

# The IGF/IGFBP system in CNS malignancy

W Zumkeller, M Westphal

## Abstract

**The insulin-like growth factor (IGF) system includes IGF-I and IGF-II, the type I and type II IGF receptors, and specific IGF binding proteins (IGFBP-1 to IGFBP-6). These factors regulate both normal and malignant brain growth. Enhanced expression of IGF-I and IGF-II mRNA transcripts has been demonstrated in gliomas, meningiomas, and other tumours. Abnormal imprinting of IGF-II occurs in gliomas, medulloblastomas, and meningiomas. Both types of IGF receptor are expressed in gliomas and, in particular, the type I IGF receptor appears to be upregulated in malignant brain tissue. Antisense IGF-I receptor mRNA induces an antitumour response, resulting in complete brain tumour regression. Clinical trials for the treatment of brain tumours in humans based on a gene transfer protocol using IGF-I receptor antisense are under way. All six IGFBPs are expressed to a variable extent in brain tumours. High concentrations of IGFBP-2 are found in cerebrospinal fluid from patients with malignant central nervous system tumours; therefore, IGFBP-2 might be a useful marker for these tumours. IGFBP-4 appears to be a negative regulator of tumour proliferation. Both in vitro and in vivo experiments suggest that the IGF system represents an important target for the treatment of malignant central nervous system tumours and the ongoing trials should provide valuable information for future therapeutic approaches.**

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Insulin-like growth factors (IGFs) are anabolic regulators in astrocytes and neurones. IGFs promote the proliferation of oligodendrocytes and myelin synthesis,<sup>1</sup> and are thought to play a pivotal role in the proliferation of brain tumours. Specific receptors for IGFs are found in central nervous system (CNS) tumours, and various IGF binding proteins (IGFBPs) are also secreted by these tumours. Sex steroids may also regulate the behaviour of certain brain tumours (such as meningiomas) at least in part through their effects on the expression of IGFs and IGFBPs.<sup>2</sup>

## IGFs

Glioma tissues showed an enhanced expression of IGF-I and IGF-II mRNA transcripts compared with normal brain.<sup>3</sup> In situ hybridisation and immunocytochemistry showed a

strong expression of both IGF-I and IGF-II mRNA molecules and of their protein products in the tumour cells of astrocytomas and meningiomas.<sup>4</sup> However, concentrations of IGFs were not raised in cerebrospinal fluid (CSF) from patients with tumours.<sup>5</sup> In anaplastic oligodendroglioma, two IGF-I cDNAs resulting from alternative splicing of the IGF-I primary transcript were identified, which encode two different precursor proteins, corresponding to Ea IGF-I and Eb IGF-I.<sup>6</sup> IGF-I greatly enhances the three dimensional growth of human glioblastomas.<sup>7</sup> Transfection of rat C6 glioma cells with an episome based vector encoding antisense IGF-I cDNA results in a loss of tumorigenicity and also regression of established brain glioblastomas when injected into the tumour.<sup>8</sup>

Both meningiomas and gliomas express IGF-II mRNA.<sup>9</sup> In meningiomas, high IGF-II mRNA expression was detected.<sup>10</sup> A significant correlation was found between a high IGF-II/IGFBP-2 ratio and anaplastic/atypical histopathology in sporadic meningiomas, indicating that higher free IGF-II values may provide a greater stimulus for proliferation.<sup>11</sup> IGF-II mRNA was found in higher amounts in benign meningiomas than in malignant glioblastomas and astrocytomas, whereas the content of immunoreactive IGF-II peptide was comparable.<sup>12</sup> IGF-II mRNA expression appears to parallel the growth rate in the LI human glioblastoma cell line.<sup>13</sup>

Upregulation of IGF-II expression is a feature of TrkA but not TrkB activation in SH-SY5Y neuroblastoma cells, suggesting that IGF-II is a component of the effector mechanism of TrkA activation.<sup>14</sup> It appears that IGF-II may counteract the programmed cell death effects of retinoic acid,<sup>15</sup> and the antiproliferative agent interferon  $\gamma$  inhibits DNA synthesis and IGF-II expression in neuroblastomas.<sup>16</sup> In addition, the cytostatic agent suramin blocks IGF-II dependent cell growth by preventing IGF-I receptor activation.<sup>17</sup>

In choroid plexus papillomas, immunoreactive IGF-II was detectable, whereas normal choroid plexus was negative for IGF-II. In addition, IGFBP-2 was positive in the endothelium and vascular media in the normal choroid plexus, whereas it was negative or weakly positive in choroid plexus papillomas.<sup>18</sup> It was concluded that IGF-II could be a useful marker for choroid plexus papilloma in differential diagnosis.<sup>19</sup>

Genomic imprinting is characterised by a differential expression of the two parental alleles of an autosomal gene. Loss of imprinting (LOI) or biallelic expression may, as an epigenetic factor, play an important role in tumorigenesis.<sup>20</sup> LOI

Martin-Luther-  
University  
Halle-Wittenberg,  
University Hospital,  
Department of  
Pediatrics,  
Ernst-Grube-Str. 40,  
06097 Halle/Saale,  
Germany  
W Zumkeller

University Hospital  
Eppendorf,  
Department of  
Neurosurgery,  
Martinistr. 52, 20246  
Hamburg, Germany  
M Westphal

Correspondence to:  
Dr Zumkeller  
walter.zumkeller@  
medizin.uni-halle.de

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of IGF-II occurs in medulloblastomas and gliomas.<sup>21, 22</sup> The normally imprinted IGF-II gene lacks imprint in the leptomeninges and choroid plexus of the brain. However, monoallelic expression for IGF-II was found in 73% of the meningiomas, which is in contrast to the lack of imprinting status of IGF-II in leptomeninges.<sup>23</sup> Abnormal imprinting of IGF-II may contribute towards tumorigenesis and the modulation of aberrant imprinting status may result in new therapeutic approaches.

### IGF receptors

The type I IGF receptor is composed of subunits and shows tyrosine kinase activity, whereas the type II IGF receptor is a monomer of 250 kDa. Receptors for IGFs are found throughout the normal brain as well as in astrocytomas and glioblastomas.<sup>24, 25</sup> Two types of IGF receptor were identified in human gliomas.<sup>25, 26</sup> IGF-I binding was significantly higher in gliomas and meningiomas compared with normal adult brain.<sup>27</sup> In addition, both enhanced binding of IGF-II to glioblastoma membranes and increased IGF-II receptor concentrations were found in glioblastomas.<sup>28</sup> The IGF-I receptor is expressed by primitive neuroectodermal tumour cell lines in vitro and an activated receptor is important for cell proliferation in vitro.<sup>29</sup> IGF-I stimulates human primitive neuroectodermal tumour cells by phosphorylation of the IGF-I receptor involving the mitogen activated protein (MAP) kinase pathway.<sup>30</sup> In MCF-7 cells, which stably express the receptor mutated at the C-terminus, decreased clonogenicity in soft agar and increased sensitivity to UV irradiation were seen.<sup>31</sup>

Mutant IGF-I receptors transfected into C6 rat glioblastoma cells can act as dominant negatives inducing apoptosis.<sup>32</sup> C6 cells expressing an antisense IGF-I receptor RNA elicit an antitumour response, leading to complete brain tumour regression.<sup>33, 34</sup>

### IGFBPs

Many tumours express IGFBPs, which regulate the bioavailability and bioactivity of IGFs. Patients with malignant CNS tumours showed increased IGFBP-2 concentrations in CSF—patients with CNS tumours and microscopically detectable malignant cells in their CSF had the highest IGFBP-2 values.<sup>35</sup> Thus, IGFBP-2 in CSF might be a useful specific marker for malignant CNS tumours. In addition, raised IGFBP-3 concentrations were found in the CSF of 70% of patients with CNS tumours and in 86% of patients with tumours and microscopically detectable malignant cells in their CSF. The IGFBP-3 protease activity in CSF was increased in 94% of those patients with CNS tumours of high grade histological malignancy.<sup>36</sup>

Meningiomas and, to a lesser degree, malignant gliomas were found to synthesise IGFBP-1, supporting the notion that IGFBPs contribute towards the growth of CNS tumours in humans.<sup>37</sup> Astrocytomas secrete various IGFBPs, including IGFBP-1.<sup>38</sup> Glioblastoma cell lines were found to express mRNA

for IGFBP-1 (42% of cell lines), IGFBP-2 (65%), IGFBP-3 (97%), IGFBP-4 (3%), IGFBP-5 (74%), IGFBP-6 (94%), and IGFBP-7 (87%) as determined by the polymerase chain reaction. In addition, membrane bound IGFBPs (44, 50, and 60 kDa) were found.<sup>39, 40</sup> C6 glioma cells stably transfected with connexin43 cDNA show a lower rate of proliferation, which was associated with high IGFBP-4 mRNA expression,<sup>41</sup> indicating that the negative modulator IGFBP-4 might be responsible for the reduced proliferative capacity of these transfected cells.

### Conclusion

The IGF system is involved in tumour growth regulation both in vitro and in vivo. These factors may act as progression and survival factors upregulating tumour growth and blocking apoptosis, respectively.<sup>42</sup> The IGF system may therefore be a target of therapeutic intervention in CNS malignancy. Gene therapy of glioblastoma in rats by an episome based transcriptional cassette expressing antisense IGF-I cDNA has proved effective.<sup>43</sup> A gene transfer protocol using IGF-I receptor antisense cDNA has progressed into human clinical trials for the treatment of brain tumours.<sup>44</sup> Clinical trials that will provide crucial information for the improvement of gene transfer technology for humans are under way and should improve the outcome for patients suffering from CNS tumours.

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