins in ES/PNET are probably cytokeratin 6,8 and 19, in addition to cytokeratin 2,3,7 and 16. Machen et al32 studied the usefulness of cytokeratin expression in differentiating ES/PNET from poorly-differentiated synovial sarcoma. He

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<th>TABLE 1: Comparison of clinicopathological parameters of cytokeratin-positive and negative tumours</th>
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<tr>
<td>Cytokeratin expression</td>
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<td>Positive (n=17)</td>
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<td>Mean patient age</td>
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<td>Sex</td>
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<td>Site of tumour</td>
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*P-value is significant (< 0.05)
showed immunoreactivity to CK19 in 15% of PNET, while none were positive for CK7. Thus, he concluded that staining for CK7 makes the diagnosis of PNET less likely among differentials. Interestingly, Ewing’s tumour expressing high molecular weight cytokeratin and p63 was recently reported by Weinreb et al.23 Therefore, our emphasis is that cytokeratin expression by ES/PNET is variable and should not be unexpected. This feature has been a source of diagnostic confusion and misdiagnoses, in many reported cases20,23,24 especially when atypical morphology and/ or diffuse cytokeratin staining is encountered.

In accordance with the results of Gu et al and Collini et al.20,22 there is no statistically significant association between cytokeratin expression and age and sex of patients or location of the primary tumour (skeletal or extraskeletal). Conversely, we found statistically significant association between cytokeratin and NSE expression. As there is also a well-known immunoreactivity to vimentin by these tumours, the combined expression of epithelial, neural and mesenchymal markers may be a reflection of developmental immaturity rather than a specific line of differentiation as suggested earlier by Sebire et al.33 We found no statistically significant association between cytokeratin expression and site of tumour (primary, metastatic or recurrent) and with chemotherapeutic treatment.

A variable frequency of cytokeratin expression in ES/PNET has been shown. Awareness of this fact is of practical importance as diffuse expression of epithelial, neural and mesenchymal markers may be a reflection of developmental immaturity rather than a specific line of differentiation as suggested earlier by Sebire et al.33 We found no statistically significant association between cytokeratin expression and site of tumour (primary, metastatic or recurrent) and with chemotherapeutic treatment.

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