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A prognostic cytogenetic scoring system to guide the adjuvant management of patients with atypical meningioma

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Background. The appropriate use of adjuvant therapy in patients with gross totally resected atypical meningioma requires an accurate assessment of recurrence risk. We sought to determine whether cytogenetic/genetic characterization may facilitate better estimation of the probability of recurrence.

Methods. We first analyzed our clinical database, including high-resolution DNA copy number data, to identify 11 common copy number aberrations in a pilot cohort of meningiomas of all grades. We summed these aberrations to devise a cytogenetic abnormality score (CAS) and determined the CAS from archived tissue of a separate cohort of 32 patients with gross totally resected atypical meningioma managed with surgery alone. Propensity score adjusted Cox regression was used to determine whether the CAS was predictive of recurrence.

Results. An association between higher CAS and higher grade was noted in our pilot cohort with heterogeneity among atypical tumors. Among the 32 patients who underwent gross total resection of an atypical meningioma, the CAS was not significantly associated with age, gender, performance status, or tumor size/location but was associated with the risk of recurrence on univariable analysis (hazard ratio per aberration = 1.52; 95% CI = 1.08–2.14; $P = .02$). After adjustment, the impact of the dichotomized number of copy aberrations remained significantly associated with recurrence risk (hazard ratio = 4.47; 95% CI = 1.01–19.87; $P = .05$).

Conclusions. The number of copy number aberrations is strongly associated with recurrence risk in patients with atypical meningioma following gross total resection and may inform the appropriate use of adjuvant radiation therapy in these patients or be useful for stratification in clinical trials.

Keywords: atypical, copy number, meningioma, recurrence, resected.

Meningioma is the most common intracranial tumor in the United States.[1,2] World Health Organization (WHO) grade II (atypical) meningioma recurs more frequently than WHO grade I (benign) meningioma,[3] and patients with subtotally resected atypical meningioma should be treated with adjuvant radiation therapy.[4–6] However, many atypical meningiomas can be gross totally resected, and whether to administer radiation to this population remains unclear. Apart from extent of resection, clinical characteristics such as age and gender and tumor-related characteristics such as tumor size and location

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have poor predictive capacity to determine which lesions will recur. Cytogenetic predictors may have significantly more promise in this regard. We sought to identify common copy number aberrations (CNAs) across all grades of meningioma and determine whether the total number of these CNAs would be useful as a cytogenetic predictor of recurrence for patients with atypical meningioma following gross total resection (GTR) in order to further segregate patients more likely to recur and thus warrant adjuvant radiation therapy.

Materials and Methods

Study Design

At our institution, we routinely collect multidimensional cancer genotyping data through formalin-fixed paraffin embedded (FFPE)–based multiplex copy number profiling (OncoCopy v1.1; Agilent array comparative genomic hybridization [aCGH] stock 1 million feature arrays) in a laboratory setting certified by the Clinical Laboratory Improvement Amendments (CLIA).[7] A minimum of 1.3 μg DNA, corresponding to $\sim 10 \times 5$ μm standard FFPE tumor sections, is obtained as part of clinical care. Patient and reference DNA (Promega) were fragmented using the fragmentation simulation method, as previously described, and hybridized to Agilent SurePrint G3 Human 1 million feature arrays.[8,9] In Agilent Workbench software, log ratios were normalized using the centralization algorithm, with a threshold score of 6.0 and bin size of 10. CNAs of 8 consecutives probes with mean log2 ratio of 0.25 (gains) and −0.35 (losses) were called using the ADM2 (aberration detection method 2) algorithm. Forty-two CNAs of known relevance to brain tumors were reported from whole genome data, including 14 arm- or chromosome-level events.[7,10] Analysis of data generated from tumor specimens and clinical variables was conducted following approval from the Dana-Farber/Harvard Cancer Center Institutional Review Board. Fisher's exact test was performed to determine the association between arm- or chromosome-level CNAs with tumor grade for those aberrations with a frequency >5 among the cohort.

We then obtained archived tissue from 32 patients with GTR of an atypical meningioma treated with surgery alone between 1997 and 2010 from a cohort for which our group had previously reported clinical outcomes.[11] No patient received radiation. OncoCopy was performed on the FFPE tissue with the selected CNAs analyzed. Patients were considered WHO grade II if one or more of the following criteria were met: (i) chordoid or clear cell histologic subtype, (ii) 4–19 mitoses per 10 high-power field, (iii) brain invasion, (iv) ≥3 of the following histologic features (even focally): cluster of small cells (high nuclear/cytoplasmic ratio), high cellularity, prominent nucleoli, sheetlike growth, spontaneous necrosis.[12,13] Of note, no patients in our cohort had chordoid/clear cell histology.

Statistical Analysis

We identified all nonredundant arm- or chromosome-level CNAs for which the frequency was >0 in the pilot cohort, including single copy loss of 1p, 4p, 6q, 7p, 10q, 11p, 14, 18q, 19q, and monosomy of 22 for each patient. Additionally, cyclin-dependent kinase inhibitor 2A was added as a single gene CNA, as it has been associated with meningioma progression,[14] and was included in the OncoCopy report while arm- or chromosome-level events involving chromosome 9 were not. The numbers of CNAs were summed for each patient and then treated as a continuous covariate thereafter. We called this continuous covariate the cytogenetic abnormality score (CAS). For example, if a patient had a single copy loss of 1p, 4p, and 10q, the CAS would be 3. Our *a priori* hypothesis was that the number of CNAs would be associated with the risk of radiographic recurrence given the correlation of histologic grade with the global number of copy aberrations.

We used the Pearson correlation to determine whether continuous covariates such as age, tumor size, and year of treatment were associated with the CAS; we used the *t*-test to determine whether categorical covariates such as gender, Karnofsky performance score (KPS), and tumor location were associated with the CAS. We used univariable Cox regression to determine whether CAS, as a continuous covariate, was associated with radiographic recurrence. All patients who recurred radiographically in this study were treated with salvage surgery and/or radiation. To adjust for potential confounding factors and other covariates of interest such as age (continuous), gender, KPS (<90 vs ≥90), year of treatment (continuous), tumor size (continuous), and tumor location (convexity vs base of skull), we used logistic regression to generate propensity scores for high versus low CAS (dichotomized about median). These propensity scores were then used as a continuous covariate in a Cox regression along with dichotomized CAS in order to determine the adjusted impact of CAS on recurrence. Such an approach allowed us to adjust for pertinent confounders while not overextending the limitations of the Cox model. We did not test whether individual CNAs were associated with recurrence as part of the initial analysis.

The median follow-up for our study was 5.0 years (after surgical resection). All *P*-values were 2-sided and a threshold of .05 was used to determine significance. Statistical analyses were performed using SAS version 9.3. Figures were created using R version 3.2.0 and ggplot2.

Results

Of the 43 patients with aCGH data available in the pilot cohort, the most common genetic abnormalities were monosomy 22 in 26 patients, 1p loss in 20, 6q loss in 7, 18q loss in 7, and monosomy 14 in 6 patients. The increasing number of chromosomal aberrations present within a tumor was correlated with increasing grade (Table 1). In addition to the specific examples listed in Table 1, the median number of mutually exclusive chromosomal aberrations increased with grade ($P < .001$). Notably, grade I and grade III tumors displayed consistently low and high CAS scores, respectively; significant heterogeneity was seen in grade II tumors (Fig. 1).

Baseline clinical characteristics of our cohort of 32 patients with GTR of an atypical meningioma are displayed in Table 2. The mean CAS was 3.2 (SD, 1.8). Notably, the 43 patients in the pilot cohort were different than the 32 patients in the cohort depicted in the primary analysis.

CAS was not significantly associated with any relevant clinical and tumor-related characteristics, including age, gender, KPS,

Table 1. Distribution of cytogenetic abnormalities in patients with meningiomas of varying grade in pilot cohort

Cytogenetic Abnormality	Grade I Without Atypical Features or High Proliferation Index ($n = 22$)	Grade I With Atypical Features or High Proliferation Index ($n = 3$)	Grade II ($n = 15$)	Grade III ($n = 3$)	*P*
Monosomy 22					.04
Present	9	2	12	3	
Absent	13	1	3	0	
1p loss					<.001
Present	3	2	12	3	
Absent	19	1	3	0	
6q loss					<.001
Present	0	0	4	3	
Absent	22	3	11	0	
18q loss					<.001
Present	0	0	4	3	
Absent	22	3	11	0	
Monosomy 14					.01
Present	0	1	4	1	
Absent	22	2	11	2	

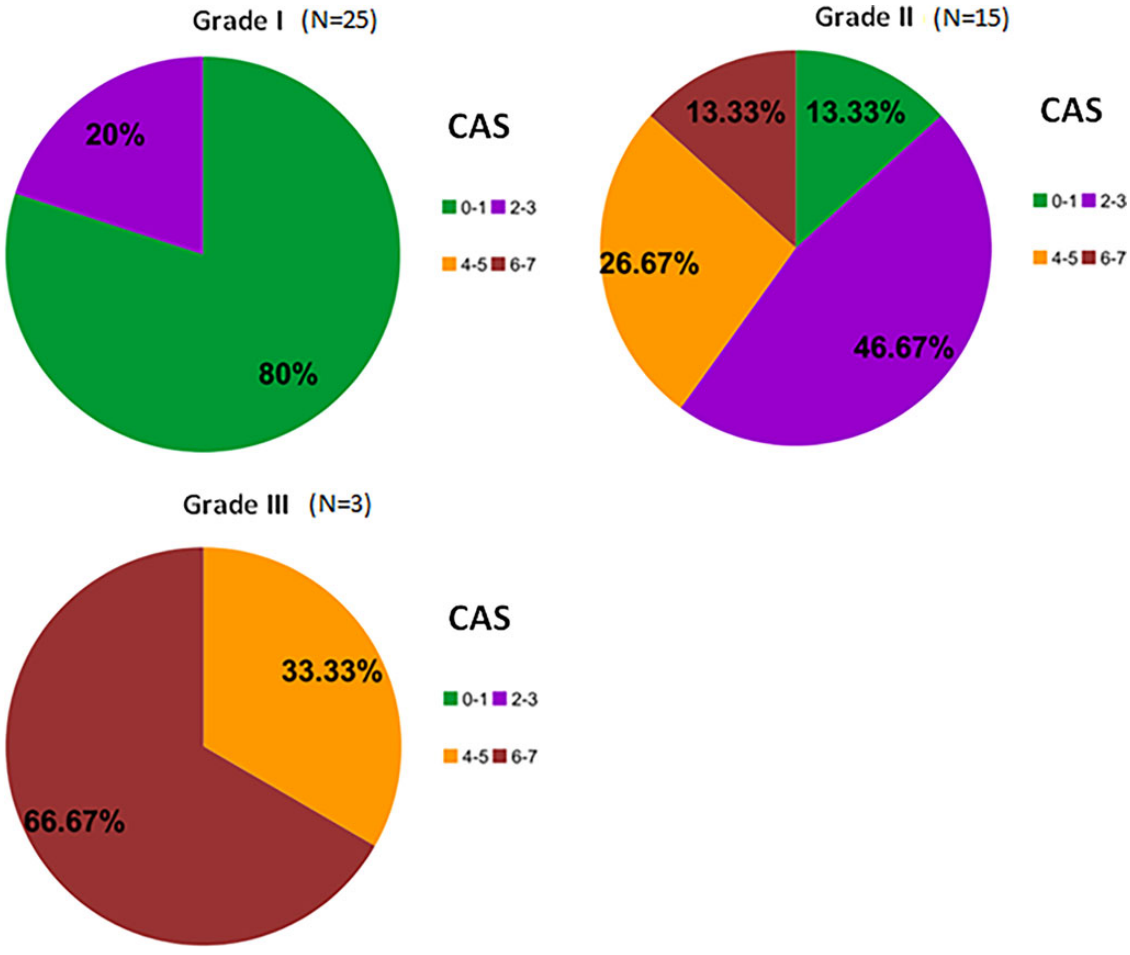

Fig. 1. Distribution of CAS by grade of meningioma.

Table 2. Baseline characteristics in clinical cohort with gross totally resected atypical meningioma

Clinical Characteristic	Distribution*
Age, y, mean (SD)	57 (17)
Gender, *N* (%)	
Male	16 (50)
Female	16 (50)
Race, *N* (%)	
White	26 (81)
Other	2 (6)
Unknown	4 (13)
Marital status, *N* (%)	
Unmarried	11 (34)
Married	21 (66)
KPS, *N* (%)	
≥90	7 (22)
<90	25 (78)
Reason for diagnosis, *N* (%)	
Symptoms	28 (88)
Incidentally discovered	4 (13)
Era of treatment, *N* (%)	
1997–2001	2 (6)
2002–2006	12 (38)
2007–2010	18 (56)
Tumor size, cm, mean (SD)	4.6 (1.9)
Site, *N* (%)	
Convexity	27 (84)
Nonconvexity	5 (16)
Cytogenetic abnormality score, mean (SD)	3.2 (1.8)

*Percentages may not add to 100 due to rounding.

Table 3. Association between cytogenetic abnormality score and variables of interest

Clinical Characteristic	Association
Age (continuous)	$r = -0.04$, $P = .82$
Gender (female vs male)	mean 3.1 vs 3.3, $P = .78$
KPS (≥90 vs <90)	mean 3.2 vs 3.1, $P = .90$
Year of treatment (continuous)	$r = -0.02$, $P = .91$
Tumor size (continuous)	$r = -0.02$, $P = .90$
Tumor location (convexity vs nonconvexity)	mean 3.5 vs 1.8, $P = .06$

year of diagnosis, tumor size, and tumor location (Table 3). The *P*-values for these associations reflected the lack of any significant association (range of *P*-values: 0.78–0.91), with the exception of tumor location, in which tumors originating in the convexity displayed a trend to a higher CAS than tumors located in the base of skull ($P = .06$).

Time to recurrence for patients in the entire cohort, stratified about median CAS, is displayed in Fig. 2. CAS was strongly associated with recurrence; every unit increase in CAS increased the risk of recurrence by ~50% (hazard ratio [HR] per unit increase = 1.52; 95% CI = 1.08–2.14; $P = .02$). No single cytogenetic abnormality used to generate CAS was independently associated with recurrence, with the exception of loss of 6q ($P = .03$). If 6q loss was excluded from the formula used to calculate CAS, the association between CAS and recurrence remained highly significant (HR per unit increase = 1.50; 95% CI = 1.03–2.19; $P = .04$). After adjustment for confounding factors and other covariates of interest using a propensity score analysis, the impact of dichotomized CAS remained significantly associated with recurrence risk (HR for high vs low CAS = 4.47; 95% CI = 1.01–19.87; $P = .05$).

Discussion

We routinely obtained whole genome copy number data on patients following surgery for meningioma. In the first part of our study, we found that the total number of CNAs increased with increasing histologic grade. Grade I tumors had few CNAs and grade III tumors had many CNAs. Interestingly, grade II tumors showed significant heterogeneity. We hypothesized that this heterogeneity would correlate with the significant variation in recurrence among patients with atypical tumors. To study this, we identified a cohort of patients in which we had previously collected extensive clinical and pathologic information. To isolate biologic factors that would be associated with recurrence, we carefully selected this cohort of patients to control for the 2 most relevant clinical factors that are associated with recurrence: extent of resection and the use of adjuvant radiation therapy.

We found that the number of CNAs in patients with atypical meningioma not receiving radiation therapy following GTR was significantly associated with the risk of recurrence. The magnitude of this relationship was found to be strong, with each additional CNA increasing the risk of recurrence by ~50%. The lack of a statistically significant result following dichotomization is likely due to the observation that the risk is relatively linear with each CNA, and we didn't have sufficient numbers of patients to overcome the reduction in study power that results from discretizing what is a linear risk association. The clinical implication of such a scenario would be that there might not be a "right" answer if cutoffs are desired to identify risk groups. High-resolution whole genome aCGH is applied in routine clinical use in our CLIA-certified laboratory, with reports available to the clinical team generally within 2 weeks of sample submission. The integration of this test into routine clinical use distinguishes our study from other studies that rely on laboratory-based research assays which are not readily accessible to clinicians and cannot be used for clinical decision making.

Our group has recently published one of the largest series describing outcomes among patients with atypical meningioma,[11] finding that patients with gross totally resected tumors recur less frequently when given adjuvant radiation. Local recurrence has been linked with mortality in patients with atypical meningioma,[15] and achieving durable local control might spare patients the morbidity associated with recurrence and additional treatment. However, radiation therapy is also associated with significant short- and long-term toxicity, including neurocognitive decline,[16,17] second malignancies,[18–20] and cerebrovascular accidents.[21–23] Therefore, it is critical to

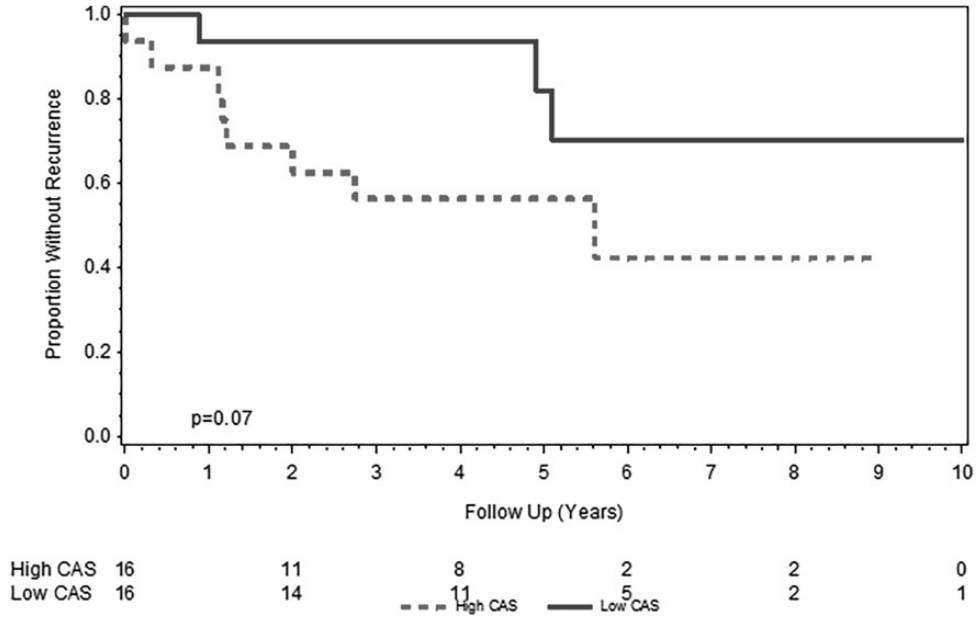

Fig. 2. Time to recurrence in patients with high vs low CAS (dichotomized around median CAS of 3.5) in patients with gross totally resected atypical meningioma.

determine which patients with atypical meningioma are at high enough risk of recurrence following GTR so that the benefits of radiation outweigh the risks; currently available clinical models cannot effectively make this discrimination. Furthermore, a more granular method for estimating risk of recurrence would also be useful in clinical trial design so that patients who are more likely to show a greater absolute magnitude of benefit with radiation can be studied independently.

Although the population of patients with atypical meningioma is heterogeneous on a cytogenetic level, no cytogenetic changes have yet been useful in regular clinical practice as prognostic or predictive factors. Some groups have found various associations of gains or losses on many chromosomes with higher grade, and some have found prognostic value of specific abnormalities within a specific grade,[24–26] though these studies are few in number and variable. Furthermore, prior studies do not have well-defined clinical endpoints or control for non-cytogenetic factors such as extent of resection and the use of adjuvant radiation, making the overall intrinsic prognostic capacity of cytogenetic aberrations unclear.

Our study should be considered in the context of its limitations. First, our sample size is relatively small given the rarity of the tumor and our exclusion of patients with subtotal resections and those who received adjuvant radiation. However, the statistical tests employed in the analysis account for sample size when determining the statistical significance of the results, and our choices for exclusion controlled for the most significant known factors predictive of recurrence. Second, the *a priori* hypothesis was that the global number of CNAs would be associated with recurrence risk. It is possible that certain chromosomal gains or losses carry more impact than others, and our analysis did not further characterize the impact of each individual chromosomal aberration. We intentionally did not test the association between each cytogenetic abnormality and recurrence as a primary analytic technique given concerns about multiple testing and the risk of generating a false positive result. Lastly, there are a small number of malignant meningioma cases in our pilot cohort ($N = 3$).

In conclusion, our study indicates that the number of CNAs is significantly associated with the risk of recurrence in patients with atypical meningioma following GTR. The CAS, a simple and clinically relevant score summarizing total CNAs, may be useful in identifying patients who are at high enough risk of recurrence to benefit from adjuvant radiation therapy and for stratification of clinical trials investigating the value of adjuvant therapy. Validation of these results in a larger patient cohort would strengthen the clinical utility of the CAS.

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Conflict of interest statement. None declared.

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