The double immunostaining of CD133 and Ki-67 favours a significant
co-localization pattern in fibroblastic subtype of meningiomas

Podwójne znakowanie immunologiczne CD133 i Ki-67 wskazuje
na ich istotną współlokализację w podtypie włóknistym oponiaków

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Abstract

Background and purpose: A unique molecular and/or cellular
marker for meningiomas, the most common intracranial
tumours, has not been identified yet.

Material and methods: We investigated the co-localization frac-
tion of CD133/Ki-67 in meningioma tissue array slide com-
posed of 80 meningioma tissue samples of various histologi-
cal variants. CD133 – a cell membrane stem cell marker – was
previously proved to be associated with the initiation and pro-
gression of intracerebral gliomas and medulloblastomas.

Results: Immunohistochemical co-localization of CD133/
Ki-67 was significantly higher in fibroblastic variant than in
meningothelial and transitional subtypes. However, since there
were only 3 atypical and 1 malignant meningioma spots in the
tumour tissue array slide, it is difficult to draw a firm conclusion
regarding the actual co-localization percentage and persistence
of CD133/Ki-67 in atypical and malignant meningiomas.

Conclusions: Far higher co-staining percentage of CD133/
Ki-67 in fibroblastic meningioma samples compared to
meningothelial subtype, a histological meningioma variant,
architectonically resembling the non-neoplastic meningeal cells,
gave us the impression that CD133 may play a role in the for-
formation and progression of fibroblastic meningioma variants.

Streszczenie

Wstęp i cel pracy: Nie określono dotąd unikalnego znacznika
molekularnego lub komórkowego dla oponiaków, naj-
częstszzych guzów wewnątrzczaszkowych. Wcześniej wykazano,
oże CD133 – znacznik błony komórkowej komórek macie-
rzystych – jest związany z zapoczątkowaniem, a także wzro-
stem wewnątrzczaszkowych glejakałów i rdzeniaków płodowych.

Materiał i metody: Zbadano odsetek współlokализacji
CD133/Ki-67 w zestawach macierzy tkankowych oponiaków,
złożonych z próbek 80 rozmaitych odmian histologicznych opo-
niaków.

 Wyniki: Immunohistochemiczna współlokализacja CD133
i Ki-67 była stwierdzana istotnie częściej w podtypie włók-
nistym oponiaka niż w podtypach meningotelioidalnym lub
przejściowym. Ze względu na małą liczbę preparatów opo-
niaków atypowych (3) oraz złośliwych (1) w badanej macie-
rzy tkankowej trudno wyciągnąć jednoznaczne wnioski do-
tyczące rzeczywistego odsetka współlokализacji i utrzymywania
się CD133/Ki-67 w oponiakach atypowych i złośliwych.

Wnioski: Znaczająco większy odsetek wspólnie występującej
reaktywności CD133/Ki-67 w preparatach oponiaka włóknis-
stego w porównaniu z podtypem meningotelioidalnym, którego
architektonika przypomina nienowotworowe komórki opon,
Introduction

Meningiomas, the most common intracranial tumors, constitute almost one-third of all brain tumors. They are thought to be of neuroectodermal origin based on the striking ultrastructural and histological resemblance to arachnoid cells [1-3]. Meningiomas were defined well cyto- genetically but they are poorly understood molecularly. Thus, histopathological grading of meningiomas does not necessarily predict its clinical course, particularly in atypical meningiomas [4]. Additionally, recent findings in molecular genetics provide strong evidence that meningiogenesis is a dynamic process with tumor suppressor NF-2 gene deletion on chromosome 22 and several other genetic aberrations including the deletion of the INK4a-ARF locus [5]. In this regard, cellular origin of meningiomas with a unique molecular marker still needs to be clarified.

For this purpose, in a meningioma tissue array consisting of 80 meningioma of various histopathological subsets, we investigated the co-localization pattern of CD133, a cell-surface antigen – a proposed molecular signature for meningioma cells with Ki-67 nuclear antigen which is expressed in dividing cells in all phases of the cell cycle except G0. Even though the exact biological function of CD133, a cell-surface antigen, is not known, it is a marker for stem and progenitor cells. CD133 was also widely expressed in cancers, including some leukaemias and brain tumors, mostly in gliomas and medulloblastomas. According to our hypothesis, meningioma cells with Ki-67 expression may have the potential to be the pluripotent meningioma initiating cells since Ki-67 antigen is mostly expressed in dividing cells and CD133, a cell-surface antigen, was proposed as a molecular marker for these candidate meningioma-initiating cells. Thus, for the first time in the literature, co-expression fraction and consistency of CD133 and Ki-67 in various meningioma subtypes were investigated immunohistochemically in our study.

Material and methods

The efficacy of the below-explained double immunostaining procedure was confirmed in several human meningioma paraffin slides (WHO grade I meningioma) with a negative control prior to proceeding to the immunostaining of the meningioma tissue array slide (Fig. 1).

For CD133 immunohistochemistry, paraffin slide consisting of 80 meningioma tissue samples (meningioma of central nerve tissue array # MG801, US Biomax, Inc., 1100, TaftSt. Rockville, MD 20850, USA) was deparaffinized and blocked for endogenous peroxidase activity with methanol containing 3% H2O2 for 15 min and for non-specific binding with universal blocking reagent (BioGenex, San Ramon, CA, USA) for 7 min at room temperature. Anti-mouse CD133 (Cat # 14-1331-80, eBioscience, Inc, San Diego, CA, USA) diluted in dilution buffer (1/100) was applied for 1 hour at room temperature in a humidified chamber. After several washes in PBS, the section was incubated with biotinylated goat anti-mouse IgG secondary antibody (1/400 dilution, Vector Lab., Burlingame, CA, USA) for 45 min followed by LSAB streptavidin-peroxidase complex (Dako, Carpinteria, CA, USA) incubation for 30 min and was rinsed with PBS. Antibody complex was visualized by incubation with diaminobenzidine (DAB) chromogen (BioGenex). After several washes in PBS, the section was incubated with a Ki-67 (Cat # RM-9106-S0; ThermoScientific, Fremont, CA, USA) rabbit monoclonal antibody (1/100 dilution) overnight at 4°C. Following the washing steps in PBS, the section was incubated with anti-rabbit biotinylated goat anti-mouse IgG secondary antibody (1/400 dilution, Vector Lab. Burlingame, CA, USA) for 45 min followed by LSAB streptavidin-peroxidase complex (Dako, Carpinteria, CA, USA) incubation for 30 min and rinsed with PBS. Antibody complex was visualized by incubation with 3-amino-9-ethylcarbazole (AEC) chromogen (Thermoscientific). The section was counterstained with Mayer’s haematoxylin (Dako), dehydrated, mounted and examined by a Zeiss-Axioplan microscope. The persistency and the validity of this finding need to be verified by further histopathological and molecular research in order to clarify the possible role of CD133 in meningiogenesis.

Key words: meningioma, fibroblastic, CD133, Ki-67, tissue array, co-localization.

Sprawia wrażenie, że CD133 może odgrywać rolę w powstawaniu i rozwoju oponiaków włóknistych. Trafność tego spostrzeżenia wymaga weryfikacji w dalszych badaniach histopatologicznych i molekularnych w celu wyjaśnienia możliwej roli CD133 w powstawaniu oponiaków.

Słowa kluczowe: oponiak, włóknisty, CD133, Ki-67, macierz tkankowa, współlokализacja.

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Fig. 1. Human meningioma paraffin trial slide double stained with CD133 and Ki-67. A, B) Total area 10×, C) negative control 20×, D) 20×, E) 40×, F-N) 100× cell staining. Arrows: double staining (CD133/Ki-67), arrowheads: CD133 staining, CD133 cytoplasmic staining with DAB (brown), Ki-67 nuclear staining with AEC (red).

Table 1. Number of specific meningioma subtypes and the mean percentage of CD133 and Ki-67 expression for each meningioma group

<table>
<thead>
<tr>
<th>Meningioma subtype</th>
<th>Number and percentage of each meningioma subtype</th>
<th>Mean % of co-localization (magnification ×40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningothelial</td>
<td>17 (22%)</td>
<td>1.50%</td>
</tr>
<tr>
<td>Fibroblastic</td>
<td>37 (49%)</td>
<td>3.67%*</td>
</tr>
<tr>
<td>Transitional</td>
<td>14 (18.5%)</td>
<td>1.60%</td>
</tr>
<tr>
<td>Psammomatous</td>
<td>2 (2.6%)</td>
<td>0.25%</td>
</tr>
<tr>
<td>Microcystic</td>
<td>2 (2.6%)</td>
<td>0.0%</td>
</tr>
<tr>
<td>Atypical</td>
<td>3 (4%)</td>
<td>1.30%</td>
</tr>
<tr>
<td>Malignant (papillary type)</td>
<td>1 (1.3%)</td>
<td>1.50%</td>
</tr>
</tbody>
</table>

*Mean percentage of co-localization is significantly higher in fibroblastic meningioma subtype than the other subtypes.

Results

Immunostaining of CD133 and Ki-67 was satisfactorily positive in 76 of 80 (95%) meningioma tissue samples in the tumour tissue array slide. Number of tumour subtypes and the mean percent of co-localization are de-
Co-localization fraction was significantly higher in fibroblastic meningioma subtypes than those of other histological variants including meningothelial, transitional, psammomatous, microcytic, atypical, and malignant (papillary) types (Figs. 2-4) \((p < 0.0001)\).

**Discussion**

Although all meningioma subsets are thought to be derived from meningothelial cells, they display a wide spectrum of histological variants. The WHO classification in 2007 reported that meningothelial, fibrous and transitional (combination of meningothelial and fibroblastic appearance) subtypes are the most frequent subtypes. While grade I meningiomas (meningothelial, fibroblastic, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmacyte-rich, metaplastic) are generally treated satisfactorily, grade II (atypical, chordoid and clear cell tumours) and grade III (anaplastic, papillary, and rhabdoid tumours) subtypes tend to behave more aggressively with higher recurrence rates \([4,6]\). Notably, it is evident that CD44, as a cell surface marker, has interactions with small cellular membrane proteins such as ezrin, moesin, and merlin, which is encoded by the NF2 gene product merlin protein with CD44 and the interaction of CD44 with cytoskeleton via ezrin, radixin, and moesin proteins – structural relatives of merlin protein. Even though the data on CD44 function in meningioma cells support the idea that CD44 contributes to the invasiveness and anaplasia of meningiomas, some authors have claimed that CD44 may have a biphasic effect on meningioma cells – that is, CD44 promotes migration of meningiomas cells in low concentrations, whereas it causes inhibition in higher concentrations \([8]\).

To our knowledge, there is no single report regarding the expression pattern of CD133 in meningioma cells. However, numerous studies have demonstrated that only CD133-positive glioblastoma multiforme (GBM) cells have tumour-initiating potential. Cancer stem cells in gliomas were first proposed by Singh et al. \([11]\) and the authors proved that only CD133-positive glioma cells have the self-renewal and self-propagating capacity. Additionally, cancer stem cells derived from GBM are capable of recapitulating original tumours when transplanted into nude mice. Taken together, CD133 plays a crucial role in GBM initiation and progression. In our study, the CD133/Ki-67 co-expression fraction was significantly higher in fibroblastic meningiomas than in other meningioma variants. However, since there were only 3 atypical and 1 malignant meningioma spots in the...
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tumour tissue array slide, it is difficult to draw a conclusion regarding the actual co-localization percentage and persistence of CD133/Ki-67 in atypical and malignant meningiomas. Nevertheless, the far higher co-staining percentage of CD133/Ki-67 in fibroblastic meningioma samples compared to meningothelial subtype, a histological meningioma variant, architecturally resembling the non-neoplastic meningeal cells, gave us the impression that CD133 may play a role in the formation and progression of fibroblastic meningioma variants.
Fig. 4. Transitional (mixed) meningioma spots in tissue micro-array slide double stained with CD133 and Ki-67. CD133 cytoplasmic staining with DAB (brown in colour) and Ki-67 nuclear staining with AEC (red in colour)

Conclusions

1. The immunohistopathologically demonstrated persistent and significant co-staining pattern of CD133/Ki-67 in fibroblastic meningioma cells still needs to be verified in various tumour samples obtained from patients with meningiomas.

2. The further investigation of the possible role of CD133 in meningiogenesis may contribute to the understanding of the diversity of meningiomas at the cellular and molecular level.

Disclosure

The authors report no conflict of interest.

References


