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CORRESPONDENCE

Prognostic significance of *NAB2–STAT6* fusion variants and *TERT* promotor mutations in solitary fibrous tumors/hemangiopericytomas of the CNS: not (yet) clear

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Grading of meningeal solitary fibrous tumors/hemangiopericytomas (SFTs/HPCs) of the central nervous system (CNS) is nowadays based on histologic criteria as described in the revised fourth edition of the WHO Classification of CNS tumors [10] or the more recently published, updated version of the Marseille Grading System (MGS) [11]. Histology-based grading of CNS SFTs/HPCs allows for discriminating subgroups with significant differences in prognosis. However, the often piece meal resection of these tumors may hamper adequate evaluation of mitotic activity and necrosis, and thereby assessment of malignancy grade. *NAB2–STAT6* fusion is the molecular hallmark of both soft tissue SFTs and CNS SFTs/HPCs, and the resulting fusion protein accumulates in the nucleus and acts as a transcriptional activator of early growth response mediated pathways with STAT6 immunohistochemistry being a very sensitive and specific tool for their diagnosis [5, 8, 12, 14]. For soft tissue SFTs, particular *NAB2–STAT6* fusion variants as well as telomerase reverse transcriptase (*TERT*) promoter mutations leading to telomerase activity and tumor cell immortalization have been reported to have prognostic value. Some studies have included CNS SFTs/HPCs in their cohort, but because of small numbers and lack of (long term) follow-up data the prognostic value of these markers for CNS SFTs/HPCs is still unclear [1–4, 6, 7, 9, 13, 15, 16].

To evaluate the prognostic value of *NAB2–STAT6* fusion variants and *TERT* promoter mutations for CNS SFTs/HPCs, we retrospectively analyzed these markers in a cohort of 136 patients with STAT6 nucleopositive CNS tumors. All tumors were graded according to the most recent WHO classification [10] and the updated MGS [11] and were analyzed for type of *NAB2–STAT6* fusion and the presence of *TERT* promoter mutation. For *NAB2–STAT6* fusion analysis, we performed reverse transcriptase polymerase chain reaction (RT-PCR) after RNA extraction and cDNA synthesis using multiple primer sets finding the most common fusion

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variants which were subsequently grouped based on their hypothesized functional effects [3, 15].

After DNA isolation, amplicons of 163 base pairs spanning *TERT* promoter hot-spot mutations at positions 1,295,228 and 1,295,250 on chromosome 5 were amplified by PCR using the primers hTERT-F CAGCGCTGCCTG AAACTC and hTERT-R GTCCTGCCCCTTCACCTT and subsequent sequencing of the products was performed. More detailed information on Materials and Methods is given in the Supplementary Information. For survival analyses, patients who died due to complications of initial therapy (e.g., surgery or radiotherapy) were excluded. Results of histological grading, analysis of *NAB2–STAT6* fusion and *TERT* promoter mutations and survival analysis are listed in Tables 1 and 2.

Eighty-seven percent of the tumors could be analyzed for *NAB2–STAT6* fusion variants using RT-PCR ($n = 106$), in the remaining 30 cases RNA quality was insufficient. Of the 106 tumors, 26% had an exon4–exon2 fusion ($n = 28$) and 56% ($n = 59$) an exon6–exon16/17 fusion. In 18% of the tumors ($n = 19$), no fusion was detected, which may

Table 1 Results of *NAB2–STAT6* fusion analysis and *TERT* promoter mutation analysis: correlation with histological grading and extradural metastatic disease

	NAB2–STAT6						TERTp			
	All	Ex4-Ex2	Ex6-Ex16/17	Neg	NA	*P* value	WT	Mutation	NA	*P* value
MGS										
1	71	24	24	7	16		43	21	7	
2	54	4	29	10	11		34	15	5	
3	11	0	6	2	3		5	3	3	
Total	136					**0.006**				0.519
WHO										
I	24	13	1	1	9		15	8	1	
II	47	11	23	6	7		28	13	6	
III	65	4	35	12	14		39	18	8	
Total	136					**< 0.0001**				0.854
Extradural metastasis										
Yes	17	0	11	3	3		15	2	0	
No	57	13	33	4	7		33	16	8	
Total	74					0.121				0.09

Significant if *p* equal or below 0.05

NA not assessable, *WT TERT*p-wildtype, *MGS* updated Marseille Grading System

Table 2 Association of *NAB2–STAT6* fusion analysis and *TERT* promoter mutation analysis with survival

	No. of patients	PFS (months)			OS (months)		
		Mean	95% CI	Log-rank	Mean	95% CI	Log-rank
NAB2–STAT6							
All	128[a]	124	93–155		346	255–436	
Ex4-Ex2	26	140	94–187		306	265–348	
Ex6-Ex16/17	55	131	83–178		307	186–428	
Neg	17	88	55–122		133	96–171	
NA	30	79	50–107		189	164–215	
				0.280			0.172
TERTp							
All	128[a]	124	93–155		346	255–436	
WT	77	126	85–166		266	151–382	
Mutation	36	111	71–151		237	198–275	
NA	15	90	93–155		272	190–356	
				0.635			0.107

PFS progression-free survival, *OS* overall survival, *NA* not assessable, *WT TERT*p-wildtype

[a]8 patients died due to complications of initial therapy (e.g., surgery or radiotherapy) and were excluded for survival analysis

partly be due to our RT-PCR approach which did not cover all possible, rarely detected *NAB2–STAT6* fusion variants.

In our cohort, the *NAB2–STAT6* fusion variants are correlated to both WHO and MGS malignancy grade: The exon6–exon16/17 fusion is significantly more frequent in WHO grade II and III and MGS grade 2 and 3 tumors ($p < 0.0001$ and $p = 0.006$, respectively).

Information regarding extradural metastatic disease was available for 74 cases, and 17 of these patients had metastases during their follow-up. In this group of 17 patients, 11 (64%) had a tumor with exon6–exon16/17 gene fusion and none an exon4–exon2 fusion. In three cases, no fusion could be detected with our RT-PCR approach and in three cases, RNA quality was insufficient. The results were not statistically significant ($p = 0.121$), possibly due to the limited number of patients with extradural metastatic disease ($n = 17$). In addition, a non-significant trend ($p = 0.172$) towards shorter overall survival was noted for the exon6–exon16/17 subgroup: of the 19 patients who died from the disease, 12 had a tumor with exon6–exon16/17 fusion and only 2 with exon4–exon2 fusion. In the remaining five cases, our fusion analysis approach did not allow for identification of the exact type of *NAB2–STAT6* fusion ($n = 3$) or could not be assessed due to poor RNA quality ($n = 2$). *NAB2–STAT6* gene fusion was not correlated to progression-free survival ($p = 0.280$).

TERT promoter mutation status could successfully be analyzed in 89% of the tumors ($n = 121$). Tumors with hot-spot mutations in this promoter region were grouped as '*TERT*p-mutant' and those without such mutations as '*TERT*p-wildtype'. In 68% of the cases, the tumor was found to be *TERT*p-wildtype ($n = 82$), and in 32% *TERT*p-mutant ($n = 39$). *TERT* promoter mutation was not significantly correlated with *NAB2–STAT6* fusion type ($p = 0.164$), WHO grade ($p = 0.854$) or MGS grade ($p = 0.519$). In addition, *TERT* promoter mutation was not significantly correlated with metastatic disease ($p = 0.090$), progression-free survival ($p = 0.635$) or overall survival ($p = 0.107$), albeit a trend was noted for less aggressive clinical course in *TERT*p-mutant tumors, which is contradictory to published results in soft tissue SFT [2, 7]. Survival curves for *NAB2–STAT6* fusion groups and the presence of *TERT* promoter mutation are provided in the Supplementary Information.

In summary, although in our relatively large cohort of CNS SFTs/HPCs the type of *NAB2–STAT6* fusion lacks significant prognostic value, tumors with an exon6–exon16/17 fusion do show a tendency towards more malignant behavior compared to tumors with exon4–exon2 fusion. This difference in aggressiveness may be explained by the presence of different functional domains within the chimeric *NAB2–STAT6* protein in different fusion groups, with, e.g., the CID domain of *NAB2* only integrated in the exon4–exon2 variant resulting in EGR1 activation and fibrosis as seen in the low-grade groups as hypothesized by Barthelmeβ et al. [3]. Furthermore, in contrast to studies on mainly extradural SFTs in which *TERT* promoter mutation was shown to indicate poor disease-free survival, in our cohort clear prognostic value of *TERT* promoter mutation status was lacking.

Based on our study we conclude that so far, histology-based grading incorporating mitotic activity and necrosis remains the best indicator of prognosis in SFTs/HPCs of the CNS. However, given the trend towards more malignant behavior in the exon6–exon16/17 fusion group future and larger studies are needed to sort out if esp. *NAB2–STAT6* fusion analysis may be of additional value in histology-based prognostic models after all.

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