Characteristics of the expression of KAI1/CD82 and PDGFRβ and their impact on glioma progression

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Abstract
The biological features of glioma cells may define their clinical outcome. Little is known about the interactions between KAI1/CD82 metastatic suppressor protein and PDGFRβ in gliomas. The aim of the study was to examine KAI1/CD82 and PDGFRβ expression in gliomas in order to find the impact of these proteins on progression of the tumors. PDGFRβ, KAI1/CD82 protein expression and mRNA of genes were evaluated on eighty four paraffin-embedded tissue of gliomas using immunohistochemical staining and RT-PCR analysis. The PDGFRβ expression was higher in IV/III than in I/II glioma grades (p = 0.0004). The level of mRNA PDGFRβ was associated with the degree of PDGFRβ immunoreactivity. Downregulation of KAI1/CD82 was associated with tumor malignancy (p = 0.007). The increased level of KAI1/CD82 gene expression (3-4-fold) was found in gliomas with strong KAI1/CD82 immunoreactivity. The parallel KAI1/CD82 and PDGFRβ expression was more significantly associated with cases in a group graded as III and IV than in a group graded as I/II (p = 0.002).

We found that a loss of KAI1/CD82 and an increase in PDGFRβ expression in gliomas relate to a progressive tumor growth. The correlation between PDGFRβ and KAI1 expression in high grade gliomas suggests that a direct or indirect interaction between these proteins might have an impact on cell motility and invasive behavior of the tumor.

Key words: gliomas, KAI1/CD82, PDGFRβ, immunohistochemistry, RT-PCR.

Introduction
Gliomas are the most frequent primary neoplasms of the central nervous system (CNS). Most of them are characterized by their extensive invasion into the brain parenchyma [17,19,20]. Grade I/II astrocytomas are slow-growing tumors without aggressive features, whereas grade III and IV gliomas possess a malignant phenotype associated with high proliferative activity and vascular formation [18,19]. Glioblastoma (grade IV) is one of the most aggressive and deadly malignant brain tumors with an average survival time of 15 months after diagnosis [8,13,18,20]. Most primary glioblastomas develop de novo but some parts of diffuse astrocytomas grade II and III may progress to grade IV as secondary glioblastomas [12,25].

The high infiltration capacity of individual glioma cells is related to the unique biological features of these cells [5,12,18]. According to some authors, the migratory behavior of glioma cells observed during the tumor progression might be a result of the active-
A number of alternations in different genes have been identified in human gliomas and some of them are involved in glioma progression [8]. Among the growth factors, the platelet-derived growth factor (PDGF) has been well described in glioblastomas [19,20].

A lot of data suggested that the PDGF receptor activates a number of downstream signal transduction pathways including PI3K/Akt/mTOR and Ras/Raf/MAPK pathways and may play an important role in both normal development and tumorigenesis of the CNS [19,20]. Overexpression of PDGF ligands and receptors are frequent events in human gliomas regardless of the tumor grade. Their expression pattern in gliomas suggests the presence of autocrine and paracrine stimulatory loops [20]. It was revealed that PDGF signaling alone may be sufficient to induce glioma formation [10]. Blocking of PDGF signaling strongly implies that PDGF signaling is necessary and sufficient to maintain the malignant phenotype in the in vitro model [31].

The specific biological mechanisms that mediate tumors’ invasive nature are still unknown [30]. Glioma progression may be compared to other solid tumor metastasis process. This process involves a molecular mechanism which requires the contribution of a multiple gene alteration [15,30]. The role of metastasis suppressor genes has been discussed very rarely in gliomas [8,19]. The most interesting is the KAI1/CD82 gene originally identified as a putative metastasis suppressor gene for prostate cancer [9]. The KAI1/CD82 gene is a member of the tetraspan transmembrane super family (TM4SF) [30]. KAI1/CD82 is expressed in various human tissues and plays an important role in cell fusion, adhesion, migration, signaling, fertilization, differentiation and invasion [11]. Downregulation of both KAI1/CD82 mRNA and protein expression is observed during progression and increased invasive behavior of tumors [7,15,30]. Several mechanisms have been proposed by which KAI1/CD82 might influence and control tumor cell behavior [29]. It was established that KAI1/CD82 plays an important role in regulating melanoma cell migration through the controlling of Rho and GTPases signaling activity [23]. Another study revealed that KAI1/CD82 might have an inhibitory role in the PI3K/AKT pathway [1,6,23]. So far, the correlation between KAI1/CD82 expression and the signal transduction pathway has been examined in breast and ovarian cancers [29].

Little is known about the interaction between the members of metastasis suppressor genes and kinase receptors in primary brain tumors and their role in glioma cell behavior. The relationship between KAI1/CD82 and tyrosine kinase receptor PDGFRβ has not been evaluated in gliomas. To investigate the possible suppressive role of KAI1/CD82 in gliomas and their influence on PDGFRβ expression we examined KAI1/CD82 and PDGFRβ expression and the relation between them in gliomas in order to find the impact of these proteins on progression of gliomas.

**Material and methods**

The study was performed on tissue sections from 84 patients diagnosed with primary gliomas hospitalized in the Clinic of Neurosurgery of the Wrocław Medical University, Poland between 2007 and 2012. Tumor tissues were obtained at initial surgery. None of the patients received any treatment before the operation. All tumors were histologically verified to confirm the diagnosis, histological type and tumor grade according to established criteria classification of the central nervous system tumors by the World Health Organization (WHO) [17]. Based on the WHO classification, gliomas were subdivided into the following groups: grade I – 6 cases (6 cases of pilocytic astrocytoma), grade II – 24 cases (22 cases of fibrillary astrocytoma, 2 cases of oligodendroglioma), grade III – 15 cases (12 cases of anaplastic astrocytoma, 3 cases of anaplastic oligodendroglioma) and grade IV – 39 cases of glioblastoma multiforme.

The study was conducted in accordance with the declaration of Helsinki. This study was approved by the Local Ethic Committee of Human Research of the Medical University of Wrocław, Poland (permission no. 37/2012). Written informed consent was obtained from all participants.

**Immunohistochemical staining**

Immunohistochemical staining (IHC) for the analyzed proteins was performed on paraffin-embedded tissue using the Universal DakoCytomation LSAB + Kit, Peroxidase procedure (LSAB+ Kit:HRP, Dako, Copenhagen, Denmark) and the following primary monoclonal antibodies: anti-KAI1 (G2) (Santa Cruz Biotechnology, USA) and anti PDGFRβ (28E1) (Cell Signaling Technology, USA).

Five-micrometer sections from one selected block from each lesion were deparaffinized and boiled for
3 x 5 minutes for each antibody in citrate buffer (pH 6.0) at 700 W in a microwave oven. After the microwave treatment, the tissue sections were slowly cooled for 20 minutes. Endogenous peroxidase reactivity was blocked with 3% H2O2 and nonspecific tissue reactions with 10% BSA (bovine serum albumin). Tissue specimens were incubated with primary antibodies (anti-KAI1 anti-PDGFRβ) overnight at 4°C. Following washing with 0.1 M Tris-buffer, pH = 7.4 (TBS), the tissue specimens were incubated with a secondary biotinylated rabbit antibody, anti-mouse IgG (Dako, Copenhagen, Denmark) and with streptavidin-horseradish peroxidase-conjugated (Dako) both for 15 minutes at room temperature. After washing with TBS, the antigen-antibody reaction was visualized by DAB (3,3′-diaminobenzidine) (Dako, Copenhagen, Denmark) as a chromogen (8 minutes, room temperature). Sections were counterstained with hematoxylin and mounted. The incubation buffer (TBS) without the primary antibody was used as a negative control. The internal positive controls were performed according to the manufacturer’s protocol.

The preparations were evaluated under a BX-51 Olympus light microscope. The localizations, distributions and intensity of immunostaining were evaluated in the tissue sections. For KAI1 and PDGFRβ membrane immunostaining was considered as positive when at least 10% of tumor cells were stained. The intensity of staining was scored as 0 for negative, + weak, ++ moderate, and +++ strong. The immunohistochemical analyses were interpreted without prior knowledge of the clinical information.

Reverse transcription polymerase chain reaction

For reverse transcription polymerase chain reaction (RT-PCR) analysis of KAI1/CD82 and PDGFRβ genes, immunopositive cases for both proteins were divided into three groups: 10-40% positive tissue, 41-70% and 71-100% positive tissue. Each group consisted of 10 cases.

We used the method of RT-PCR for analysis of KAI1/CD82 and PDGFRβ gene expression. RNA was isolated from the freezing tissue of 30 patients with gliomas (which revealed a different level of KAI1/CD82 and PDGFRβ expression in IHC staining) according to the method of RNeasy Plus Mini from Qiagen. The reverse transcription was performed with QuantiTect Reverse Transcription kit (Qiagen). The estimation of KAI1/CD82 and PDGFRβ gene expression was performed in Rotor-Gene TM. The reaction mixture for RT-PCR volume of 25 μl contained 2 μl complementary DNA (cDNA) and 2 μl gene sequences of primers for KAI1/CD82 (Hs_CD82_1_SG Quant Tect Primer Assay Qiagen), PDGFRβ (Forward – 5'-AAT-GTCTCCACGCACCTTCGT-3' 489-509, Reverse – 3'-AGC-GGATGGTGTAAGGCAATA-5') (1177-1156) and reagents Rotor-Gene SYBR Green Master Mix and RNase-free water. The standard curve plotted on the basis of the reference gene peptidyl prolyl isomerase C (cyclophilin C, CYCC, Hs PPIA Quant Tect Primer Assay, Qiagen) with a different concentration; diluted 10-, 100- and 1000-fold. The RT-PCR reaction consisted of one cycle PCR initial activation step of 95°C for 5 seconds, followed by 40 cycles of 95°C for 5 seconds and 60°C of 10 seconds.

Statistical analysis

Correlations between KAI1 and PDGFRβ expression and glioma grade malignancy were statistically studied by χ2 test. Associations between KAI1 and PDGFRβ expression were analyzed by Spearman’s rank correlation. Differences were considered as significant when p ≤ 0.05.

Results

PDGFRβ expression and analysis of mRNA PDGFRβ levels in gliomas

Membrane PDGFRβ expression was found in 44/84 (52.3%) gliomas. The majority of cases revealed PDGFRβ immunopositivity in 10-40% of tumor tissue. In the vast majority of gliomas, the immunoreactivity for PDGFRβ was observed in the group of cells distributed in different parts of tumor tissue (Fig. 1). The strong reactivity for PDGFRβ (70-100% positive tissue) was found only in 9.09% of cases. In glioblastomas PDGFRβ expression was stronger than in fibrillary astrocytomas and oligodendrogliomas (p = 0.0001). The PDGFRβ expression revealed differences in IV/III tumor grades versus I/II glioma grades (p = 0.0004) (Fig. 2). Similar differences were found for the mean value for PDGFRβ expression between low grade gliomas (I and II) [GI 1.666 ± 4.082 (SD), GII 6.25 ± 19.067 (SD)] compared with high grade gliomas (III and IV) [GIII 20.666 ± 22.834 (SD), GIV 31.794 ± 28.457 (SD)], p = 0.001. In gliomas showing PDGFRβ immunoreactivity of 70-100% of tumor tissue, the level of mRNA PDGFRβ was higher (increased 3-5-fold) compared to
cases with PDGFRβ immunoreactivity of 40-70% of positive cases (increased 1.5-2 fold).

**KAI1/CD82 expression and analysis of mRNA KAI1/CD82 levels in gliomas**

KAI1/CD82 immunostaining was observed on the membrane and in the cytoplasm of tumor cells in 75.0% of gliomas. The membrane staining dominated and was found in a different range of tumor cells (10-100% tumor tissue) in individual gliomas. Only 17.4% of cases showed strong immunoreactivity for KAI1/CD82 ranging between 70% and 100% positive tissue (Fig. 3). Statistical differences were observed for KAI1/CD82 expression between GI/II vs. GIII/IV (p = 0.01) (Fig. 4). Similarly, the mean value of KAI1/CD82 expression in I/II [GI 58.333 ± 23.1666 (SD), GII 52.916 ± 32.900 (SD)] and III/IV [GIII 30.666 ± 28.652 (SD), GIV 25.128 ± 27.991 (SD)] tumor grades showed significant differences (p = 0.001). Comparing the extent of KAI1 immunoreactivity in relation to tumor grades, a significant downregulation of KAI1/CD82 protein expression in III/IV compared with I/II tumor grades was found (p = 0.007) (Fig. 5). The increased level of KAI1/CD82 gene expression (3-4-fold) was observed in gliomas with immunoreactivity for KAI1/CD82 above 50% of tumor tissue with strong intensity of staining defined as +++.

No correlation between KAI1/CD82 and PDGFRβ expression was observed in the whole group of gliomas (p ≤ 0.05). Moreover, the parallel KAI1/CD82 and PDGFRβ expression was observed more frequently in a group graded as III and IV than in a group graded...
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Discussion

The common feature of malignant brain tumors is local invasiveness to the surrounding tissue. Specific chemotactic signaling pathways are involved in the regulation of tumor cell motility [22]. In agreement with earlier data [14,16,21,31], we found the PDGFRβ expression more frequently in grade III and IV than in grade I and II gliomas. Some authors suggest that the autocrine and paracrine stimulation of PDGFRβ could play an essential role in glial tumorigenesis [4,31]. It was revealed that during glioma progression the PDGFRβ stimulation can induce the activation of different signaling receptors like EGFR, Notch which lead to dedifferentiation of glioma cells, and increase cell motility and malignancy of the tumor [8,31].

The correlation of PDGFRβ expression with the WHO high grade gliomas observed in the current study is partly linked to other data which suggest that this receptor is a crucial factor which regulates angiogenesis and might indirectly facilitate dissemination of tumor cells from primary tumor mass and invade normal brain tissue [21]. The role of PDGFRβ in invasion of tumor cells was revealed by inhibition of cell migration by suppression of PDGFRβ tyrosine phosphorylation [16]. Based on previous reports and our data we postulate that a high PDGFRβ expression in low grade gliomas might characterize the subset of gliomas with biological aggressive behavior and suggest that depending on the PDGFRβ signaling pathway, activation is possible in an early oncogenic event in gliomas [14]. Similarly to other tumors such as breast or liver tumors, a high PDGFRβ expression in gliomas might contribute to the decreased cell-cell adhesion and promote metastatic capacity of tumor cells [22]. Some data indicate that PDGFRβ overexpression is associated with epithelial-to-mesenchymal transition (EMT) [22]. Moreover, the PDGFRβ expression following EMT transient state may induce the metastatic process [27]. Additionally, in this group

![Fig. 5. The extent of KAI1 immunoreactivity related to glioma grades. Significant downregulation of KAI1/CD82 protein expression was found between gliomas graded as III/IV compared with I/II tumor grades (p = 0.007).](image)

![Fig. 6. The parallel KAI1/CD82 and PDGFRβ expression dominated in gliomas graded as III and IV than in gliomas graded as I/II. Observed differences were statistically significant (p = 0.002).](image)
of tumors a high expression of chitinase-like protein (YKL40) was found [2]. The authors suggested that YKL40 positivity contributes to progression of glioblastoma [2]. In gliomas with a high tumor grade the EMT transient state is often observed and in these tumors both mRNA PDGFRβ and its protein are highly expressed [18]. Authors claim that glioblastoma cells which have mesenchymal features and possess a high PDGFRβ expression indicate that this biological feature might characterize the group of tumors with increased cell plasticity [14]. This observation might be compared with our previous study which showed that the E-cadherin expression was weaker or absent in high than low grade gliomas [3]. In the current study, the PDGFRβ expression was analyzed in the same set of gliomas and obtained data related to the E-cadherin expression (unpublished data).

There are no data describing the role of KAI1/CD82 expression in gliomas by other authors. In the present study we analyzed the mRNA KAI1/CD82 gene and its protein gliomas in order to show the impact of KAI1/CD82 expression on glioma growth. Some authors found that loss of the KAI1/CD82 protein expression is associated with the invasive growth of tumors and metastasis [7,15]. In gliomas the invasive growth is associated with the migration of tumor cells to the surrounding tissue, so the question is whether KAI1/CD82 may suppress the migratory function of tumor cells in gliomas similarly to colorectal carcinoma [27].

The current study found a high KAI1/CD82 expression in low and loss in high grade gliomas. These results are consistent with previous data that KAI1/CD82 expression is associated with increased invasive behavior of solid tumors [7,26,29]. In analyzed glioma specimens overexpression of KAI1/CD82 was observed in glioma cells without malignancy features regardless of the tumor grade whereas the majority of malignant cells were negative for KAI1/CD82 immunostaining. The different pattern of KAI1/CD82 expression observed in this study might reflect the high degree of intratumoral heterogeneity of the primary tumor that progression and metastasis are spawned by selective subclone of cells [28]. Recently, data revealed that upregulation of microRNA-210 induced cell proliferation and migration of glioblastoma cells [32]. We postulate that KAI1/CD82 protein expression in gliomas might play a similar role to that in tumors with metastasis to other organs [7,23]. Firstly, KAI1/CD82 as a metastasis suppressor protein might inhibit the migratory ability of glioma cells, so we suggest that a high expression of KAI1/CD82 in low grade gliomas might reduce the risk of secondary glioblastoma development. Secondly, the other function of KAI1/CD82 protein could be considered in gliomas like regulation by microRNA. An experimental study has shown that KAI1/CD82 is able to inhibit the signaling PI3K/AKT pathway in breast, bladder, and melanoma cancer cell lines [23,24]. Taking into account that the PI3K/AKT pathway is often activated by PDGFR receptors during glioma progression, we suggest that the suppression of the PI3K/AKT pathway by KAI1/CD82 protein might limit the proliferation and spread of glioma cells [19,23].

The present study is the first one to investigate the association between KAI1/CD82 protein and PDGFRβ expression in gliomas. Interestingly, we found that KAI1/CD82 expression is closely related to PDGFRβ expression in high grade glioma malignancy. Our results demonstrate that parallel expression of both biomarkers in glioblastomas might identify the cases where KAI1/CD82 might lead to a reduction in PDGFRβ activity and probably inhibit angiogenesis dependent on PDGFRβ expression. Our observations are partly consistent with the studies reporting that the KAI1/CD82 attenuated pathway depends of receptor tyrosine kinase (RTKs) activity [7,24,29]. Based on earlier reports which describe the association between KAI1/CD82 and surface receptor responsible for a different signaling pathway we suggest that such mechanism may occur in gliomas [31].

Conclusions

We found that a loss of KAI1/CD82 and an increase in PDGFRβ expression in gliomas relate to a progressive tumor growth. A correlation between PDGFRβ and KAI1/CD82 expression in high grade gliomas suggests that a direct or indirect interaction between these proteins might influence the cell motility and invasive behavior of the tumor.

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Disclosure

Authors report no conflict of interest.
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