The Significance of Immunohistochemical Expression of Merlin, Ki-67, and p53 in Meningiomas

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Abstract: Meningiomas are one of the most common CNS tumors whose appearance is closely linked to *NF2* gene product merlin. Tumor markers Ki-67 and p53 play established role in tumor progression which should be analyzed in close association with merlin expression. The aim of this study was to investigate the immunohistochemical expression of merlin in meningiomas, correlation with Ki-67 and p53, and to determine the association of these results with histologic grade and subtype. The histologic sections of 170 patients with totally resected meningiomas, between January 2000 and December 2010, were classified according to WHO, immunohistochemically stained for Ki-67, p53, and merlin, and analyzed using light microscope. Ki-67 median was 5.6 times higher in group of patients with negative merlin than in those with positive merlin ($P = 0.05$). Statistically significant correlation of merlin with p53 was found ($P < 0.001$). Merlin expression between 2 combined groups (meningothelial/secretory and fibroblastic/transitional) was statistically significant ($P = 0.002$). By comparing merlin expression and p53 levels, statistically significant difference was found ($P = 0.017$). In the group with positive merlin and negative p53 as well as positive merlin and low p53, meningothelial/secretory subtypes of meningiomas were more common. In combination of negative merlin and negative p53 as well as negative merlin and high p53, there were more meningiomas of fibroblastic/transitional subtype. There was no statistically significant correlation between merlin and tumor grade ($P = 0.420$). There is undeniable influence of merlin on the development and the proliferative ability of meningioma subtypes. Significant role of p53 pathway was confirmed.

Key Words: meningioma, merlin, Ki-67, p53

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Meningiomas are common tumors of the central nervous system that originate from the meningeal coverings of the brain and the spinal cord. They account for about 30% of all primary brain tumors with an adjusted annual incidence of about 4 to 5 per 100,000 individuals, most commonly reported in elderly patients with peak incidence in the seventh decade of life.[1–3] Meningioma initiation is closely linked to the inactivation of one of the highly conserved protein 4.1 superfamily, neurofibromatosis type 2 (*NF2*) gene product merlin/schwannomin. Approximately 60% of sporadic meningiomas are caused by the loss of heterozygosity (LOH) on chromosome 22q12 where the NF2 tumor suppressor gene is localized. Losses on 22q12.2 region encoding the tumor suppressor merlin, represent the most common genetic alterations in early meningioma formation.[4] A number of studies suggest that merlin has a critical role in controlling cell growth and motility. Uncontrolled proliferation is one of the characteristic of malignant tumors. Mouse embryonic fibroblasts with merlin defects are associated with abnormal cell growth and motility through the destabilization of adherens junctions. Expression of wild-type merlin leads to reduced tumor growth and decreased cell motility.[5] Multiple factors are involved in tumor progression and one of the important factors is mutation of tumor suppressor gene *p53*. Mutation of *p53* gene results in metabolically stable abnormal protein that accumulates in the nucleus, reaching the level to be easily detected by immunostains. Expression of p53 protein in cells is an indicator of possible mutation in *p53* gene itself or the defective ubiquitin pathway.[6,7] Expression of p53 was found in 10% to 88% of meningiomas but their role in pathogenesis is still uncertain. Data suggest a role for the p53 pathway in the progression of meningiomas in which NF2 is inactivated, and highlights the importance of accounting for NF2LOH in future studies of meningiomas and the p53 pathway.[8]

Ki-67 antigen is a protein expressed only in proliferative phase of cell cycle. It is considered to be the most reliable proliferative marker predicting tumor behavior and can be detected on formalin-fixed paraffin-embedded tissue section.[9,10] Merlin expression might have impact on proliferative activity in meningiomas, according to high proliferative activity observed in meningiomas with merlin-negative expression.

The aim of this study was to investigate the immunohistochemical expression of merlin in meningiomas, correlation with Ki-67 and p53, and to determine the association of these results with histologic grade and subtype.

MATERIALS AND METHODS

Histologic specimens of 170 patients [70 (41%) men and 100 (59%) women] with totally resected meningiomas, diagnosed at the Department of Pathology, Split University Hospital Centre, between January 2000 and December 2010, were retrospectively reviewed. All histologic and immunohistochemical (IHC) tumor slides were evaluated by experienced pathologist (G.F.), and regrouped as benign, atypical, and anaplastic meningiomas according to the WHO classification of tumors of the central nervous system.[3]

The surgical specimens were fixed in 10% buffered neutral formalin, immediately after surgery, entirely sampled, embedded in paraffin, and 5 μm sections were stained with hematoxylin and eosin. Representative slide of each case was selected for additional IHC analysis of p53, Ki-67, and merlin, performed on automated Ventana stainer (Ultra Benchmark Ventana, Tucson, AZ).

For merlin analysis, tissue sections were heated at 95°C in W-CAP TEC buffer pH 8.0 (Bio Optica, Milano, Italy) for 20 minutes for antigen retrieval. The primary antibody NF2-Merlin, ab84550 (rabbit polyclonal to NF2/Merlin IgG isotype, immunogen affinity purified; Abcam, UK), was detected using rabbit polyclonal-epitope mapping at NF2 terminus, abCAM (dilution of 1:75 at temperature of 37°C for 32 min), with the serous ovarian cancer used as positive control.

DO-7 antibody (Dako, Denmark) at dilution 1:100 was used for p53 detection. Planocellular cervical cancer was used as positive control. It was incubated for 32 minutes and after that heated in CC1 solution for 36 minutes at 95°C. The same procedure was performed for Ki-67 for which MIB-1 antibody (Dako), with tonsillar tissue as positive control, at dilution 1:200 was used.

Immunoreactivity was detected using Ultra view universal DAB detection Kit (Ventana Medical Systems Inc., Tuscon, AZ). The nuclei were counterstained with hematoxylin.

Tissue sections were evaluated using Olympus BX41 light microscope with ×40 objective equipped with an ocular grid. Merlin staining (usually cytoplasmatic and membranous) was considered positive when present in >10% of the neoplastic cells (Fig. 1). For Ki-67 and p53, cells with brown nuclear staining were considered positive. Ki-67 and p53 expressions were defined as percentage of positive cells, determined by using Cell D1 Image analysis (Olympus; Soft Imaging System GmbH, Munster, Germany) by counting at least 1000 tumor cells in fields with the largest number of positive cells.

The data obtained were analyzed using Statistics for Windows Release 12.0 (Statsoft, Tulsa, OK). All P values <0.05 were considered statistically significant. p53 percentages were statistically grouped into 3 categories (0 = none, ≤25 = low, and >25 = high). Combination of merlin positivity/negativity with p53 levels were also created and statistically analyzed.

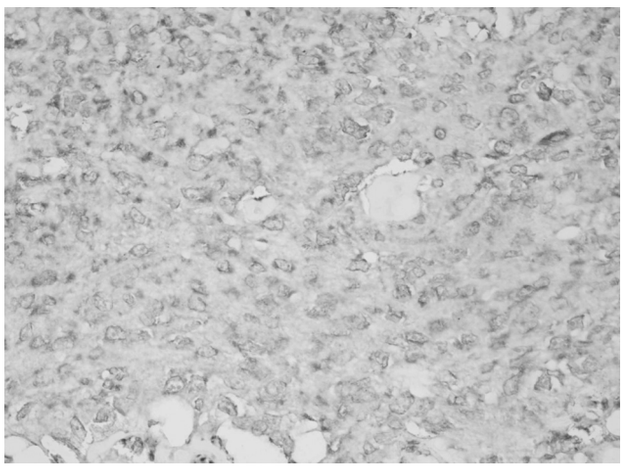

FIGURE 1. Merlin cytoplasmic staining with focally visible paranuclear granular dots in meningothelial meningioma.

RESULTS

Of total number of 170 meningiomas, 142 (83.5%) were classified as tumor grade I, 17 (10%) as grade II, and 11 (6.5%) as grade III (Table 1).

There was no statistically significant correlation between merlin and tumor grade ($\chi^2 = 1.74$; $P = 0.420$) and between number of tumors with expressed merlin between grade I and grade II + III ($\chi^2 = 1.73$; $P = 0.189$).

Median for Ki-67 was 5.6 times higher in the group of patients with negative merlin expression than in those with positive merlin expression ($z = 1.96$; $P = 0.05$).

Statistically significant correlation regarding merlin expression and p53 was found ($\chi^2 = 15$; $P < 0.001$) (Table 1). In the group with negative p53, there were 1.8 times more patients with negative merlin expression. The same result was obtained in group with high p53 where there were 2.5 times more patients with negative merlin expression. In the group with low p53, there were 2 times more patients with positive expressed merlin.

Statistically significant influence was found regarding predictive significance of merlin expression in formation of specific subtypes ($P = 0.002$) (Table 1).

There were 2.3 times more tumors with positive expression of merlin in meningothelial and secretory subtype group and 1.8 times more tumors with negative expression of merlin in fibroblastic and transitional subtype ($\chi^2 = 9.5$; $P = 0.002$).

Statistically significant difference in p53/merlin combination groups and subtypes was found ($\chi^2 = 13.8$; $P = 0.017$).

In the group with positive merlin and negative p53, there was 1.7 times more meningothelial and secretory meningiomas than fibroblastic/transitional as well as in

TABLE 1. Correlation Between Tumour Grade and Subtype, Ki-67 and p53 Levels With Merlin Expression

	n (%)		
	Merlin +	**Merlin −**	***P***
Tumour grade			
I	57 (40)	85 (60)	
II	9 (53)	8 (47)	
III	6 (54)	5 (46)	
Total	72 (42)	98 (58)	0.420*
Ki-67	2.1 (0-42.1)	11.8 (0-24.7)	0.05†
p53			
0	32 (36.4)	56 (63.6)	
≤25	28 (68.3)	13 (31.7)	
> 25	12 (29.3)	29 (70.7)	< 0.001*
Subtype (N = 145)			
Meningothelial (17) and secretory (10)	19 (70.4)	8 (29.6)	
Fibroblastic (63) and transitional (55)	42 (35.6)	76 (64.4)	0.002*

*χ^2 test.
†Kruskal-Wallis test.

combination where merlin was positive and p53 was low. While in the combination of negative merlin and negative p53 as well as negative merlin and high p53, there were more meningiomas of fibroblastic/transitional subtype than meningothelial and secretory.

DISCUSSION

The deficiency or loss of the *NF2* gene product, merlin, plays an important role in tumor development. The majority of the previous studies have indicated the NF2 protein inactivation as an early tumorigenic event in sporadic and NF2 syndrome-associated meningiomas.[11–13]

Merlin expression does not show difference according to clinicopathologic subsets and is not connected with histopathologic features predicting unfavorable outcomes.

Buccoliero et al[14] compared *NF2* gene expression in the different meningioma grades and they did not note a significant difference despite tendency to decrease from grade I to grade III.

In our study there was no significant difference in merlin expression between meningioma grades, which is consistent with results of Buccoliero and colleagues. On the contrary, merlin expression varies significantly according to subtype of meningioma.[14]

Chang et al[8] found that NF2 loss was associated with higher grade tumors, but only in men. Significant correlation of NF2 loss with higher graded tumors in men was not found in our study.

First results date back to 1995, when Wellenreuther et al[15] found *NF2* gene mutation in 83% of transitional and 70% of fibroblastic, but only in 25% of meningothelial meningiomas. Further results obtained later endorsed such differences in gene NF2 mutations.[16,17] Results of our study show significantly higher expression of merlin in meningothelial and secretory meningiomas compared with group of fibroblastic and transitional meningiomas, which is consistent with previous results.[16,17]

Merlin expression, determined by IHC analysis, depends not only to genetic mutation; it can degradate by abnormal activation of μ-calpain due to His proteolytic activity in some meningiomas.[18]

According to our results and the supporting results of previous studies regarding dependent or independent NF2 meningiomas pathogenesis, the occurrence of subtypes of fibroblastic and transitional meningiomas compared with meningothelial and secretory meningiomas was 4 times higher when merlin was not expressed.[18]

Pathogenesis of initiation, both in NF2 associated and in 60% sporadic meningiomas, is linked to the inactivation of the *NF2* gene and negative expression of merlin. In such circumstances, the likelihood of occurrence of transitional and fibroblastic subtype is significantly higher. According to this, merlin expression is significant predictor of occurrence of specific types of meningioma. Regarding the merlin expression, we analyzed coincidental proliferation characteristics of meningiomas.

Ki-67 antigen is protein expressed only in proliferative phase of cell cycle. It is considered to be the most reliable proliferative marker predicting tumor behavior.[9,10] Proliferative activity of meningiomas with negative merlin expression is more pronounced. Previously reported values of Ki-67 in benign and atypical meningiomas are estimated as 3% for benign and 8% for atypical meningiomas. We established high Ki-67 values in meningiomas with negative merlin expression, up to 5 times higher compared with meningiomas with positive merlin expression. High levels of proliferation are usually found in higher grade meningiomas that tend to be more aggressive.

Results of a study by James et al[19] on cell cultures of human meningiomas and arachnoidal cells suggest that merlin mediates cell growth by maintaining appropriate cytoskeletal organization and cell to cell communication, and also that merlin inactivation may lead to premature activation of cell senescence programs that may restrict the growth of benign meningioma in vivo.

Our findings of higher Ki-67 values in meningiomas with negative merlin expression are contradictory to results of James and colleagues. Expression of merlin in meningiomas with lower proliferative values would be consistent with several observations of cellular overproliferation and loss of contact cell inhibition in several different cell lines lacking merlin, or conversely inhibition of many signaling pathway with merlin overexpression.[20–23]

Tumor suppressor gene p53 is a protein that inhibits the proliferation of tumor cells by variety of mechanisms. Mutations of the *p53* gene have been reported to be rare in meningiomas and are mainly associated with malignant histology. Previously it was shown that NF2 and p53 double mutant mice had a higher level of tumor induction than each of the single mutant mice.[24,25]

However, considering the IHC expression of merlin and p53, we established a statistically significant correlation. Meningiomas with positive merlin expression had lower p53. These results show a tendency that IHC-detectable p53 activity might have influence in regulation of the expression of merlin.

In our study, within the group of meningiomas with positive merlin expression, there were only 16% of the tumors with p53 levels >25%, and intermediate levels were more common. Those results would indicate a moderate p53 activity in the presence of merlin. Lowest and the highest p53 levels were found more often in the group of tumors with negative merlin expression. In this group of tumors a high expression of p53, with values >25%, was found. The p53 values in the group of tumors with negative merlin expression was 2.4 times higher and significantly more presented in fibroblastic and transitional subtypes.

Kim and colleagues found a strong correlation between the levels of p53 and merlin in different brain tumor samples, using immunoblotting analysis. They showed that positive merlin expression increased the p53 stability by inhibiting the mdm2-mediated degradation of p53, which accompanied the increase in the p53-dependent transcriptional activity, and could be a positive regulator of p53 in terms of tumor suppressor activity.[25] Chang et al[8] investigated prolin72 incidence of the p53 codon in a cohort of 92 sporadic meningiomas and its association with histologic grade and NF2LOH, and they found a significant association only when considering subgroups of meningiomas with or without NF2LOH. The results of our study also suggest a role of the p53 pathway in the progression of meningiomas through inactivation of NF2.

We suppose that significant influence of the p53 pathway might exist in fibroblastic and transitional subtypes because of the lack of merlin suppressor activity. In cases with positive merlin expression, the role of p53 pathway is less pronounced. These observations remain for the further investigations and confirmation.

CONCLUSIONS

There is undeniable influence of merlin on the development and the proliferative ability of meningioma subtypes. Lack of merlin is accompanied by an increased proliferative ability of meningiomas and significant role of p53 pathway. Our findings contribute to better understanding of meningiomas pathogenesis and their future clinical behavior.

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