Correlation of leptin receptor expression with BMI in differential grades of human meningiomas

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Abstract. Meningioma is one of the most common primary brain tumor, especially in postmenopausal women. The most important risk factors include radiation, primary head injury or genetic alterations, however it is currently unclear why postmenopausal women are predominantly affected. The aim of the present study was to evaluate leptin receptor (LEPR) expression and body mass index (BMI) in patients with meningiomas of differential grades. Specimens of 158 meningiomas were classified as either G1 (low-grade meningiomas, n=114) or G2/G3 (high-grade meningiomas, n=44). Immunohistochemistry was performed to assess LEPR expression. The mean BMIs of the female and male patient groups were 28.43±5.29 and 23.93±4.66, respectively. Mean BMI was significantly higher in the female group, by ~4.50 kg/m^2. Patient age significantly correlated with LEPR expression, with the highly positive (++) and positive (+) groups having mean ages of 62.3±12.07 and 52.3±13.04, respectively. A strong positive correlation (r=0.73) was observed between leptin receptor expression and BMI, with the LEPR (++) group having a mean BMI of 30.11±4.49, compared to 22.12±2.48 for the LEPR (+) group. Furthermore, in the low-grade meningioma group, mean BMI was higher in female patients than male patients (28.13±5.54 and 25.38±4.57, respectively; P=0.01). Additionally, there was strong positive correlation between BMI and leptin receptor expression in the low-grade meningioma group (r=0.69). For the high-grade meningioma group, mean BMI was 29.49±4.26 and 21.76±3.98 in female and male patients, respectively, and LEPR expression strongly correlated with BMI in this group (r=0.80). The present study demonstrates a correlation between patient BMI, age, and LEPR expression status in low- and high-grade meningiomas. Our results indicate that in addition to endogenous hormones, such as estrogen or progesterone, or fatty tissue-associated proinflammatory cytokines, LEPR expression status may be a risk factor for meningioma growth and progression.

Introduction

Meningiomas are the second most common primary brain tumor. The majority are biologically benign, however, 5-15% are malignant, with features of brain and skull infiltration, and ultimately lead to death. Meningioma is the most common primary brain neoplasm in postmenopausal women (1). The major risk factors for developing meningioma include head injury, female gender, and previous irradiation, as well as genetic alterations, such as those observed in Li Fraumeni syndrome and neurofibromatosis type I patients (2). In addition, numerous endogenous risk factors are proposed to be associated with meningioma development. A number of previous studies propose that meningioma risk increases with increased weight and body mass index (BMI). Risk of meningioma may also be influenced by the expression of sex hormones (3). Over the last decade, numerous types of tumors have been correlated with obesity, including colorectal and breast cancer, and uterus adenocarcinoma (4-6).

A previous study has demonstrated an association between meningioma risk and higher weight, greater BMI, and lower levels of physical activity (7). The mechanism of BMI and cancer association remains to be elucidated, however, previous studies suggest that BMI may affect cancerogenesis through sex hormone levels and insulin resistance, which may be relevant to meningioma (1,8). Other studies claim that BMI is only relevant in postmenopausal age, however, BMI at 30 or 18 years was not associated with an increased meningioma risk, suggesting that current or recent hormonal or metabolic effects of BMI around the time of diagnosis may be more important than any historical measurements (1). Recent data demonstrates that increased total physical activity is negatively associated with the concentrations of estrone, estradiol, and androstendione in postmenopausal women, and is positively associated with increased insulin sensitivity (7). The BMI-meningioma connection has been discussed in recent studies, and a number of the studies reveal a 40-60% increase in meningiomas in individuals with the highest BMIs compared to those with the lowest (9-11). Meningioma associated with obesity may explain cases in which the most common risk factors are absent (such as radiation or injury).

Women are more commonly affected by meningiomas, and therefore, there may an association with increased BMI in postmenopausal women (compared with men), as well as the effect of circulating hormones (estrol, estradiol), insulin resistance [with elevated insulin growth factor 1 (IGF-1)], and inflammatory response factors (1). The relationship between BMI and meningioma may involve the mediation of other hormonal factors, such as leptin.

Leptin, discovered in 1994, is a product of the LEP gene (also known as OB). In humans, LEP is located on the 7 alpha chromosome, and contains three exons separated by two introns. Leptin is a hormone synthesized by fatty tissue, and is responsible for the regulation of physiological processes, such as the modulation of appetite and thermogenesis, as well as pathological processes (9). A number of studies have demonstrated that the expression of the leptin receptor (LEPR, also known as ObR) correlates with the presence of leptin, suggesting that leptin-dependent malignancy may be regulated through autocrine and paracrine mechanisms (5,9). Leptin receptor isoforms are expressed throughout the central nervous system and a number of peripheral tissues, and six isoforms have been described previously. All LEPRs belong to the cytokine receptor family, which signal through janus kinases (JAKs) and signal transducers and activators of transcription (STAT) (5,9,12). Overexpression of leptin and leptin receptor has been demonstrated previously in human cancers, including breast, colorectal, endometrial and prostate (9,12). Furthermore, previous studies have demonstrated the involvement of leptin in cancer cell migration, invasion, and vascular endothelial growth factor-independent angiogenesis (4). Additionally, leptin may activate the pathways of growth factors, such as epidermal growth factor or insulin-like growth factor-1 (12).

The aim of the present study was to evaluate LEPR expression in human meningiomas of differential grades and explore whether this correlates with the BMI of the meningioma patients.

Materials and methods

Patient specimens. In total, 158 surgically removed meningioma specimens were obtained during craniotomy, from patients at the University Hospital in Bialystok (Bialystok, Poland), following receipt of their written consent. Specimens were examined in the Department of Neurosurgery, Medical University of Bialystok (Bialystok, Poland), and were obtained from 2000 to 2013. The specimens were fixed in RCL2® solution (ALPHELYS, Plaisir, France) and then routinely embedded in paraffin blocks. Slides were stained with hematoxylin and eosin, and the meningioma grade was evaluated by a pathologist as G1 (low-grade) or G2/G3 (high-grade), according to the WHO classification Lyon 2007 (13). Ethical approval for the current study was granted by the ethics committee of the Medical University of Bialystok.

BMI calculation and classification. The BMI of the patients included in the study was calculated using the following formula: $BMI = weight / (height^2)$. The BMI results were divided into the following six groups: <18.5 was classified

as underweight, 18.5 to 24.9 as normal weight, 25 to 29.9 as overweight, 30 to 34.9 as slight obesity (I°), 35 to 39.9 as medium obesity (II°), >40 was classified as pathological obesity (III°).

Immunohistochemistry for leptin receptor. Immunohistochemistry was performed to assess LEPR expression in the meningioma specimens obtained from the patients. Following deparaffinization and rehydration, epitope retrieval was performed using EnVision Flex Target Retrieval Solution (Dako, Glostrup, Denmark) in high pH conditions. Endogenous peroxidase activity was blocked by incubating the sections in methanol and 3% hydrogen peroxidase for 20 min. The slides were then incubated with a polyclonal goat anti-mouse IgG, suitable for detecting the C-terminus of all human LEPR isoforms (Ob-R antibody (M-18); cat no. sc-1834; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The primary antibody was incubated overnight at 4°C, and antibody-epitope complexes were visualized using the EnVision FLEX, High pH (Link) system (Dako) and 3,3'-diaminobenzidine (Dako) for 10 min.

Appropriate positive and negative controls were used for the immunohistochemistry. Negative controls used nonimmunized IgG in place of the LEPR primary antibody. Human skeletal muscle tissue with cytoplasmic staining of myocytes was used as a positive control. The slides were then counterstained with hematoxylin and examined under a light microscope (BX45, Olympus Corp., Tokyo, Japan). Evaluation of the LEPR status of the patient specimens using the results of the immunohistochemistry was performed by two independent pathologists. Cells stained positively for LEPR were counted in 10 representative high-power fields and classified as follows: Negative (-) with ≤10% of positive cells, positive (+) with 11-49% of positive cells (focal, moderate expression), and highly positive (++) with ≥50% positive cells (strong and diffuse expression). Counts were made in a set of 10 random fields with x20 magnification (8).

Statistical analysis. The Chi square test and Spearman correlation were used in the current study. Statistical analyses were performed using SYSTAT version 12 software (Systat Software GmbH, Erkrath, Germany). P<0.05 was considered to represent a statistically significant difference.

Results

Classification of patient meningiomas. The present study included 158 meningioma cases: 114 were classified as low-grade (G1) and 44 were classified as high-grade (both G2 and G3). Of the low-grade meningiomas, 27 were fibrous, 8 menigothelial, 78 transitional, and 1 angiomatous. In the high-grade group, 2 cases were anaplastic, 1 clear cell, and 1 chordoid type; the remaining specimens were atypical menigiomas. Patient age ranged from 26-88 years (mean 58.3), and there were 108 female and 50 male patients.

Patient BMI. The mean BMI in the female patient group was 28.43±5.294, and 23.93±4.66 in the male group. The BMI of the female group was significantly higher than that of the males, by ~4.50 (P=0.001). Furthermore, two patients

Table I. Patient gender and leptin receptor expression status.

Gender	(-) Negative, n (%)	(+) Positive, n (%)	(++) Highly positive, n (%)	Total, n (%)
Female	1 (0.64)	23 (14.56)	84 (53.16)[a]	108 (68.35)
Male	4 (2.53)	34 (21.52)	12 (7.59)[a]	50 (31.65)
Total	5 (3.17)	57 (36.08)	96 (60.75)	158 (100.0)

[a]P=0.001.

Table II. Grade of meningioma and leptin receptor expression status.

Grade	(-) Negative, n (%)	(+) Positive, n (%)	(++) Highly positive, n (%)	Total, n (%)
Low	5 (3.17)	36 (22.79)	73 (46.20)[a]	114 (72.15)
High	0 (0.00)	21 (13.29)	23 (14.56)[a]	44 (27.85)
Total	5 (3.17)	57 (36.08)	96 (60.76)	158 (100.0)

[a]P=0.084.

Table III. Leptin receptor expression status in BMI groups.

BMI Group	(-) Negative, n (%)	(+) Positive, n (%)	(++) Highly positive, n (%)	Total, n (%)
Underweight	-	2 (1.27)	-	2 (1.27)
Normal weight	4 (2.53)	45 (28.48)[a]	9 (5.70)	58 (36.71)
Overweight	-	10 (6.33)	44 (27.85)[a]	54 (34.18)
I° Obesity	1 (0.63)	-	33 (20.89)[a]	34 (21.52)
II° Obesity	-	-	9 (5.70)	9 (5.70)
III° Obesity	-	-	1 (0.63)	1 (0.63)
Total	5 (3.16)	57 (36.08)	96 (60.76)	158 (100.0)

[a]P=0.001. BMI, body mass index.

were underweight, 58 patients were normal weight, 54 were overweight, 44 were obese (34 had I degree of obesity, 9 had II degree, and 1 patient had pathological obesity).

Leptin receptor expression correlates with age and gender. Although only a weak positive correlation was found between patient age and BMI (r=0.37), a statistically significant difference in mean patient age was identified between leptin receptor expression groups. In meningioma specimens with strong and diffuse expression (++) the mean age of patients was 62.3±12.07, and in group with only focal expression (+), the mean age was 52.3±13.04 (P=0.001).

Leptin receptor expression is significantly higher in female patients compared to males. In ~78% of meningioma specimens in the female patient group, LEPR expression was identified as highly positive, strong and diffuse (++), whereas this was the case for only ~24% of specimens in the male group, with 68% of male specimens exhibiting only focal, moderate expression (+) of LEPR (Table I).

Leptin receptor expression does not correlate with meningioma grade. No statistically significant difference in leptin receptor expression was identified between the low- and high-grade meningioma groups (P=0.084). However, in the low-grade meningioma group, 73 out of 114 cases, (64%) exhibited strong and diffuse LEPR expression, and 23 of 44 (52.3%) high-grade meningiomas also exhibited strong and diffuse LEPR expression (Table II; Fig. 1).

Elevated leptin receptor expression correlates with a high BMI. A strong positive correlation was observed between leptin receptor expression and BMI in the examined groups. The mean BMI of the (+) LEPR expression group
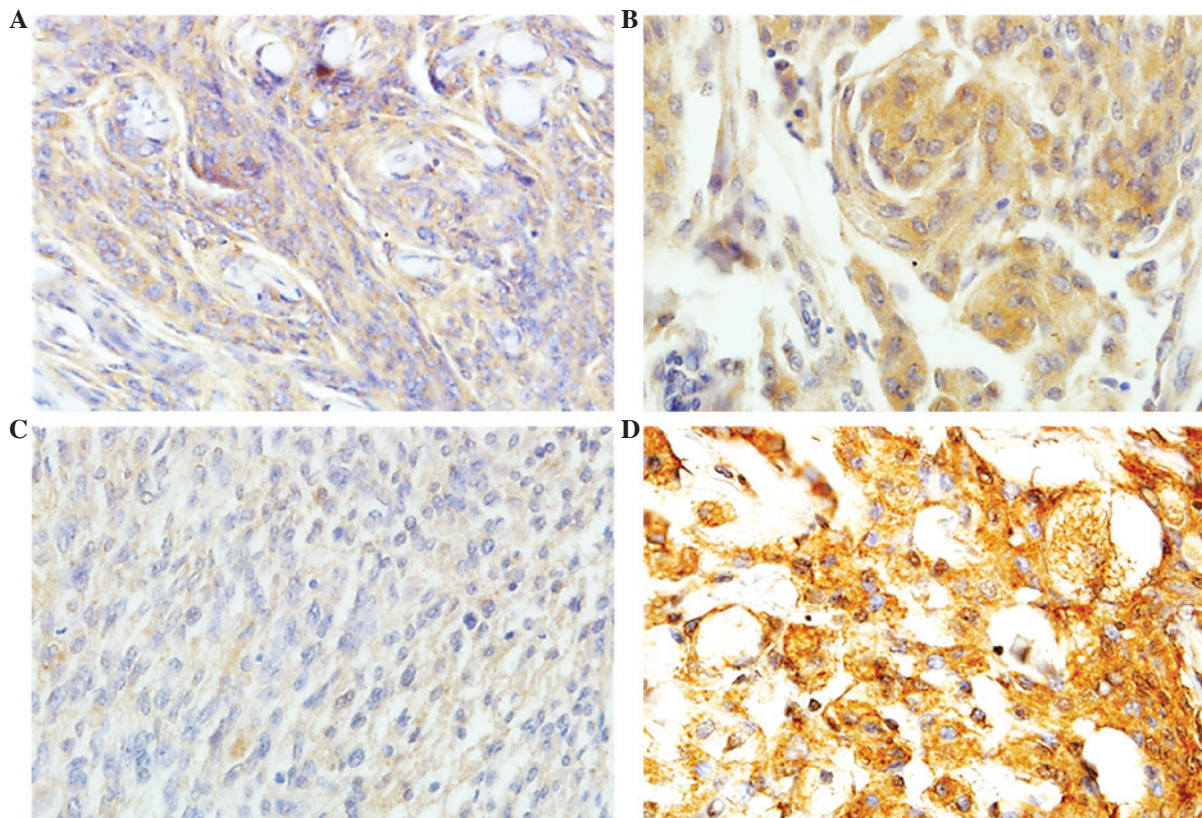
Figure 1. (A) Low-grade (G1), transitional meningioma with strong and diffuse LEPR expression. Magnification, x200. (B) Low-grade (G1), menigiomatous meningioma with LEPR expression. Magnification, x400. (C) High-grade (G3), anaplastic meningioma with focal LEPRexpression. Magnification, x200. (D) High-grade (G2), atypical meningioma with strong and diffuse LEPR expression. Magnification, x200. LEPR; leptin receptor.

was 22.12±2.48, whereas that of the (++) LEPR expression group was 30.11±4.49. This indicates that a higher BMI is associated with elevated LEPR expression (r=0.73; P=0.001; Table III).

BMI association with grading and patients age and gender. In the low-grade meningioma group, 84 patients were females, aged from 27-88 years (mean 60.3), with a mean BMI of 28.13±5.54, and 30 were males, aged from 26-71 years (mean 58.5), with a mean BMI of 25.38±4.57. In this group, there was a strong positive correlation between patient BMI and leptin receptor expression (r=0.69). The high-grade meningioma group consisted of 24 female patients, aged 28-76 years (mean 57.0) with a mean BMI of 29.49±4.26, and 20 male patients, aged 26-79 (mean 50.80) with a mean BMI of 21.76 ±3.98. LEPR expression strongly correlated with BMI this group (r=0.80).

Discussion

Obesity plays a complex role in a number of different of human neoplasms. Increased BMI is associated with more aggressive progression and reduced survival of a number of cancers, especially breast cancer (5,7). It is also known that obesity is associated with increased inflammation, angiogenesis, and increased levels of many growth regulators, such as leptin (5). A previous study has demonstrated the presence of LEPR in breast cancer, and that high serum leptin levels are associated with a poor prognosis (4). Leptin expression is positively

correlated with body weight, BMI, and total body fat. Increased serum leptin levels and overexpression of LEPR in tissues is associated with an elevation in IGF-1 levels, increased cell proliferation and angiogenesis, and decreased cell death (9). Furthermore, increased detection of LEPR in ovarian cancer correlates with decreased patient survival, and elevated leptin expression levels (compared with those of healthy or preoperative patients) has been reported in hepatocellular cancer and prostate cancer patients, although in pancreatic cancer patients, leptin expression appears unchanged (5,6,9).

In the present study, the correlation between BMI and leptin expression was investigated, together with clinicopathological features. A statistically significant correlation was observed between patient gender and BMI. The mean BMI was highest in the group of menopausal and postmenopausal women with meningioma. Our results are similar to those presented in the previous literature. Benson *et al* (14) demonstrated that BMI is associated with tumor incidence in the central nervous system, with an increased risk of ~20% per 10 kg/m^2 increase in BMI. Additionally, Michaud *et al* (7) observed a positive association between BMI and meningioma risk. Johnson *et al* (1) demonstrated a correlation between lack of physical activity, BMI, height, and meningioma risk in older women, and also Wiedmann *et al* (15) confirmed these results by demonstrating an association between obesity and meningioma risk in women.

A number of previous studies support a possible correlation between sex hormones (such as estrogens and progesterone) and meningioma development, however, current data concerning LEPR expression in brain tumors, especially

meningiomas, is lacking. Some of these previous studies implicate endogenous estrogen in meningioma development. Schildkraut *et al* (16) observed that increased BMI associated with a 2-fold increased risk of developing meningioma. The authors concluded that endogenous estrogen-associated factors, such as a high BMI, may increase the risk of developing meningioma. Jhawar *et al* (3) have also proposed that increased BMI and endogenous estrogens are important contributors to an increased risk of meningioma.

While the association between BMI and meningioma risk may be mediated by hormonal factors, other factors may be involved, such as the inflammatory factors tumor necrosis factor α, interleukin 6, and C-reactive protein (10). In addition, the overexpression of IGF-I, IGF-II and IGF receptor 1 has been previously reported in meningiomas (17).

Although our current study did not observe a statistically significant association between leptin receptor expression and meningioma grading, high LEPR expression was observed in the low-grade meningioma group compared to the high-grade group (46.2% and 14.5%, respectively). Importantly, LEPR expression was associated with BMI in both low- and high-grade meningiomas groups. Patients that were assessed as being overweight or obese, and with either low- or high-grade meningiomas, had significantly increased LEPR expression compared with those patients assessed as normal weight or underweight. These results are partly supported by Menghi *et al* (18), who investigated LEPR expression in meningiomas, and identified that high expression of the LEPR was significantly higher in low-grade meningiomas than high-grade, and it may serve as a relevant prognostic marker to define progression risk in meningioma patients. In support of this finding, Knerr *et al* (19) observed high LEPR expression in meningiomas.

In conclusion, the present study demonstrates a correlation between patient BMI, age, and LEPR expression in low- and high-grade meningiomas. Our results demonstrate that in addition to endogenous hormones such as estrogen or progesterone, or fatty tissue-associated, proinflammatory cytokines, LEPR expression status may be an important risk factor for meningioma growth and progression

References

1. Johnson DR, Olson JE, Vierkant RA, Hammack JE, Wang AH, Folsom AR, Virnig BA and Cerhan JR: Risk factors for meningioma in postmenopausal women: Results from the Iowa Women's Health Study. Neuro-oncol 13: 1011-1019, 2011.
2. Alexiou GA, Markoula S, Gogou P and Kyritsis AP: Genetic and molecular alterations in meningiomas. Clin Neurol Neurosurg 113: 261-267, 2011.
3. Jhawar BS, Fuchs CS, Colditz GA and Stampfer MJ: Sex steroid hormone exposures and risk for meningioma. J Neurosurg 99: 848-853, 2003.
4. Fiorio E, Mercanti A, Terrasi M, Micciolo R, Remo A, Auriemma A, Molino A, Parolin V, Di Stefano B, Bonetti F, *et al*: Leptin/HER2 crosstalk in breast cancer: In vitro study and preliminary in vivo analysis. BMC Cancer 8: 305, 2008.
5. Grossmann ME, Ray A, Nkhata KJ, Malakhov DA, Rogozina OP, Dogan S and Cleary MP: Obesity and breast cancer: Status of leptin and adiponectin in pathological processes. Cancer Metastasis Rev 29: 641-653, 2010.
6. Park J and Scherer PE: Leptin and cancer: From cancer stem cells to metastasis. Endocr Relat Cancer 18: C25-C29, 2011.
7. Michaud DS, Bové G, Gallo V, Schlehofer B, Tjønneland A, Olsen A, Overvad K, Dahm CC, Teucher B, Boeing H, *et al*: Anthropometric measures, physical activity, and risk of glioma and meningioma in a large prospective cohort study. Cancer Prev Res (Phila) 4: 1385-1392, 2011.
8. Riolfi M, Ferla R, Del Valle L, Piña-Oviedo S, Scolaro L, Micciolo R, Guidi M, Terrasi M, Cetto GL and Surmacz E: Leptin and its receptor are overexpressed in brain tumors and correlate with the degree of malignancy. Brain Pathol 20: 481-489, 2010.
9. Nalabolu MR, Palasamudram K and Jamil K: Adiponectin and leptin molecular actions and clinical significance in breast cancer. Int J Hematol Oncol Stem Cell Res 8: 31-40, 2014.
10. Rajaraman P: Hunting for the causes of meningioma--obesity is a suspect. Cancer Prev Res (Phila) 4: 1353-1355, 2011.
11. Shao C, Bai LP, Qi ZY, Hui GZ and Wang Z: Overweight, obesity and meningioma risk: A meta-analysis. PLoS One 9: e90167, 2014.
12. Uddin S, Hussain AR, Khan OS and Al-Kuraya KS: Role of dysregulated expression of leptin and leptin receptors in colorectal carcinogenesis. Tumour Biol 35: 871-879, 2014.
13. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P: The 2007 WHO Classification of tumours of the central nervous system. Acta Neuropathol 114: 97-109, 2007.
14. Benson VS, Pirie K, Green J, Casabonne D and Beral V; Million Women Study Collaborators: Lifestyle factors and primary glioma and meningioma tumours in the Million Women Study cohort. Br J Cancer 99: 185-190, 2008.
15. Wiedmann M, Brunborg C, Lindemann K, Johannesen TB, Vatten L, Helseth E and Zwart JA: Body mass index and the risk of meningioma, glioma and schwannoma in a large prospective cohort study (The HUNT Study). Br J Cancer 109: 289-294, 2013.
16. Schildkraut JM, Calvocoressi L, Wang F, Wrensch M, Bondy ML, Wiemels JL and Claus EB: Endogenous and exogenous hormone exposure and the risk of meningioma in men. J Neurosurg 120: 820-826, 2014.
17. Baxter DS, Orrego A, Rosenfeld JV and Mathiesen T: An audit of immunohistochemical marker patterns in meningioma. J Clin Neurosci 21: 421-426, 2014.
18. Menghi F, Orzan FN, Eoli M, Farinotti M, Maderna E, Pisati F, Bianchessi D, Valletta L, Lodrini S, Galli G, *et al*: DNA microarray analysis identifies CKS2 and LEPR as potential markers of meningioma recurrence. Oncologist 16: 1440-1450, 2011.
19. Knerr I, Schirl C, Horbach T, Stuppy A, Carbon R, Rascher W and Dötsch J: Maturation of the expression of adrenomedullin, endothelin-1 and nitric oxide synthases in adipose tissues from childhood to adulthood. Int J Obes 29: 275-280, 2005.