EUROPEAN JOURNAL OF CANCER 46 (2010) 3307–3315

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journal homepage: www.ejconline.com

Prognostic impact of adenomatous polyposis coli gene expression in osteosarcoma of the extremities

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ARTICLE INFO

Article history:
Received 12 February 2010
Received in revised form 27 May 2010
Accepted 2 June 2010
Available online 30 June 2010

Keywords:
Cadherin
β-Catenin
Adenomatous polyposis coli
Osteosarcoma

ABSTRACT

Purpose: To evaluate the impact of adjuvant chemotherapy on the outcome of osteosarcoma of the extremities, and to identify prognostic factors using the expression of adenomatous polyposis coli (APC), cadherin and β-catenin Wnt-signalling markers.
Methods: The clinical, demographic, anatomic and pathological factors including a detailed analysis of the immunohistochemical expression of cadherin, β-catenin and APC were retrospectively examined in 97 patients with osteosarcoma of the extremities (metastatic and non-metastatic at diagnosis), treated with surgery and/or chemotherapy from 1985 to 2000.
Results: APC immunoreactivity showed a statistically significant association with age and serum alkaline phosphatase levels ($p = 0.025$ and $p = 0.038$). When survival was the endpoint of multivariate analysis, race segregated patients with poor survival as did lack of cadherin expression. For overall survival, cadherin immunoreactivity and the interaction between APC expression and response to adjuvant chemotherapy were significant ($p = 0.012$ and $p < 0.001$). No significant clinical association was evident with immunohistochemical expression of cadherin, β-catenin.
Conclusion: Lack of expression of cadherin was a significant variable to overall and disease-free survival. Significantly, positive APC immunoreactivity and adjuvant chemotherapy were associated with a favourable treatment response. Studies using newer immunohistochemical markers within the Wnt-signalling pathway may guide the development of more appropriate therapeutic targets for future individualised treatment.

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.06.004

1. Introduction

Before 1970, osteosarcoma treatment consisted of primary tumour surgery, usually amputation, conservative surgery being restricted to patients with linear tumoural growth and small involvement of soft tissues.[1] About 80–90% of these patients used to develop metastasis within 5 months time.[2] After the effective chemotherapy advent, an increase in cure rates from 10% to 15% for 60–70%, better quality of life, less mutilating surgeries and functional preservation surgery[1] were achieved, although prognosis in patients with metastatic disease at diagnosis is still poor (15% of the cases) with cure rate from 10% to 15%.[3] Molecular mechanisms underlying disease progression currently are largely unknown. Despite intensive effort, the outcome of patients with osteosarcoma has not improved significantly during the past decade.

The APC/cadherin–catenin complex is part of the Wnt-signalling pathway and changes in expression of these markers may be associated with invasion and metastasis, and patient outcome. Moreover, Wnt/APC/cadherin/β-catenin pathways are involved in osteosarcoma prognosis,[4] and may also provide promising avenues for more targeted therapies in treatment.[5–8] Previous studies have demonstrated that β-catenin signalling is de-regulated in over 70% of human osteosarcoma,[4] and modulation of Wnt and β-catenin has been shown to influence motility and invasion in osteosarcoma cell lines.[9]

Anatomic, pathological and clinical factors are generally sub-optimal to predict the evolution and therapeutic response, the discovery of new prognostic factors being of extreme importance. Even lactic dehydrogenate (LDH) and alkaline phosphatase (ALP), known important prognostic factors to predict aggressiveness and poor outcomes, are not sufficient to define response to therapy. They are frequently associated with higher tumour size and worse prognosis. In this study we have evaluated the role of three Wnt pathway proteins by assessing pan-cadherin, β-catenin and adenomatous polyposis coli (APC) antibody immunoexpression in the primary tumour.

2. Methods

2.1. Osteosarcoma tumour samples

A retrospective analysis has been performed using tissues and clinical information from 97 patients admitted from 1985 to 2000 at Medical and Research Center of Cancer Hospital A.C. Camargo, São Paulo, Brazil, with conventional osteosarcoma of the extremities with or without metastatic disease at diagnosis. Patients with multifocality or polyostotic osteosarcoma were excluded. Clinical staging was performed according to the TNM Staging System for the classification of malignant tumours, in which T represents size of tumour, N represents lymph node involvement and M represents distant metastasis.[10]

Tumours were classified as osteoblastic, chondroblastic or fibroblastic based on the predominant cell type and the intercellular material. The Huvos grading system was used to evaluate chemotherapy-induced necrosis. Grades III and IV (⩾90% necrosis) indicate favourable and grades I and II (<90% necrosis), unfavourable histological response to chemotherapy.

Tumour imaging was carried out by computerised tomography or nuclear magnetic resonance (according to the indication and availability at that time), and the investigation of lung and bone metastases by computerised tomography and scintigraphy, respectively. Patients were submitted to chemotherapy protocols based on cisplatin and doxorubicin.

2.2. Immunohistochemistry technique

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections. The 4 μm sections were de-waxed before all reactions being performed automatically in an auto-stainer (DAKO®) employing Flex Plus as immunohistochemical visualisation system. Polyclonal pan-cadherin (broad spectrum antibody, reactive with the C-terminal of N-Cadherin, and multiple cadherins), dilution 1/3000 (Sigma, USA C3678); Polyclonal APC, dilution 1/600 (Santa Cruz – SC 896); Monoclonal β-catenin (clone 14), dilution 1/1000 (BD Transduction – 610154) were used as primary antibodies (Table 1). The negative control was obtained by omitting the primary antibody (Fig. 1A).

A tumour was considered positive for pan-cadherin when more than 10% of the tumour cells presented moderate to strong labelling (+2 or +3) at the cell membrane. APC was considered positive when cytoplasmic or nuclear stain was observed in more than 10% of the stained cells, and β-catenin, when there was cytoplasmic or nuclear membrane stain in more than 10% of tumour cells.

2.3. Statistical analysis

Statistical analysis was applied by using Windows Statistical Package for Social Science (SPSS) version 12.0. Associative analyses of quantitative expression variables of APC, pan-cadherin and β-catenin and of the presence of metastasis were performed with the bicaudal chi-square test or Fisher test.

Patients with metastasis at diagnosis were excluded from the disease-free survival analysis. Overall survival time was calculated as the period from the beginning of the treatment to the last observed objective information (for alive patients in that date) or to the deceased date. Alive patients in the date of the last information were censored. Patients without information for a period twice superior than stipulated for return consultation were considered lost to follow-up at the date of the last information and have contributed to survival curve to that date and considered under censorship.

Table 1 – Immunohistochemistry technique: primary antibody, clones, dilution and supplier.

Antibodies	Dilution	Supplier – Code
Polyclonal anti-human c-erbB-2	1/500	DAKO –AO485
Polyclonal pan-cadherin	1/3000	SIGMA C3678
Polyclonal APC	1/600	SCruz – SC 896
Monoclonal β-catenin (clone 14)	1/1000	BD – 610154

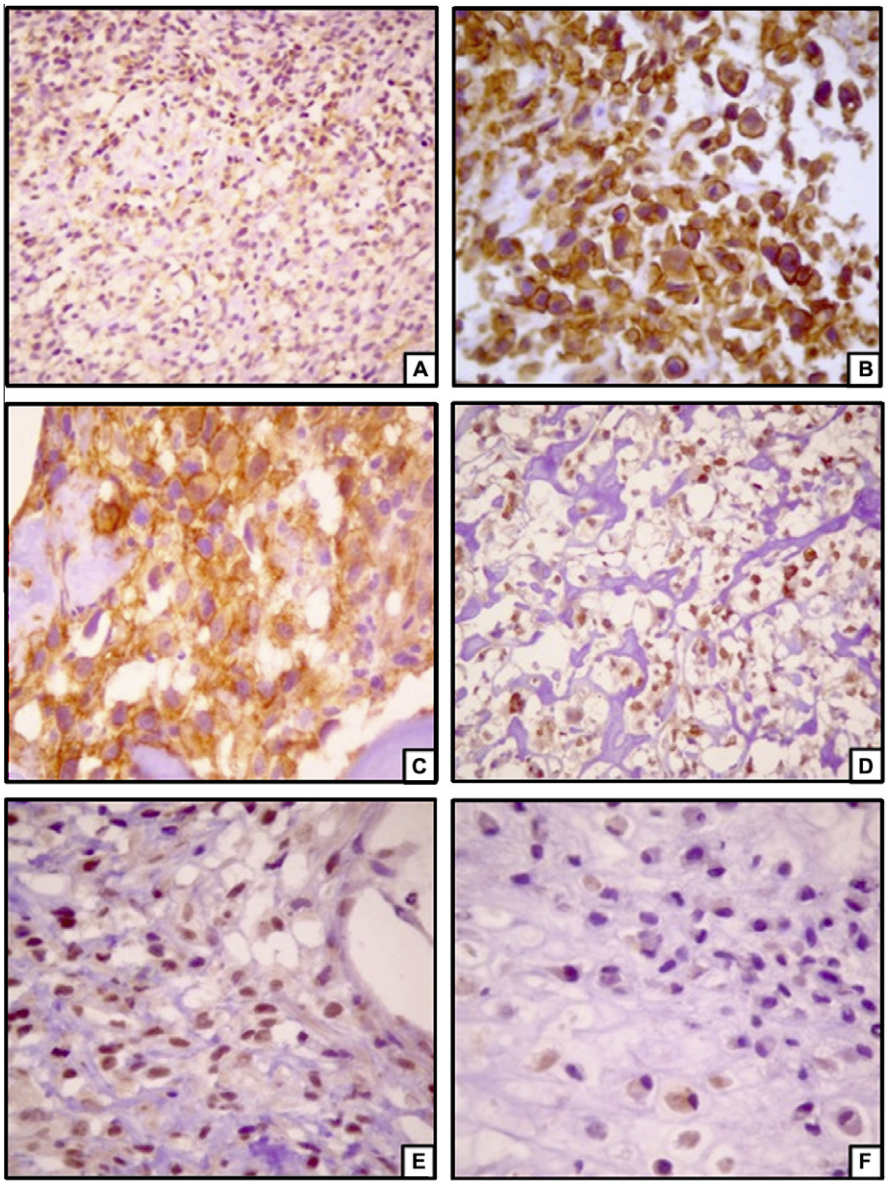

Fig. 1 – Photomicrography of conventional osteosarcoma of the extremities with: (A) The negative control (400×); (B) membrane immunoreactivity for pan-caderin. (200×); (C) positive cytoplasmatic immunoreactivity for β-catenin. (400×); (D) positive nuclear immunoreactivity for β-catenin (200×); (E) positive nuclear immunoreactivity for APC (400×) and (F) positive cytoplasmatic immunoreactivity for APC (400×).

Survival rates were calculated using Kaplan–Meier methods and the log rank test was used in order to compare the outcome estimates. The COX proportional hazards model was used to assess the independent prognostic factors for survival. The final result for the multivariate models, logistic and COX regression was obtained by the stepwise forward selection strategy. A p value of <0.05 was considered significant.

2.4. *Ethics*

This work has been approved by the ethical committee of the institution in which it was performed. All subjects gave informed consent to this work.

3. Results

Age of patients ranged from 6- to 70-years-old with a median of 16 years. Sixty-four patients (66%) were under 18-years-old. Seventy-six patients (78.48%) were white and 21 (21.6%) were non-white. Fifty-five patients were male (56.7%) and 42 (43.38%) were female.

The primary tumour location was in the femur in 49 patients (50.5%); tibia in 27 (27.8%); humerus in 16 (16.5%); fibula in 3 (3.1%) and radius in 2 (2.1%).

According to TNM 2002 clinical staging, 5 patients (5.2%) were staged as T1 and 68 (70.1%) as T2. There was no reference to T staging in 24 (24.7%) cases. All patients were labelled

N0 at the initial presentation. Eighty-three patients were considered as M0 (85.6%) and 14 (14.4%) as M1. The histological type was osteoblastic in 70 cases (72.2%), chondroblastic in 20 cases (20.6%) and fibroblastic in seven cases (7.2%).

Serum alkaline phosphatase level was determined in 61 cases (62.9%) ranging from 28 to 4696 U/l considered above normal value (NV: 50–136 U/l) in 70.5% of the cases. Serum lactic dehydrogenase level determined in 51 cases (52.6%) ranged from 96 to 854 U/l and 62.7% were above normal levels (NV: 100–190 U/l).

Surgery was used in 91 cases: conservative in 62 (63.9%) and amputation in 29 (29.9%). Eleven patients did not receive neoadjuvant chemotherapy, and 21 did not receive adjuvant chemotherapy. Only 7 patients received neither neoadjuvant nor adjuvant chemotherapy. To compare the effects of differing chemotherapy, neoadjuvant and adjuvant chemotherapy were evaluated in two groups: (1) not submitted to chemotherapy and (2) submitted to chemotherapy. This simple two-way comparison was possible because there was no difference between the Huvos necrosis degree and the survival based on follow-up times varying from 2.14 to 217.9 months with a median of 44.7 months.

Ten patients (10.3%) were classified as Huvos I, 39 patients (40.2%) as Huvos II, 18 (18.6%) as Huvos III and 11 patients (11.3%) as Huvos IV for histological response. There was no description of the necrosis degree in 13 cases (13.4%). Six cases (6.2%) did not undergo surgery. Patients were classified in (a) good response to chemotherapy (grade III/IV) and (b) poor response to chemotherapy (grade I/II).

Fifty-six out of 97 patients (57.7%) developed metastases: 14 patients (14.4%) at diagnosis and in 42 patients (43.3%) during follow-up. The disease-free interval in those who subsequently developed metastasis varied from 3.19 to 62.4 month, with 20.6 month average and 15.6 months median. In 34 of these cases (80.9%) the metastasis was identified before 24 months and, in 2 cases, after the fifth year. Thirty-eight (39.2%) out of 56 metastatic cases (57.7%) presented metastasis exclusively in the lung, 14 of them (14.4%) presented in lung and, concomitantly, in other organs. Four cases (4.1%) presented metastasis in other organs apart from lung. The second metastatic site was bone, totalling 8 cases (14.2%). At the end of the follow-up, 31 patients (32%) were alive and disease free, 45 (46.4%) died due to cancer and 21 (21.6%) were out of follow-up. Among those who lost out of follow-up, 13 of them (62%) had been through, at least, 5-year time follow-up.

The pan-cadherin immunoreactivity was negative in 3 cases (3.1%) and positive in 94 cases (96.9%) (Fig. 1B). The immunoreactivity for β-catenin was negative in 13 cases (13.4%). Cytoplasm immunostaining was observed in 74 cases (76.3%), nuclear immunostaining was observed in 2 (2.1%) and nuclear and cytoplasm immunostaining was positive in 1 case (1.0%) (Fig. 1C and D). Seven cases (7.2%) could not be evaluated due to technical artifacts. No association was found between the immunoreactivity for pan-cadherin and β-catenin with clinical, demographic, anatomic and pathological variables.

The APC immunoreactivity was negative in 28 cases (28.9%), positive in 65 (67%) (Fig. 1E and F). Four cases (4.1%)

Table 2 – Immunoreactivity of APC according to the clinical, demographic and anatomopathological variables in patients with osteosarcoma of the extremities.

Variable	Category	APC (–)[a] *n* (%)	APC (+)[a] *n* (%)	*p*
Age	Up to 18 years	14(22.6)	48(77.4)	0.025
	Plus than 18 years	14(45.2)	17(54.8)	
Race	White	22(29.7)	52(70.3)	0.875
	No white	6(31.6)	13(68.4)	
Gender	Male	17(32.7)	35(67.3)	0.650
	Female	11(26.8)	30(73.2)	
Serum levels of lactic dehydrogenase (U/L)[a]	Normal value	2(11.1)	16(88.9)	0.054
	Above the normal	11(36.7)	19(63.3)	
Serum levels of alkaline phosphatase (U/L)[a]	Normal value	8(47.1)	9(52.9)	0.038
	Above the normal	8(20.0)	32(80.0)	
Staging CT[a]	T1	2(40.0)	3(60.0)	0.646
	T2	20(30.8)	45(69.2)	
Metastasis at the diagnosis	Negative M	27(33.8)	53(66.3)	0.099
	Positive M	1(7.7)	12(92.3)	
Metastasis[b]	Negative	12(29.3)	29(70.7)	0.876
	Positive	16(30.8)	36(69.2)	
Necrosis grade (HUVOS)[a]	I and II	15(31.3)	33(68.8)	0.806
	III and IV	8(28.6)	20(71.4)	
Histological type	Osteoblastic	20(29.9)	47(70.1)	0.931
	No osteoblastic	8(30.8)	18(69.2)	

[a] Cases with not estimable APC were excluded.
[b] Metastasis at any moment.

could not be evaluated due to technical artifacts. No association among APC immunoreactivity with clinical, anatomic, and pathological demographic variables was found. However, there was a positive and significant correlation between APC immunoreactivity and age and alkaline phosphatase serum level ($p = 0.025$ and $p = 0.039$, respectively).

Five-year disease-free survival in 83 cases metastasis-free at diagnosis was of 49.0%. The disease-free follow-up ranged from 2.3 to 178.6 month, with an average of 48.4 and a median of 32.4 months.

When considering clinical, anatomic, pathological and demographic factors together with evaluated treatment and immunohistochemistry variables, only race ($p = 0.028$) significantly influenced the disease-free survival. The pan-cadherin immunoreactivity was statistically significant ($p < 0.001$) only for the disease-free survival, despite the small casuistic and the short follow-up time (maximum of 4.6 months) (Table 2).

In multivariate model pan-cadherin and race were independent factors to the disease-free survival, HR = 32.6; CI 95% [1.9–554.8] and HR=2.1; CI 95% [1.1–4.1], respectively ($p < 0.05$).

Five-year overall survival rate was 55.25%. For the 56 metastasis cases, when evaluated after metastasis diagnosis, it was 28.46%. (26.8% for the 14 cases with metastasis at diagnosis and 29.2% for the 42 cases that developed metastasis during the follow-up) The follow-up time of the 14 cases with metastasis at diagnosis varied from 2.6 to 217.9 months, and from 2.1 to 155 months for the 42 cases that developed metastasis.

Clinical, anatomic, pathological and demographic variables presented no statistically significant association with the overall survival. HUVOS score, a well-known prognostic factor, when correlated with overall survival and disease-free survival was not statistically significant either.

Table 3 – Cumulated probability of disease-free survival (DFS) in 5 years in agreement with variables clinical-demographic, anatomopathological, treatment-related and Immunohistochemistry variables in 83 patients with osteosarcoma of the extremities without metastases at the diagnosis.

Variable	Category	n[a]	Disease-free survival in 5 years (%)	p(log rank)
Age	Up to 18 years	53	45.9	0.772
	>18 years	30	55.2	
Race	White	65	56.1	0.028[b]
	No white	18	23.7	
Gender	Male	46	44.4	0.567
	Female	37	54.7	
Serum levels of lactic dehydrogenase[a]	Normal value	16	42.0	0.759
	Above the normal	27	52.0	
Serum levels of alkaline phosphatase[a]	Normal value	16	51.3	0.776
	Above the normal	36	45.3	
Staging CT[a]	T1	5	75.0	0.338
	T2	58	49.9	
Necrosis grade[a] (HUVOS)	I and II	43	50.9	0.572
	III and IV	26	61.2	
Histological type	Osteoblastic	23	58.3	0.325
	No osteoblastic	60	45.3	
Neoadjuvant chemotherapy	No	8	37.5	0.360
	Yes	75	56.4	
Adjuvant chemotherapy	No	16	56.9	0.877
	Yes	67	47.7	
Surgery type[a]	Conservative	57	55.4	0.070[b]
	Amputation	23	32.2	
c-erbB-2[a]	Negative	66	53.3	0.091
	Positive	15	38.9	
Pan-cadherin	Negative	2	#	<0.001[b]
	Positive	81	49.6	
APC[a]	Negative	27	40.4	0.150[b]
	Positive	53	56.4	
β-Catenin[a]	Negative	75	48.3	0.244
	Positive	2	100.0	

[a] Excluded cases without information.
[b] Variables selected for COX regression.

Table 4 – Cumulative probability of overall survival in 5 years in agreement with the immunoreactivity of c-erbB-2, pan-cadherin, APC, β-catenin and treatment-related variables in 97 patients with osteosarcoma of the extremities.

Variable	Category	n[a]	Overall survival in 5 years (%)	p(log rank)
c-erbB-2	Negative	78	53.7	0.445
	Positive	16	63.5	
Pan-cadherin	Negative	3	0.0	<0.001[b]
	Positive	94	57.0	
APC	Negative	28	36.9	0.051[b]
	Positive	65	63.2	
β-Catenin	Negative	87	54.1	0.177[b]
	Positive	3	100.0	
Neoadjuvant chemotherapy	No	10	46.7	0.239
	Yes	87	56.3	
Adjuvant chemotherapy	No	20	39.3	0.023[b]
	Yes	77	59.2	
Surgery type[a]	Conservative	62	60.8	0.194[b]
	Amputation	29	50.1	

[a] Excluded cases without information.
[b] Variables selected for COX regression.

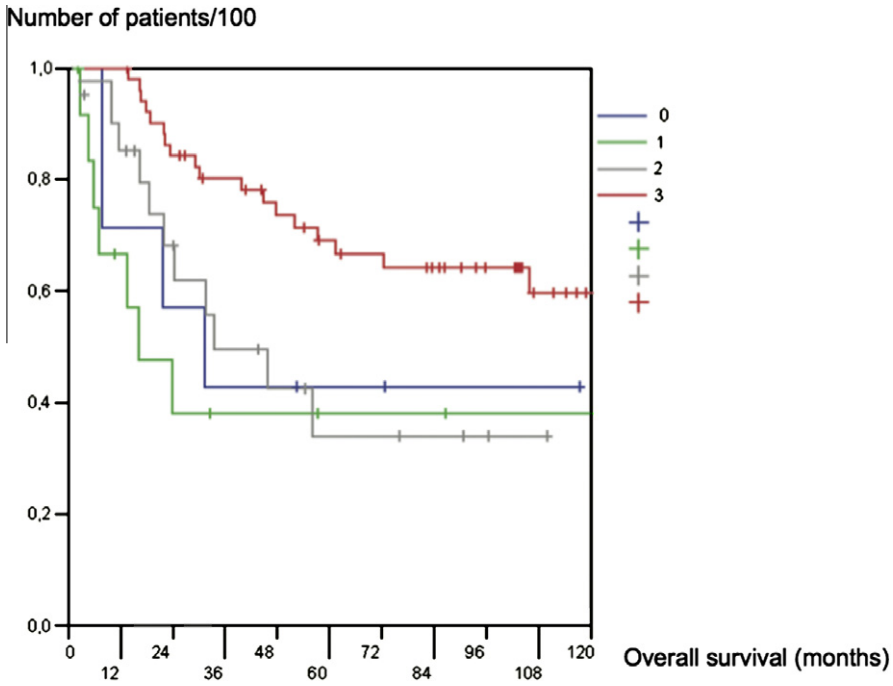

Fig. 2 – Overall survival of 4 groups of 97 patients with osteosarcoma of the extremities whose association between APC immunostaining result and post surgery chemotherapy is explicit below:

Group	APC immuno-staining	Post surgery chemotherapy	Number of cases	Overall survival (5 years)
Group 0	Negative	Negative	7	42.8
Group 1	Positive	Negative	21	38.1
Group 2	Negative	Positive	13	34.0
Group 3	Positive	Positive	52	69.9

Pan-cadherin immunoreactivity showed statistical significance for overall survival ($p < 0.001$) among the immunohistochemical markers. The APC immunoreactivity was close to the statistical significance ($p = 0.051$). (Table 3) and only adjuvant chemotherapy has presented statistical significance ($p = 0.023$) for overall survival, when considering variables related to the treatment.

During the multivariate model analysis for overall survival it was observed that both APC and adjuvant chemotherapy showed interaction between them. Thus the analysis of interaction between APC and adjuvant chemotherapy was performed.

For the variables interaction APC immunoreactivity and adjuvant chemotherapy, different survival status could be observed, depending on the APC immunoreactivity. So, they were stratified in four groups as shown in Table 4 and Fig. 2.

When the multivariate model was used with selected variables, the immunoreactivity for pan-cadherin (HR = 0.028; CI 95% [0.005–0.148]) and the APC immunoreactivity with adjuvant chemotherapy interaction (HR = 0.438; CI 95% [0.230–0.833] were the predictable factors for overall survival ($p < 0.05$).

4. Discussion

In this study, we retrospectively examined the expression levels of three proteins involved in wnt signalling in 97 osteosarcomas of the extremities using demographic, pathologic and immunophenotypic parameters in the context of standard clinical outcome end-points. Whilst there was no correlation between pan-cadherin or β-catenin expression and other prognostic factors, we demonstrate that APC immunoreactivity correlated with age and serum alkaline phosphate levels. Significantly, when survival was the endpoint, multivariate analysis suggested that race segregated patients with poor survival, as did lack of cadherin expression. Finally, there appeared to be an interaction between APC immunoreactivity and adjuvant chemotherapy which impacted prognosis. This latter observation suggests that APC expression may be considered as a surrogate biomarker for differential treatment response in osteosarcoma.

In relation to ethnic group, the Memorial Sloan Kettering Cancer Center experience has reported worst disease-free survival and OS for black race due to a worse response to chemotherapy.[2] In our analysis, no correlation between the race and other variables was found, but race was also a significant variable for disease-free survival in uni- and multivariate analysis.

Serum alkaline phosphatase and lactic dehydrogenase abnormal levels are usually associated with advanced disease or metastasis[12,13] and to increased recurrence risk and death.[14] In this series no difference among patients with or without metastatic disease was found, showing no correlation with advanced disease, disease-free and overall survivals.

Metastasis at diagnosis is considered an adverse prognostic factor, mainly when compared with patients that developed metastasis after six months.[15] In this present study, there are 14 patients with metastatic disease at diagnosis (14.4%). The median time for metastasis diagnosis during the follow-up was 15.6 months, and in 80.4% it has occurred in the first 2 years. The survival of the group that has developed metastasis during the follow-up was similar to that of the group with metastatic disease at diagnosis (23.9% × 29.8% in 5 years) when this was evaluated just after the metastasis diagnosis.

Chemotherapy necrosis-induced index is considered as a predictive factor for disease-free survival[16] and it is related to metastases, local recurrence or both.[3] In this series it was not a significant variable for disease-free survival and overall survival, possibly due to the small number of good responders. Some authors attributed different responses and biological potentials,[14] chemosensitivities and sometimes different vascularisation,[5] to histological subtype.[3,16–18] In our study, the most frequent histological type was osteoblastic followed by chondroblastic, and no difference was found in necrosis degree, disease-free and overall survival among histological subtypes. We excluded patients with multifocality or polyostotic osteosarcoma in order to standardise the groups since patients with this histological type of tumour present distinct outcomes.

In this study, 86 patients were submitted to chemotherapy, both in neoadjuvant or adjuvant period. Adjuvant chemotherapy demonstrated a significant benefit in OS that was independent of the treatment regimen. When analysing neoadjuvant and adjuvant chemotherapy, the Pediatric Oncology Group[8] observed benefits in both groups, with no advantage for disease-free survival in neoadjuvant chemotherapy. Nowadays some investigators propose the use of neoadjuvant chemotherapy with careful consideration of established prognosticators and risk factors. This offers the possibility of increasing the use of conservative surgery, with no impact on overall or disease-free survivals.[2,19–21]

Cadherins are membrane glycoproteins that act as molecules of cell adhesion, responsible for the maintenance of the tissue integrity[22] and also for the progression of carcinoma, promoting alteration in the morphology, invasion and metastasis.[23] There is little knowledge about cadherin participation in mesenchymal tumours. According to Kashima et al.[24] several forms of cadherins may be detected in osteoblasts by RT-PCR, however, to the protein level only the cadherin-11 and the N-cadherin can be detected. Pan-cadherin in this series was expressed in almost all cases and did not present any relationship with clinical, anatomic, pathological or demographic factors. However, no expression of pan-cadherin was related to the univariate and multivariate analyses of the disease-free survival ($p < 0.001$ [HR 127.68 (IC 95% = 11.29–1143.24)] and the overall survival ($p < 0.001$ [HR 0.028 (IC 95% = 0.005–0.148). In spite of the small number of patients (3 cases), all pan-cadherin-negative died and the pan-cadherin-positive group had a survival of 57%. This finding is consistent with the observation by Kashima et al.[24] that overexpression of cadherins suppresses metastasis of osteosarcoma. Cadherin levels may also be indirectly related to the differentiation grade and to the functional state of these cells.[25] New prospective studies with larger number of cases are necessary for more conclusive results.

β-Catenin is a crucial part of the Wnt- and E-cadherin-signalling pathways, which are involved in tumourigenesis. Dys-

regulation of these pathways allows β-catenin to accumulate and translocate to the nucleus, where it may activate oncogenes. Such a nuclear accumulation can be detected by immunohistochemistry. Cytoplasmic and/or nuclear accumulation of the β-catenin protein has been observed by other authors in over 70% of all their cases.[4] The β-catenin in this series demonstrated a similar high frequency of expression, but no association with clinical, anatomic, pathological or demographic variables, disease-free and overall survival. It is reported that β-catenin, APC and cadherin are strongly expressed in osteoclasts and osteoblasts and that this expression is lost when the bone cells differentiate in osteocytes, and their expression in osteosarcoma is similar to that in osteoblasts of normal bone.[25] However, positive cadherins and β-catenin in osteocytes were identified in nidus of the osteoid osteoma and in the osteosarcomas cells. It is not known if this fact represents a transition period in the differentiation of osteoblasts in osteocytes or a phenomenon of cellular transformation. It seems that the negativity of β-catenin is an initial event in the genesis of sarcomas.[25]

There is only one report in the literature, with a small series of patients that associates APC and osteosarcoma. APC was positive in 100% of the sample (nine cases), and the author concludes that abnormalities in this can be important in the formation of bone tumours and in clinical evolution.[25] In our casuistic no association between APC immunoreactivity and clinicodemographic variables was observed, except age ($p = 0.025$), where there was a greater positivity in patients up to 18 years. Due to the few data regarding the role of APC in osteosarcoma, it is not possible to compare these results. Maybe it is an additional data to explain best prognosis in patients in this age group.

When the interaction between APC and adjuvant chemotherapy and the selected patients in the four groups were evaluated, a survival difference favouring the APC-positive group and the positive adjuvant chemotherapy was found. These data suggest that patients who present APC immunoreactivity have larger benefit with the adjuvant chemotherapy, reflecting a larger survival rate of 69.9% ($p = 0.002$) compared to the other groups. And the patients with loss of the APC expression, independent of the adjuvant chemotherapy, present an unfavourable prognosis. These findings suggest the role of APC in the regulation of the osteoblasts, and osteoclasts function underscores how Wnt signalling and its interaction with catenin–cadherin pathways may be involved in the observed differential responses. It is noteworthy that *in vitro* modulation of Wnt and β-catenin expression levels have been shown to influence both motility and invasion of osteosarcomas.[9] Abnormalities or difference in expression of Wnt proteins may be important in the formation and evolution and chemotherapy response of bone tumours, and our findings reinforce the interest in understanding Wnt signalling and its impact on therapeutic response.[5]

When considering immunohistochemical variables and those variables related to the treatment only the pan-cadherin immunoreactivity ($p < 0.001$) and the interaction of the adjuvant chemotherapy with the APC immunoreactivity were significant for the overall survival in univariate and multivariate analyses ($p < 0.001$ [HR 0.028 (IC 95% = 0.005–0.148) and ($p = 0.012$ [HR 0.438 (IC 95% = 0.230–0.833).

There is a lack of information on the association between APC and osteosarcoma, what makes these findings important, mainly for suggesting a group that can have larger benefit with chemotherapy, this group being eventually treated with more aggressive schemes outlines. This emphasises the importance of the identification of groups that, independent on the chemotherapy scheme, present poor response, since these subgroups should be introduced to new therapeutic modalities, new drugs and participation in clinical assays. So, new prospective studies with analysis of these immunohistochemical markers (APC and pan-cadherin) and larger study groups are important for validation of these data.

Growth factors and Wnt-signalling pathway proteins have been examined in a wide variety of tumours, predominantly carcinomas, and examination of sarcomas has been relatively limited to date. This detailed analysis of prognostic factors in osteosarcoma of the extremities using expression of APC, cadherin and β-catenin Wnt-signalling markers draws attention to the importance of this pathway in bone tumour progression and response. Moreover, the significant interaction between APC immunoreactivity and the use of adjuvant chemotherapy suggest that knowledge of APC expression will be important for improving overall survival levels in osteosarcoma in the future as a possible target for therapy.

Conflict of interest statement

None declared.

Acknowledgement

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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