Expression of Ki-67 antigen in nonfunctioning pituitary adenomas: correlation with growth velocity and invasiveness

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Object. The cell cycle–dependent nuclear antigen Ki-67 is related to growth potential in a variety of tumors. Elevated expression of Ki-67 was previously shown in recurrent pituitary adenomas; however, it has remained unclear whether this expression is related to the growth velocity or invasive behavior of these tumors. The aim of this study was to determine the correlation of Ki-67 antigen expression, growth velocity, and invasiveness in nonfunctioning pituitary adenomas.

Methods. Between April 1998 and April 2002, 23 patients with nonfunctioning pituitary adenomas who had participated in an observation period in which multiple computerized tomography and magnetic resonance imaging studies had been performed were surgically treated in our department. Tumor volumes were assessed using a stereological method based on the Cavalieri principle. The growth rate was calculated for each patient. Expression of Ki-67 antigen was examined using the monoclonal antibody MIB-1.

The assessed growth velocity of the adenomas was best described by a linear growth model. The correlation between Ki-67 expression and growth rate was highly significant. Rapidly growing adenomas (> 0.07% daily increase in size) were found to have a Ki-67 labeling index (LI) exceeding 1.5%, whereas all five adenomas with a very slow growth rate (< 0.02% daily increase in size) had a Ki-67 LI lower than 1.5%. No correlation was found between the growth rate and the invasive character of the adenomas.

Conclusions. Expression of Ki-67 antigen is significantly correlated to the growth velocity of pituitary adenomas. Invasive behavior is a feature independent of proliferative activity. The extent of Ki-67 expression is helpful for clinical decision making and routine assessment of Ki-67 is recommended during the histopathological workup of pituitary adenomas.

Abbreviations used in this paper: CT = computerized tomography; LI = labeling index; MR = magnetic resonance.
Clinical Material and Methods

Patient Population

Between April 1998 and April 2002, 95 patients underwent surgery at our department for nonfunctioning pituitary adenomas. In 23 of these patients, tumor growth was documented before surgery by at least two neuroimaging studies obtained at different times. None of these 23 patients had undergone a course of radiotherapy and all 23 patients were enrolled in the study. Twenty of them underwent repeated transsphenoidal surgery after a residual adenoma had been kept under observation over a certain period of time by performing repeated CT or MR imaging studies. All 20 patients had residual tumor following previous surgical intervention(s); no true tumor recurrences were surgically treated during the study period. In five patients who had undergone more than two surgeries, only the observation period between the most recent surgery and the preceding operation was included. The remaining three patients underwent primary surgery and preoperative tumor growth was documented over a period of 34, 36, and 83 months, respectively. The mean age of our patients was 51 years (range 33–70 years). Fifteen patients were men and eight were women. No clinical or biochemical signs of hormonal hypersecretion by the tumor were found in our patients.

Volumetric Measurement and Invasiveness of the Tumor

All available CT scans and MR images were retrieved from the neuroradiological department and from radiological practices. All contiguous and parallel slices through the entire tumor were scanned (ScanJet 6100C/T; Hewlett-Packard, Palo Alto, CA) into a personal computer at a 150-dpi resolution. For the MR images, coronal sections were used for calculation of tumor volumes. For the CT scans, only the original axial sections and no reconstructions were used for volumetric measurements; the CT sections were no thicker than 3 mm.

In 12 patients the course of the disease was documented by MR imaging alone, and in seven patients tumor growth was documented by both CT and MR imaging. In the remaining four patients the course of the disease was documented by CT scanning alone, but a recent MR image was also available before surgery, allowing the evaluation of tumor invasion to be based on MR imaging criteria.

Tumor volumes were calculated using a stereological method according to the Cavalieri principle. Using this method, the three-dimensional tumor volume was calculated from two-dimensional images. The tumor contour was outlined on each sectional plane and the area (square centimeters) was calculated by a computerized program.

Invasiveness are clinically far more meaningful than histological signs of dural invasion.

Immunohistochemical Analysis Using the MIB-1 Monoclonal Antibody

The tissue sample obtained at the last surgical procedure was used for the assessment of Ki-67 expression, that is, Ki-67 expression was measured at the end of the observation period. The tissue was fixed in 4% phosphate-buffered formaldehyde for 24 hours and embedded in Paraplast, after which 5-μm-thick sections were cut and mounted on Superfrost Plus slides (Gerhard Menzel Glasbearbeitungswerk GmbH & Co., Braunschweig, Germany). Hematoxylin and eosin and reticulin fiber stain were applied and immunohistochemical detection of pituitary hormones was performed for a routine diagnostic evaluation. An immunohistochemical study for the proliferation-associated antigen Ki-67 was performed after antigen retrieval by microwave treatment in citrate buffer (pH 6, 2 × 10 minutes), blocking of endogenous peroxidase with 0.3% H2O2 in distilled water, and preincubation with 5% heat-inactivated normal goat serum. The primary antibody (anti–Ki-67 monoclonal [clone MIB-1]; Dianova, GmbH, Hamburg, Germany) was used as an undiluted working solution. Bound antibodies were visualized using a biotinylated secondary antibody and peroxidase-conjugated avidin–biotin complex (Vectastain; Vector Laboratories, Burlingame, CA), according to the manufacturer’s instructions, followed by 3,3′-diaminobenzidine. The sections were washed, dehydrated, and mounted in Entellan (Merck, Darmstadt, Germany). Positive controls...
were performed on lymphoreticular tissue (lymph node, tonsil). Negative controls were treated identically by omitting the incubation with the primary antibody. The percentage of Ki-67–positive nuclei (the Ki-67 LI) was determined by counting approximately 1500 to 2500 nuclei of tumor cells at 400-fold magnification.

Statistical Analysis

The amount of tumor growth was determined from different volumes over time (see Fig. 2). The regression line through the individual points was calculated and used to define the growth velocity of each adenoma. The growth rate of each adenoma (expressed as the daily percentage of increase in size) was calculated at the end of the observation period and before the surgical procedure, during which a tissue sample was collected for Ki-67 immunostaining. The growth rate was correlated with the Ki-67 LI by performing a linear regression analysis. The Pearson correlation coefficient was used to determine the level of statistical significance. The dependence of the Ki-67 LI and the growth rate on the invasiveness of the adenomas was analyzed using the Mann–Whitney U-test. A statistically significant difference was defined as a probability value lower than 0.05.

Results

In all 23 patients, tumor size was documented at different points of time. The patients underwent CT scanning or MR imaging between two and 10 times during the observation period. On average, the patients underwent 5.2 imaging studies. The observation period varied between 15 and 143 months (mean 54 months). In the majority of cases, the resulting growth pattern was best described by a linear growth model. Representative cases are shown in Fig. 2. Each point represents the tumor volume at a certain observation time. The regression line used for calculation of growth rate is shown. In only two cases (Cases 18 and 23) did we find accelerating growth, which resembled a logarithmic growth pattern. Figure 3 presents the MR images of a rapidly growing adenoma during the observation period.

The mean tumor volume at the time of surgery and Ki-67 assessment was 6.1 cm³ (range 1.5–22.1 cm³).

The mean Ki-67 LI was 1.75% (range 0.45–3.2%). Figure 4 shows histopathological sections following Ki-67 immunostaining in two cases, one with a low Ki-67 LI (Fig. 4 upper) and the other with a high Ki-67 LI (Fig. 4 lower). Immunopositive nuclei were clearly distinguished from immunonegative nuclei.

Fig. 3. Magnetic resonance images documenting a rapidly growing pituitary adenoma. Coronal MR imaging sections demonstrate tumor progression during a 22-month-long observation period.
Among the 23 adenomas, the increase in tumor size varied between 0 and 0.115% per day. The scatterplot in Fig. 5 shows a comparison of Ki-67 LIs and growth rates. The correlation of Ki-67 LIs to growth rates, as assessed using the Pearson correlation coefficient, was highly significant ($p < 0.001$). The correlation coefficient was 0.67. Figure 5 demonstrates that slowly growing adenomas ($< 0.02\%$ daily increase in size) yielded Ki-67 LIs lower than 1.5% and rapidly growing adenomas ($> 0.07\%$ daily increase in size) yielded Ki-67 LIs higher than 1.5%. Rapid growth with a daily increase of greater than 0.07% equals a yearly increase that is greater than 25% of the adenoma volume. In those adenomas with moderate growth velocities (0.02–0.07% daily increase in size), a wide range of Ki-67 LIs was observed.

Clinically, all tumors were nonfunctioning adenomas. In 21 adenomas, the histopathological diagnosis was null-cell adenoma. The remaining two adenomas were silent gonadotroph adenomas. The Ki-67 LIs of the two gonadotroph adenomas were 1.2 and 1.98%, respectively. These silent gonadotroph adenomas belonged to the group of tumors with moderate growth velocity.

Twelve of the 23 adenomas were clinically noninvasive and all of them were completely removed during the last surgical procedure. In 11 adenomas, gross tumor invasion was evident. In all cases of invasive tumor, the cavernous sinus was invaded. Additional invasion of the sphenoid sinus was encountered in three of these cases. The Ki-67 LIs were not significantly different regardless of whether the adenomas were invasive (Fig. 6 left). The mean Ki-67 LI of noninvasive adenomas was 1.89% (range 0.6–3.2%); a slightly lower mean Ki-67 LI of 1.6% (range 0.45–2.7%) was found in invasive adenomas.

Similarly, the growth rate did not statistically differ between noninvasive and invasive adenomas (Fig. 6 right). A mean daily growth rate of 0.045% was found in noninvasive adenomas and a rate of 0.041% in invasive adenomas.

**Discussion**

The correlation of the proliferation-parameter Ki-67 nuclear antigen to various biological features of pituitary adenomas has been previously studied. Controversy surrounds the question of whether a difference in Ki-67 immunostaining exists between hormone-secreting and -nonsecreting pituitary adenomas. Although some authors have found no difference,1,13,29 others have shown a higher Ki-67 growth fraction in hormone-secreting adenomas15,16,26 or significantly higher Ki-67 LIs only in adrenocorticotropic hormone-secreting adenomas.20 No clear correlation has been found between tumor size and Ki-67 immunostaining.1,13,19,25,27 A significantly higher Ki-67 proliferation index has been found in adenomas with subsequent regrowth1,11,22 and in recurrent adenomas.25,27 Mizoue, et al.,22 have shown a higher mean Ki-67 LI by using the MIB-1 antibody in eight patients who experienced rapid regrowth of subtotally or partially resected adenomas within 2 years.

Aggressive behavior and increased proliferative activity are generally accepted features of recurrent tumors, and increased Ki-67 LIs in recurrent adenomas may reflect increased growth potential in these tumors. Recurrent adenomas often represent a heterogeneous group, however. Series of so-called recurrent tumors include aggressively or slowly growing adenomas, true recurrences following complete removal, or regrowth of residual adenomas. The invasive character of these lesions might also account for the occurrence of a relapse, and tumor invasion has been shown to be the strongest predictor of recurrence.37 Hence, the finding of
an elevated Ki-67 LI in recurrent adenomas only provides indirect evidence that Ki-67 correlates with the growth velocity of adenomas. Furthermore, Ki-67 expression shows a significant overlap between recurrent and nonrecurrent adenomas.1,27

Only two groups8,18 have directly compared growth velocity and Ki-67 expression in nonfunctioning adenomas. Luedecke and colleagues18 have assessed the increase in the largest tumor diameter. They only found Ki-67 (MIB-1) positivity among adenomas growing more than 1 mm per year. Furthermore, two thirds of their tumors with a high growth rate demonstrated low or no Ki-67 positivity. Ekramullah and colleagues8 assessed tumor-doubling time in 14 regrowing adenomas and showed a significant inverse correlation with Ki-67 staining indices. We have determined the growth velocity of 23 adenomas in patients by performing serial neuroimaging studies. Tumor growth was best described by a linear growth model in the majority of our cases. The growth rate was expressed as the daily percentage of increase in the size of the adenomas. The results were correlated with the proliferation parameter Ki-67. The results of this study demonstrate a highly significant correlation of Ki-67 expression and growth rate in nonfunctioning pituitary adenomas. All rapidly growing adenomas with a daily growth rate exceeding 0.07% were found to have Ki-67 LIs greater than 1.5%. Rapid growth with a daily increase of more than 0.07% equals a yearly increase greater than 25% of the adenoma volume. Slowly growing adenomas (< 0.02% daily increase) were found to have Ki-67 LIs lower than 1.5%. Among adenomas with a moderate growth velocity (0.02–0.07% daily increase), an overlap with both high and low Ki-67 expression was found.

Apparently, a Ki-67 LI of 1.5% is a clinically useful cutoff value. If a Ki-67 LI exceeding 1.5% is detected in a surgical specimen, a higher risk of rapid tumor regrowth exists. On the other hand, rapid regrowth is unlikely if the Ki-67 LI is lower than 1.5%. This finding is in agreement with the suggestion from previous studies25 that a proliferating cell index exceeding 1.5% correlates with rapid regrowth and the likelihood of recurrence. Abe and coworkers1 found a regrowth rate of 50% in adenomas with MIB-1 indices exceeding 1.5%, and a regrowth rate of only 16% in those adenomas with indices lower than 1.5%. In our opinion, the Ki-67 LI represents an additional piece of information that is helpful for clinical decision making. An adenoma with a high Ki-67 LI is at risk to regrow early. It may need to be watched more closely or may be treated with radiotherapy. On the other hand, an adenoma with a low Ki-67 LI may remain quiescent for years. Nevertheless, because of the overlap of Ki-67 LIs, particularly in those adenomas with moderate growth velocity, the prognosis cannot be predicted on the basis of the Ki-67 LI alone.

It is still controversial whether Ki-67 expression correlates with invasive behavior of pituitary adenomas.1,3,5,10,11,13,15,16,19,25,26,28,29 An elevated Ki-67 LI has been shown in microscopically invasive pituitary adenomas, compared with noninvasive adenomas.13,15,20 Interestingly, Landolt and coworkers19 found no difference in Ki-67 expression when invasiveness was judged by clinical criteria rather than by microscopic examination of the dura mater. Higher Ki-67 LIs have been described in adenomas that invade the cavernous sinus.10,19 In contrast, Yokoyama, et al.,23 found no differences in Ki-67 expression regardless of whether the cavernous sinus was involved. Buchfelder and coworkers5 showed a significant difference in Ki-67–positive cells when noninvasive microadenomas and invasive macroadenomas were compared. Thapar and colleagues26 reported finding a significantly higher Ki-67 LI in adenomas that

![Graphs demonstrating the Ki-67 LIs (left) and growth rates (right) of noninvasive and invasive nonfunctioning pituitary adenomas.](image-url)
displayed clinical gross invasion. These authors concluded that invasion is a feature of more rapidly growing adenomas. In contrast, a number of other studies failed to show a significant difference in Ki-67 LIIs between invasive and noninvasive adenomas. We cannot readily account for these discordant results in the literature. Our study supports the notion that Ki-67 antigen expression reflects growth potential rather than the invasive character of an adenoma. We present the first study in which growth velocity was directly compared with the invasive character of the adenomas. Growth velocity was not different regardless of whether the adenomas were clinically invasive. Our study provides evidence that Ki-67 is correlated to the growth velocity, but not to the invasive behavior of nonfunctioning pituitary adenomas.

Conclusions

We conclude that Ki-67 antigen expression is a significant predictor of growth potential in pituitary adenomas. Therefore, we recommend the routine assessment of Ki-67 expression in pituitary adenomas during the histopathological workup. It appears that growth velocity and invasion are independent biological features of pituitary adenomas.

References


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