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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 współlokalizację 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 fraction of CD133/Ki-67 in meningioma tissue array slide composed of 80 meningioma tissue samples of various histological variants. CD133 – a cell membrane stem cell marker – was previously proved to be associated with the initiation and progression 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 formation and progression of fibroblastic meningioma variants.

Streszczenie

Wstęp i cel pracy: Nie określono dotąd unikalnego znacznika molekularnego lub komórkowego dla oponiaków, najczęstszych guzów wewnątrzczaszkowych. Wcześniej wykazano, że CD133 – znacznik błony komórkowej komórek macierzystych – jest związany z zapoczątkowaniem, a także wzrostem wewnątrzczaszkowych glejaków i rdzeniaków płodowych.
Materiał i metody: Zbadano odsetek współlokalizacji CD133/Ki-67 w zestawach macierzy tkankowych oponiaków, złożonych z próbek 80 rozmaitych odmian histologicznych oponiaków.
Wyniki: Immunohistochemiczna współlokalizacja CD133 i Ki-67 była stwierdzana istotnie częściej w podtypie włóknistym oponiaka niż w podtypach meningotelialnym lub przejściowym. Ze względu na małą liczbę preparatów oponiaków atypowych (3) oraz złośliwych (1) w badanej macierzy tkankowej trudno wyciągnąć jednoznaczne wnioski dotyczące rzeczywistego odsetka współlokalizacji i utrzymywania się CD133/Ki-67 w oponiakach atypowych i złośliwych.
Wnioski: Znacząco większy odsetek wspólnie występującej reaktywności CD133/Ki-67 w preparatach oponiaka włóknistego w porównaniu z podtypem meningotelialnym, którego architektonika przypomina nienowotworowe komórki opon,

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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, współlokalizacja.

Introduction

Meningiomas, the most common intracranial tumours, constitute almost one-third of all brain tumours. 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 cytogenetically 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 tumour 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 meningiomas 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 G_0. 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 tumours, 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% H_2O_2 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