DNA Methylation Profiling Identifies Distinct Clusters in Angiosarcomas

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ABSTRACT

Purpose: DNA methylation profiling has previously uncovered biologically and clinically meaningful subgroups within many tumor types, but was not yet performed in angiosarcoma. Angiosarcoma is a rare sarcoma with very heterogeneous clinical presentations, which may be based on differences in biological background. In this exploratory study, DNA methylation profiling of 36 primary angiosarcoma samples from visceral, deep soft tissue, radiation-induced, and UV-induced localizations was performed.

Experimental Design: Primary angiosarcoma formalin-fixed paraffin-embedded samples from visceral, soft tissue, radiation-induced, and UV-induced origin were collected from a nationwide search for angiosarcoma in the Netherlands. DNA was extracted for methylation profiling with the Illumina Infinium MethylationEPIC array. Quality control assessment and unsupervised hierarchical clustering were performed. Copy-number profiles were generated and analyzed for chromosomal stability. Clinical data were obtained from the Netherlands Cancer Registry.

Results: DNA methylation profiling by unsupervised hierarchical clustering of 36 angiosarcoma samples (6 visceral, 5 soft tissue, 14 radiation-induced, 11 UV-induced) revealed two main clusters (A and B), which were divided into four subclusters. The clusters largely corresponded with clinical subtypes, showing enrichment of UV-induced cases in cluster A1 and radiation-induced cases in cluster A2. Visceral and soft tissue cases almost exclusively fell into cluster B. Cluster A showed significantly increased chromosomal instability and better overall survival (22 vs. 6 months, $P = 0.046$) compared with cluster B.

Conclusions: In this novel methylation profiling study, we demonstrated for the first time four different angiosarcoma clusters. These clusters correlated with clinical subtype, overall survival, and chromosomal stability.

Introduction

Angiosarcoma is a rare sarcoma with endothelial properties with an incidence of about 1.5 in 1,000,000 persons per year (1). Clinical presentation is heterogeneous as angiosarcoma can be localized in any organ or tissue. Angiosarcoma may occur both sporadically or secondary to predisposing factors such as radiotherapy, UV exposure, or chronic lymphedema. Current angiosarcoma classification is based on clinical characteristics, including site of origin, etiology, or a combination of both. The reported prevalence of these different clinical subtypes highly depends on the subsets of patients per study and is therefore difficult to estimate. In some studies, angiosarcoma of deep soft tissue or organs are most prevalent (50%; ref. 2), whereas others report UV-associated AS of head and neck region (43%; ref. 3) or radiation-induced angiosarcoma of the breast (35%; ref. 4) to be most common. Dependent on previous treatments in case of secondary angiosarcoma, the mainstay of localized angiosarcoma treatment consists of surgery, if indicated, (neo)adjuvant radiotherapy and/or doxorubicin-based or taxane single-agent chemotherapy (5). Although clinical subtype has been described as a prognostic factor for survival or response to taxane treatment, this correlation remains inconclusive (4, 6–8). Some studies, for instance, found inferior survival for patients with radiation-induced angiosarcoma (9), whereas other, larger studies reported no difference between sporadic and radiation-induced angiosarcoma (10, 11). UV-induced angiosarcoma of the head and neck region have been associated with unfavorable survival by some (6, 12), but others demonstrated no difference in survival between clinical subgroups at all (2, 13). Overall, the large variety in clinical subtype definitions and different composition of angiosarcoma cohorts between studies might explain the discrepancies in reported outcomes and prognostic factors.

Increased understanding of angiosarcoma biology could help to clarify whether clinical subtypes relate to a distinct biological background, and to optimize future treatment strategies. Until now, the identification of specific genetic alterations such as mutations or gene amplifications has proven difficult to correlate with clinical subtypes, with the exception of MYC amplification which is present in the vast majority of radiation-induced angiosarcoma (14–16). Analysis of chromosomal aberrations by array-comparative genomic hybridization (CGH) did reveal a difference in chromosomal stability in five sporadic angiosarcoma cases (visceral and soft tissue origin; ref. 17).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2020;26:93–100

doi: 10.1158/1078-0432.CCR-19-2180

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Translational Relevance

With our results, we provide preclinical data supporting the hypothesis that biologically different subtypes of angiosarcoma indeed exist. We demonstrate distinct clusters of patients with angiosarcoma, which largely correspond with clinical subtype. Furthermore, we show that these clusters differ significantly in terms of median overall survival and chromosomal stability. These findings could offer a next step toward a more specific diagnosis for patients with angiosarcoma, possibly enabling clinicians to provide more precise prognostic information in the future. In addition, dividing patients with angiosarcoma into clusters could contribute to more effective clinical research by selecting only those patients who are most likely to benefit from a specific treatment. For example, we found MGMT hypermethylation was present exclusively in one of the four clusters. Further exploration of this target could provide additional treatment options for these particular patients and ultimately improve patient survival.

Two cases showed a complex profile with a substantial number of alterations, whereas the other three harbored none to a few alterations. However, these findings have not yet been confirmed in a larger series. Clustering based on RNA expression profiles of 22 primary and secondary angiosarcoma samples showed that radiation-induced and post lymphedema angiosarcoma fell into a distinct cluster, and that most primary angiosarcoma samples clustered together as well (18). In this study, head and neck angiosarcoma was considered primary angiosarcoma.

DNA methylation is an epigenetic modification that is strongly associated with gene expression regulation (19). In that way, DNA methylation patterns reflect both the cell of origin and gene expression changes associated with different tumor types. In recent years, novel methods for tumor class discovery and classification have been developed on the basis of genome-wide DNA methylation patterns (20, 21). These improved classifications offer perspectives in terms of tumor diagnosis as well as risk stratification and treatment possibilities.

So far, DNA methylation in AS has only been investigated in relation to the tumor suppressor gene cyclin-dependent kinase inhibitor 2A (CDKN2A, also known as INK4A/ARF), demonstrating hypermethylation of the CDKN2A (p16INK4A) promoter in a majority of sporadic liver angiosarcoma (22).

In this study, we aimed to investigate whether clinically relevant different angiosarcoma subtypes can be discriminated by genome-wide DNA methylation patterns. For this purpose, we analyzed 36 angiosarcoma primary tumors of four different clinical subgroups (radiation-induced, UV-induced, soft tissue, and visceral angiosarcoma) by Illumina MethylationEPIC arrays.

Materials and Methods

Tissue collection

A nationwide search for angiosarcoma cases diagnosed between 1989 and 2015 in the Netherlands was performed through PALGA (Dutch nationwide network and registry of histo- and cytopathology; ref. 23). Slides and appropriate blocks when available ($n = 656$) and pathology reports were obtained from 37 pathology labs throughout the Netherlands and reviewed by an expert pathologist (U.E. Flucke), yielding 479 confirmed angiosarcoma cases. These cases were divided into clinical subgroups based on the available clinical data and pathology reports. As a pilot, we randomly selected 43 angiosarcoma primary tumors available for methylation profiling, consisting of 17 radiation-induced, 11 UV-induced, 7 soft tissue, and 8 visceral angiosarcoma. radiation-induced cases developed due to previous radiotherapy and were all located in the breast area, whereas the UV-induced cases originated in sun-exposed skin of the scalp. Visceral cases were all primarily located in an organ (e.g., liver, thyroid, gastrointestinal), and soft tissue cases were by definition located in the deep soft tissues.

Clinical data

Clinical data were obtained through a query of angiosarcoma cases diagnosed between 1989 and 2015 in the nationwide Netherlands Cancer Registry. These data were linked to the data from PALGA. If applicable, data from original pathology reports were used to complement the clinical database. Ethical approval for the study was obtained from the local certified Medical Ethics Committee of the Radboud University Medical Center, Nijmegen, the Netherlands (file number 2016-2686).

DNA extraction

H&E slides were examined to select areas with the highest available tumor content. DNA was extracted from these areas in formalin-fixed paraffin-embedded tissue blocks. Extraction was performed using the automated Maxwell system (Promega). DNA concentrations were measured using SYBR green.

EPIC array, methylome clustering, and copy-number profiling

The Illumina Infinium MethylationEPIC array was used to obtain DNA methylation profiles of at least 850,000 methylation sites across the genome (Illumina) according to the manufacturer's instructions at the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ), Heidelberg, Germany. Methylation levels were quantified using the beta value, calculated as the ratio of intensities between methylated (M) and unmethylated (U) alleles.

To judge data quality of raw DNA methylation bead array data, a quality control (QC) report was generated for individual samples based on three existing R-packages (Minfi, Meffil, and ENmix; refs. 24–27). The QC report included six sample independent probes, nine sample dependent probes, beta value distribution plot, and quantified quality scores for the beta value distribution. Within each QC report, all failures for sample independent or dependent control probes were summed and samples with more than 20 failing quality scores were excluded from further analyses.

Intensity values were converted to beta values, after which missing values were imputed using probabilistic principal component analysis from the R package pcaMethods (version 1.60.0; ref. 28). The imputation was followed by filtering out all probes representing non-CpG sites, reference SNPs, or probes that are targeting chromosome X or Y (build 37). The resulting dataset was used to select features representing the 1% most varying probes. The feature selection was used as input to heatmap3 (version 1.1.1) with default settings (29). To examine the stability of a cluster dendrogram, bootstrapping with $n = 1,000$ was performed with the package pvclust (version 2.00; refs. 30, 31; see full description of DNA methylation calculation and bootstrapping method in Supplementary Material).

Copy-number assessment for segmental or entire chromosomal changes was done manually based on array data by a proprietary algorithm based on the R package conumee after additional baseline correction (https://github.com/dstichel/conumee).

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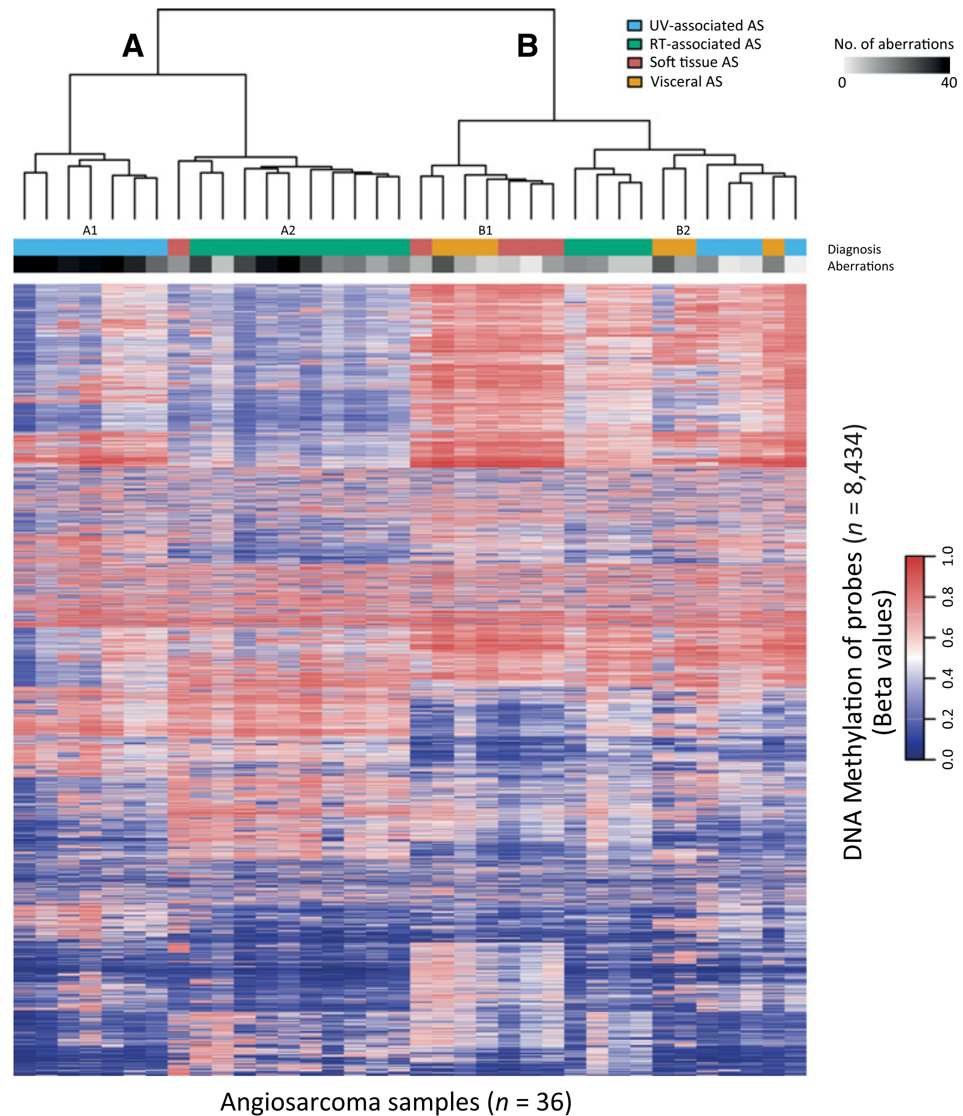

Figure 1.
Unsupervised hierarchical clustering. Unsupervised hierarchical clustering of methylation levels of 8,434 probes (1% most varying). The level of DNA methylation (beta value) is represented with a color scale. The clinical subgroup is indicated by color. The number of chromosomal aberrations per case is represented with a color scale. AS, angiosarcoma; RT, radiation-induced; UV, UV-induced.

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Statistical analysis

Differences between clinical subgroups and clusters were determined using the appropriate statistical tests (χ^2, one-way ANOVA with Bonferroni correction for multiple comparisons). Univariate survival analyses were performed using the Kaplan–Meier method, applying the log-rank test to detect differences between survival curves. Correlation between age and chromosomal stability was assessed using bivariate Pearson correlation. All statistical analyses were performed with IBM SPSS Statistics, version 25.0.0.1.

Promoter methylation status determination

Methylation of MGMT was calculated using MNPpredict_mgmt from R package MNP (version 11b4; refs. 32, 33), which is based on the methylation status of probes cg12981137 and cg12434587. Methylation status of CDKN2A was accessed by retrieving methylation status of all probes designed in the promoter region CHR9:21974934-21976355 (build 37).

Methylation-specific PCR MGMT

Bisulfite conversion of 200 ng DNA was performed using the EZ DNA Methylation-Direct Kit (catalog no. D5020, Zymo Research, Inc.) according to the manufacturer's protocol. Subsequently, the DNA was amplified by two PCR reactions, one specific for the methylated MGMT promoter (MGMT-M) and one specific for the unmethylated MGMT promoter (MGMT-U), which serves as a control. PCR was performed using the AmpliTaq Gold 360 Master Mix (Applied Biosystems) with the following PCR program: 95°C for 10 minutes; 95°C for 30 seconds; 58°C for 30 seconds; 72°C for 1 minute, 40 cycles; and 72°C for 7 minutes. Primers specific for unmethylated MGMT (5-TGTGTTTTTAGAATGTTTTGTGTTTTGAT-3, 5-CTACCACCATCCCAAAAAAAAAACTCCA-3) or methylated MGMT (5- GTTTTTAGAACGTTTTGCGTTTCGAC-3, 5-CACCGTCCCGAAAAAAAACTCCG-3) were used. PCR products were analyzed by agarose gel electrophoresis. Bisulfite-converted DNA from patients with glioblastoma with and without MGMT methylation was used as positive and negative control, respectively.

Results

A total of 43 primary AS tumor samples were analyzed for genome-wide array-based DNA methylation. Four cases were excluded because of low tumor percentage (<50%). On the basis of the QC report, another three samples were excluded from further analysis. This resulted in 36 samples for clustering and copy-number variation analysis (14 radiation-induced, 11 UV-induced, 5 soft tissue, and 6 visceral angiosarcoma). Median age for the entire cohort was 74 years and did not differ significantly between the clinical subgroups (Supplementary Table S1). The male-to-female ratio was 1:1.6 for the entire cohort; the median overall survival was 9.0 months [95% confidence interval (CI), 0.5–17.6 months]. Demographic and clinical characteristics of the 36 assessed cases were representative of the total group of angiosarcoma cases with median age 70 years, male-to-female ratio 1:1.9, and median overall survival 12.8 months (95% CI, 10.1–15.5 months), respectively. The ratio between secondary (radiation-induced/UV-induced) and primary (soft tissue/visceral) cases was also representative (1:0.4) for both cohorts.

Clustering

After unsupervised hierarchal clustering on the 36 primary samples, two main clusters were observed (A and B, both $n = 18$), which were each subdivided into two separate clusters (A1, A2 and B1, B2; **Fig. 1**). These clusters consisted of 7 (A1, B1) and 11 (A2, B2) patients and were found to be stable by bootstrapped clustering. Cluster A1 consisted exclusively of UV-induced-associated cases, whereas A2 primarily consisted of radiation-induced-induced cases. Cluster B1 had both visceral and soft tissue cases, and cluster B2 contained radiation-induced, UV-induced, and visceral angiosarcoma. Median age did not differ between the four clusters ($P = 0.774$). When comparing the two main clusters A and B, median overall survival was significantly longer in cluster A than in cluster B (22.2 vs. 5.5 months, $P = 0.046$; **Fig. 2**).

Chromosomal stability

We assessed the number of chromosomal gains and losses per case by copy-number profile analysis (**Fig. 3**). An average of 20 aberrations per case was found, ranging from 3 to 39 aberrations. The number of aberrations did not differ significantly between clinical subtypes (**Fig. 4A**) and did not correlate with age ($P = 0.889$). Analysis of chromosomal stability in relation to the clusters revealed a significantly higher number of chromosomal aberrations in cluster A (27.5 ± 9.8 SD) compared with cluster B (12.0 ± 6.9 SD; $P < 0.001$; **Fig. 4B**). Between the four clusters, the mean number of aberrations differed significantly with the exception of cluster B1 compared with B2 (**Fig. 4C**). Within the UV-induced-associated cases, those in cluster A1 were significantly more unstable, harboring a higher number of aberrations compared with the UV-induced cases in cluster B2 (34.4 ± 5.4 SD vs. 7.3 ± 6.6 SD, $P < 0.001$; **Fig. 4D**). These unstable UV-induced cases (cluster A1) showed a trend toward favorable survival compared with the more stable UV-induced cases in cluster B2 (median overall survival, 22.1 vs. 3.1 months, $P = 0.071$; **Fig. 4F**). As for the radiation-induced cases, the number of aberrations was significantly higher for the radiation-induced cases in cluster A2 compared with the radiation-induced cases in cluster B2 (23.8 ± 9.7 SD vs. 4.9 ± 2.5 SD, $P = 0.045$; **Fig. 4E**). Median overall survival did not differ between these two groups (22.8 vs. 10.2 months, $P = 0.627$; **Fig 4G**).

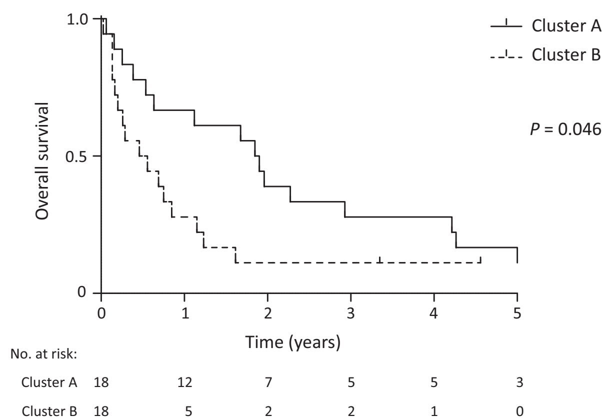

Figure 2.
Overall survival clusters A and B. Kaplan–Meier curves showing the difference in 5-year overall survival between clusters A and B.

Specific alterations

CDKN2A loss

By copy-number profile analysis, specific loss of CDKN2A was found in 19% (7/36) of angiosarcoma and was associated with the non–RT-induced clinical subtypes, as CDKN2A was lost in 7 of 22 (32%) of non–radiation-induced tumors versus 0/14 radiation-induced tumors ($P = 0.029$). In cluster A, only 1 of 18 cases (6%) harbored specific CDKN2A loss, compared with 6 of 18 (33%) cases in cluster B ($P = 0.088$). Analysis of the CDKN2A promoter methylation status showed that the CDKN2A promoter was unmethylated in all samples (results not shown).

MGMT promoter methylation

Methylation status of the O6-methylguanine-DNA methyltransferase (MGMT) promoter region was specifically studied because of its potential clinical relevance. In our cohort, methylation of the MGMT promoter based on probes cg12981137 and cg12434587 was present in 19% (7/36) of cases, consisting of 4 soft tissue (80%) and 3 visceral (50%) angiosarcoma (Supplementary Table S1). All 7 methylated cases fell into cluster B1 (7/7, 100%). Validation was performed by PCR for 3 cases, of which 2 were methylated and 1 unmethylated according to the MethylationEPIC array data. The array results were confirmed in all three cases (Supplementary Fig. S1).

MYC amplification

As expected, all of the radiation-induced cases harbored MYC amplification by copy-number assessment (14/14, 100%).

Discussion

In this study, we identified four distinct clusters of angiosarcoma that were largely correlated with clinical subtype. Interestingly, UV-associated cases fell into 2 clusters (A1 and B2). Radiation-induced cases also fell into 2 different clusters (A2 and B2), suggesting that both these clinical subtypes may consist of two different subgroups. This might be related to chromosomal instability, as the number of aberrations significantly differed between UV-induced cases in clusters A1 and B2, and between radiation-induced cases in clusters A2 and B2. Overall, a remarkably higher number of chromosomal aberrations were observed in cluster A compared with cluster B. This may be explained by the fact that cluster A almost exclusively consists of secondary (radiation-induced and UV-induced) angiosarcoma cases.

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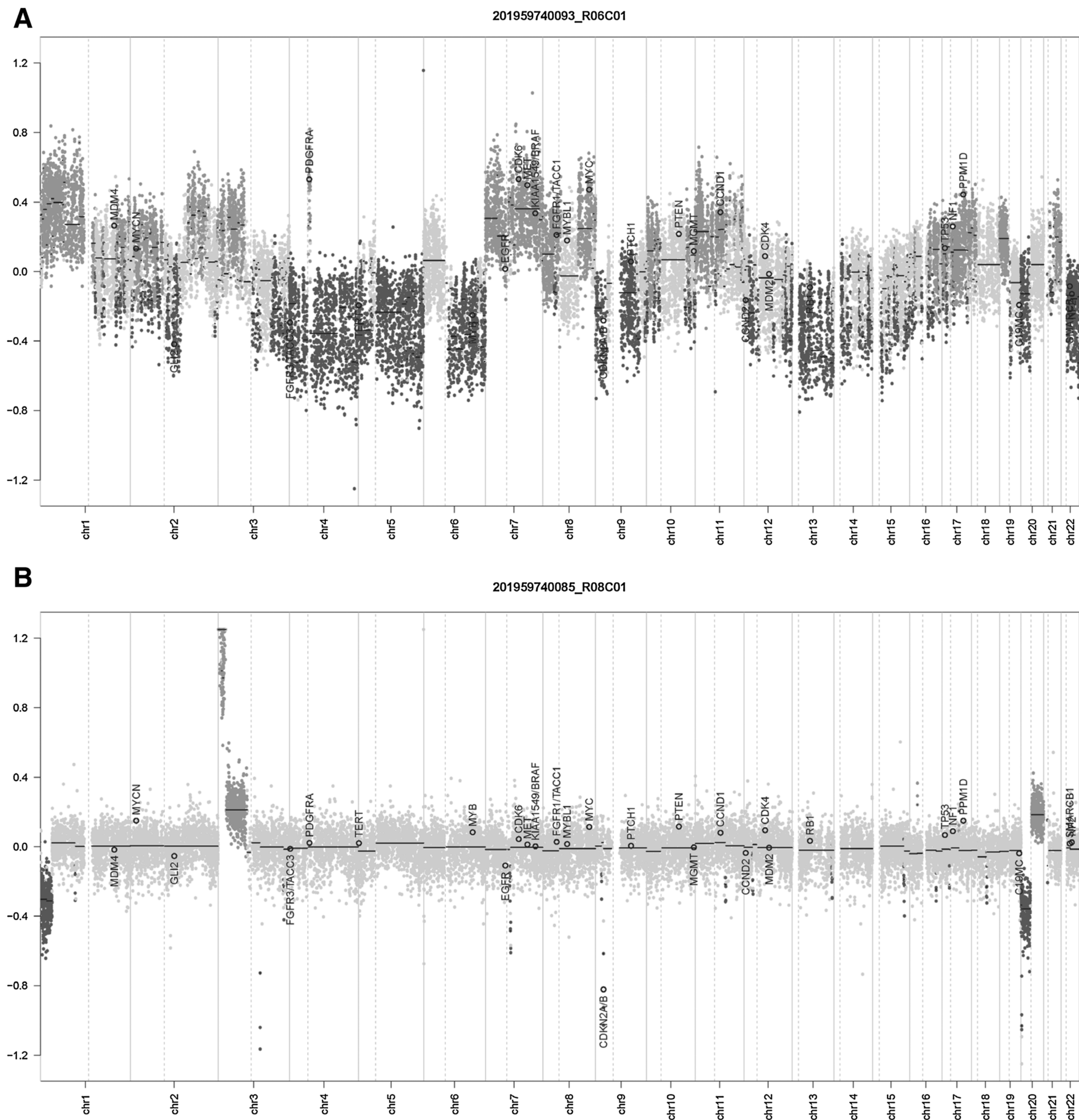

Figure 3.

Examples of copy-number profiles. **A,** Example of an unstable UV-associated angiosarcoma. **B,** Example of a stable UV-associated angiosarcoma.

Both UV-induced exposure and radiotherapy are known inducers of chromosomal instability (34, 35).

The availability of clinical data allowed us to investigate the correlation between clusters and patient survival. Even in this relatively small sample, survival of patients in cluster A was significantly more favorable than in cluster B. Whether this difference is due to the higher level of chromosomal instability in cluster A is uncertain. So far, the correlation between chromosomal instability and prognosis in cancer has not been fully established. Most studies report worse prognosis for patients with unstable tumors, based on the development of therapy-resistant cell populations within a tumor due to chromosomal instability (36). However, other results indicate that extreme chromosomal instability was correlated to better prognosis, which could be due to the fact that excessive chromosomal instability itself can be lethal to tumor cells, or lead to increased vulnerability to anticancer therapy (37). In addition, the correlation between chromosomal instability and prognosis might also vary between different tumor subtypes (38). For instance in synovial sarcoma, chromosomal instability was strongly associated with shorter metastasis-free survival (39), whereas response to neoadjuvant chemotherapy did not correlate with chromosomal stability (40).

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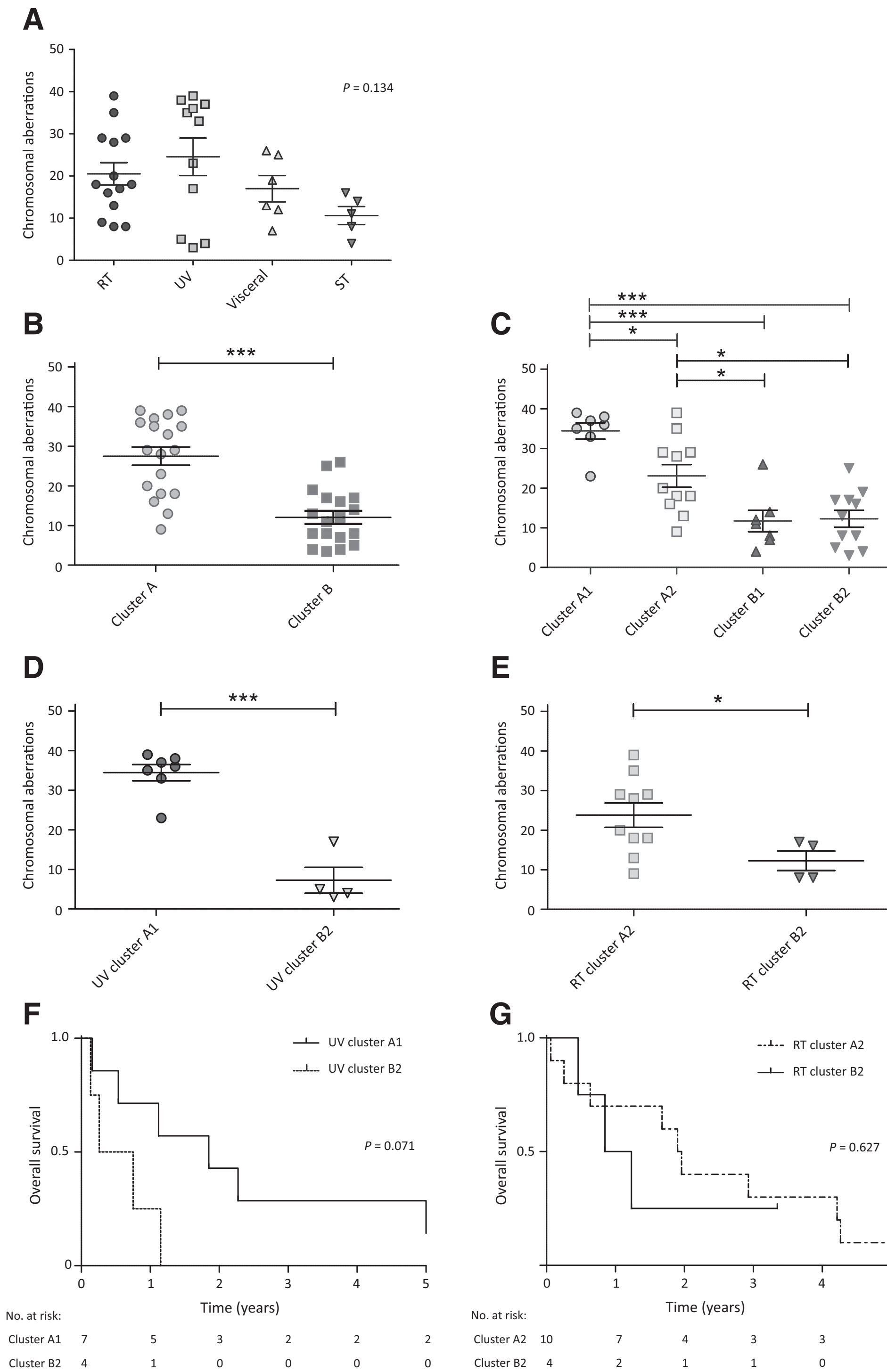

Figure 4.

Number of chromosomal aberrations per sample and overall survival. **A,** Chromosomal aberrations per clinical subgroup; **B,** per cluster A and B; **C,** per cluster A1-B2. **D,** UV-associated cases, cluster A1 vs. B2. **E,** RT-associated cases, cluster A2 vs. B2. **F,** Kaplan-Meier curves showing the difference in 5-year overall survival of UV-associated cases in cluster A1 vs. B2. **G,** Kaplan-Meier curves showing the 5-year overall survival of RT-associated cases in cluster A2 vs. B2.

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Although chromosomal instability was found to be more frequent among adult patients with synovial sarcoma compared with pediatric patients (39), in our cohort angiosarcoma chromosomal stability did not correlate with age. Because of limited patient numbers, we could not achieve enough power in Kaplan–Meier curves to assess the exact correlation between treatment, chromosomal stability, and outcome.

We are the first to perform genome-wide DNA methylation profiling in AS. Previous clustering analysis based on RNA expression of 22 angiosarcoma cases did show two angiosarcoma clusters (18). One cluster mainly contained secondary angiosarcoma cases (radiation-induced and postlymphedema), whereas most primary angiosarcoma cases fell into the second cluster. These results partly correspond with our findings, although head and neck angiosarcoma, which we considered to be secondary angiosarcoma because of its strong relationship with UV exposure, were considered primary angiosarcoma in this study and did not cluster

together with radiation-induced cases. In addition, correlation with survival was not reported in the previous study.

The only DNA methylation research in angiosarcoma performed so far has focused exclusively on the *CDKN2A* gene, which encodes cell-cycle–regulatory proteins p16(INK4A) and p14(ARF; ref. 22). Down-regulation of p16(INK4A) is found in malignant progression, is significantly correlated with reduced patient survival, and may be explained by DNA hypermethylation in sarcoma cells (41). In a total of 19 sporadic liver angiosarcoma, promoter hypermethylation of p16 (INK4A) was shown to be present in the majority (63%, 12/19) of cases, resulting in loss of p16 protein expression evaluated by IHC (22, 42). CDKN2A promoter hypermethylation can also result in loss of the tumor suppressor protein p14(ARF), which was the case in 5 of 19 (26%) of sporadic liver angiosarcoma cases (22). In our study, we found CDKN2A loss in 7 cases by copy-number variation (CNV) analysis. However, specific analysis of the CDKN2A promoter region revealed that all our samples were unmethylated. The observed loss of CDKN2A in the CNV profiles is therefore likely to be caused by another mechanism, for example, genomic deletion or mutation. Loss of CDKN2A could be of clinical significance in terms of novel targeted treatment options as CDKN2A is a negative regulator of cell-cycle–regulatory proteins CDK4 and CDK6. Therefore, tumors harboring CDKN2A loss might benefit from treatment with CDK4/6 inhibitors such as palbociclib (43).

In this study, for the first time, MGMT methylation was studied in angiosarcoma and revealed MGMT promoter methylation in 19% (7/36) of cases. The *MGMT* gene encodes for an enzyme involved in DNA repair, and silencing of MGMT by methylation renders tumor cells sensitive to treatment with alkylating agents such as temozolomide and dacarbazine (44, 45). Promoter methylation of MGMT has shown to predict sensitivity to temozolomide in glioblastoma and is therefore routinely being tested in clinical practice (46). Interestingly, in our study, methylation was limited to soft tissue (4/5, 80%) and visceral angiosarcoma (3/6, 50%), and all MGMT-methylated cases fell exclusively into cluster B1. MGMT promoter methylation has been described in 13% to 43% of soft-tissue sarcomas such as undifferentiated pleomorphic sarcomas and liposarcomas (47, 48). Clinically, application of temozolomide in MGMT-methylated sarcomas has only been described in case reports and a small case series (49–51). The first clinical study in patients with sarcoma is currently ongoing, evaluating MGMT status of patients with Ewing sarcoma receiving temozolomide and irinotecan (NCT03542097). In angiosarcoma, the presence of MGMT promoter methylation in a part of soft tissue and visceral cases supports research into temozolomide as a potential treatment option for these particular patients.

Our study has several limitations. Multivariable analysis with regard to survival and prognostic factors was not feasible due to small patient numbers. Clinical information was retrieved from the Netherlands Cancer Registry, which offers comprehensive registration since 1989. As a result, data regarding radiotherapy given before 1989 or radiotherapy applied for benign indications might be missing in incidental cases, which may have affected the optimal selection of radiation-induced angiosarcoma. In addition, survival data did not include cause of death; therefore, disease-specific survival could not be assessed. Further research is warranted to confirm our findings, starting with analysis of additional visceral and soft tissue cases to improve statistical power. Next, validation in a larger and independent angiosarcoma cohort is needed to further establish the differences between clusters including clinical prognosis and chromosomal stability.

In conclusion, with this exploratory study, we demonstrate for the first time four distinct angiosarcoma clusters by DNA methylation profiling. We showed that the two main angiosarcoma clusters were significantly associated with chromosomal instability and prognosis. Furthermore, our findings may provide a rationale for the exploration of novel treatment options for particular angiosarcoma patient groups. Our results support the hypothesis that angiosarcoma heterogeneity is indeed present at a biological level. Further exploration of DNA methylation patterns in angiosarcoma could lead to more specific classification and ultimately enable treatment tailored to the individual patient with angiosarcoma.

Disclosure of Potential Conflicts of Interest

A. von Deimling reports receiving royalties for tumor-specific antibodies against BRAFV600E and IDH1R132H. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

This study was funded by the German Cancer Aid (grant number: 70112499; to C. Koelsche and A. von Deimling) and the Dutch foundation Honderdduizend Keer een Tientje' (to W.T.A. van der Graaf and Y.M.H. Versleijen-Jonkers).

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Received July 3, 2019; revised August 19, 2019; accepted September 23, 2019; published first September 27, 2019.

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References

1. National Cancer Intelligence Network. Soft tissue sarcoma incidence and survival: tumours diagnosed in England between 1985 and 2009. London, United Kingdom: NCIN; 2012. http://www.ncin.org.uk/cancer_type_and_topic_specific_work/cancer_type_specific_work/sarcomas/.
2. Shon W, Jenkins SM, Ross DT, Seitz RS, Beck RA, Ring BZ, et al. Angiosarcoma: a study of 98 cases with immunohistochemical evaluation of TLE3, a recently described marker of potential taxane responsiveness. J Cutan Pathol 2011;38: 961–6.
3. Mark RJ, Poen JC, Tran LM, Fu YS, Juillard GF. Angiosarcoma. A report of 67 patients and a review of the literature. Cancer 1996;77:2400–6.
4. Fayette J, Martin E, Piperno-Neumann S, Le Cesne A, Robert C, Bonvalot S, et al. 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.
5. Weidema ME, Versleijen-Jonkers YMH, Flucke UE, Desar IME, van der Graaf WTA. Targeting angiosarcomas of the soft tissues: a challenging

effort in a heterogeneous and rare disease. Crit Rev Oncol Hematol 2019;138:120–31.
6. Wang L, Lao IW, Yu L, Wang J. Clinicopathological features and prognostic factors in angiosarcoma: a retrospective analysis of 200 patients from a single Chinese medical institute. Oncol Lett 2017;14:5370–78.
7. Schlemmer M, Reichardt P, Verweij J, Hartmann JT, Judson I, Thyss A, et al. Paclitaxel in patients with advanced angiosarcomas of soft tissue: a retrospective study of the EORTC soft tissue and bone sarcoma group. Eur J Cancer 2008;44:2433–6.
8. Farid M, Ong WS, Lee MJ, Hartmann JT, Judson I, Thyss A, et al. Cutaneous versus non-cutaneous angiosarcoma: clinicopathologic features and treatment outcomes in 60 patients at a single Asian cancer centre. Oncology 2013;85:182–90.
9. Merfeld E, Gabani P, Spraker MB, Zoberi I, Kim H, Van Tine B, et al. Clinical outcomes and prognostic features of angiosarcoma: significance of prior radiation therapy. Clin Oncol (R Coll Radiol) 2019;31:232–41.
10. Yin M, Wang W, Drabick JJ. Prognosis and treatment of non-metastatic primary and secondary breast angiosarcoma: a comparative study. BMC Cancer 2017;17:295.
11. Buehler D, Rice SR, Moody JS, Rush P, Hafez GR, Attia S, et al. Angiosarcoma outcomes and prognostic factors: a 25-year single institution experience. Am J Clin Oncol 2014;37:473–9.
12. Scott MT, Portnow LH, Morris CG, Marcus RB Jr, Mendenhall NP, Mendenhall WM, et al. Radiation therapy for angiosarcoma: the 35-year University of Florida experience. Am J Clin Oncol 2013;36:174–80.
13. Lahat G, Dhuka AR, Hallevi H, Xiao L, Zou C, Smith KD, et al. Angiosarcoma: clinical and molecular insights. Ann Surg 2010;251:1098–106.
14. Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP, et al. Recurrent CIC gene abnormalities in angiosarcomas: a molecular study of 120 cases with concurrent investigation of PLCG1, KDR, MYC, and FLT4 gene alterations. Am J Surg Pathol 2016;40:645–55.
15. Guo T, Zhang L, 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.
16. Fraga-Guedes C, Andre S, Mastropasqua MG, Botteri E, Toesca A, Rocha RM, et al. Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of MYC gene amplification and protein expression. Breast Cancer Res Treat 2015;151:131–40.
17. Verbeke SL, de Jong D, Bertoni F, Sciot R, Antonescu CR, Szuhai K, et al. Array CGH analysis identifies two distinct subgroups of primary angiosarcoma of bone. Genes Chromosomes Cancer 2015;54:72–81.
18. Antonescu CR, Yoshida A, Guo T, Chang NE, Zhang L, Agaram NP, et al. KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors. Cancer Res 2009;69:7175–9.
19. Kulis M, Esteller M. DNA methylation and cancer. Adv Genet 2010;70:27–56.
20. Rohrich M, Koelsche C, Schrimpf D, Capper D, Sahm F, Kratz A, et al. Methylation-based classification of benign and malignant peripheral nerve sheath tumors. Acta Neuropathol 2016;131:877–87.
21. Sturm D, Orr BA, Toprak UH, Hovestadt V, Jones DTW, Capper D, et al. New brain tumor entities emerge from molecular classification of CNS-PNETs. Cell 2016;164:1060–72.
22. Weihrauch M, Markwarth A, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Abnormalities of the ARF-p53 pathway in primary angiosarcomas of the liver. Hum Pathol 2002;33:884–92.
23. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol 2007;29:19–24.
24. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014;30:1363–9.
25. Fortin JP, Triche TJ Jr, Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. Bioinformatics 2017;33:558–60.
26. Min JL, Hemani G, Davey Smith G, Relton C, Suderman M. Meffil: efficient normalization and analysis of very large DNA methylation datasets. Bioinformatics 2018;34:3983–9.
27. Xu Z, Niu L, Li L, Taylor JA. ENmix: a novel background correction method for Illumina HumanMethylation450 BeadChip. Nucleic Acids Res 2016;44:e20.
28. Stacklies W, Redestig H, Scholz M, Walther D, Selbig J. pcaMethods–a bioconductor package providing PCA methods for incomplete data. Bioinformatics 2007;23:1164–7.
29. S. Zhao YG, Sheng Q, Shyr Y. Heatmap3: an improved heatmap package, 2015. https://cran.r-project.org/web/packages/heatmap3/index.html.
30. Suzuki R, Shimodaira H. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. Bioinformatics 2006;22:1540–2.
31. Suzuki R, Shimodaira H. Hierarchical clustering with P-values via multiscale bootstrap resampling; 2006. https://cran.r-project.org/web/packages/heatmap3/index.html.
32. Bady P, Delorenzi M, Hegi ME. Sensitivity analysis of the MGMT-STP27 model and impact of genetic and epigenetic context to predict the MGMT methylation status in gliomas and other tumors. J Mol Diagn 2016;18:350–61.
33. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature 2018;555:469–74.
34. Dahle J, Kvam E. Induction of delayed mutations and chromosomal instability in fibroblasts after UVA-, UVB-, and X-radiation. Cancer Res 2003;63:1464–9.
35. Bakhoum SF, Cantley LC. The multifaceted role of chromosomal instability in cancer and its microenvironment. Cell 2018;174:1347–60.
36. Vargas-Rondón N, Villegas VE, Rondón-Lagos M. The role of chromosomal instability in cancer and therapeutic responses. Cancers 2018;10:4.
37. Birkbak NJ, Eklund AC, Li Q, McClelland SE, Endesfelder D, Tan P, et al. Paradoxical relationship between chromosomal instability and survival outcome in cancer. Cancer Res 2011;71:3447–52.
38. McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C. Cancer chromosomal instability: therapeutic and diagnostic challenges. EMBO Rep 2012;13:528–38.
39. Lagarde P, Przybyl J, Brulard C, Pérot G, Pierron G, Delattre O, et al. Chromosome instability accounts for reverse metastatic outcomes of pediatric and adult synovial sarcomas. J Clin Oncol 2013;31:608–15.
40. Chakiba C, Lagarde P, Pissaloux D, Neuville A, Brulard C, Pérot G, et al. Response to chemotherapy is not related to chromosome instability in synovial sarcoma. Ann Oncol 2014;25:2267–71.
41. Knosel T, Altendorf-Hofmann A, Lindner L, Issels R, Hermeking H, Schuebbe G, et al. Loss of p16(INK4a) is associated with reduced patient survival in soft tissue tumours, and indicates a senescence barrier. J Clin Pathol 2014;67:592–8.
42. Tannapfel A, Weihrauch M, Benicke M, Uhlmann D, Hauss J, Wrbitzky R, et al. p16INK4A – alterations in primary angiosarcoma of the liver. J Hepatol 2001;35:62–67.
43. Elvin JA, Gay LM, Ort R, Shuluk J, Long J, Shelley L, et al. Clinical benefit in response to palbociclib treatment in refractory uterine leiomyosarcomas with a common CDKN2A alteration. Oncologist 2017;22:416–21.
44. Dolan ME. Structure, function, and inhibition of O6-alkylguanine-DNA alkyltransferase. Prog Nucleic Acid Res Mol Biol 1995;51:167.
45. Amatu A, Sartore-Bianchi A, Moutinho C, Belotti A, Bencardino K, Chirico G, et al. Promoter CpG island hypermethylation of the DNA repair enzyme MGMT predicts clinical response to dacarbazine in a phase II study for metastatic colorectal cancer. Clin Cancer Res 2013;19:2265–72.
46. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997–1003.
47. Jakob J, Hille M, Sauer C, Ströbel P, Wenz F, Hohenberger P. O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation is a rare event in soft tissue sarcoma. Radiat Oncol 2012;7:180.
48. Kim JI, Suh JT, Choi KU, Kang HJ, Shin DH, Lee IS, et al. Inactivation of O6-methylguanine-DNA methyltransferase in soft tissue sarcomas: association with K-ras mutations. Hum Pathol 2009;40:934–41.
49. Kinoshita M, Yamada A, Sawa D, Kamimura S, Miyachi M, Moritake H. Successful treatment of metastatic alveolar rhabdomyosarcoma with MGMT gene promoter methylation by temozolomide-based combination chemotherapy. Pediatr Blood Cancer 2018;65:e26750.
50. Tanaka H, Sasayama T, Nishihara M, Arai A, Kawamura A, Kanomata N, et al. Brain metastasis of undifferentiated sarcoma and response to temozolomide treatment. Case report. Neurol Med Chir 2010;50:689–93.
51. Ferriss JS, Atkins KA, Lachance JA, Modesitt SC, Jazaeri AA. Temozolomide in advanced and recurrent uterine leiomyosarcoma and correlation with o6-methylguanine DNA methyltransferase expression: a case series. Int J Gynecol Cancer 2010;20:120–5.

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