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Analysis of Clinical and Molecular Factors Impacting Oncologic Outcomes in Undifferentiated Pleomorphic Sarcoma

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Abstract

Background—Undifferentiated pleomorphic sarcomas (UPS) present a diagnostic and therapeutic challenge. Identification of prognostic molecular markers is required for the discovery of novel treatment approaches. The aim of this study was to correlate clinicopathologic variables, expression of tyrosine kinase receptors, and markers of cell cycle progression and survival with oncologic outcomes.

Methods—A tissue microarray containing 208 primary UPS samples was analyzed by immunohistochemistry for protein markers and *in situ* hybridization for microRNA. Staining results were correlated with clinicopathologic features and oncologic outcomes. Univariate and multivariate analyses were conducted to assess associations between expression of protein markers, mi-RNA and outcome.

Results—At a median follow-up of 3.9 years (9 years for survivors), 5-year disease-specific survival (DSS) was 63%. Clinical variables associated with improved DSS included age < 61 years, tumor size < 10 cm, margin-negative resection and sporadic-tumor status. At the protein level, loss of cyclin D1 ($p=0.06$), pEGFR ($p=0.023$), pIGF-1R ($p=0.022$), and PTEN ($p<0.001$) and overexpression of AXL ($p=0.015$) were associated with reduced DSS on univariate analysis. Ki67, PCNA and pEGFR were more highly expressed in sporadic UPS than radiation-associated (RA-UPS) while RA-UPS samples expressed higher levels of both phosphorylated and total IGF-1R.

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Discussion—Cyclin D1, AXL and PTEN are associated with cancer-specific outcomes and warrant further investigation in UPS. The differences in protein expression in sporadic versus RA-UPS may indicate that the activated molecular signaling nodes may be different for each specific histology and could also explain the aggressive phenotype seen in RA-UPS when compared to the sporadic lesions.

Keywords

Undifferentiated pleomorphic sarcoma; UPS; MFH; Radiation

Introduction

Soft tissue sarcomas comprise a large, heterogeneous group of rare tumors, originating from varied mesenchymal cell types. Undifferentiated pleomorphic sarcoma (UPS) presents a unique diagnostic challenge, as there is no clear origin of histogenesis. These tumors are largely morphologically high grade with a complex karyotype.¹

Margin-negative surgical resection remains the mainstay of treatment. Despite aggressive surgery, local recurrences occur in 13–42% of patients and nearly 31–35% will develop metastatic disease.^{2–4} Although the addition of chemotherapy has shown some benefit in meta-analyses, these responses are marginal at best, highlighting the need for improved therapeutic options for UPS patients.^{5,6} The use of radiation therapy is associated with reduced local recurrences in high grade sarcomas; however, approximately 3–5% of UPS arise in a prior site of therapeutic radiation for an unrelated malignancy and are termed radiation-associated UPS (RA-UPS), making radiation therapy controversial in this subset of the patients due to the comorbidities of re-irradiation^{7,8}. Therefore, a greater understanding of the molecular biology of UPS, both sporadic and radiation-associated, is necessary to facilitate the discovery of novel treatment approaches in the future.

Advanced age, large tumors, and radiation-associated tumors have been associated with reduced survival in patients with UPS.⁴ While recent studies have described genomic rearrangements and gene expression profiles of UPS tumors, the expression patterns and prognostic potential of specific proteins and microRNAs (miRNAs) have not yet been extensively investigated.^{9–12} Previously, Tomita *et al*¹³ found that high levels of activated (phosphorylated) AKT (pAKT), a key step in the phosphatidylinositol-3-kinase/mammalian target of rapamycin (PI3K/mTOR) pathway, correlated with poorer overall (OS) and disease-specific survival (DSS) outcomes. Similarly, in previous studies, we found that a subset of UPS tumors expressed elevated pAKT levels which was associated with poorer DSS outcomes.¹⁴

Recent evidence suggests that miRNA-mediated gene regulation interconnects with the AKT pathway, forming an AKT–microRNA (miRNA) regulatory network. In this network, the miRNA and PI3K/AKT/mTOR pathway work together to exert their cellular functions. Thus, to better understand these interactions we evaluated some miRNAs that have been associated to the PI3K/AKT/mTOR network and have been found to be relevant prognostic indicators of survival in other sarcomas¹⁵ and UPS^{16–19}.

Therefore, in the current study, the protein expression levels of the PI3K/mTOR pathway components were examined, including downstream effectors and upstream activating receptor tyrosine kinases (RTKs) as well as miRNAs for their association with patient survival outcomes. Furthermore, we evaluated the potential prognostic associations of several miRNAs indicated to be differentially expressed in UPS.

Methods

Clinical database and tissue microarray construction

With the approval of the Institutional Review Board (IRB) of The University of Texas MD Anderson Cancer Center (UTMDACC), the UTMDACC Pathology Archive was searched for formalin-fixed, paraffin embedded (FFPE) sporadic and radiation-associated UPS specimens. UPS diagnoses were confirmed by a UTMDACC sarcoma pathologist and in the context of multidisciplinary tumor board review. A tissue microarray (TMA) consisting of 168 sporadic and 60 RA-UPS tumor samples was constructed as previously described²⁰, with duplicate 1.2 mm cores included from each tissue block. The TMA was used for protein and miRNA analysis.^{14,21} MDM2 immunohistochemistry or fluorescence *in situ* hybridization was performed on all samples prior to inclusion in this study to exclude liposarcoma histology. A clinical database was populated by collecting clinicopathologic variables including patient age and gender; tumor size, grade, and location; and the use of chemo- or radiotherapy. Local recurrence was considered as any recurrence at the primary site without concomitant metastasis. For the outcomes analysis, only primary tumor tissue with sufficient clinical history were included (total cohort: $n = 208$; RA-UPS: $n = 35$; sporadic UPS: $n = 173$). Therefore, nine (4%) primary samples lacking adequate corresponding clinical history, 8 (4%) recurrent samples, and 3 (1%) metastatic samples were excluded.

Immunohistochemistry and in situ hybridization

Immunohistochemical (IHC) studies were performed using commercially available antibodies, following standard automated and manual protocols. Immunostaining for pAKT, AKT, pS6RP, S6RP, and p4EBP1 was performed by the histology core facility at The Virginia Harris Cockrell Cancer Research Center at Science Park histology core. Immunohistochemistry for Ki67, PCNA, Cyclin D1, CD31, p53, c-KIT, pEGFR, IGF-1R, PTEN, PDGFR α , and PDGFR β was performed by the UTMDACC Research Histopathology Facility.¹⁴ *In situ* hybridization was performed for miR-1, miR-133a, miR-182 and miR-183 by the RNA Center at UTMDACC.²²

Immunostaining for pIGF-1R, pMEK, MEK, pMET, and AXL was conducted in our laboratory as follows. Tumor specimens were deparaffinized in xylene and rehydrated using a graded ethanol series. Sections were subjected to antigen retrieval at 100°C for 45 minutes in Tris-EDTA, pH 8 and endogenous peroxidase blocking in 3% H₂O₂ in PBS for 12 minutes. Primary antibody incubation took place at 4°C overnight using pIGF-1R rabbit monoclonal antibody #3024 diluted 1:25, pMEK rabbit monoclonal antibody #2338 diluted 1:50, MEK rabbit polyclonal primary antibody #9122 diluted 1:50, pMET rabbit monoclonal antibody #3077 diluted 1:100, or AXL rabbit monoclonal antibody #4566 diluted 1:100

(Cell Signaling Technology). Primary antibody was visualized using the 4plus two-step HRP detection system (Biocare Medical) and diaminobenzidine. Sections were counterstained with hematoxylin and mounted with Permount (Fisher).

Scoring was performed by 2 independent sarcoma pathologists (GAA and WLW). The percentage of tumor cells expressing the marker was recorded and an intensity score was assigned (0 = no expression; 1 = low expression; 2 = intermediate expression; 3 = high expression). Cutoffs for survival analysis stratification were assigned based on median expression tendency. A negative or positive expression classification was assigned to markers (Ki67, Cyclin D1, p53, AXL, c-kit, pEGFR, IGF-1R, PTEN, pS6RP, and p4EBP1) with low median expression (0–30% of cells positive for stain); samples were considered positive if 10% of cells were stained. A negative/low or high expression classification was assigned to markers (PCNA, CD31, pIGF-1R, pMET, MET, pAKT, AKT, S6RP, PDGFRa, PDGFRb, pMEK, MEK, miR-1, miR-133a, miR-183, and miR-182) with moderate to high median expression (40–100% of cells positive for stain); samples were classified as negative/low expression (< 60% of cells positive for stain) or high expression (>60% of cells positive). Studies have shown that PI3K pathway-associated kinases such as AKT translocate within the cell and that subcellular localization often dictates protein function adding complexity to the functional repertoire of the PI3K pathway²³; therefore, we make a distinction between nuclear and cytoplasmic staining.

Statistics

Fisher's exact test was used to differentiate protein and mi-RNA expression between sporadic-UPS and RA-UPS. Univariate Kaplan and Meier methods were used to estimate overall survival (OS), disease-specific survival (DSS), local recurrence-free survival (LRFS), and metastasis-free survival (MFS). OS and DSS time were measured from date of pathological confirmation of UPS to date of death owing to any cause or date of sarcoma-specific death, respectively. LRFS and MFS time was measured from the date of surgical resection to time of pathological or radiographic determination of recurrent or metastatic disease, respectively. Multivariate Cox proportional hazard regression models were constructed to assess associations between marker expression, clinical variables, and survival outcomes. Variables with a *p* value of < 0.1 in univariate analysis were fitted to multivariate models. A two-tailed *p* value of < 0.05 was accepted as statistically significant. All analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY).

Results

UPS patient and tumor characteristics

Patient characteristics and tumor variables corresponding to the 208 primary UPS samples (*patients, n = 208*) are summarized in Table 1. Median age at diagnosis was 64 years and 63% of the cohort was comprised of males. Primary sites of origin included the extremities (73%), trunk or retroperitoneum (24%), and head and neck (3%). The median tumor size was 7.5 cm and the majority of tumors were high grade (76%). Sixty-five (31%) patients were treated with systemic chemotherapy and 118 (57%) received radiation therapy. As we have previously reported differences in clinical outcomes among patients with sporadic

versus RA-UPS⁴, we also analyzed the current data within the context of disease setting (Table 1).

Protein and miRNA expression in UPS and association with survival outcomes

After a median follow-up period of 3.9 years (range, 0.1–20.75 years; for survivors: 9 years, range 0.2–20.7), local recurrence following resection was observed in 27% ($n = 56$) of patients. Distant metastasis occurred in 63 (30%) patients. Median time to local and distant recurrence was 0.9 years (range, 0.1–13.7 years) and 0.8 years (range, 0.1–16.3 years), respectively. The local recurrence rate was higher in RA-UPS compared to sporadic UPS (43% versus 24%, respectively; $p = 0.019$). The metastasis rate was not significantly different between RA-UPS and sporadic UPS (34% versus 29%, respectively; $p = 0.353$). Univariate and multivariate analysis of clinical and protein markers associated with reduced risk of local recurrence included sporadic tumors, margin-negative resection and retained PTEN expression (Table 2). Loss of PTEN and tumors > 10cm were associated with increased distant recurrence on univariate and multivariate analysis (Table 2).

Five-year overall survival was 54.7% and disease-specific survival was 63% (data not shown). Univariate analysis of clinical factors for DSS resulted in the identification of age < 61 years at diagnosis, tumor size < 10 cm, margin-negative resection and sporadic tumors were associated with improved DSS (Table 3; Fig. 1 A–C). Expression of cytoplasmic AXL and loss of PTEN were also associated with reduced DSS [hazard ratio (HR) = 0.46 95% confidence interval (CI), 0.25–0.86; $p = 0.015$ and HR = 10.3; CI, 4.55–23.4; $p = 0.015$, respectively] (Fig. 1 D–F). Although not statistically significant, we observed that tumors expressing high levels of cyclin D were associated with a better survival (5-year DSS 78% versus 58%, $p = 0.060$). When evaluated in a multivariate model, only age < 61 years at diagnosis, tumor size < 10 cm, sporadic tumors, and retained PTEN were associated with improved DSS (Table 3). Previously, we evaluated miRNA expression in several UPS cell lines and found that expression of miR-1 and miR-133a was the most significantly decreased while the expression of miR-182 and miR-183 was strongly increased when compared to the human mesenchymal stem cell control (data not shown). However, in this study we did not observe any prognostic significance for the four miRNAs.

Although RA-UPS is associated with poorer survival outcomes than sporadic UPS⁴, currently no molecular distinctions between the two disease settings have been identified. Therefore, we examined protein and miRNA expression patterns separately. Both Ki67 and PCNA, markers of cell proliferation, were more highly expressed in sporadic UPS than RA-UPS (Table 4). In addition, more sporadic UPS samples were positive for pEGFR expression, while RA-UPS samples expressed higher levels of both phosphorylated and total IGF-1R when compared to the sporadic lesions.

Discussion

There is no clear origin of histogenesis for UPS; therefore, this histological entity represents a unique diagnostic and therapeutic challenge. Surgical resection with negative margins remains the mainstay of treatment for these tumors^{1,24} Subtype-specific benefit of neoadjuvant or adjuvant therapies has not yet been demonstrated. Therefore, it is critical to

not only identify novel targets for therapeutic interventions but evaluate potential biomarkers to predict response to therapy and survival outcomes.^{4–6,24,25} Previously, we have demonstrated that radiation-associated disease, tumor size, and advanced age are negative clinical prognosticators for UPS.⁴ However, little is known about the molecular dysregulations in UPS. In the current study, we evaluate the expression profiles of numerous protein biomarkers including components of the PI3K/mTOR and MAPK pathways as well as upstream receptor tyrosine kinases (RTKs) and specific miRNAs in a relatively large cohort of sporadic and radiation-associated UPS tumors. Although immunohistochemistry is typically used to rule out other high grade sarcomas, few studies have specifically examined whether there is a difference between sporadic and radiation-associated lesions. We identified cyclin D1, cytoplasmic AXL and PTEN as potential markers of prognosis for patients with UPS. In addition, different protein expression profiles between sporadic and radiation-associated UPS.

Although RA-UPS is associated with worse clinical outcomes, the molecular mechanisms governing these differences are currently unknown.^{4,26} In this study, we demonstrate differences in protein expression between sporadic and RA-UPS. Positive pEGFR expression was more readily detected in sporadic UPS, while phosphorylated and total IGF-1R were more highly expressed in RA-UPS. While preclinical data have supported the development of anti-IGF-1R therapies, the majority of clinical trials evaluating these therapeutic interventions in large, unselected patient populations have reported low efficacy.^{27,28} The identification and validation of biomarkers, such as pIGF-1R and other members of the IGF pathway, suggests that there may be subgroups that would derive clinical benefit from anti-IGF-1R therapy. The tumor suppressor PTEN, a negative regulator of the PI3K/mTOR pathway, is frequently inactivated in human cancer.^{29,30} In addition, loss of function is associated with increased pathway activity, advanced disease, and worse patient outcome in numerous cancer types.²⁹ PTEN was the only biomarker significantly associated with recurrence and survival outcomes in our cohort. Previous studies have described decreased PTEN expression in pleomorphic sarcomas, including UPS, as a result of the loss of chromosome 10q in some samples.^{9,31} However, only 4% of patients demonstrated loss of PTEN suggesting that activation of the AKT/mTOR pathway occurs independently of PTEN protein loss in the majority of this subset of tumors.

Oncogenic PI3K/mTOR pathway activation can also occur through the inappropriate or unregulated activation of upstream RTKs. In our study, we found overexpression of the RTK, AXL. Others have shown that activation of AXL can promote multiple tumorigenic responses including cell survival, proliferation, migration, and adhesion.^{32–34} Overexpression of AXL has been associated with worse prognosis in many cancer types, including Ewing's and osteosarcoma.^{32,35–37} Anti-AXL monoclonal antibodies have shown promise in preclinical models of Ewing's sarcoma^{33,35} and one such therapy is currently being investigated in a phase II trial in lung cancer patients.³⁸ In the current study, overexpression of cytoplasmic AXL was associated with worse DSS (5-yr: 75% versus 50%, $p = 0.015$) in univariate analysis, which supports the further consideration of anti-AXL therapy in UPS patients.

There are several limitations to the current study, namely the retrospective nature of the analysis, variable use of chemotherapy and radiation therapy, and the limited number of UPS samples available for evaluation. However, to our knowledge, this is one of the largest UPS-centric retrospective studies examining potential biomarkers to date. Here we confirmed that the clinical variables of age, tumor size, negative margins, and radiation-associated disease indicate increased recurrence and poor survival outcomes. Additionally, we demonstrated the potential of PTEN loss associated with worsened patient outcomes. Furthermore, we propose that pIGF-1R expression may differentiate disease setting and may be a useful therapeutic target in UPS.

In conclusion, cyclin D1, AXL and PTEN are associated with cancer-specific outcomes and warrant further investigation in UPS. Differences in protein expression in sporadic versus RA-UPS may explain biologic differences in oncologic outcomes.

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Synopsis

Sporadic and radiation-associated undifferentiated pleomorphic sarcomas (UPS) demonstrate differences in protein expression. PTEN status is associated with disease-specific survival in UPS.

Figure 1.

Univariable Kaplan-Meier survival curves of disease-specific survival stratified by (A) age at diagnosis ($p = 0.001$; HR 0.41, 95% CI 0.24–0.67), (B) disease setting ($p < 0.001$; HR 0.37, 95% CI 0.22–0.63), (C) index tumor size ($p = 0.002$; HR 0.48, 95% CI 0.30–0.77), (D) cyclin D1 expression ($p = 0.060$; HR 1.73, 95% CI 0.98–3.05), (E) AXL expression ($p = 0.015$; HR 0.46, 95% CI 0.25–0.86), and (F) PTEN expression ($p < 0.001$; HR 10.3, 95% CI 4.55–23.4).

Table 1

Clinicopathologic Characteristics of Patients with Undifferentiated Pleomorphic Sarcoma by Radiation-Associated Status

Variable	Entire Cohort	Sporadic	RAS
Entire Cohort	208 (100.0%)	173 (83.2%)	35 (16.8%)
Gender			
Male	131 (62.9%)	114 (65.9%)	17 (48.6%)
Female	77 (37.0%)	59 (34.1%)	18 (51.4%)
Age			
Median (range)	64.0 (17–87)	65.0 (22–87)	62.0 (17–87)
Tumor size, cm			
Median (range)	7.5 (0.9–35.0)	8.0 (1.0–35.0)	6.0 (0.9–30.0)
Grade			
Low	6 (2.8%)	4 (2.3%)	2 (5.7%)
Intermediate	38 (18.2%)	38 (21.9%)	0 (0.0%)
High	157 (75.5%)	130 (75.1%)	27 (77.1%)
Unknown	7 (3.4%)	1 (0.6%)	6 (17.1%)
Location			
Head & Neck	6 (2.9%)	0 (0.0%)	6 (17.1%)
Trunk/Retroperitoneum	50 (24.0%)	30 (17.3%)	20 (57.1%)
Extremity	152 (73.1%)	143 (82.7%)	9 (25.7%)
Depth			
Superficial	28 (13.5%)	25 (14.5%)	3 (8.6%)
Deep to investing fascia	180 (86.5%)	148 (85.5%)	32 (91.4%)
Therapy received			
Chemotherapy	65 (31.2%)	57 (32.9%)	8 (22.9%)
Radiation	118 (56.7%)	99 (57.2%)	19 (54.3%)
Resection Margins			
R0	145 (69.7%)	119 (68.8%)	26 (74.3%)
R1	57 (27.4%)	51 (29.5%)	6 (17.1%)
R2	3 (1.4%)	1 (0.6%)	2 (5.7%)
Unknown	3 (1.4%)	2 (1.2%)	1 (2.9%)

Table 2

Univariate and Multivariate Analysis for Overall and Disease-specific Survival in Patients with Undifferentiated Pleomorphic Sarcoma

Covariate, (indicator)	Overall Survival			Disease-specific survival		
	Univariable		Multivariable (n = 123) [†]		Multivariable (n = 129) [†]	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, (<61) v. 61	0.36 (0.23–0.57)	<0.001 [*]	0.17 (0.08–0.38)	<0.001 [*]	0.41 (0.24–0.67)	0.001 [*]
Sex, male v. female	1.13 (0.74–1.69)	0.555			1.21 (0.75–1.98)	0.430
Setting, sporadic v. RAS	0.44 (0.28–0.69)	<0.001 [*]	0.36 (0.16–0.79)	0.011 [*]	0.37 (0.22–0.63)	<0.001 [*]
Size, <10 cm v. 10 cm	0.51 (0.34–0.76)	0.001 [*]	0.45 (0.24–0.84)	0.012 [*]	0.48 (0.30–0.77)	0.002 [*]
Clinical markers						
Depth, superficial v. deep	0.78 (0.43–1.42)	0.415			0.63 (0.29–1.84)	0.253
Grade, low-intermediate v. high	0.22 (0.31–1.58)	0.132			0.29 (0.04–2.06)	0.285
Chemotherapy, no v. yes	0.92 (0.59–1.45)	0.732			0.93 (0.55–1.54)	0.763
Radiation, no v. yes	1.04 (0.70–1.54)	0.830			1.04 (0.59–1.69)	0.851
Margins, negative v. positive	0.61 (0.40–0.91)	0.016 [*]	0.24 (0.24–0.84)	<0.001 [*]	0.62 (0.38–1.00)	0.051 [*]
					0.26 (0.11–0.62)	0.002 [*]
Ki67, negative v. positive	0.75 (0.48–1.19)	0.228			0.90 (0.52–1.56)	0.904
Cyclin D1, negative v. positive	1.40 (0.89–2.21)	0.145			1.73 (0.98–3.05)	0.060 ^{**}
CD31, negative-low v. high	1.26 (0.51–3.14)	0.617			1.10 (0.39–3.07)	0.850
p53, negative v. positive	0.81 (0.52–1.26)	0.350			0.86 (0.51–1.45)	0.562
AXL Receptor, negative v. positive	0.66 (0.40–1.06)	0.085 ^{**}	0.91 (0.46–1.83)	0.797	0.46 (0.25–0.86)	0.015 [*]
c-kit, negative v. positive	1.38 (0.66–2.87)	0.391			1.43 (0.57–3.58)	0.447
pEGFR, negative v. positive	0.69 (0.35–1.35)	0.278	1.94 (1.09–3.43)	0.023 [*]	0.58 (0.26–1.30)	0.186
pGFIR, negative-low v. high	0.92 (0.47–1.78)	0.804	0.49 (0.27–0.90)	0.022 [*]	0.59 (0.27–1.33)	0.208
Protein markers						
IGF1R, negative v. positive	0.67 (0.41–1.07)	0.091 [*]	0.87 (0.42–1.79)	0.706	0.65 (0.37–1.13)	0.130
pMET, negative-low v. high	0.97 (0.38–2.51)	0.955			0.88 (0.30–2.55)	0.814
PTEN, negative v. retained	7.70 (3.47–17.1)	<0.001 [*]	6.73 (2.48–18.3)	<0.001 [*]	10.3 (4.55–23.4)	<0.001 [*]
pAKT (<i>Cytoplasmic</i>), negative-low v. high	0.83 (0.53–1.32)	0.434			0.74 (0.43–1.26)	0.261
pAKT (<i>Nuclear</i>), negative-low v. high	0.86 (0.55–1.35)	0.520			1.05 (0.62–1.78)	0.854
AKT (<i>Cytoplasmic</i>), negative-low v. high	1.04 (0.65–1.67)	0.861			1.15 (0.64–2.05)	0.629
					20.8 (6.34–68.2)	<0.001 [*]

Covariate, (indicator)	Overall Survival				Disease-specific survival			
	Univariable		Multivariable (n = 123) [†]		Univariable		Multivariable (n = 129) [†]	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
AKT (<i>Nuclear</i>), negative-low v. high	0.61 (0.39–0.96)	0.034 [*]	0.78 (0.39–1.54)	0.468	0.83 (0.47–1.45)	0.827		
pS6RP, negative v positive	1.23 (0.77–1.96)	0.380			1.58 (0.88–2.86)	0.128		
S6RP, negative-low v. high	0.78 (0.46–1.35)	0.379			0.67 (0.35–1.31)	0.241		
P4EBP1 (<i>Cytoplasmic</i>), negative v positive	0.97 (0.62–1.50)	0.879			1.04 (0.61–1.76)	0.890		
miR-1, negative-low v. high	0.83 (0.47–1.49)	0.537			0.76 (0.38–1.52)	0.437		
miR-133a, negative-low v. high	0.69 (0.39–1.23)	0.213			0.82 (0.39–1.69)	0.585		
miRNA markers								
miR-183, negative-low v. high	0.79 (0.45–1.37)	0.397			0.89 (0.46–1.74)	0.736		
miR-182, negative-low v. high	0.89 (0.42–1.89)	0.759			0.92 (0.36–2.38)	0.863		

^{*} Significant at level of $p < 0.05$

^{**} Significant at level of $p < 0.1$

[†] Patients without expression scores for all molecular variables included in the multivariate analysis are excluded

Univariate and Multivariate Analysis for Local Recurrence-free and Metastasis-free Survival in Patients with Undifferentiated Pleomorphic Sarcoma

Table 3

Covariate, (indicator)	Local recurrence-free survival			Metastasis-free survival		
	Univariable		Multivariable (n = 145) [†]	Univariable		Multivariable (n = 175) [†]
	HR (95%CI)	P-value		HR (95%CI)	P-value	
Age, (<61) v. 61	0.78 (0.46–1.34)	0.372		0.59 (0.35–1.00)	0.051 **	0.53 (0.28–1.00)
Setting, sporadic v. RAS	0.38 (0.21–0.69)	0.002 *	0.29 (0.15–0.56)	0.75 (0.40–1.41)	0.378	
Size, <10 cm v. 10 cm	0.68 (0.39–1.2)	0.149		0.41 (0.25–0.68)	0.001 *	0.36 (0.19–0.66)
Depth, superficial v. deep	0.81 (0.37–1.79)	0.600		0.61 (0.26–1.42)	0.250	
Grade, low-intermediate v. high	0.91 (0.22–3.77)	0.898		0.31 (0.04–2.24)	0.245	
Chemotherapy, no v. yes	0.87 (0.49–1.52)	0.619		1.18 (0.67–2.05)	0.569	
Radiation, no v. yes	0.78 (0.46–1.35)	0.782		1.23 (0.75–2.02)	0.409	
Margins, negative v. positive	0.51 (0.29–0.87)	0.013 *	0.45 (0.24–0.87)	0.71 (0.42–1.18)	0.187	
Ki67, negative v. positive	1.25 (0.67–2.35)	0.480		0.88 (0.49–1.55)	0.653	
Cyclin D1, negative v. positive	0.97 (0.53–1.79)	0.928		1.19 (0.67–2.11)	0.549	
CD31, negative-low v. high	1.19 (0.37–3.87)	0.773		0.62 (0.24–1.58)	0.317	
p53, negative v. positive	0.86 (0.48–1.56)	0.624		1.02 (0.58–1.81)	0.936	
AXL Receptor, negative v. positive	0.71 (0.37–1.37)	0.309		0.58 (0.31–1.11)	0.100	
c-kit, negative v. positive	1.17 (0.46–2.98)	0.741		1.12 (0.45–2.84)	0.803	
pEGFR, negative v. positive	0.63 (0.32–1.20)	0.161		1.23 (0.64–2.39)	0.534	
pIGFIR, negative-low v. high	0.60 (0.32–1.15)	0.126		0.86 (0.43–1.71)	0.660	
IGFIR, negative v. positive	0.57 (0.30–1.09)	0.092 **	0.72 (0.37–1.39)	0.80 (0.43–1.49)	0.482	
pMET, negative-low v. high	1.66 (0.39–7.12)	0.492		3.00 (0.39–22.8)	0.287	
PTEN, negative v. retained	6.25 (2.18–17.9)	0.001 *	5.29 (1.77–15.9)	8.68 (3.33–22.7)	<0.001 *	7.98 (2.89–22.0)
pAKT (<i>Cytoplasmic</i>), negative-low v. high	0.89 (0.48–1.68)	0.732		0.64 (0.36–1.11)	0.636	
pAKT (<i>Nuclear</i>), negative-low v. high	0.85 (0.46–1.56)	0.603		0.96 (0.55–1.67)	0.878	
AKT (<i>Cytoplasmic</i>), negative-low v. high	1.56 (0.80–3.04)	0.192		1.00 (0.56–1.81)	0.982	
AKT (<i>Nuclear</i>), negative-low v. high	0.91 (0.48–1.70)	0.909		0.76 (0.43–1.36)	0.357	
pS6RP, negative v. positive	1.54 (0.80–2.95)	0.193		1.24 (0.69–2.26)	0.472	

Covariate, (indicator)	Local recurrence-free survival			Metastasis-free survival		
	Multivariable (n = 145) [‡]			Multivariable (n = 175) [‡]		
	Univariable	HR (95%CI)	P-value	Univariable	HR (95%CI)	P-value
S6RP, negative-low v. high		0.77 (0.37–1.60)	0.479		0.83 (0.42–1.62)	0.577
P4EBP1 (<i>Cytoplasmic</i>), negative v positive		0.92 (0.51–1.69)	0.798		1.04 (0.59–1.82)	0.883
miRNA markers	miR-1, negative-low v. high	1.87 (0.83–4.21)	0.132		0.79 (0.36–1.75)	0.572
	miR-133a, negative-low v. high	0.75 (0.33–1.66)	0.472		1.12 (0.46–2.69)	0.804
	miR-183, negative-low v. high	1.14 (0.52–2.49)	0.740		0.65 (0.31–1.35)	0.246
	miR-182, negative-low v. high	1.00 (0.34–2.95)	0.992		2.36 (0.56–9.93)	0.242

* Significant at level of $p < 0.05$

** Significant at level of $p < 0.1$

[‡] Patients without expression scores for all molecular variables included in the multivariate analysis are excluded

Table 4

Protein and miRNA Expression by Radiation-Associated Status.

Variable	Overall Median (range)	Sporadic			Radiation-Associated			P-value
		N	Negative	Positive ^o	N	Negative	Positive	
Ki67	10.0 (0.0–80.0)	131	39.7%	60.3%	29	72.4%	27.6%	0.001
Cyclin D1	0.0 (0.0–50.0)	137	58.4%	41.6%	30	50.0%	50.0%	0.261
p53	10.0 (0.0–90.0)	137	47.4%	52.6%	39	37.9%	62.1%	0.234
AXL (<i>Cytoplasmic</i>)	10.0 (0.0–90.0)	114	50.0%	50.0%	22	36.4%	63.6%	0.174
c-kit	0.0 (0.0–70.0)	141	87.2%	12.8%	31	93.5%	6.5%	0.257
pEGFR	50.0 (0.0–100.0)	131	19.1%	80.9%	31	54.8%	45.2%	<0.001
IGF1R	50.0 (0.0–100.0)	120	75.0%	25.0%	30	46.7%	53.3%	0.003 *
PTEN	2 (0–3) *	144	3.5%	96.5%	31	6.5%	93.5%	0.360
pS6RP	10.0 (0.0–90.0)	142	63.4%	36.6%	31	61.3%	38.7%	0.490
p4EBP1 (<i>Cytoplasmic</i>)	5.0 (0.0–90.0)	140	57.1%	42.9%	29	48.3%	51.7%	0.251
		N	Negative-low	High **	N	Negative-low	High	
PCNA [†]	90.0 (0.0–100.0)	128	1.6%	98.4%	29	24.1%	75.9%	<0.001
CD31	2 (1–3) *	132	90.9%	9.1%	24	100.0%	0.0%	0.124
pIGF-IR	50.0 (0–100.0)	86	70.9%	29.1%	30	20.0%	80.0%	<0.001
pMET	30.0 (0.0–70.0)	35	91.4%	8.6%	29	79.3%	20.7%	0.152
MET [‡]	90.0 (40.0–100.0)	116	0.9%	99.1%	29	0.0%	100%	0.800
pAKT (<i>Cytoplasmic</i>)	50.0 (0.0–90.0)	134	66.4%	33.6%	29	51.7%	48.3%	0.101
pAKT (<i>Nuclear</i>)	60.0 (0.0–100.0)	134	46.3%	53.7%	29	48.3%	51.7%	0.502
AKT (<i>Cytoplasmic</i>)	50.0 (0.0–90.0)	127	63.8%	36.2%	30	56.7%	43.3%	0.301
AKT (<i>Nuclear</i>)	50.0 (0.0–90.0)	127	67.7%	32.3%	30	50.0%	50.0%	0.055
S6RP	70.0 (0.0–100.0)	139	25.9%	74.1%	31	16.1%	83.9%	0.181
PDGFRa [‡]	90.0 (40.0–100.0)	144	0.7%	99.3%	31	3.2%	96.8%	0.324
PDGFRb [‡]	100.0 (60.0–100.0)	139	0.0%	100%	30	0.0%	100%	1.000
pMEK [‡]	100.0 (30.0–100.0)	89	3.4%	96.6%	30	3.3%	96.7%	0.737
MEK [‡]	100.0 (0.0–100.0)	98	4.1%	95.9%	29	0.0%	100%	0.350

Variable	Overall Median (range)	Sporadic	Radiation-Associated			P-value
miR-1	50.0 (0.0–90.0)	64	43.8%	23	52.2%	0.462
miR-133a	40.0 (0.0–90.0)	64	32.8%	25	64.0%	0.481
miR-183	40.0 (0.0–90.0)	74	33.8%	25	64.0%	0.512
miR-182	30.0 (0.0–90.0)	62	12.9%	29	72.4%	0.080

* Intensity score

[†] Not evaluated in survival analysis due to homogeneously high expression.

[‡] Samples were considered positive if there was labeling present in 10% of cells.

[§] Samples were considered to have high expression if there was labeling in >60% of cells.