Clin Transl Oncol (2009) 11:548-551
DOI 10.1007/s12094-009-0401-3

RESEARCH ARTICLES

Expression of E-cadherin, ß-catenin and topoisomerase IIα in leiomyosarcomas

Pinelopi N. Gogou · Anna Batistatou · Emilios E. Pakos · Nikiforos Apostolikas · Dimitrios Stefanou · Pericles G. Tsekeris

Received: 13 January 2009 / Accepted: 30 May 2009

Abstract
Introduction The expression of E-cadherin, β-catenin and topoisomerase II has been associated with clinical outcome of several cancers including sarcomas. We aimed to evaluate the expression of these markers in leiomyosarcomas (LMS).
Materials and methods Paraffin blocks of 19 primary, non-metastatic LMS were analysed immunohistochemically for the expression of the above-mentioned markers with a cut-off level for positivity of 20% of cell staining.
Results Expression of E-cadherin was negative in all LMS. Nuclear expression of β-catenin was also negative in all cases, while positive cytoplasmic β-catenin expression was observed in approximately half of the patients. The majority of LMS had expression of topoisomerase IIα, although only in 10 patients was this expression in more than 20% of tumour cells. From the analysed factors, tumour size was statistically significantly correlated with relapse-free survival.
Conclusions Further evidence with larger series is required in order to determine the implication of these markers in LMS.

Keywords Leiomyosarcomas · E-cadherin · β-Catenin · Topoisomerase IIα

Introduction

Cadherins are multifamily transmembranous glycoproteins that mediate homophilic, calcium-dependent adhesion. They are specifically associated with the junction region [1] and they are implicated in processes such as tissue morphogenesis in complex organisms and tumorigenesis [2]. E-cadherin is expressed by epithelial cells and is thought to be a tumour suppressor gene and a morphogenic factor in epithelial tumours. It has been subjected to extensive investigation, and reduced or absent E-cadherin has been associated with decreased differentiation, invasion and/or metastases in several malignancies [3, 4], including certain sarcomas [5].

β-Catenin is a member of the armadillo family of proteins that is involved in tumorigenesis through two distinct pathways [6]. Firstly, β-catenin functions in the adherens junction where it interacts with E-cadherin at the cell surface, forming a cadherin–catenin unit and through β-catenin links to the actin cytoskeleton [7]. Secondly, β-catenin is a key effector of the Wnt signalling cascade, which regulates cell proliferation and differentiation [8].

Finally, topoisomerase IIα is a cell cycle related intranuclear protein, which separates chromosomes at the end of mitosis [9, 10]. Topoisomerase IIα has been noted as

P.N. Gogou · E.E. Pakos · P.G. Tsekeris
Department of Radiotherapy
University of Ioannina, Medical School
Ioannina, Greece

A. Batistatou · D. Stefanou
Department of Pathology
University of Ioannina, Medical School
Ioannina, Greece

N. Apostolikas
Department of Pathology
St Savvas Anticancer-Oncologic Hospital
Athens, Greece

E.E. Pakos (✉)
Neohoropoulo, PO Box 243
Ioannina 45500, Greece
e-mail: epakos@yahoo.gr

a novel proliferation marker, with nuclear expression observed mainly in the S, G2 and M phases of the cell cycle [9, 10]. Overexpression of topoisomerase IIα is reported to correlate with drug resistance. Multidrug resistance is closely correlated to an unfavourable prognosis in various human cancers [11–13].

Based on the implication of the above-mentioned markers in clinical outcome of several cancers, we aimed to evaluate the expression of E-cadherin, β-catenin and topoisomerase IIα in leiomyosarcomas (LMS) by immunohistochemistry, to determine the potential relationship between them and to explore possible correlations with clinical outcome in LMS patients.

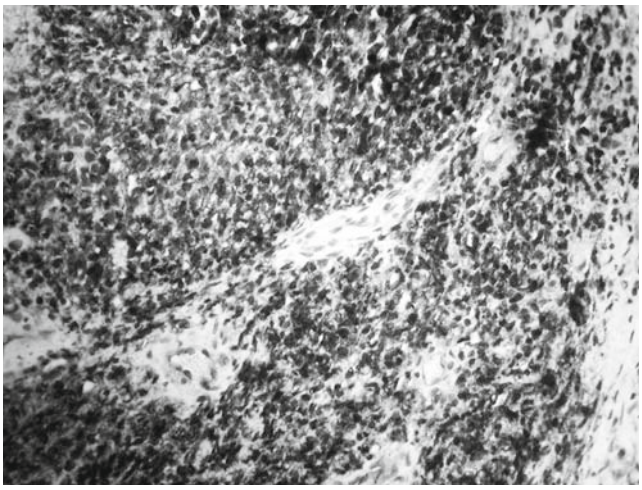

Fig. 1 Cytoplasmic high β-catenin expression (DAB ×400)

Materials and methods

Patients

Paraffin blocks of 19 primary, non-metastatic LMS that were treated surgically with radical tumour excision between 1990 and 2000 were retrieved from the archives of the Ioannina University Hospital, Ioannina, Greece and the St Savvas Hospital, Athens, Greece. The location of LMS was the retroperitoneum in 3 cases, the pelvis in 11 cases and the extremities in 5 patients. The median age of patients was 67 years with a range from 19 to 86 years. Fifteen of the patients were females. All patients had negative surgical margins according to the histopathology report. Two LMS were of low grade, 6 of intermediate grade and 11 of high grade. The median maximum tumour size was 75 mm (range from 15 to 230 mm). No patient received any treatment preoperatively, while adjuvant treatment was given postoperatively in 11 patients: two patients received chemotherapy and 9 patients received radiotherapy. Clinical data regarding the development of local recurrences, distal metastases and death were recorded for each patient.

Immunohistochemistry

Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using the EnVision System (DAKO Corp, the Netherlands), and the monoclonal antibodies: E-cadherin (CM170B, Biocare Medical, California), β-catenin (DBS, Menarini, Greece) and topoisomerase Iα (Ki-S1, DAKO). Briefly, 4-μm-thick tissue sections were deparaffinised in xylene, rehydrated through graded concentrations of alcohol and heated in a microwave oven for 2 cycles of 15 min each at 300 W, in citrate buffer, for antigen retrieval. Endogenous peroxidase activity was blocked with H_2O_2 solution in methanol (0.01 M) for 30 min. After washing with PBS for 5 min, the primary antibodies CM170B (dilution 1:50), β-catenin (dilution 1:50) and topoisomerase Iα (dilution 1:50) were applied for incubation (30 min at room temperature). Then the slides were washed for 10 min with PBS and were visualised with the EnVision system (DAKO) using diaminobenzidine tetrahydrochloride as a chromogen (Sigma Fast DAB tablets, St. Louis, MO). Finally, all sections were counterstained with haematoxylin. As a negative control, the first antibody was substituted with normal mouse immunoglobulin of the same class.

The evaluation of E-cadherin, β-catenin and topoisomerase IIα was performed with a semiquantitative method. The expression was recorded as "absent" when no cell expressed the above markers, "slight" when the expression ranged from 1% to 20% of tumour cells, "moderate" for 21% to 50% expression and "strong" when the expression was over 50% of tumour cells. For β-catenin we examined both nuclear and cytoplasmic staining. A level of over 20% cell staining was regarded as the cut-off level of a tumour expressing the above markers.

The association of E-cadherin, β-catenin, topoisomerase IIα and various clinical factors with disease progression (development of local recurrence or metastasis) and with survival was examined using proportional hazards models. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 14.0, Chicago, IL, USA).

Results

E-cadherin, β-catenin and topoisomerase IIα expression in LMS

None of the examined LMS exhibited E-cadherin expression. Similarly, none of the LMS showed positive nuclear β-catenin expression. Cytoplasmic β-catenin expression was seen in 11 cases (57.9%). One patient had slight expression of only 1% of tumour cells and was considered β-catenin negative. Ten patients had positive cytoplasmic β-catenin expression ranging from 80% to 100% of tumour

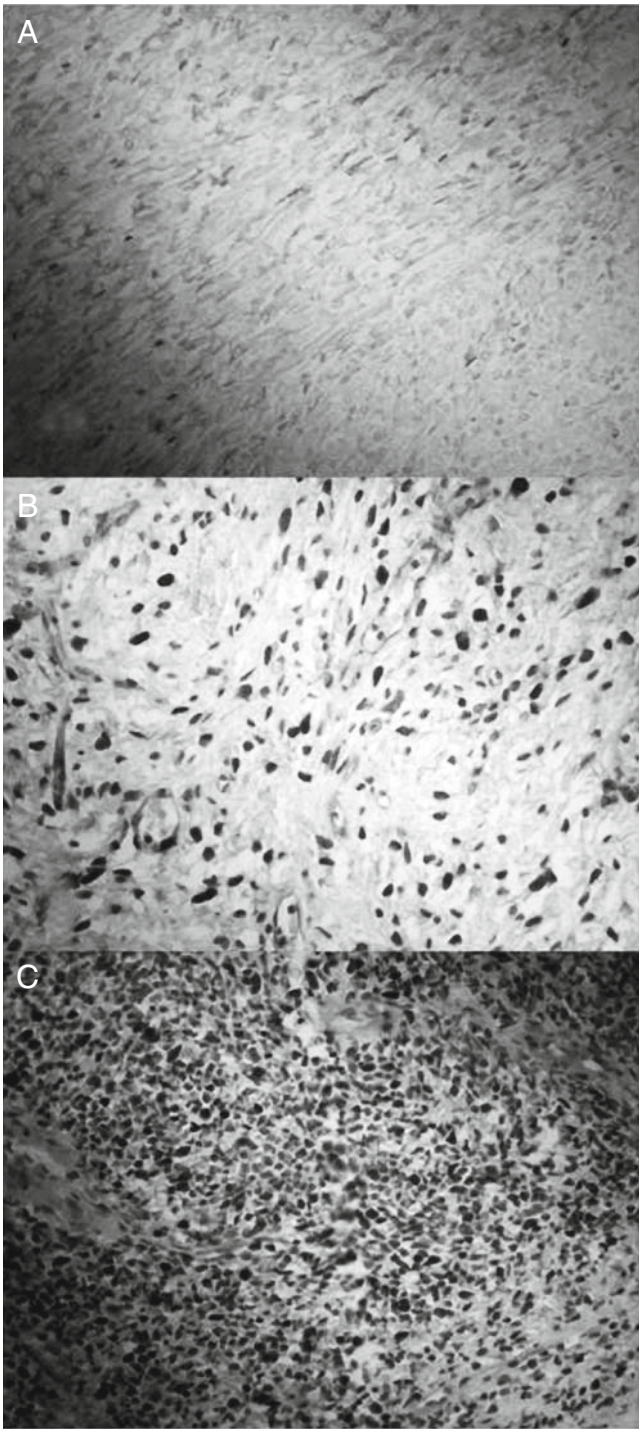

Fig. 2 Nuclear topoisomerase IIα expression, in the corresponding cases. **a** Low expression; **b** moderate expression; **c** high expression (DAB ×400)

cells (Fig. 1). Topoisomerase IIα was expressed in all but 2 LMS. In seven this expression was slight (ranging from 1% to 20%), while in 10 patients there was moderate to strong expression ranging from 30% to 100%, considered positive (Fig. 2).

Clinical outcome

All 19 LMS included in our study had available clinical data during the follow-up period. The mean follow-up period for these patients was 36 months (range from 3 to 204 months). Nine out of the nineteen patients (47%) developed local recurrences within a mean time of 8.6 months of diagnosis (range 1–18 months). Five patients (26.3%) developed distal metastases within a mean time of 13.8 months (range from 1 to 42 months) and 17 patients died during the follow-up period (3–85 months).

Among the examined clinical parameters, only tumour size was a statistically significant factor associated with recurrence-free survival. The hazard ratio was 1.15 (95% confidence intervals: 1.03–1.28] (p=0.01). No other factors were correlated with the development of local recurrence or metastasis or death. Positive β-catenin expression was not associated with recurrence-free survival (p=0.82), metastasis-free survival (p=0.70) or overall survival (0.69). Similarly, positive topoisomerase IIα expression was not associated with the above metrics (p values of 0.89, 0.81, 0.64 respectively). Similar, results were obtained when different cut-off levels of β-catenin and topoisomerase IIα expression were used.

Discussion

The absence of E-cadherin expression in all our cases of LMS suggests that E-cadherin protein may not be correlated with the establishment and maintenance of cellular architecture in LMS. Until now there has been only limited data regarding E-cadherin expression in LMS. Yoo et al. found that all ten LMS were negative for E-cadherin, but it was of interest that two out of ten unexpectedly expressed E-cadherin at the cell-to-cell boundaries [14]. It is uncertain whether the presence of E-cadherin in these samples actually reflects epithelioid differentiation. E-cadherin is expressed by certain types of soft tissue sarcomas, especially those with epithelioid features such as rhabdomyosarcomas and synovial sarcomas [15]. In accordance with our findings, Sato et al. found that all LMS were negative for E-cadherin expression [15].

The level of β-catenin is continuously reduced under normal conditions in the cytoplasm. Increased β-catenin cellular accumulation is the effect of either wnt-signals or mutations of the APC protein or mutations of the β-catenin itself. The first two pathways result in cytoplasmic β-catenin accumulation and finally to its aberrant accumulation to the nucleus [16]. The nuclear accumulation is the sequence of β-catenin mutations [17] that accumulate in the nucleus resulting in increased proliferation and/or inhibition of apoptosis representing the pivotal mechanisms of tumorigenesis and progression. Similarly to the present study, no nuclear accumulation of β-catenin in LMS was reported by other studies [6]. Nuclear accumulation has been demonstrated

in synovial sarcomas, osteosarcomas, liposarcomas and malignant fibrous histiocytomas [6, 18].

Finally, the current study demonstrated that the vast majority of LMS expressed topoisomerase IIα, probably implying the aggressive behaviour of this type of tumour. Our results are in accordance with the study of Gaumann et al., where all cells of LMS were positive for topoisomerase IIα [19]. Topoisomerase IIα is involved in cell growth and division and is related to DNA repair and therefore upregulation of this molecule provides neoplastic cells having high topoisomerase IIα expression with a survival advantage. It has been reported that a high labelling index of topoisomerase IIα in malignant tumours generally predicts aggressive behaviour [20].

Inevitably, the present study has a limited sample size and therefore possible associations of β-catenin and topoisomerase IIα with the development of recurrences, metastases or death could have been missed. Larger population studies should be the future goal in order to delineate the expression of these markers in LMS and to explore possible associations with clinical outcome.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455
2. Halbleib JM, Nelson WJ (2006) Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. Genes Dev 20:3199–3214
3. Muller S, Su L, Tighiouart M et al (2008) Distinctive E-cadherin and epidermal growth factor receptor expression in metastatic and nonmetastatic head and neck squamous cell carcinoma: predictive and prognostic correlation. Cancer 113:97–107
4. Miyanaga A, Gemma A, Ando M et al (2008) E-cadherin expression and epidermal growth factor receptor mutation status predict outcome in non-small cell lung cancer patients treated with gefitinib. Oncol Rep 19:377–383
5. Saito T, Oda Y, Sakamoto A et al (2000) Prognostic value of the preserved expression of the E-cadherin and catenin families of adhesion molecules and of beta-catenin mutations in synovial sarcoma. J Pathol 192:342–350
6. Ng TL, Gown AM, Barry TS et al (2005) Nuclear beta-catenin in mesenchymal tumors. Mod Pathol 18:68–74
7. Ilyas M, Tomlinson IP (1997) The interactions of APC, E-cadherin and beta-catenin in tumour development and progression. J Pathol 182:128–137
8. van Es JH, Barker N, Clevers H (2003) You Wnt some, you lose some: oncogenes in the Wnt signaling pathway. Curr Opin Genet Dev 13:28–33
9. Iino K, Sasano H, Yabuki N et al (1997) DNA topoisomerase II alpha and Ki-67 in human adrenocortical neoplasms: a possible marker of differentiation between adenomas and carcinomas. Mod Pathol 10:901–907
10. Tanoguchi K, Sasano H, Yabuki N et al (1998) Immunohistochemical and two-parameter flow cytometric studies of DNA topoisomerase II alpha in human epithelial ovarian carcinoma and germ cell tumor. Mod Pathol 11:186–193
11. Depowski PL, Rosenthal SI, Brien TP et al (2000) Topoisomerase IIalpha expression in breast cancer: correlation with outcome variables. Mod Pathol 13:542–547
12. Larbcharoensub N, Leopairat J, Sirachainan E et al (2008) Association between multidrug resistance-associated protein 1 and poor prognosis in patients with nasopharyngeal carcinoma treated with radiotherapy and concurrent chemotherapy. Hum Pathol 39:837–845
13. Haber M, Smith J, Bordow SB et al (2006) Association of high-level MRP1 expression with poor clinical outcome in a large prospective study of primary neuroblastoma. J Clin Oncol 24:1546–1453
14. Yoo J, Park S, Kang CS et al (2008) Expression of E-cadherin and p53 proteins in human soft tissue sarcomas. Arch Pathol Lab Med 126:33–38
15. Sato H, Hasegawa T, Abe Y et al (1999) Expression of E-cadherin in bone and soft tissue sarcomas: a possible role in epithelial differentiation. Hum Pathol 30:1344–1349
16. Bhattacharya B, Dilworth HP, Iacobuzio-Donahue C et al (2005) Nuclear beta-catenin expression distinguishes deep fibromatosis from other benign and malignant fibroblastic and myofibroblastic lesions. Am J Surg Pathol 29:653–659
17. Carlson JW, Fletcher CD (2007) Immunohistochemistry for beta-catenin in the differential diagnosis of spindle cell lesions: analysis of a series and review of the literature. Histopathology 51:509–514
18. Hasegawa T, Yokoyama R, Matsuno Y et al (2001) Prognostic significance of histologic grade and nuclear expression of beta-catenin in synovial sarcoma. Hum Pathol 32:257–263
19. Gaumann A, Tews DS, Mentzel T et al (2003) Expression of drug resistance related proteins in sarcomas of the pulmonary artery and poorly differentiated leiomyosarcomas of other origin. Virchows Arch 442:529–537
20. Endo H, Hirokawa M, Ishimaru N et al (2004) Unique cell membrane expression of topoisomerase-II alpha as a useful diagnostic marker of liposarcoma. Pathol Int 54:145–150