Metastatic glioblastoma cells use common pathways via blood and lymphatic vessels

Przerzutujące komórki glejaka wielopostaciowego wykorzystują typowe drogi naczyń krwionośnych i limfatycznych

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Abstract

Generally, gliomas do not metastasize. Therefore, larger series are not available to investigate the pathways of tumour spread. Here, we present the case of a young man with a glioblastoma multiforme WHO grade IV and distant metastases in several tissues.

The glioblastoma multiforme WHO grade IV of a young male patient recurred within a very short time along the surgical resection pathway within the temporalis muscle. After removal of the tumour bulk, the patient developed a distant intracranial tumour lesion around the contralateral ventricular system and a pulmonary tumour. Later on, the patient underwent an operation on a facial lesion representing a local extracranial glioblastoma recurrence and containing metastases within lymph nodes and lymphatic vessels.

Our case report indicated a lymphatic pathway of metastasis, which could be demonstrated by our histopathological analysis. We suggest that altered gene expression stimulated by glioblastoma-environment interaction altered the properties of glioblastoma cells, whether caused by a spontaneous genetic shift or induced by factors provided by the extracranial tissue.

Streszczenie

Glejaki na ogół nie dają przerzutów. Trudno w związku z tym zebrać większe serie przypadków, na podstawie których można by badać drogi szerzenia się tych guzów. W pracy przedstawiono przypadek młodego mężczyzny chorego na glejaka wielopostaciowego (stopień IV wg WHO), u którego wystąpiły odległe przerzuty do kilku różnych tkanek.

U młodego mężczyzny z glejakiem wielopostaciowym (stopień IV wg WHO) w krótkim czasie po operacji wystąpił na-wrót guza wzdłuż drogi dostępu chirurgicznego w mieściu skroniowym. Po usunięciu masy guza, u chorego rozwinął się wewnątrzczaszkowo-guz w miejscu odległym – przykomorowo po stronie przeciwnej, oraz kolejny guz – w płucach. W późniejszym czasie pacjent był również operowany z powodu zmiany na twarzy, która okazała się miejscową zewnętrzczaszkową wznową guza, z przerzutami w obrębie węzłów chłonnynych i naczyń limfatycznych.

Opisany przez autorów przypadek wskazuje na limfogenną drogę szerzenia się przerzutów, co wykazano w badaniu histopatologicznym. Autorzy stwierdzają, że zmieniona ekspresja genów, pobudzana przez interakcję komórek glejaka wie-

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Introduction

Human glioblastomas are well known as the most malignant human gliomas. Among all brain tumours, the glioblastomas comprise the majority of primary brain tumours in the elderly. Often described as systemic CNS disease with a high risk of local recurrence despite surgery, irradiation, and chemotherapy, they metastasize only in single cases outside the CNS, with a reported incidence of less than 2% [1]. Possible reasons for their low propensity to metastasize outside the CNS are the absence of lymphatic vessels within the brain and the blood-brain barrier without any communication between intracranial and extracranial perivascular spaces. According to the literature, nearly all reported cases of metastatic glioblastomas are associated with previous brain surgery of the primary tumour. This would support the suggestion that the glioblastoma cells need some contact with the systemic lymphatic vessels or the blood circulation to distantly metastasize. Here we present a case of a young man suffering from a right temporal glioblastoma multiforme. Months after the initial surgery, we observed metastases in the right temporalis muscle being followed by distant lymph node metastases and pulmonary glioblastoma manifestation. We could show the glioblastoma to change its phenotype and proliferation index.

Case report

A 36-year-old man experienced a generalized epileptic seizure in January 2006. Cranial magnetic resonance imaging (MRI) was performed at this time, showing no pathology at all. Five months later, he was admitted to our hospital after he noticed unspecified disorders of vision, memory, and coordination. He presented an increased intracranial pressure without focal neurological deficits. A new cranial MRI demonstrated an intracerebral haemorrhage on the right side with a perifocal oedema. After intravenous administration of gadolinium-DTPA, an underlying tumour was identified. Macroscopically total tumour removal was performed and revealed a necrotic, partly vascularized and bleeding tumour. The histopathological work-up resulted in the diagnosis of a glioblastoma multiforme with locally different tissue features. Four weeks later, tumour bed irradiation in combination with temozolomide chemotherapy was started [2]. Temozolomide monotherapy was performed thereafter including 200 mg of temozolomide per m² body surface for the first five days per 28-day cycle. Three months later, the patient presented with a swollen mass within the right temporal muscle without any other complaints. With the intention of removing an old haematoma, surgery was performed but revealed a tumour within the temporal muscle instead of the anticipated haematoma. A tumour biopsy was taken and could be diagnosed as a metastasis of the glioblastoma multiforme. Therefore a second local operation was done, but complete resection of the tumour proved to be impossible. At this time, the cranial MRI visualized a new glioblastoma lesion in the wall of the contralateral ventricle. At the same time, a pulmonary lesion was observed in a standard chest X-ray and a subsequent thoracic computed tomography (CT). Therefore, control imaging was performed; it presented progressive multiple lesions in both lungs confirming a metastatic disease. Within only four weeks, the local tumour mass in the temporalis muscle enlarged and the patient urgently requested facial surgery, which was performed under palliative conditions. The removed tissue mass included cervical lymph nodes that were removed as well. Subsequent histopathological work-up again revealed glioblastoma metastases. After systemic chemotherapy, the patient finally died 15 months after the initial cranial glioblastoma surgery.

Imaging studies

The initial cranial MRI after the epileptic seizure five months prior to the glioblastoma manifestation did not show any pathology (Figs. 1A-B). The preoperative...
MRI visualized a large right-sided temporal lesion with irregular and ring-like uptake of gadolinium-DTPA in the T1-weighted sequences after administration of the contrast medium (Figs. 1D-F). The bleedings into the tumour bed were visualized with a methemoglobin sensitive sequence (Fig. 1C). The early postoperative MRI confirmed macroscopically total removal of the tumour burden. Because of histopathological proof of an extracranial glioblastoma metastasis within the temporalis muscle, new imaging was performed. It showed a tumourous lesion within the right sided temporalis muscle (Fig. 1L) which was surgically removed a few days later (Fig. 1M). At the same time, intracerebral spread of the glioblastoma was observed to the frontal lateral ventricle of the left side (Fig. 1N). Despite temozolomide therapy, the patient developed further cerebral tumour progression and metastases being visualized on chest X-ray, chest CT, and cranial MRI (Fig. 2). The glioblastoma spread to the right edge of the mandible was visualized in MRI with irregular contrast enhancement (Fig. 3).

Fig. 1. (A, B) T1-weighted and T2-weighted transversal sections of the cranial MRI after an epileptic seizure without any further neurological deficits. (C) MRI of the patient’s tumour revealed intratumoural bleeding. (D-F) MRI of the tumour with T1-weighted sequences after the intravenous administration of gadolinium-DTPA. The typical feature of irregular, rim-like contrast enhancement is clearly visible. Additionally, the centre of the tumour is hypointense; that could correspond to the tumour necrosis. (G) H&E stains showed the typical glioblastoma features of neoangiogenesis, tumour necroses, pseudopalisading, cellular pleomorphism, and nuclear atypia in the first tumour (arrowheads). Bar indicates 500 µm. (H) Ki67 staining (arrowheads) showed a proliferation index of about 12% within the primary glioblastoma. Bar indicates 100 µm. (I) Strong and regular immunoreactivity was observed for GFAP (arrowhead). Bar indicates 100 µm. (K) MAP-2 was almost negative (arrowhead) in the primary tumour manifestation. Bar indicates 100 µm. (L) T1-weighted imaging revealed the tumour manifestation within the temporal muscle that was initially thought to be a haematoma. The tumour mass enhances with gadolinium. (M) The photograph of the intraoperatively removed muscle and tumour tissue demonstrated the extension of about 5 cm. (N) The MRI imaging with T1-weighted sequences and gadolinium administration revealed a further intracerebral glioblastoma spread during ongoing extracranial metastasis. (P) H&E staining of the intramuscular glioblastoma metastasis displays the same features of the intracranial glioblastomas (arrowhead). Bar indicates 500 µm. (Q) The Ki67 index rose massively within the intramuscular glioblastoma metastasis (arrowheads). Bar indicates 100 µm. (R) Strong GFAP positivity is maintained within the glioblastoma manifestation within the temporalis muscle (arrowhead). Bar indicates 100 µm. (S) Massive MAP-2 immunopositivity (arrowhead) is observed and is a clear contrast to the initially MAP-2 negative intracranial glioblastoma. Bar indicates 100 µm.
The glioblastoma specimens of our patient were stained with eosin and haematoxylin (H&E). They were immunohistochemically investigated with antibodies against Ki-67, glial fibrillary acidic protein (GFAP) and microtubule-associated protein 2 (MAP-2). All primary antibodies were purchased from DakoCytomation (Glostrup, Denmark).

H&E staining revealed the typical glioblastoma features of neoangiogenesis, tumour necroses, pseudopalisading, cellular pleomorphism, and nuclear atypia (Fig. 1G) in the first intracerebral glioblastoma manifestation. Immunostaining with a Ki-67 antibody showed a proliferation rate of approximately 12% (Fig. 1H). The immunochemistry with the GFAP antibody showed a strong and regular immunoreaction (Fig. 1J), whereas MAP-2 was almost negative within the tumour tissue (Fig. 1K). The immunohistochemical investigation of the intramuscular glioblastoma metastasis showed contrary results. GFAP remained strongly positive (Fig. 1R), but the expression pattern of MAP-2 changed significantly towards a massive expression (Fig. 1S). The proliferation rate switched to a much higher index of about 50% as visualized by Ki-67 immunostains (Fig. 1Q), whereas the H&E stains showed the typical picture of a glioblastoma (Fig. 1P). The fourth glioblastoma manifestation was located in the right masseter muscle and the right mandible with intermingled muscle and tendinous tissue and was furthermore demonstrated in local cervical lymph nodes (Figs. 3A-D, 4A-F). Analysis revealed aggregation of glioblastoma cells located in lymphatic vessels (Fig. 4A-F). GFAP remained strongly positive, whereas MAP-2 was strongly positive in comparison to the MAP-2 immunonegativity of the primary tumour. The proliferation rate was estimated to be about 15% (Fig. 3A-D).
Discussion

Extracranial metastases of a glioblastoma multiforme are rare. Though there has been no evidence of how glioblastoma metastasize outside the CNS, cases of pulmonary and pleural (60%), lymph node (51%), bony (30%), and liver (22%) metastases have mainly been described [3]. Other targets for metastases are the kidney, spleen, and heart [3-5]. In principle, continuous glioblastoma growth along surgical pathways, lymphatic spread, and haematogenous metastasis could occur. Single cases have been reported that described glioblastoma growth outside the CNS after surgical intervention such as stereotactic biopsy or...
craniotomy [6-9]. Scalp tumour masses or cutaneous tumour tissue was observed in these cases. Glioblastoma cells could also spread via pre-existing cavities of the cerebrospinal fluid (CSF), as was shown for cases with spinal cord metastasis [10–12]. This phenomenon is also well known for the spread of glioblastoma cells within the ventricular system, as was observed in our case. In addition to that, some cases with extracranial metastases have been described that followed artificial CSF pathways along ventriculo-peritoneal shunt systems [13]. Some publications report tumour spreading into local lymph nodes and the parotid gland [6,7,14,15].

With our case presentation we could show that glioblastoma cells are able to invade lymphatic vessels, implicating that glioblastoma cells are able to act like any other metastatic carcinoma cells. Additional metastases within the lungs and the mediastinum also proved the haematogenous pathway to be used by

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Fig. 4. (A) The spread of glioblastoma cells is shown to occur within lymphatic vessels as illustrated in the H&E stain. Bar indicates 500 µm. (B) The CD34 immunohistochemistry shows the CD34 positive lymphatic vessel wall that surrounds the glioblastoma tissue. The magnification is seen in the inset. Bar indicates 500 µm. (C) D-2-40 staining displays the tumour mass surrounded by lymphatic endothelium (also see inset). Bar indicates 500 µm. (D–F) The glioblastoma tissue still showed GFAP immunopositivity also in the metastatic localization in the mandible. Bars indicate 500 µm (D, E) and 100 µm (F).
the glioblastoma cells in our case. Almost all reported cases of extracranial metastases were associated with previous surgery, suggesting that perioperative tumour spread and cutaneous infiltration could be more frequent than described [7,13-18]. In conclusion, this could cause the glioblastoma cells to get in contact with blood vessels and lymphatic vessels outside the CNS. Further tumour cell adaptation via change in gene expression could enable the glioblastoma cells to express exactly those structures that are necessary for lymphogenous or haematogenous spread. On the other hand, there are reports about glioblastoma metastases without any previous surgery [19,20]. The metastatic pathway was thought to involve the venous system and the dural sinuses [19,20].

The histopathological appearance of the glioblastoma tissue significantly changed in our patient. The proliferation rate increased massively; a proliferation rate of about 50% was shown by Ki67 labelling. This indicates an increased biological aggressiveness of the metastatic glioblastoma in comparison to the primary tumour. In addition to that, the metastatic tumour tissue strongly expressed MAP-2 after the initial MAP-2 negativity of the primary glioblastoma. The reasons for the phenotypic change of malignant tumours are not known yet. A mutation of the genetic material of a subpopulation of glioblastoma cells seems likely and could be the reason for a higher aggressiveness and the tumour’s ability to resist irradiation and chemotherapy. Only recently, a case was described with an extracranially located secondary glioblastoma, which changed its histology significantly and exhibited features of a more malignant tumour [21,22]. This suggests the existence of stimulating factors in the extracranial environment of the glioblastoma metastasis. These factors could induce the expression of receptors, integrins, or matrix proteins that enable the glioblastoma cells to attach and invade lymphatic vessels followed by distant lymph node metastases as in our case.

Although surgery – stereotaxis or open surgery – is done in the majority of patients, extracranial metastasis of glioblastoma multiforme remains a rare event. Our case report indicated a lymphatic pathway of metastasis, which could be demonstrated by our histopathological analysis. We suggest that altered gene expression stimulated by glioblastoma-environment interaction altered the properties of glioblastoma cells, whether caused by a spontaneous genetic shift or induced by factors provided by the extracranial tissue. Because glioblastoma multiforme is able to change its protein expression and its biological behaviour, we should recognise glioblastoma multiforme as a systemic disease.

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Disclosure

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References