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Alterations of p53 and PIK3CA/AKT/mTOR Pathways in Angiosarcomas: A Pattern Distinct from Other Sarcomas with Complex Genomics

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Abstract

Background—The p53 and the PIK3CA/AKT/mTOR pathways are frequently altered in sarcoma with complex genomics such as leiomyosarcoma (LMS) or undifferentiated pleomorphic sarcoma (UPS). The scale of genetic abnormalities in these pathways remains unknown in angiosarcoma.

Methods—We investigated the status of critical genes involved in the p53 and the PIK3CA/AKT/mTOR pathways in a series of 62 AS.

Results—The mutation and deletion rates of *TP53* were 4% and 0, respectively. p53 overexpression was detected by immunohistochemistry in 49% of cases and was associated with inferior disease-free survival. Although p14 inactivation or HDM2 overexpression are frequent in LMS and UPS and could substitute for *TP53* mutation or deletion, such alterations were rare in angiosarcomas. pS6K and/or p-4eBP1 overexpression was observed in 42% of cases suggesting frequent activation of the PIK3CA/AKT/mTOR pathway in angiosarcomas. Activation was not related to intragenic deletion of *PTEN*, an aberration that is frequent in LMS and UPS, but absent in angiosarcomas.

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Conclusion—Angiosarcomas constitute a distinct subgroup among sarcomas with complex genomics. Although *TP53* mutation and *PTEN* deletion are frequent in LMS and UPS, these aberrations are rarely involved in the pathogenesis of AS.

Keywords

angiosarcoma; *TP53*; *PIK3CA*; *PTEN*; *MDM2*; *P14*; *BRAF*; *NRAS*

INTRODUCTION

Angiosarcomas (AS) represent a rare (<2%) subgroup of soft tissue sarcomas (STS) characterized by an aggressive clinical behavior and occurring not only in different anatomical locations, but also in distinct clinical settings, such as *de novo* (primary AS), or after radiation therapy or chronic lymphedema (secondary AS) ¹. Radical surgery and adjuvant radiotherapy when indicated represent the cornerstone of treatment for patients with localized disease. However, despite an adequate loco-regional treatment, half or more of patients will develop metastatic relapse and will die of disease ². The genetic aberrations involved in AS genesis are still poorly understood. Until recently, data were limited to case reports or small case series showing a complex pattern of numerical and structural genetic aberrations ³⁻¹¹. We and others have recently identified *MYC* amplification as a recurrent genetic aberration in secondary AS ^{12, 13}, but no specific recurrent event has been reported in primary AS to date.

Alterations of *TP53* or *PTEN* are among the most frequent genetic events in human cancer and the role of their combined inactivation in tumorigenesis has been well examined in several contexts, including lymphoma, prostate cancer, glioblastoma, and medulloblastoma ¹⁴⁻²⁰. Interestingly, the p53 and the PIK3CA/AKT/mTOR pathways are frequently altered in sarcoma with complex karyotypes. Mutations and/or deletions of the *TP53* gene have been recently shown to occur in approximately 80% of leiomyosarcomas (LMS), 70% of undifferentiated pleomorphic sarcoma (UPS), and 60% of pleomorphic liposarcomas ²¹⁻²³. Large deletions on chromosome 10 including the tumor suppressor gene *PTEN* have also been observed in approximately 40-50% of LMS and 20-25% of UPS ^{24, 25}. Moreover, *in vitro* and *in vivo* studies ^{25, 26} suggest the crucial role of the PIK3CA/AKT/mTOR pathway in LMS genesis. Due to its rarity, studies regarding the incidence of p53 and PIK3CA/AKT/mTOR pathway aberrations in AS have used heterogeneous methods and are based on single case reports or small case series ²⁷⁻³⁵. Therefore, we investigated the status of key genes involved in these pathways in a series of primary and secondary AS by sequencing, array CGH and immunohistochemistry.

PATIENTS AND METHODS

Patient and samples

Sixty-two AS (30 primary and 32 secondary) were included in this study on the basis of availability of tumor material for genetic studies. The clinico-pathologic characteristics are listed in Table 1. Diagnoses were established according to the World Health Organization Classification of Tumors ¹. This study was approved by the institutional review board (IRB-protocol 02-060).

Array Comparative Genomic Hybridization (CGH)

Genomic DNA was isolated from frozen tumor tissue by phenol/chloroform extraction and quality was confirmed by spectrophotometry and electrophoresis. Array CGH analysis was performed as described previously (Thomas et al., 2011). Briefly, tumor and reference DNA

samples were labeled with Cyanine-3-dUTP and Cyanine-5-dUTP, respectively, by random priming (Agilent Enzymatic Labeling Kit, Agilent Technologies, Santa Clara, CA). The reference sample comprised a pool of DNA from multiple clinically healthy donors (Promega, Madison, WI) of the same gender as the AS patient. The labeled probes were combined and hybridized to a ~180,000-feature, genome-wide oligonucleotide CGH array (design 052252, Agilent Technologies). Arrays were scanned at 3µm resolution using an Agilent G2565CA scanner. Image data were processed with Feature Extraction version 10.10 and Genomic Workbench version 6.5 (Agilent Technologies). Data were filtered to exclude probes exhibiting non-uniform hybridization or signal saturation and were normalized using the centralization algorithm with a threshold of six. The ADM2 algorithm was used to define DNA copy number aberrations using a 'three probes minimum' filter and a threshold of six with a fuzzy zero correction, with genomic gain and loss defined as $\log_2$ test:reference DNA signal intensity >0.2 and <-0.2 , respectively. A genomic copy number amplification was defined as a region within which the $\log_2$ ratio of tumor DNA to reference DNA exceeded 2.0.

Real-Time Quantitative Reverse Transcription-PCR

One µg of total RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) at 25°C for 10 min, 37°C for 120 min, 85°C for five min and hold at 4°C. Twenty ng/µl of resultant cDNA was used in a quantitative-PCR using an 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) and pre-designed TaqMan ABI Gene expression Assays (Hs_00234753_m1 for *HDM2*; Hs_99999189_m1 for *P14/CDKN2A*). Amplification was carried out at 95°C for 10 min, and 40 cycles (95°C for 15 sec, 60°C for one min). To calculate the efficiency of the PCR and to assess the sensitivity of each assay, we also performed a five point standard curve (80, 26.67, 8.88, 2.96, and 0.98 ng/ul). Triplicates CT values were averaged and amounts of target were interpolated from the standard curves and normalized to *GAPDH* (reference gene).

Mutation Screening

The mutational status of *TP53* (exon 2 to exon 11), *PIK3CA* (exons 3, 10, 20), *BRAF* (exon 15) and *NRAS* (exons 2 and 3) was assessed by direct sequencing of genomic DNA. Protocols and primers are available on request. Sequence analysis was performed with Applied Biosystems Sequence Scanner™ v1.0.

Enhanced Mismatch Mutation Detection Analysis (EMMA) and Copy Number Analysis by Quantitative Multiplex PCR (QMP)

A screening of genomic variants of *PTEN* was performed by using Enhanced Mismatch Mutation Detection Analysis (EMMA), an electrophoretic heteroduplex analysis based on the use of a matrix increasing the electrophoretic mobility between homoduplex and heteroduplex³⁶. Primers were designed to include flanking intronic sequences (at least 50 base-pairs from splices sites) and to avoid known polymorphism and prevent mis-priming (primer sequences are available upon request). *PTEN* coding sequences were divided into nine amplicons, analyzed in three multiplex PCRs. EMMA was performed as previously described using EMMA polymer and according to the manufacturer's instructions³⁷. Amplicons showing abnormal profiles were sequenced and analyzed on the ABI PRISM 3130XL Genetic Analyser using the Collection and Sequence Analysis software package (Applied Biosystems, Courtaboeuf, France). Nucleotide position was indicated on the basis of the coding sequence NM_000314.4 and according to the recommended guidelines (available at <http://www.emqn.org/emqn.php> and www.hgvs.org/mutnomen).

Quantitative multiplex PCR (QMP) was carried out with 200 ng of tumor DNA sample, measured on a NanoDrop (ND-1000, Labtech International, France). All primers were designed in order to include flanking intronic sequences and prevent the detection of the processed pseudogene (*PTENI*) that presents 98.6% homology to the cDNA of *PTEN* (the sequences of primers are available upon request). Amplicon sizes ranged from 111-347 bp. Exons of the *PTEN* gene were analyzed by two assays consisting of four and five *PTEN* amplicons, one amplicon of two genes proximal to *PTEN* (*PAPSS2* and *KILLIN*) and amplicons of the *MSH2* and *BRCA1* genes used as internal control for copy number analysis. Multiplex PCRs were performed with the Qiagen Multiplex PCR kit, according to the manufacturer's recommendations (Qiagen, Courtaboeuf, France), and 1 µl of 10X primer mix (final concentration from one to 10 µmol/L of each primer), one of them carrying a 6-FAM label. Eight per cent DMSO was added for the assay including *PTEN* exon 1 and *KILLIN* amplicons. Amplification was performed as recommended by the manufacturer (Qiagen) and 24 cycles were performed for quantitative PCR. DNA fragments generated by QMP were separated by capillary electrophoresis on the ABI 3130XL Genetic Analyser (Applied Biosystems). Data were analyzed with the GeneMapper Software V4.0 and copy number variation was evaluated for each amplicon by calculating the relative peak height for each sample with respect to the mean value of the relative peak height observed in all samples.

Immunohistochemistry

Immunohistochemistry (IHC) for p53 (1:800, DO7, Dako, Carpinteria, CA), HDM2 (1:50, clone# 1F2, Calbiochem), p14 (1:1000, clone 4C6/4, Cell Signaling, Danvers, MA), p-S6K (1:50, clone Ser235/236, Cell Signaling), p-4eBP1 (1:500, clone Thr37/46, Cell Signaling), and p44/p-ERK (1:50; clone Thr202/Tyr204, Cell Signaling) was performed according to manufacturer's recommendations. The immunoreactivity scoring was counted as the percentage of nuclear staining per 10 high-power fields, in several areas, regardless of staining intensity. A 20% cutoff value for detection of positive nuclear reactivities was selected for all antibodies.^{38, 39}

Statistical analysis

Descriptive statistics were used to show the distribution of variables in the population. Differences between groups were evaluated by χ^2 test or Fisher's exact test for categorical variables and t test for continuous variables. The statistical analysis of baseline demographics and clinical outcome is based on all data available up to the cut-off date, 31 August 2011. Disease-free survival was defined as the length of time after diagnosis during which patients had no evidence of disease and was estimated with the use of the Kaplan–Meier method.

RESULTS

TP53 gene alterations are very rare in angiosarcomas

TP53 deletions have been reported to be frequent events in sarcomas with complex karyotypes, especially LMS and UPS. To assess the frequency of this aberration in AS, we analyzed 18 tumors (6 primary and 12 secondary) by array CGH, none of which exhibited copy number loss of the *TP53* gene. We then analyzed the mutational status of *TP53* by sequencing exons 2-11 on genomic DNA in 52 tumors. We found a *TP53* mutation in only two cases (4%) (A138S exon 5, R238Q exon 7), which were not present in the corresponding germline DNA from the same patient. Both cases with *TP53* mutation occurred in patients with a secondary breast AS developed in a previously irradiated field (AS10, AS17). Both tumors carried *MYC* amplification and one of them a *KDR* A1065T exon 24 mutation (AS17), as previously reported 40. Moreover, we observed a R72P

polymorphism in *TP53* exon 4 in 30 cases (58%), including 19 secondary and 11 primary AS. P53 protein expression was then assessed by IHC in 46 cases. Twenty-three tumors (50%) were negative for p53 expression and 23 (50%) were positive (including the two tumors with *TP53* mutation). There was no significant difference of p53 IHC status between primary and secondary AS. Moreover, there was no correlation between the presence of the R72P polymorphism and protein expression.

p53 protein expression correlates with disease-free survival in angiosarcoma

Since p53 protein status assessed by IHC was previously associated with outcome in several tumor types, including sarcomas with complex genomics, we decided to analyze its correlation with clinical behavior in AS. We found that p53 positivity (defined as >20% nuclear positivity) was significantly associated with worse disease-free survival: 3.4 months (95% CI: 0.8-6) versus 14.9 months (95% CI: 2.5-27.4), $p = 0.002$ (Figure 1).

p14 inactivation or HDM2 overexpression does not substitute for TP53 inactivation in angiosarcoma

Since p14 is implicated in p53 degradation by proteasome through its interaction with HDM2, we analyzed the *p14* and *MDM2* copy number alterations by array CGH and their expression level (real-time PCR, IHC) in order to identify the potential loss of p14 expression or HDM2 overexpression, which could compensate for a lack of *TP53* inactivation. However, no deletion of the *CDKN2A* locus encoding for *P14* or *MDM2* amplification was observed by array CGH. Real-time RT-PCR was performed for 19 tumors (10 secondary AS and nine primary AS) and did not reveal any significant difference in *P14* or *MDM2* expression between primary and secondary AS or between AS and other vascular tumors (data not shown). Immunohistochemistry was performed for 43 tumors. We observed P14 and HDM2 expression in 36 tumors out of 40 (89%) and 6 tumors out of 40 (15%) with interpretable results, respectively. We found a concordance between the presence/absence of p14 overexpression and absence/presence of HDM2 expression in 81% of cases.

No PTEN intragenic mutations or deletions are detected in angiosarcoma

PTEN deletion is a frequent event in sarcomas with complex karyotypes. In order to assess the incidence of this genomic aberration in AS, we analyzed a series of 26 tumors using QMP to detect gross genomic rearrangements and by array CGH to detect smaller deletions. Six cases revealed a duplication of all the exons of *PTEN*, whereas eight cases revealed deletion of all the exons. No case with intragenic rearrangement or deletion was identified. These results indicate the presence of large genomic imbalance involving chromosome 10 but do not favor *PTEN* as a target gene. We then used EMMA in order to detect point mutations that may inactivate the function of *PTEN* and substitute the absence of specific deletion. We did not find *PTEN* mutations in this series of AS. We analyzed the expression of *PTEN* gene using published expression data from a series of 21 AS and 3 other vascular tumors previously studied on the U133A Affymetrix platform⁴⁰. Although the expression of *PTEN* was relatively low, it was uniform across the samples and similar between AS and other vascular tumors.

The PIK3CA/AKT/mTOR pathway is activated in a subset of angiosarcoma

Previous studies suggested a crucial role of the PIK3CA/AKT/mTOR pathway in the malignant transformation of endothelial cells. Although, *PTEN* did not appear to be specifically altered in AS we hypothesized that the PIK3CA/AKT/mTOR pathway was activated through alternative mechanisms in at least a subset of AS. We therefore performed further immunohistochemical analyses to evaluate the expression of p-S6K and p-4eBP1 (4E-binding protein 1), two downstream targets of mTORC1. We also evaluated the

activation of the ERK pathway due to its multiple interactions with the PIK3CA/AKT/mTOR pathway and its potential involvement in the phosphorylation of S6 ribosomal protein. We found that 17 tumors out of 40 AS (42%) were positive for pS6K and/or p-4eBP1. We observed also that 12 tumors of 39 (31%) were positive for p44/p-ERK. Eleven of the 17 tumors positive for p-S6K and/or p-4eBP1 were also positive for p44/p-ERK (Pearson correlation coefficient 0.6; $P=0.02$). Then we investigated the incidence of point mutations in *PIK3CA* by screening this gene for 13 common hot spot mutations in exons 3, 10 and 20 in 33 cases of AS (22 secondary and 11 primary). We found a recurrent truncating frame shift mutation in exon 10 of the *PIK3CA* gene (S553fs) in 13 cases (39%) including five primary and eight secondary AS. This mutation was then confirmed by PCR using mutant-specific allele exon 10 primers (Fwd: 5'-CAGTTACTATTCTGTGACTGGTGT-3'; Rev: 5'-AGATTTTCTATGGACCACAGG-3', Fig. S1). In order to explore further the biological significance of this unusual mutation, we sequenced the cDNA of three mutated cases with available RNA. However, no mutation was identified suggesting that the mutant *PIK3CA* transcript undergoes a nonsense-mediated decay and therefore is not detectable at the cDNA level (Fig. S1). Moreover, there was no correlation between the *PIK3CA* mutational status and the p-S6K and/or p-4eBP1 immunohistochemistry results.

NRAS and BRAF are not mutated in angiosarcomas

Given the high proportion of AS being positive for p44/ERK IHC in our series and the potential role of RAS dysregulation in abnormal vascular growth control, we hypothesized that a subset of AS are associated with mutation of the *BRAF* or *NRAS* genes. We screened *BRAF* exon 15 and *NRAS* exons 2 and 3 in 52 cases of AS, however, no mutations were identified at these hot spots.

DISCUSSION

Recent studies investigating the TP53 pathway in large series of sarcomas with complex genomics (LMS, UPS and pleomorphic liposarcomas) have shown that *TP53* alterations are highly recurrent in this group of disease with up to 80% of cases exhibiting at least one *TP53* alteration (mutation and/or deletion)²¹⁻²³. Our results showing deletion and mutation rates of 0% and 4%, respectively, indicate that AS display a different *TP53* alteration profile than the majority of other sarcomas with complex karyotypes. Our findings are in agreement with previous smaller scale reports suggesting that *TP53* mutations are uncommon in AS^{28, 30}.

Interestingly, a P72R polymorphism in exon 4 was detected in 58% of the analyzed cases. Unlike the deleterious tumor-associated P53 mutations, which occur in about 98% of cases in the DNA-binding-domain of the protein, single nucleotide polymorphisms are expected to be phenotypically silent. The R72P polymorphism occurs in the polyproline domain, which lies between the N-terminal transactivating domain and the DNA-binding domain of p53. This polymorphism has been associated with a modulation of the pro-apoptotic potential of p53 and cancer susceptibility⁴¹. However, the level of evidence is poor due to important methodological issues and several meta-analyses failed to demonstrate a risk between the R72P polymorphism and any cancer⁴¹. Interestingly, a high incidence of this polymorphism has been recently reported in atypical vascular lesions occurring after radiotherapy³⁵. In our series, we have observed this SNP in both primary and secondary AS suggesting the absence of any link between this polymorphism and the etiology of AS.

The rarity of deleterious *TP53* mutations or deletions in AS does not necessarily imply that the TP53 pathway is intact and functional in these tumors. p14 acts on the TP53 pathway by interacting with HDM2, an E3 ubiquitin ligase targeting p53 for degradation by the

proteasome. This results in a stabilization and activation of p53. Loss of p14 expression and/or HDM2 overexpression are frequent in sarcomas and have been shown to substitute for p53 inactivation in tumors without *TP53* gene aberrations. Our results indicate that these genetic aberrations are not commonly involved in AS genesis. Despite these results, we observed a high proportion of AS displaying p53 overexpression. Normal p53 protein has an extremely short half-life and it is not detected by IHC. However, following genotoxic stress, the p53 protein level increases rapidly due to stabilization through post-transcriptional mechanisms. In contrast, mutation of the *TP53* gene results in nuclear accumulation of the abnormal protein, which has a longer half-life and can be detected by IHC. Based on the assumption that only stable (presumably mutated) protein will be expressed, TP53 IHC is often used as a surrogate to predict the presence of *TP53* mutations. In our study, however, we observed a high level of discordance between the IHC and the sequencing results. Although the two cases harboring a *TP53* mutation were found to be positive upon immunostaining, 21 cases harboring wild-type *TP53* stained also positive. Such discrepancies have already been reported in several studies suggesting that IHC is not a reliable tool for *TP53* mutation screening^{42, 43}. One hypothesis is that this overexpression is the result of specific oncogenic stresses leading to stabilization of the wild-type protein. In this case, we would expect to observe an improved outcome as the result of increased tumor apoptosis, which was not the case. A second hypothesis is that the nuclear accumulation of p53 is dependent on mechanisms different from mutation and reflects functional changes. Interestingly, p53 overexpression by IHC has been identified as a strong prognostic factor in many human cancers, including soft-tissue sarcomas^{42, 43}. Our data indicate that this biomarker has also a prognostic value in AS.

We have also shown that in contrast with other sarcomas with complex genomics, *PTEN* is neither deleted nor mutated in AS. Moreover, our expression data showing similar level of *PTEN* expression across the AS cases and other vascular tumors suggest that epigenetic silencing or transcriptional defect of *PTEN* is unlikely. All together, our results are in agreement with previous reports showing that constitutional biallelic inactivation of *PTEN* was associated with vascular malformation, but not with malignant vascular tumors⁴⁴.

Despite the lack of PTEN alteration in AS, we have identified an activation of the PIK3CA/AKT/mTOR pathway in a significant proportion of cases. Several studies have already demonstrated the crucial role of PIK3CA/AKT/mTOR signaling in endothelial cell homeostasis⁴⁵. Moreover, embryonal fibroblasts transformed with the *PI3KCA* gene have been shown to induce AS at the site of injection in chicken^{46, 47}. Interestingly, we have found a recurrent frameshift mutation affecting the helical domain of PI3KCA in about 40% of cases. The same mutation has been recently reported at a low incidence rate in one case of large B cell lymphoma⁴⁸ and one case of synovial sarcoma⁴⁹. Our results showing that this mutation is not detectable at cDNA level strongly suggest that this aberration does not have functional consequence. Therefore, further studies are needed to identify the mechanisms involved in the activation of PIK3CA/AKT/mTOR activation in AS. Indeed, the patterns of regulation of the PIK3CA/AKT/mTOR pathway in cancer cells are very complex and cross-talk with several other pathways. For instance, it is now recognized that PI3KCA and MAPK signals converge upstream and downstream of mTORC1⁵⁰. Interestingly, we found a good correlation between the activation of these two pathways in our series with the majority of tumors positive for p-S6K and/or p-4eBP1 being also positive for p44/p-ERK. Overall, our findings may have therapeutic implications, specifically that PI3KCA and/or mTOR antagonists represent a promising approach in the systemic treatment of AS, as already suggested by a recent *in vitro* study and preliminary clinical data^{51, 52}. They suggest also that, besides the inhibition of VEGFR receptors, the inhibition of MAPK signaling may be a relevant approach in AS as suggested by preclinical data⁵³ and may also contribute to the recently described clinical activity of multi-target tyrosine kinase inhibitors such as

sorafenib in AS ⁵⁴. Moreover, synergy is suggested with the combination of mTOR and MAPK inhibitors ⁵⁰.

In summary, we have shown here that AS represents a distinct subgroup among sarcomas with complex karyotypes. Although *TP53* mutation and *PTEN* deletion are frequent in LMS and UPS, these aberrations are not involved in the pathogenesis of AS. However, AS are characterized by a complex pattern of alterations of the TP53 and PIK3CA/AKT/mTOR pathways that is not dependent on the *MYC* amplification status. These results underscore the complex coordinated regulation of the survival and growth-control pathways in AS. Our results represent an important basis for further investigations with the aim of determining how the activities of these pathways are coordinated in AS and for the design of new therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Fletcher, CD.; Unni, K.; Mertens, F. World Health Organization Classification of Tumours Pathology and Genetics of Tumours of Soft Tissue and Bone (2002). IARC Press. G; Lyon: 2002.
2. Fayette J, Martin E, Piperno-Neumann S, Le Cesne A, Robert C, Bonvalot S, Ranchere D, Pouillart P, Coindre JM, Blay JY. Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases. *Ann Oncol.* 2007; 18:2030–6. [PubMed: 17974557]
3. Mandahl N, Jin YS, Heim S, Willen H, Wennerberg J, Biorklund A, Mitelman F. Trisomy 5 and loss of the Y chromosome as the sole cytogenetic anomalies in a cavernous hemangioma/angiosarcoma. *Genes Chromosomes Cancer.* 1990; 1:315–6. [PubMed: 2278963]
4. Kindblom LG, Stenman G, Angervall L. Morphological and cytogenetic studies of angiosarcoma in Stewart-Treves syndrome. *Virchows Arch A Pathol Anat Histopathol.* 1991; 419:439–45. [PubMed: 1750189]
5. Gil-Benso R, Lopez-Gines C, Soriano P, Almenar S, Vazquez C, Llombart-Bosch A. Cytogenetic study of angiosarcoma of the breast. *Genes Chromosomes Cancer.* 1994; 10:210–2. [PubMed: 7522046]
6. Van den Berg E, Van Oven MW, de Jong B, Dam A, Wiersema J, Dijkhuizen T, Hoekstra HJ, Molenaar WM. Comparison of cytogenetic abnormalities and deoxyribonucleic acid ploidy of benign, borderline malignant, and different grades of malignant soft tissue tumors. *Lab Invest.* 1994; 70:307–13. [PubMed: 8145525]
7. Cerilli LA, Huffman HT, Anand A. Primary renal angiosarcoma: a case report with immunohistochemical, ultrastructural, and cytogenetic features and review of the literature. *Arch Pathol Lab Med.* 1998; 122:929–35. [PubMed: 9786357]
8. Wong KF, So CC, Wong N, Siu LL, Kwong YL, Chan JK. Sinonasal angiosarcoma with marrow involvement at presentation mimicking malignant lymphoma: cytogenetic analysis using multiple techniques. *Cancer Genet Cytogenet.* 2001; 129:64–8. [PubMed: 11520569]

9. Zu Y, Perle MA, Yan Z, Liu J, Kumar A, Waisman J. Chromosomal abnormalities and p53 gene mutation in a cardiac angiosarcoma. *Appl Immunohistochem Mol Morphol*. 2001; 9:24–8. [PubMed: 11277410]
10. Baumhoer D, Gunawan B, Becker H, Fuzesi L. Comparative genomic hybridization in four angiosarcomas of the female breast. *Gynecol Oncol*. 2005; 97:348–52. [PubMed: 15863129]
11. Cao Y, Zou SM, Zhang KT, Lu N, Liu Y, Feng L, Wen P, Han NJ, Lin DM. Genetic alterations in pulmonary epithelioid hemangioendothelioma and epithelioid angiosarcoma. *Histol Histopathol*. 2011; 26:491–6. [PubMed: 21360442]
12. Manner J, Radlwimmer B, Hohenberger P, Mossinger K, Kuffer S, Sauer C, Belharazem D, Zettl A, Coindre JM, Hallermann C, Hartmann JT, Katenkamp D, Katenkamp K, Schoffski P, Sciort R, Wozniak A, Lichter P, Marx A, Strobel P. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. *Am J Pathol*. 2010; 176:34–9. [PubMed: 20008140]
13. Guo T, Chang NE, Singer S, Maki RG, Antonescu CR. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. *Genes Chromosomes Cancer*. 2011; 50:25–33. [PubMed: 20949568]
14. Mao JH, Wu D, Perez-Losada J, Nagase H, DelRosario R, Balmain A. Genetic interactions between Pten and p53 in radiation-induced lymphoma development. *Oncogene*. 2003; 22:8379–85. [PubMed: 14627978]
15. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C, Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*. 2005; 436:725–30. [PubMed: 16079851]
16. Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer*. 2006; 6:184–92. [PubMed: 16453012]
17. Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. *Genes Dev*. 2008; 22:436–48. [PubMed: 18281460]
18. Kwon CH, Zhao D, Chen J, Alcantara S, Li Y, Burns DK, Mason RP, Lee EY, Wu H, Parada LF. Pten haploinsufficiency accelerates formation of high-grade astrocytomas. *Cancer Res*. 2008; 68:3286–94. [PubMed: 18451155]
19. Zheng H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, Perry SR, Tonon G, Chu GC, Ding Z, Stommel JM, Dunn KL, Wiedemeyer R, You MJ, Brennan C, Wang YA, Ligon KL, Wong WH, Chin L, DePinho RA. p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature*. 2008; 455:1129–33. [PubMed: 18948956]
20. Puzio-Kuter AM, Castillo-Martin M, Kinkade CW, Wang X, Shen TH, Matos T, Shen MM, Cordon-Cardo C, Abate-Shen C. Inactivation of p53 and Pten promotes invasive bladder cancer. *Genes Dev*. 2009; 23:675–80. [PubMed: 19261747]
21. Perot G, Chibon F, Montero A, Lagarde P, de The H, Terrier P, Guillou L, Ranchere D, Coindre JM, Aurias A. Constant p53 pathway inactivation in a large series of soft tissue sarcomas with complex genetics. *Am J Pathol*. 2010; 177:2080–90. [PubMed: 20884963]
22. Ghadimi MP, Liu P, Peng T, Bolshakov S, Young ED, Torres KE, Colombo C, Hoffman A, Broccoli D, Hornick JL, Lazar AJ, Pisters P, Pollock RE, Lev D. Pleomorphic liposarcoma: Clinical observations and molecular variables. *Cancer*. 2011
23. Ito M, Barys L, O'Reilly T, Young S, Gorbacheva B, Monahan J, Zumstein-Mecker S, Choong PF, Dickinson I, Crowe P, Hemmings C, Desai J, Thomas DM, Lisztwan J. Comprehensive mapping of p53 pathway alterations reveals an apparent role for both SNP309 and MDM2 amplification in sarcomagenesis. *Clin Cancer Res*. 2011; 17:416–26. [PubMed: 21159888]
24. Yang J, Du X, Chen K, Ylipaa A, Lazar AJ, Trent J, Lev D, Pollock R, Hao X, Hunt K, Zhang W. Genetic aberrations in soft tissue leiomyosarcoma. *Cancer Lett*. 2009; 275:1–8. [PubMed: 18649996]
25. Gibault, L. PhD thesis. University Paris Descartes; Paris: 2010. Recurrent genomic alterations in sarcomas with complex genomics: PTEN and DKK1 loss of expression, mechanisms and biological consequences. (Print)

26. Hernando E, Charytonowicz E, Dudas ME, Menendez S, Matushansky I, Mills J, Socci ND, Behrendt N, Ma L, Maki RG, Pandolfi PP, Cordon-Cardo C. The AKT-mTOR pathway plays a critical role in the development of leiomyosarcomas. *Nat Med.* 2007; 13:748–53. [PubMed: 17496901]
27. Hollstein M, Marion MJ, Lehman T, Welsh J, Harris CC, Martel-Planche G, Kusters I, Montesano R. p53 mutations at A:T base pairs in angiosarcomas of vinyl chloride-exposed factory workers. *Carcinogenesis.* 1994; 15:1–3. [PubMed: 8293534]
28. Soini Y, Welsh JA, Ishak KG, Bennett WP. p53 mutations in primary hepatic angiosarcomas not associated with vinyl chloride exposure. *Carcinogenesis.* 1995; 16:2879–81. [PubMed: 7586214]
29. Naka N, Tomita Y, Nakanishi H, Araki N, Hongyo T, Ochi T, Aozasa K. Mutations of p53 tumor-suppressor gene in angiosarcoma. *Int J Cancer.* 1997; 71:952–5. [PubMed: 9185695]
30. Zietz C, Rossle M, Haas C, Sendelhofert A, Hirschmann A, Sturzl M, Lohrs U. MDM-2 oncoprotein overexpression, p53 gene mutation, and VEGF up-regulation in angiosarcomas. *Am J Pathol.* 1998; 153:1425–33. [PubMed: 9811333]
31. Amo Y, Masuzawa M, Hamada Y, Katsuoka K. Serum concentrations of vascular endothelial growth factor in angiosarcomas with and without p53 gene mutation. *Acta Derm Venereol.* 2002; 82:373–4. [PubMed: 12430738]
32. Weihrauch M, Bader M, Lehnert G, Koch B, Wittekind C, Wrbitzky R, Tannapfel A. Mutation analysis of K-ras-2 in liver angiosarcoma and adjacent nonneoplastic liver tissue from patients occupationally exposed to vinyl chloride. *Environ Mol Mutagen.* 2002; 40:36–40. [PubMed: 12211074]
33. Domfeh AB, Fichera M, Hunt JL. Allelic loss of 3 different tumor suppressor gene loci in benign and malignant endothelial tumors of the head and neck. *Arch Pathol Lab Med.* 2006; 130:1184–7. [PubMed: 16879020]
34. Tate G, Suzuki T, Mitsuya T. Mutation of the PTEN gene in a human hepatic angiosarcoma. *Cancer Genet Cytogenet.* 2007; 178:160–2. [PubMed: 17954274]
35. Santi R, Cetica V, Franchi A, Pepi M, Cesinaro AM, Miracco C, Paglierani M, De Giorgi V, Delfino C, Difonzo EM, Pimpinelli N, Bianchi S, Sardi I, Santucci M, Massi D. Tumour suppressor gene TP53 mutations in atypical vascular lesions of breast skin following radiotherapy. *Histopathology.* 2011; 58:455–66. [PubMed: 21323968]
36. Weber J, Miserere S, Champ J, Looten R, Stoppa-Lyonnet D, Viovy JL, Houdayer C. High-throughput simultaneous detection of point mutations and large-scale rearrangements by CE. *Electrophoresis.* 2007; 28:4282–8. [PubMed: 17990260]
37. Caux-Moncoutier V, Castera L, Tirapo C, Michaux D, Remon MA, Lauge A, Rouleau E, De Pauw A, Buecher B, Gauthier-Villars M, Viovy JL, Stoppa-Lyonnet D, Houdayer C. EMMA, a cost- and time-effective diagnostic method for simultaneous detection of point mutations and large-scale genomic rearrangements: application to BRCA1 and BRCA2 in 1,525 patients. *Hum Mutat.* 2011; 32:325–34. [PubMed: 21120943]
38. Cordon-Cardo C, Reuter VE. Alterations of tumor suppressor genes in bladder cancer. *Semin Diagn Pathol.* 1997; 14:123–32. [PubMed: 9179973]
39. Mallofre C, Castillo M, Morente V, Sole M. Immunohistochemical expression of CK20, p53, and Ki-67 as objective markers of urothelial dysplasia. *Mod Pathol.* 2003; 16:187–91. [PubMed: 12640096]
40. Antonescu CR, Yoshida A, Guo T, Chang NE, Zhang L, Agaram NP, Qin LX, Brennan MF, Singer S, Maki RG. KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors. *Cancer Res.* 2009; 69:7175–9. [PubMed: 19723655]
41. Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. *Nat Rev Cancer.* 2009; 9:95–107. [PubMed: 19165225]
42. Das P, Kotilingam D, Korchin B, Liu J, Yu D, Lazar AJ, Pollock RE, Lev D. High prevalence of p53 exon 4 mutations in soft tissue sarcoma. *Cancer.* 2007; 109:2323–33. [PubMed: 17429838]
43. Robles AI, Harris CC. Clinical outcomes and correlates of TP53 mutations and cancer. *Cold Spring Harb Perspect Biol.* 2010; 2:a001016. [PubMed: 20300207]
44. Caux F, Plauchu H, Chibon F, Faivre L, Fain O, Vabres P, Bonnet F, Selma ZB, Laroche L, Gerard M, Longy M. Segmental overgrowth, lipomatosis, arteriovenous malformation and epidermal

- nevus (SOLAMEN) syndrome is related to mosaic PTEN nullizygosity. *Eur J Hum Genet.* 2007; 15:767–73. [PubMed: 17392703]
45. Dimmeler S, Zeiher AM. Akt takes center stage in angiogenesis signaling. *Circ Res.* 2000; 86:4–5. [PubMed: 10625297]
 46. Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tsichlis PN, Cantley LC, Roberts TM, Vogt PK. Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3-kinase. *Science.* 1997; 276:1848–50. [PubMed: 9188528]
 47. Aoki M, Batista O, Bellacosa A, Tsichlis P, Vogt PK. The akt kinase: molecular determinants of oncogenicity. *Proc Natl Acad Sci U S A.* 1998; 95:14950–5. [PubMed: 9843996]
 48. Baohua Y, Xiaoyan Z, Tiecheng Z, Tao Q, Daren S. Mutations of the PIK3CA gene in diffuse large B cell lymphoma. *Diagn Mol Pathol.* 2008; 17:159–65. [PubMed: 18382359]
 49. Barretina J, Taylor BS, Banerji S, Ramos AH, Lagos-Quintana M, Decarolis PL, Shah K, Socci ND, Weir BA, Ho A, Chiang DY, Reva B, Mermel CH, Getz G, Antipin Y, Beroukhi R, Major JE, Hatton C, Nicoletti R, Hanna M, Sharpe T, Fennell TJ, Cibulskis K, Onofrio RC, Saito T, Shukla N, Lau C, Nelander S, Silver SJ, Sougnez C, Viale A, Winckler W, Maki RG, Garraway LA, Lash A, Greulich H, Root DE, Sellers WR, Schwartz GK, Antonescu CR, Lander ES, Varmus HE, Ladanyi M, Sander C, Meyerson M, Singer S. Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy. *Nat Genet.* 2010; 42:715–21. [PubMed: 20601955]
 50. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. *Oncogene.* 2008; 27:5527–41. [PubMed: 18794886]
 51. Schuetze SM, Baker LH, Maki RG. Sirolimus reduced tumor-related morbidity and resulted in biochemical and radiographic response in patients with progressive sarcoma. *J Clin Oncol.* 2006; 24:9503.
 52. Bundscherer A, Vogt T, Kohl G, Landthaler M, Hafner C. Antiproliferative effects of rapamycin and celecoxib in angiosarcoma cell lines. *Anticancer Res.* 2010; 30:4017–23. [PubMed: 21036716]
 53. LaMontagne KR Jr, Moses MA, Wiederschain D, Mahajan S, Holden J, Ghazizadeh H, Frank DA, Arbiser JL. Inhibition of MAP kinase kinase causes morphological reversion and dissociation between soft agar growth and in vivo tumorigenesis in angiosarcoma cells. *Am J Pathol.* 2000; 157:1937–45. [PubMed: 11106566]
 54. Maki RG, D'Adamo DR, Keohan ML, Saulle M, Schuetze SM, Undevia SD, Livingston MB, Cooney MM, Hensley ML, Mita MM, Takimoto CH, Kraft AS, Elias AD, Brockstein B, Blachere NE, Edgar MA, Schwartz LH, Qin LX, Antonescu CR, Schwartz GK. Phase II study of sorafenib in patients with metastatic or recurrent sarcomas. *J Clin Oncol.* 2009; 27:3133–40. [PubMed: 19451436]

Figure 1.

Immunohistochemical (IHC) results for p53 (A,B,C), p14 (D), HDM2 (E) and prognostic impact of p53 expression (F). (A,B) Diffuse and strong nuclear reactivity for p53 in a secondary AS associated with a *TP53* mutation and *KDR* mutation (A: AS17, 200x) and a primary AS without *TP53* mutation (B: AS59, 200x). (C) Absence of expression of p53 in a case of secondary AS (AS19, 200x). (D) Diffuse nuclear reactivity for p14 in a secondary AS (AS20, 200x). (E) Absence of expression of HDM2 in a case of secondary AS (AS39, 200x). (F) Kaplan Meier curve for disease-free survival according to p53 IHC status (blue line: negative; green line: positive).

Figure 2. Immunohistochemical (IHC) results for p-S6K (A), p-4eBP1 (B) and p44/p-ERK (C). Diffuse and strong reactivity for pS6K (A, 200x), p-4eBP1 (B, 200x) and p44/p-ERK (C, 200x) in a primary angiosarcoma (AS9).

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Table 1

Clinical and pathologic characteristics of angiosarcoma patients

Case ID	Age	Gender	Previous radiation therapy	Chronic Lymphedema	Primary location
AS3 ^δ	58	M	No	No	Femur
AS4	63	F	Yes	No	Breast
AS5	82	F	No	No	Thigh
AS6	49	F	No	No	Humerus
AS9 ^δ	70	M	No	No	Thigh
AS10 ^δ	70	F	Yes	No	Breast
AS11 ^δ	50	M	No	No	Spleen
AS12	66	M	No	No	Kidney
AS13	67	F	No	Yes	Forearm
AS15 ^δ	61	F	Yes	No	Breast
AS14	40	F	No	No	Breast
AS16	34	M	No	No	Pelvis
AS17	74	F	Yes	No	Breast
AS19	62	F	Yes	No	Breast
AS20 ^δ /AS21	56	F	Yes	No	Breast
AS22	67	F	No	No	Breast
AS25	70	F	Yes	No	Chest wall
AS26	34	F	No	No	Breast
AS27	37	F	No	No	Breast
AS28	54	M	No	No	Pelvis
AS29 ^δ	75	F	Yes	No	Breast
AS30 ^δ	38	F	Yes	No	Head and Neck
AS31	79	M	No	No	Scalp
AS32 ^δ	76	F	Yes	No	Breast
AS33	69	M	No	No	Thigh

Case ID	Age	Gender	Previous radiation therapy	Chronic Lymphedema	Primary location
AS34	43	F	No	No	Breast
AS36	77	F	No	No	Breast
AS37	71	F	No	No	Mediastinum
AS38 ^δ	74	F	No	Yes	Forearm
AS39 ^δ	84	M	No	Yes	Arm
AS44	63	F	Yes	No	Breast
AS45	66	F	Yes	No	Breast
AS47	65	F	Yes	No	Breast
AS48	74	F	Yes	No	Breast
AS49	43	F	Yes	No	Chest wall
AS56	64	F	No	No	Breast
AS57	66	F	Yes	No	Thigh
AS58	74	M	No	No	Scalp
AS59	79	M	No	No	Nostril
AS61	83	F	Yes	No	Breast
AS62	52	F	No	No	Liver
AS66	77	M	No	No	Femur
AS68 ^δ	80	F	Yes	No	Breast
AS69	60	F	Yes	No	Breast
AS70 ^δ	38	F	No	No	Breast
AS71	41	F	Yes	No	Spleen
AS72	66	F	No	Yes	Breast
AS73 ^δ	83	F	Yes	No	Breast
AS75	80	M	No	No	Head and Neck
AS76 ^δ	76	M	Yes	No	Bladder
AS77	73	M	No	No	Retroperitoneum
AS79	48	F	No	No	Spine
AS83	65	M	Yes	No	Breast

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Case ID	Age	Gender	Previous radiation therapy	Chronic Lymphedema	Primary location
AS85	76	F	Yes	No	Breast
AS100	60	M	No	No	Chest wall
AS101	62	F	Yes	No	Breast
AS109	63	M	No	No	Spleen
AS117	38	F	Yes	No	Head and Neck
AS123 ^δ	76	F	Yes	No	Breast
AS124 ^δ	57	M	No	No	Head and Neck
AS125 ^δ	73	F	No	No	Breast

^δ tumors studied by aCGH.