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의학석사 학위논문

Expression of c-Met  
in Invasive Meningioma

침윤성 뇌수막종에서  
c-Met 의 발현

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침윤성 뇌수막종에서  
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in Invasive Meningioma

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# Expression of c-Met in Invasive Meningioma

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## ABSTRACT

**Introduction:** Meningiomas are divided into three grades according to 2007 WHO classification and most meningiomas are benign (WHO grade I). However, meningiomas show high recurrence rate even after curative tumor removal and invasiveness of tumor may contribute to the high recurrence rate. Recently, c-Met, HGF, AQP1 and NKCC1 have been reported to be involved in cancer invasion. The aim of this study was to elucidate whether protein expressions of c-Met, HGF, AQP1 and NKCC1 were associated with clinicopathologic variables as well as brain and bone/scalp invasion in large scale of meningiomas.

**Methods:** We examined immunohistochemical expression of c-Met, HGF, AQP1 and NKCC1 in 198 cases of meningiomas treated with curative tumor removal (Simpson grade I or II). Kaplan – Meier analyses were used to evaluate whether patients with meningioma had a different recurrence free survival depending on c-Met, HGF, AQP1 and NKCC1 expression status.

**Results:** c-Met<sup>-High</sup> was observed in 18.9% (35/185) without brain invasion and 46.2% (6/13) meningiomas with brain invasion. c-Met<sup>-High</sup> significantly correlated with brain invasion. ( $P = 0.030$ ) And it was also observed in 18.4% (32/174) of meningiomas without bone/scalp invasion and in 37.5% (9/24) of meningiomas with bone/scalp invasion. There was a tendency for increased c-Met<sup>-High</sup> in meningiomas with bone/scalp invasion compared with meningiomas without bone/scalp invasion, although statistical significance was not reached. ( $P = 0.055$ ) HGF<sup>-High</sup> did not show statistical association with brain invasion or bone/scalp invasion. ( $P = 0.222$ ,  $P = 0.108$ , respectively) On

the other hand, AQP1<sup>-High</sup> showed significant inverse correlation with brain invasion. (31.9% [59/187] vs. 0% [0/11],  $P = 0.011$ ) But AQP1<sup>-High</sup> showed no significant difference in bone/scalp invasion. ( $P = 0.812$ ) NKCC1 did not show statistical association with brain invasion or bone/scalp invasion. ( $P = 0.598$ ,  $P > 0.9$ , respectively) c-MET<sup>-high</sup> showed shorter recurrence free survival ( $93.467 \pm 8.211$  months) than c-MET<sup>-low</sup> ( $96.131 \pm 1.911$  months) however, it did not reach statistical significance. ( $P = 0.139$ ) There was no association of HGF<sup>-high</sup>, AQP1<sup>-High</sup> and NKCC1<sup>-High</sup> expression with recurrence free survival.

**Conclusions:** We demonstrated that c-Met<sup>-High</sup> was associated with brain invasion of meningiomas and that c-Met expression could be a useful predictive marker for meningioma recurrence.

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**Keywords:** invasive meningioma, c-Met, brain invasion

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## **Introduction**

Meningioma is a common intracranial tumor arising from meningotheial (arachnoid) cells. Meningiomas are divided into 15 histologic subtypes and 3 grades such as benign (grade I), atypical (grade II), and anaplastic (grade III). (1) Most of meningiomas are benign, corresponding to WHO grade I and have a favorable outcome. Whereas, grade II atypical meningiomas and grade III anaplastic meningiomas have less favorable outcomes. (2, 3) Meningiomas, even benign cases, have a high recurrence rate after curative surgical treatment and it has been estimated to recur in 7-25%, 29-52% and 50-94% in grade I, II and III, respectively. (4)

Several markers, such as proliferation index, vascular density marker and expression of sex hormonal receptor are suggested to predict the recurrence of tumor. However it is generally accepted that both histopathological features including histologic subtypes and clinical data have a limitation to be used as reliable markers predicting tumor recurrence due to low accuracy. (5) Previous studies reported an association between meningioma with brain or bone invasion and higher tumor recurrence rate. (6, 7) However, the mechanism of its invasion has not been well established.

c-Met is a receptor tyrosine kinase that, on binding of its ligand, hepatocyte growth factor (HGF) is phosphorylated and subsequently activates signaling pathway of cell proliferation, migration. c-Met/HGF signaling pathway was first described as an oncogene in the 1980s. This pathway has

been known to induce tumor cell proliferation, motility and invasion as well as to promote angiogenesis in several human cancers, such as breast carcinomas, lung carcinomas and hepatocellular carcinomas. (8, 9) In meningioma, expression of c-Met/HGF has been reported to have diverse relationship with tumor recurrence, angiogenesis, histologic subtypes and invasiveness of meningioma. Those reports were based on the limited number of samples and different methods such as enzyme-linked immunosorbent assay, immunohistochemistry or RT-PCR. (10-12) To date, little is known about their expression in invasive meningiomas.

Ion channels and transporters that affect cell shape and movement also have been suggested that these are involved in neoplastic cell migration and metastasis. (13) Aquaporins are a family of small transmembrane water channels that have a major role in transcellular and transepithelial water transport. To date, 13 distinct subtypes of aquaporin have been identified in tissue and organism. Several types of aquaporins have been demonstrated in the central nerve system. (14, 15) Aquaporin 1 is mainly present in choroid plexus epithelium and it is important in the formation of cerebrospinal fluid. (16) Recently, it has been identified that aquaporin 1 is strongly expressed in tumor cells and capillaries at the invasion site and dural attachment of meningiomas. (14, 17) These suggest that AQP1 may participate in invasion of tumor cells of meningiomas.

Na-2K-Cl cotransporter isoform 1 (NKCC1) is a membrane-bound active cotransporter encoded by the *SLC12* gene family. NKCC1 mediates

potassium and chloride into the cell and it is associated with cerebral edema and seizure disorder. (14) Several studies revealed that NKCC1 channels are associated with neoplastic cell division, apoptosis and migration in several neoplasms including meningioma. (16, 18) Mahlon D. Johnson *et al.* showed that NKCC1 was also widely expressed in meningioma cells invading dura. (14) These results suggest that NKCC1 and AQP1 may participate in invasion of tumor cells of meningiomas.

Taken together, we aimed to elucidate whether protein expressions of c-Met, HGF, AQP 1 and NKCC1 were associated with clinicopathologic variables as well as brain and bone/scalp invasion in large scale of meningiomas.

## **Materials and method**

### **Meningioma cases**

Formalin-fixed, paraffin-embedded archival tissue samples from 198 patients who underwent curative surgical resection (Simpson grade I and II) of meningioma between August 2003 and May 2013 were collected from the databases of the Department of Pathology, Seoul National University Bundang Hospital. Clinical and pathologic data were obtained by reviewing medical records and pathology reports. Two pathologists (G. Choe and S. Yun) independently reviewed the hematoxylin and eosin-stained (H&E) slides, confirmed the diagnosis according to the 2007 WHO classification, and classified the histological subtypes and grading of meningioma. All 198 cases of curative tumor removal consisted of 113 Simpson grade I and 85 Simpson grade II. Among 113 cases with complete tumor removal (Simpson grade I), 100 cases were included for the recurrence free survival analysis, excluding 5 cases for the reason of lack of follow up data and 8 cases for the reason of post-operative chemotherapy and radiotherapy. All patients were followed regularly after surgery. The recurrence-free survival (RFS) was calculated from the time of surgery to the first suspected recurrence. The evidence of tumor recurrence was given by a computerized tomography scan or magnetic resonance image showing a meningioma in a location contiguous with the previous operation site.

## **Demographic data**

The clinicopathologic features are summarized in Table 1. The 198 patients consisted of 50 (25.3%) male and 148 (74.7%) female, and their median age was 59 years (range: 12 to 86). According to 2007 WHO grading, the 198 cases were subdivided into 3 groups as follows: 184 benign meningiomas (grade I); 12 atypical meningiomas (grade II); and 2 anaplastic meningiomas (grade III). Of these, bone/scalp invasion was observed in 24 cases, brain invasion in 13 cases, and both bone/scalp invasion and brain invasion in 4 cases. Histologically, 198 cases consisted of meningothelial type ( $n = 70$ ), transitional type ( $n=54$ ), fibrous type ( $n=28$ ), angiomatous type ( $n=19$ ), psammomatous type ( $n=4$ ), microcystic type ( $n=1$ ), metaplastic type ( $n=1$ ), chordoid type ( $n=1$ ), atypical type ( $n=11$ ), rhabdoid type ( $n=1$ ) and anaplastic type ( $n=1$ ). (Table 1)

## **Construction of tissue microarray (TMA)**

We chose one representative tumor block in each case and we harvested on core with a diameter of 3 mm from the most representative tumor areas of the donor block. The cores were precisely arranged into new recipient TMA blocks using a trephine apparatus according to the protocols described previously. (19)

## **Immunohistochemistry**

Immunohistochemical staining was carried out using TMA according

to the method described previously. (19) Briefly, 4  $\mu\text{m}$ -thick sections were transferred to poly-L-lysine-coated adhesive slides and dried, deparaffinized, and rehydrated, and the slides were subjected to heat-induced antigen retrieval. The following antibodies were used: AQP 1 (1:100, mouse monoclonal antibody, Clone B-11, Santa Cruz Biotechnology, USA), c-Met (pre-dilution, rabbit monoclonal antibody, Ventana Medical Systems, Inc, USA), HGF $\alpha$  (1:100, rabbit polyclonal antibody, Santa Cruz Biotechnology, USA) and NKCC1 (1:100, goat polyclonal antibody, Santa Cruz Biotechnology, USA) according to the manufacturer's instructions. The sections were incubated with appropriate reagents from the Dako real envision detection system and were counterstained with Meyer hematoxylin.

The scoring of each antibody was done on the basis of the cytoplasmic and/or membrane-staining intensity, as follows: score of “0” for no distinct immunoreactivity or weak staining in <10% of tumor cells; score of “1+” if 10% to 25% of tumor cells were immunoreactive; score of “2+” if 25% to 50%; score of “3+” if greater than 50% of tumor cells were positive. (14) AQP1 were moderately expressed in the cytoplasm of vascular endothelial cells within meningiomas, and this endothelial expression was used as internal control. For statistical convenience, the expression of each marker was referred as a dichotomous covariate, namely, c-MET<sup>-low</sup> (score of 0 and 1+) versus c-MET<sup>-high</sup> (score of 2+ and 3+); HGF<sup>-low</sup> (score of 0 and 1+) versus HGF<sup>-high</sup> (score of 2+ and 3+); AQP1<sup>-low</sup> (score of 0 and 1+) versus AQP1<sup>-high</sup> (score of 2+ and 3+); NKCC1<sup>-low</sup> (score of 0 and 1+) versus

NKCC1<sup>-high</sup> (score of 2+ and 3+).

### **Ethics statements**

The study was conducted according to the World Medical Association's Declaration of Helsinki.

### **Statistical analysis**

All statistical analyses were conducted using the Statistical Package for the Social Sciences software (version 21.0, SPSS Inc., Chicago, IL, USA). The association between protein expression of each antibody and the categorical variables was assessed using the chi-square test or Fisher's exact test, if appropriate. Kaplan-Meier survival curves for Recurrence free survival were plotted for each antibody and the survival comparison was determined using the log-rank test. All tests were two-tailed and statistical significance was determined as *P* values <0.05.

## Results

### **Clinical characteristics according to brain invasion**

Among 198 cases of meningiomas, brain invasion was observed in 13 cases (6.6%). Median age of the cases with brain invasion was 56 years (range: 12 to 77). Male patients with brain invasion were 14% (7/50) and female patients with brain invasion were 4.1% (6/148). Brain invasion was found more frequently in male patients. ( $P = 0.022$ ) According to the WHO grade, brain invasion was found in 4.3% (8/184) of the benign meningiomas, 33.3% (4/12) of the atypical meningiomas and 50% (1/2) of the anaplastic meningiomas, respectively. Brain invasion showed significant association with the WHO grade. ( $P < 0.001$ ) However, there was no significant difference between brain invasion and Simpson grade. (Table 2)

### **Clinical characteristics according to bone/soft tissue invasion**

Bone and/or soft tissue invasion was observed in 24 cases of meningiomas which consisted of five males (10%; 5/50) and 19 females (12.8%; 19/148). Median age of patients with bone/soft tissue invasion was 65.5 years (range: 42-83). According to the WHO grade, bone/soft tissue invasion was found in 10.9% (20/184) of the benign meningiomas, 33.3% (4/12) of the atypical meningiomas, respectively. No bone/soft tissue invasion was found in two cases of the anaplastic meningiomas. ( $P = 0.060$ ) There was no significant difference between bone/soft tissue invasion and Simpson grade. (Table 3)

### **Expression of c-Met and HGF in meningioma according to WHO grade and histologic subtypes**

c-Met<sup>-High</sup> and HGF<sup>-High</sup> were found in 20.7% (41/198) and 13.6% (27/198) of meningiomas, and c-Met<sup>-High</sup>/HGF<sup>-High</sup> was observed in 8.6% (17/198) of meningiomas. According to the WHO grade, c-Met<sup>-High</sup> was found in 20.1% (37/184) of the benign meningiomas, 25% (3/12) of the atypical meningiomas and 50.0% (1/2) of the anaplastic meningiomas, respectively. However, there was no significant difference between c-Met<sup>-High</sup> and WHO grading. ( $P = 0.543$ ) HGF<sup>-High</sup> was found in 14.7% (27/184) of the benign and no HGF<sup>-High</sup> was noted in atypical and anaplastic meningiomas.

Expression of c-Met and HGF showed no significant correlation with histologic subtypes of meningiomas. (Table 4)

### **Expression of AQP1 and NKCC1 in meningiomas according to WHO grade and histologic subtypes**

AQP1<sup>-High</sup> and NKCC1<sup>-High</sup> were found in 29.8% (59/198) and 93.4% (185/198) of meningiomas, respectively. NKCC1<sup>-High</sup> was observed in 93.5% (172/184) of the benign meningiomas, 91.7% (11/12) of the atypical meningiomas and all anaplastic meningiomas (2/2). However, AQP1<sup>-High</sup> was observed only in 32.1% (59/184) of benign meningiomas and no AQP1<sup>-High</sup> was noted in both atypical meningiomas (12 cases) and anaplastic meningiomas (2 cases).

Expression of AQP1 and NKCC1 showed no significant correlation

with histologic subtypes of meningiomas. (Table 5)

### **Association of the expression of c-Met, HGF, AQP1 and NKCC1 with brain invasion**

Among 198 cases, c-Met<sup>-High</sup> was observed in 18.9% (35/185) meningiomas without brain invasion and in 46.2% (6/13) meningiomas with brain invasion, respectively. c-Met<sup>-High</sup> significantly correlated with brain invasion. ( $P = 0.030$ ) On the other hand, HGF<sup>-High</sup> and c-Met<sup>-High</sup>/HGF<sup>-High</sup> were found in 14.6% (27/185) and 7.6% (14/185) of meningiomas without brain invasion. HGF<sup>-High</sup> and c-Met<sup>-High</sup>/HGF<sup>-High</sup> were not observed in meningiomas with brain invasion. HGF<sup>-High</sup> or c-Met<sup>-High</sup>/HGF<sup>-High</sup> did not show statistical association with brain invasion. ( $P = 0.222$ ,  $P = 0.605$ , respectively).

AQP1<sup>-High</sup> was observed in 31.9% (59/185) meningiomas without brain invasion, and AQP1<sup>-High</sup> was not observed in any meningiomas with brain invasion. Interestingly, AQP1<sup>-High</sup> inversely correlated with brain invasion. ( $P = 0.017$ ) On the other hand, NKCC1<sup>-High</sup> was found in 93.5% (173/185) of meningiomas without brain invasion, and in 92.3% (12/13) of meningiomas with brain invasion, respectively. In addition, AQP<sup>-High</sup>/NKCC1<sup>-High</sup> was observed in 27.6% (51/185) of meningiomas without brain invasion. And it was not observed in any meningiomas with brain invasion. AQP<sup>-High</sup>/NKCC1<sup>-High</sup> had significant inverse correlation with brain invasion. ( $P = 0.023$ ) (Table 6)

## **Association of the expression of c-Met, HGF, AQP1 and NKCC1 with bone/scalp invasion**

c-Met<sup>High</sup> was observed in 18.4% (32/174) meningiomas without bone/scalp invasion and 37.5% (9/24) meningiomas with bone/scalp invasion. There was a tendency for increased c-Met<sup>High</sup> in meningiomas with bone/scalp invasion compared with meningiomas without bone/scalp invasion; however, statistical significance was not reached. ( $P = 0.055$ ) HGF<sup>High</sup>, AQP1<sup>High</sup> and NKCC1<sup>High</sup> were found in 12.1% (21/174), 29.3% (51/174) and 93.1% (162/174) of meningiomas without bone/scalp invasion, respectively and in 25.0% (6/24), 33.3% (8/24), 95.8% (23/24) of meningiomas with bone/scalp invasion, respectively.

HGF<sup>High</sup>, AQP1<sup>High</sup> and NKCC1<sup>High</sup> were not significantly correlated with bone/scalp invasion. ( $P = 0.108$ ,  $P = 0.812$ ,  $P > 0.95$ , respectively) In addition, c-Met<sup>High</sup>/HGF<sup>High</sup> was found in 20.8% (5/24) of meningiomas with bone/scalp invasion and in 5.2% (9/174) of meningiomas without bone/scalp invasion, respectively. c-Met<sup>High</sup>/HGF<sup>High</sup> had significant association with bone/scalp invasion. ( $P = 0.016$ ) (Table 6)

## **Tumor recurrence free survival analysis according to c-Met, HGF, AQP1 and NKCC1 expression**

To identify whether completely removed meningiomas (Simpson grade I) had a different recurrence free survival (RFS) depending on c-Met, HGF, AQP1 and NKCC1 expression status, we performed univariate analysis

in 100 cases of Simpson grade I meningiomas. In current study, median follow up period was 26.7 months (range: from 1.1 to 106.2), and 4 cases (4%) suffered tumor recurrence.

Among 17 cases with c-MET<sup>high</sup>, 2 (11.8%) cases experienced recurrence, whereas 2 (2.4%) of 83 cases with c-MET<sup>low</sup> suffered recurrence. Cases with c-MET<sup>high</sup> showed shorter recurrence free survival ( $93.467 \pm 8.211$  months) than those of c-MET<sup>low</sup> ( $96.131 \pm 1.911$  months); however, it did not reach statistical significance. ( $P = 0.139$ ) However, expressions of HGF<sup>High</sup>, AQP1<sup>High</sup>, NKCC1<sup>High</sup>, c-Met<sup>High</sup>/HGF<sup>High</sup> and AQP<sup>High</sup>/NKCC1<sup>High</sup> were not correlated with RFS. (Figures 2 and 3)

## Discussion

We set out to determine whether expression of c-Met, HGF, AQP1 and NKCC1 were associated with invasiveness of meningioma and its clinical implication. In present study, c-Met<sup>High</sup> was correlated with brain invasion and bone/scalp invasion. To the best of our knowledge, it is first report in large scale of meningiomas of East Asian patients.

c-Met is a receptor tyrosine kinase (RTK) and it is well known as a proto-oncogene. In development and wound tissue, c-Met, which is expressed in liver, pancreas, prostate etc., regulates many cellular processes including cell proliferation, motility and cell survival. HGF is the known ligand of c-Met RTK. (8, 9, 20) In previous studies, c-Met/HGF signaling pathway or c-Met overexpression have been demonstrated to have strong relationship with tumor cell proliferation, motility, invasion, tumor angiogenesis and poor prognosis. Therapeutic agent targeting c-Met TKR and HGF has recently received attention. (8, 20, 21) Meningiomas, as previously described, show high recurrence rate even after curative resection of tumor. And the recurrence rate depends on several prognostic factors including invasiveness of tumor. As invasive meningiomas show poor prognosis, the identification of its mechanism may be useful in management of meningiomas. (22, 23) Several studies showed the association of c-Met/HGF expression and its clinical significance. Most implicated the association between c-Met/HGF expression and tumor recurrence. In Martinez-Rumayor *et al.*'s study (10), it revealed

that co-expression of c-Met/HGF related to cell proliferation and recurrence of meningiomas using immunohistochemistry. Kim *et al.*'s study (12) also showed that expression of HGF and co-expression of c-Met/HGF were associated with histologic grade of and recurrence of meningiomas by RT-PCR. Yet, Karja *et al.* and Lamszus *et al.* (3, 11) reported that HGF was not related to tumor recurrence using ELISA and immunohistochemistry. Few studies demonstrate the association of c-Met/HGF with brain and bone invasion. This present study provides data on expression of c-Met and HGF in a large number of meningiomas and relationship with brain and bone/scalp invasion. c-Met<sup>High</sup> was significantly associated with brain invasion. And it also showed a tendency for increased c-Met<sup>High</sup> in meningiomas with bone/scalp invasion. But HGF<sup>High</sup> didn't show any significant difference with invasiveness of meningiomas. Recent studies revealed c-Met signaling cascade facilitates invasion of cancer. Downstream cascade signaling of activated c-Met by either autocrine or paracrine interaction leads the dissociation of tumor cells with surrounding stromal tissue. It results tumor cell invasion. (8, 9, 20, 24) Our study supports the result that c-Met is closely related to invasion of tumor. There were limits in this study that few cases of several histologic subtypes and WHO grades were included. Nevertheless, these results suggest that c-Met may participate in invasion of tumor.

The aquaporins are a family of transmembrane water channel proteins that participate in the molecular pathway for transcellular and transepithelial water transport. In brain, AQP1 is expressed in choroid plexus

epithelium and may involve in regulation of cerebrospinal fluid. (15, 25, 26) The current studies showed that AQP 1 is upregulated in several cancer such as colon cancer, breast cancer, lung cancer and brain tumors and that it may play a role in tumor cell invasion and migration. (13, 27, 28) AQP1 expression induced osmotic water influx and this leads to increased hydrostatic pressure, causing cell shape change and cell membrane protrusion at the leading edge in tumors. (25, 29) These processes are suggested that they may facilitate cancer invasion and spreading. (25) NKCC1, which transport 1 sodium, 1 potassium and 2 chloride ions into the cells, is important in maintaining cytoplasmic volume. Recently, it has been suggested that NKCC1 may work in modulating cell volume and promote migration in tumor. (13, 30) Haas *et al.* showed that NKCC1 expression was localized to the leading area of migrating cells and pharmacologic inhibition of NKCC1 reduced tumor cell migration in gliomas. (31) In meningiomas, a few results were reported based on a small number of cases. Nagashima *et al.* (17) found that AQP1 expression was seen at the dura invading front of meningioma by immunohistochemical analysis (n=7). Johnson *et al.*'s results (n=36) showed that AQP1 and NKCC1 were detected in meningioma cells and capillaries invading dura and bone by Western blot and immunohistochemical analysis. (14) These results suggest that AQP1 and NKCC1 participate in meningioma invasion. However, in this study, AQP1<sup>-High</sup> and AQP1<sup>-High</sup>/NKCC1<sup>-High</sup> tended to decrease in meningiomas with brain invasion. There was no significant difference between AQP1<sup>-High</sup> and meningiomas with or without bone/scalp invasion. NKCC1 also was not

associated with brain and bone/scalp invasion. These findings are not consistent with the results of previous studies. In this study, we used the most representative tumor area for tissue microarray, not invading edge. Thus, AQP1 may involve in another tumor biology in meningioma or APQ1 may accelerate invasion only in minor tumor cell population of invading site inferring intra-tumor heterogeneity. To the best of our knowledge, it has not been reported that AQP1 expression show an association to better prognosis. Further studies are needed to clarify the mechanisms responsible for the inverse correlation of APQ1 expression in meningiomas.

We also evaluated a possible association of c-Met, HGF, AQP1, NKCC1 expression with disease recurrence. In this study, we demonstrated c-Met<sup>high</sup> only showed tendency to be associated with shorter recurrence free survival. In general, the recurrence of meningioma was mainly occurred within 2 years after surgical treatment and up to 94% of patients with meningiomas experience recurrence within 5 years follow up. (32) However, vast majority of meningiomas are slowly growing tumors and completely removed benign meningiomas recurred at a rate of 19% in 20 years follow up. (33) Thus, the results of recurrence rate was limited because of lack of long period of follow up (median follow up time: 26.7 months) in this study and further studies are needed to elucidate the association between c-Met overexpression and recurrence free survival.

In summary, our results demonstrate that c-Met is associated with brain invasion of meningiomas and that c-Met expression could be a useful

predictive marker for meningioma recurrence.

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**Table 1. Clinicopathologic characteristics of meningioma cases**

<b>Characteristic</b>	<b>Patients</b>	
	<b>No.</b>	<b>%</b>
<b>Age</b>	median age : 59 (range: 12 - 86)	
<b>Sex</b>		
Male	50	25.3
Female	148	74.7
<b>WHO grade</b>		
Grade I	184	92.9
Grade II	12	6.1
Grade III	2	1
<b>Histologic subtype</b>		
Meningothelial	70	35.4
Transitional	54	27.3
Fibrous	28	14.1
Angiomatous	19	9.6
Psammomatous	4	2
Microcystic	8	4
Metaplastic	1	0.5
Chordoid	1	0.5
Atypical	11	5.6
Rhabdoid	1	0.5
Anaplastic	1	0.5
<b>Simpson grade</b>		
Simpson 1	113	57.1
Simpson 2	85	42.9

**Table 2. Summary of brain invasion in meningiomas**

<b>Characteristic</b>	<b>Brain invasion</b>		<b>P</b>
	<b>yes (n=13)</b>	<b>no (n=185)</b>	
<b>Age</b>	median : 56 (range : 12-77)	median : 59 (range : 27-86)	
<b>Sex</b>			
Male	7 (14%)	43 (86%)	0.022
Female	6 (4.1%)	142 (95.9%)	
<b>WHO grade</b>			
Grade I	8 (4.3%)	176 (95.7%)	<0.001
Grade II	4 (33.3%)	8 (66.7%)	
Grade III	1 (50%)	1 (50%)	
<b>Simpson grade</b>			
Simpson 1	10 (8.8%)	103 (91.2%)	0.158
Simpson 2	3 (3.5%)	82 (96.5%)	

**Table 3. Summary of bone/soft tissue invasion in meningiomas**

<b>Characteristic</b>	<b>Bone/soft tissue invasion</b>		<b>P</b>
	<b>yes (n=24)</b>	<b>no (n=174)</b>	
<b>Age</b>	median : 65.5 (range : 42-83)	median : 58.5 (range : 12-86)	
<b>Sex</b>			
Male	5 (10%)	45 (90%)	0.803
Female	19 (12.8%)	129 (87.2%)	
<b>WHO grade</b>			
Grade I	20 (10.9%)	164 (89.1%)	0.06
Grade II	4 (33.3%)	8 (66.7%)	
Grade III	0 (0%)	2 (100%)	
<b>Simpson grade</b>			
Simpson 1	14 (12.4%)	99 (87.6%)	>0.95
Simpson 2	10 (11.8%)	75(88.2%)	

**Table 4. Expression of c-Met and HGF in meningiomas according to WHO grade and histologic subtypes**

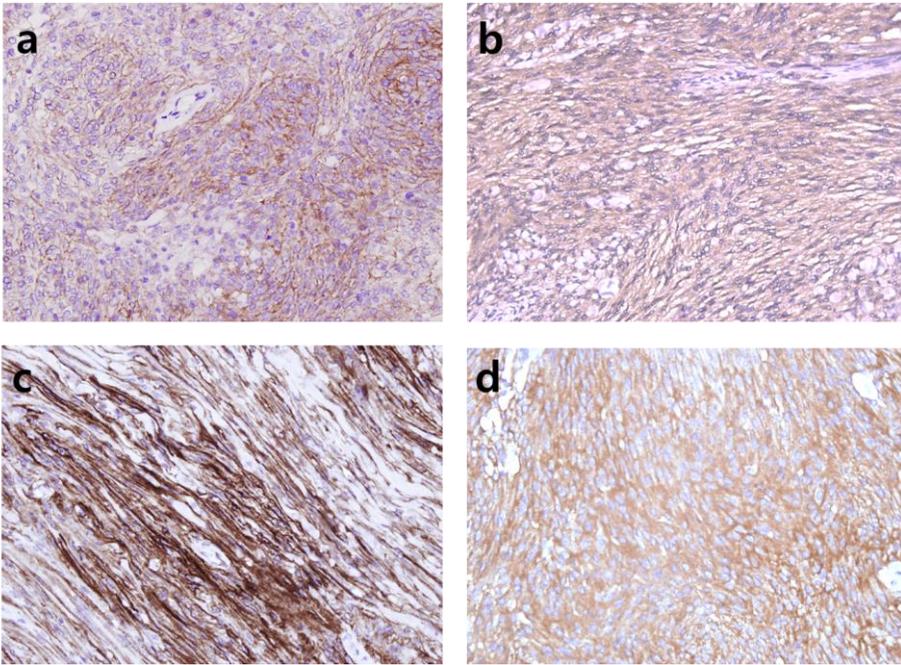
	c-Met <sup>-High</sup>		HGF <sup>-High</sup>		c-Met <sup>-High</sup> / HGF <sup>-High</sup>	
	No.	%	No.	%	No.	%
<b>WHO grade</b>						
Benign (Grade I)	37	20.1	27	14.7	14	7.6
Atypical (Grade II)	3	25	0	0	0	0
Anaplastic (Grade III)	1	50.5	0	0	0	0
<i>P value</i>	<i>0.543</i>		<i>0.304</i>		<i>0.564</i>	
<b>Histologic subtype</b>						
Meningothelial (n=70)	27	38.6	13	18.6	10	14.3
Transitional (n=54)	3	5.6	5	9.3	1	1.9
Fibrous (n=28)	3	10.7	5	17.9	1	3.6
Angiomatous (n=19)	2	10.5	2	10.5	0	0
Psammomatous (n=4)	1	25	1	25	1	25
Microcystic(n=8)	1	12.5	1	12.5	1	12.5
Metaplastic(n=1)	0	0	0	0	0	0
Chordoid (n=1)	0	0	0	0	0	0
Atypical (n=11)	3	27.3	0	0	0	0
Rhabdoid (n=1)	0	0	0	0	0	0
Anaplastic (n=1)	1	100	0	0	0	0

**Table 5. Expression of AQP1 and NKCC1 in meningiomas according to WHO grade and histologic subtypes**

	AQP1 <sup>-High</sup>		NKCC1 <sup>-High</sup>		AQP1 <sup>-High</sup> / NKCC1 <sup>-High</sup>	
	No.	%	No.	%	No.	%
<b>WHO grade</b>						
Benign (Grade I)	59	32.1	172	93.5	51	27.7
Atypical (Grade II)	0	0	11	91.7	0	0
Anaplastic (Grade III)	0	0	2	100	0	0
<i>P value</i>	<i>0.041</i>		<i>0.904</i>		<i>0.073</i>	
<b>Histologic subtype</b>						
Meningothelial (n=70)	7	10	67	95.7	6	8.6
Transitional (n=54)	30	55.6	48	88.9	25	46.3
Fibrous (n=28)	19	67.9	26	92.9	17	60.7
Angiomatous (n=19)	0	0	18	94.7	0	0
Psammomatous (n=4)	2	50	4	100	2	50.0
Microcystic(n=8)	0	0	8	100	0	0
Metaplastic(n=1)	1	100	1	100	1	100
Chordoid (n=1)	0	0	1	100	0	0
Atypical (n=11)	0	0	10	90.9	0	0
Rhabdoid (n=1)	0	0	1	100	0	0
Anaplastic (n=1)	0	0	1	100	0	0

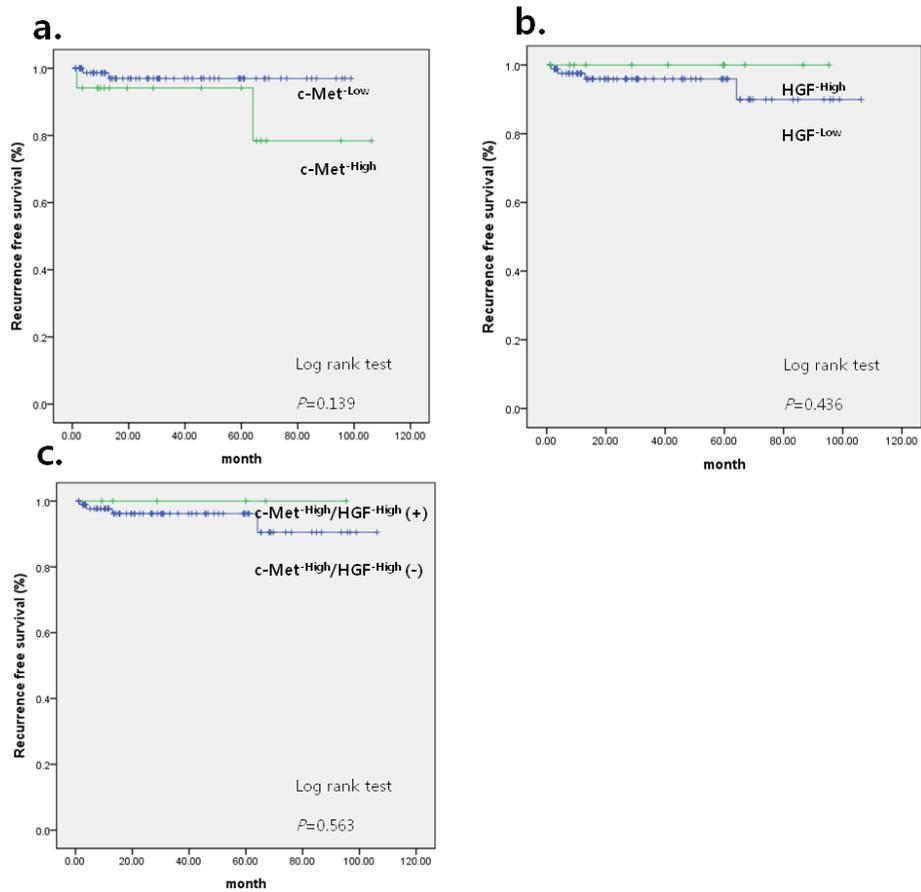
**Table 6. Association of the expression of c-Met, HGF, AQP1 and NKCC1 with brain invasion and bone/scalp invasion**

	Brain invasion		<i>p</i>	Bone/scalp invasion		<i>p</i>
	Negative	Positive		Negative	Positive	
<b>c-Met</b> (n=198)						
c-Met <sup>-Low</sup>	150	7	<i>0.030</i>	142	15	<i>0.055</i>
c-Met <sup>-High</sup>	35	6		32	9	
<b>HGF</b> (n=198)						
HGF <sup>-Low</sup>	158	13	<i>0.222</i>	153	18	<i>0.108</i>
HGF <sup>-High</sup>	27	0		21	6	
<b>c-Met<sup>-High</sup>/HGF<sup>-High</sup></b> (n=198)						
Negative	171	13	<i>0.605</i>	165	19	<i>0.016</i>
Positive	14	0		9	5	
<b>AQP1</b> (n=198)						
AQP1 <sup>-Low</sup>	128	11	<i>0.011</i>	123	16	<i>0.686</i>
AQP1 <sup>-High</sup>	59	0		51	8	
<b>NKCC1</b> (n=198)						
NKCC1 <sup>-Low</sup>	12	1	<i>0.598</i>	12	1	<i>&gt;0.95</i>
NKCC1 <sup>-High</sup>	173	12		162	23	
<b>AQP1<sup>-High</sup>/NKCC1<sup>-High</sup></b> (n=198)						
Negative	134	13	<i>0.023</i>	130	17	<i>0.803</i>
Positive	51	0		44	7	



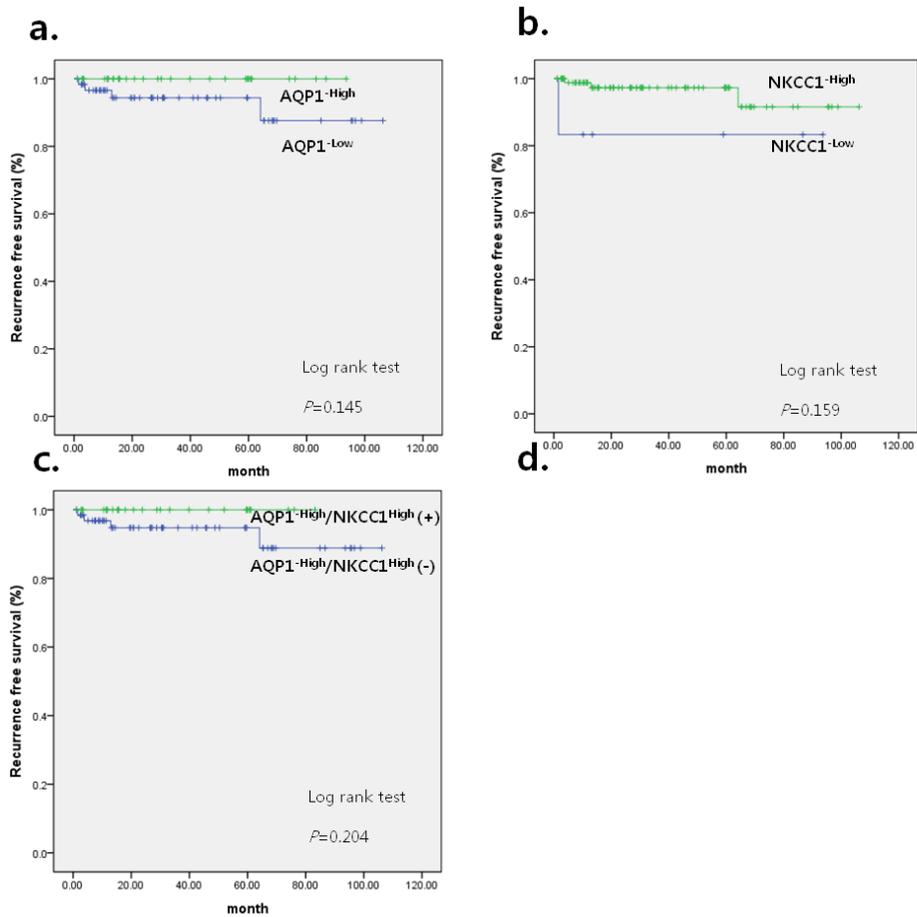
**Figure 1. Immunohistochemical staining in meningiomas.**

**a.** c-Met<sup>High</sup> (original magnification x400). **b.** HGF<sup>High</sup> (original magnification x 400). **c.** AQP1<sup>High</sup> (original magnification x 400). **d.** NKCC1<sup>High</sup> (original magnification x 400).



**Figure 2. Kaplan-Meier curves for recurrence free survival according to the expression of c-MET and/or HGF**

- a.** Analysis by c-Met expression status
- b.** Analysis by HGF expression status
- c.** Analysis by c-Met/HGF co-expression status



**Figure 3. Kaplan-Meier curves for recurrence free survival according to the expression of AQP1 and/or NKCC1**

**a.** Analysis by AQP1 expression status **b.** Analysis by NKCC1 expression status **c.** Analysis by AQP1/NKCC1 co-expression status

## 국문 초록

# 침윤성 뇌수막종에서 c-Met의 발현

**연구 배경:** 뇌수막종은 WHO classification에 따라 세 개의 grade로 나뉘며, 대부분의 뇌수막종은 양성(WHO grade I)으로 분류된다. 하지만 뇌수막종은 근치적 절제술을 받은 경우에 있어서도 높은 재발율을 보이며, 종양의 침윤성 여부는 이러한 재발율과 연관되어 있다. 최근 연구에서 c-Met, HGF, AQP1, NKCC1이 다양한 종류의 암종에서 침윤기전에 관여하는 것으로 밝혀졌다. 그런데 뇌수막종에서 c-Met, HGF, AQP1, NKCC1 발현에 따른 임상병리학적 특성, 침윤성 및 재발율과의 관계에 대해서는 많이 알려진 바가 없다.

**재료 및 방법:** 2003에서 2013년까지 분당 서울대학교병원에서 근치적 수술(Simpson grade 1, 2)을 받은 298례의 뇌수막종을 대상으로 면역조직화학검사를 통해 c-Met, HGF, AQP, NKCC1의 발현 여부를 평가하였고, 임상병리학적 특성 및 종양의 침윤성 여부와 상관 분석을 시행하였다. 또한 Simpson grade 1의 수술치료를 받은 100례의 뇌수막종에서 c-Met, HGF, AQP1, NKCC1의 발현에 따른 뇌수막종의 재발과의 관계를 분석하였다.

**결과:** 면역조직화학검사상 c-Met<sup>-High</sup> 발현이 있는 경우, 뇌수막종에서 뇌 침윤이 동반되는 경우가 많았으며, (46.2% vs. 18.9%,  $P = 0.030$ ), 주변 골연부조직으로의 침윤이 좀더 빈번하게 관찰되었다. (37.5% vs. 18.4%,  $P = 0.055$ ) 반면, HGF<sup>-High</sup> 발현은 뇌 침윤 및 주변 골연부조직으로의 침윤과는 유의한 관계를 보이지 않았다. AQP1<sup>-High</sup> 발현은 뇌 침윤이 동반된 경우 31.9%에서 관찰되었으며, 뇌 침윤과 역상관관계를 보였다. ( $P = 0.011$ ) 하지만, AQP1<sup>-High</sup> 발현은 주변 골 연부조직으로의 침윤과 유의한 관계를 보이지 않았으며 ( $P = 0.686$ ), NKCC1<sup>-High</sup> 발현 역시 뇌 침윤 및 주변 골연부조직으로의 침윤과 유의한 관계를 보이지 않았다. ( $P = 0.598$ ,  $P > 0.95$ ) 한편, c-Met<sup>-High</sup> 발현하는 뇌수막종의 경우 c-Met<sup>-Low</sup> 발현하는 경우에 비해 recurrence free survival이 짧은 경향을 보이고 있었다. (11.8% vs. 2.4%,  $P = 0.139$ ) 그러나 HGF, AQP1, NKCC1 혹은 c-Met/HGF 발현은 뇌수막종의 recurrence free survival과는 유의한 관계를 보이지 않았다.

**결론:** 뇌수막종에서 c-Met의 면역조직화학적 발현은 뇌수막종의 침습성과 관련이 있었으며, c-Met 발현이 뇌수막종의 재발을 예측하는 유용한 표지자로 이용될 수 있음을 시사하였다.

**주요어:** c-Met, 침윤성 뇌수막종

**학번:** 2012-21747