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Genomic imprinting of *IGF2* and *H19* in human meningiomas

S. Müller [a], D. Zirkel [a], M. Westphal [a], W. Zumkeller [b,*]

[a] *Department of Neurosurgery, Laboratory for Brain Tumour Biology, University Hospital Eppendorf, Hamburg, Germany*
[b] *German Cancer Research Centre, Division of Cytogenetics, Heidelberg, Germany*

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Abstract

A number of genes, including *IGF2* and *H19*, are normally imprinted with preferential expression of the paternal or maternal allele, respectively. Loss of imprinting (LOI) of *IGF2* and *H19* is found in a number of tumours, suggesting that LOI of *IGF2* and/or *H19* may play an important role in tumorigenesis. The *IGF2* gene codes for a fetal growth factor and the *H19* gene is likely to act as an RNA with an antitumour effect. We investigated the imprinting status of *IGF2* and *H19* in human meningiomas. The normally imprinted *IGF2* gene lacks imprint in the leptomeninges and choroid plexus of the brain. To examine the imprinting status of *IGF2* and *H19* in human meningiomas we used the ApaI polymorphism in exon 9 for the *IGF2* gene and the AluI polymorphism in exon 5 for the *H19* gene. In total, 24 meningiomas of WHO grade I, II and III were analysed. 15 meningiomas (63%) were informative for the ApaI polymorphism in the *IGF2* gene. Monoallelic expression (MAE) for *IGF2* was found in 11 out of 15 tumours (73%) which is in contrast to the lack of imprinting status of *IGF2* in leptomeninges. Ten cases (42%) were heterozygous for the *H19* gene and biallelic expression was found in 3 out of 10 meningiomas (30%). These results indicate that modulation of the imprinting status of *IGF2* and *H19* may play an important role for the development of meningiomas.

Keywords: IGF2; H19; Meningioma; Imprinting

1. Introduction

Meningiomas are composed of neoplastic meningothelial cells and represent between 13 and 26% of primary intracranial tumours. Meningiomas are often multiple in patients with neurofibromatosis type 2 (NF2) and other families with a hereditary predisposition to meningioma [1]. Although patients with meningiomas are most commonly middle-aged, children are also affected showing a tendency towards the more aggressive forms. The WHO classification comprises grade I meningiomas, grade II atypical meningiomas and grade III anaplastic (malignant) meningiomas. Various subtypes, with meningothelial (arachnoidal), fibrous and transitional meningiomas being by far the most common, are included in the classification [2].

Genomic imprinting is a process by which the two parental alleles of an autosomal gene are differentially expressed [3]. Loss of imprinting (LOI) or biallelic expression may as an epigenetic factor play a pivotal role in tumorigenesis [4]. This is supported by the observation that patients with Beckwith-Wiedemann syndrome (BWS) presented LOI in their non-tumorigenic tissues, indicating that LOI precedes the development of cancer and is not merely an epiphenomenon [5,6]. Approximately 70% of Wilms' tumours, frequently associated with BWS, showed LOI of *IGF2* resulting in an increased gene dosage of *IGF2* [7]. In mouse, *IGF2* is expressed at high levels in fetal life, but its expression is down-regulated soon after birth [8]. Parental imprinting leads to the inactivation of the maternal allele of *IGF2* in fetal tissues, except in the CNS, where both alleles are functional [3]. In fact, *IGF2* is not parentally imprinted in the leptomeninges and choroid plexus [9]. LOI of *IGF2* occurs in medulloblastomas but can also occur in normal fetal cerebellum indicating that its occurrence in medulloblastomas may reflect the embryonal nature of the tumour rather than representing a primary pathogenetic mechanism [10].

* Corresponding author. Current address: Martin-Luther University Halle-Wittenberg, Children's Hospital Medical Centre, Department of Haematology/Oncology, Ernst-Grube-Str. 40, 06097 Halle/Saale, Germany. Tel.: +49-345-557-2053; Fax: +49-345-557-2389.

E-mail address: walter.zumkeller@medizin.uni-halle.de (W. Zumkeller).

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The majority of gliomas revealed a loss of imprinting for *IGF2* but maintained imprinting for *H19* in all cases suggesting that LOI of *IGF2* but not *H19* plays a role in the development of human gliomas [11].

The *H19* gene is widely expressed during the development of the human and mouse foetus [12–14] and is located near the *IGF2* gene on chromosome 11p15.5 [15]. The gene codes for an RNA which is spliced and polyadenylated [16]. In contrast to earlier reports, the H19 transcript is associated with polysomes and a possible *trans*-function of the *H19* gene has been suggested as there appears to be a reciprocal correlation in *trans* between cytoplasmic H19 and IGF2 mRNA levels [17]. H19 is thought to act as a tumour suppressor because overexpression of the gene results in the abolition of tumorigenicity of the G401 embryonic rhabdomyosarcoma cell line in nude mice [18]. Furthermore, decreased expression of the *H19* gene is associated with the transition of a normal kidney cell to nephroblastoma [6]. In addition, downregulation of *H19* was found in an adrenocortical carcinoma cell line when compared with normal fetal adrenal gland tissue [19]. In some tumours with loss of imprinting of *IGF2*, the expression of the *H19* gene is totally abolished whereas no change of expression was seen in tumours with normal imprinting [6].

Since the IGF system modulates tumorigenesis and apoptosis [20], epigenetic modification of the *IGF2* gene may have a profound impact on the gene transcription level. Previous studies revealed high IGF2 mRNA levels in meningiomas [21,22]. Recently, a high ratio of IGF2/IGF-binding protein-2 mRNA has been described as a marker for anaplasia in meningiomas [23]. Thus, we investigated whether modulation of imprinting of both *IGF2* and *H19* may be involved in the development of meningiomas.

2. Patients and methods

2.1. Patients and tissues

Twenty-four meningiomas were obtained from the Department of Neurosurgery at the University Hospital Eppendorf, Hamburg, Germany. Tumour samples were immediately frozen in liquid nitrogen and stored at −70°C until use. Grading and staging was performed according to the criteria established by WHO [2].

2.2. DNA and RNA extraction

Genomic DNA was extracted from meningiomas using the Qiamp Kit (Qiagen, Hilden, Germany). Total RNA of cells and tumour samples was isolated by homogenisation with Tri Star Reagent™ (acid-guanidinium method), followed by phase separation with chloroform and precipitation with isopropanol. The RNA was treated with RNase-free DNase (Boehringer-Mannheim, Mannheim, Germany) for at least 3 h. Reverse transcription was carried out using random primers and following the manufacturer's instructions in the 'Ready to go kit' (Pharmacia, Uppsala, Sweden).

2.3. PCR reaction and restriction fragment length polymorphism (RFLP)

The PCR reactions contained 100 ng cDNA or genomic DNA (gDNA), 0.2 units of AmpliTaq Gold Polymerase (Perkin-Elmer, Weiterstadt, Germany), 0.2 mM Jump Start dNTPs, 1×PCR buffer, 20 pmol sense primer and 20 pmol antisense primer in 20 μl and were performed in a thermal cycler (Perkin-Elmer) at the following temperatures:

IGF2: 94°C for 16 min, 30 cycles at 94°C for 1 min, at 55°C for 1 min and at 72°C for 2 min, followed by 72°C for 10 min. To check the quality of the PCR products before restriction analysis, one tenth of the reaction was size separated by electrophoresis on a 2% agarose gel.

H19: 94°C for 16 min, 35 cycles at 94°C for 40 s, at 66°C for 40 s and at 72°C for 40 s, followed by 72°C for 10 min. To check the quality of the PCR products before restriction analysis, one tenth of the reaction was size separated by Visigel™ Separation Matrix (Stratagene, Heidelberg, Germany) electrophoresis.

The presence of a polymorphic pattern was examined in each sample using an ApaI polymorphism for *IGF2* [7] and an AluI polymorphism for the *H19* gene [24]. *IGF2* gDNA and cDNA were amplified using sense primer 5′-CTTGGACTTTGAGTCAAATTGGC-3′ and antisense primer 5′-CCTCCTTTGGTCTCAAATTGG-3′ as described by Tadokoro and colleagues [25]. The PCR product size was a 236 bp fragment; after ApaI restriction 236 bp (no restriction site), and 173 and 63 bp fragments were detectable when a restriction site was there. The 63 bp fragment was not detectable by gel electrophoresis. Thus, in cases of heterozygosity there were two fragments on the gel of 236 and 173 bp. *H19* gDNA and cDNA were amplified using sense primer 5′-CTTTACAACCACTGCACTACCTG(AC)C-3′ and antisense primer 5′-GATGGTGTCTTTGACGTTGGGC-TGA-3′ as described by Yballe and colleagues [26]. The PCR product size from gDNA was 174 bp fragment; after AluI restriction 174 and 130 bp fragments were detectable (in cases of heterozygosity). Using cDNA, the product size was 95 bp; after AluI restriction 95 and 51 bp fragments were detectable (in cases of heterozygosity).

3. Results

We analysed 24 human meningiomas for monoallelic or biallelic expression of *IGF2* and *H19* respectively

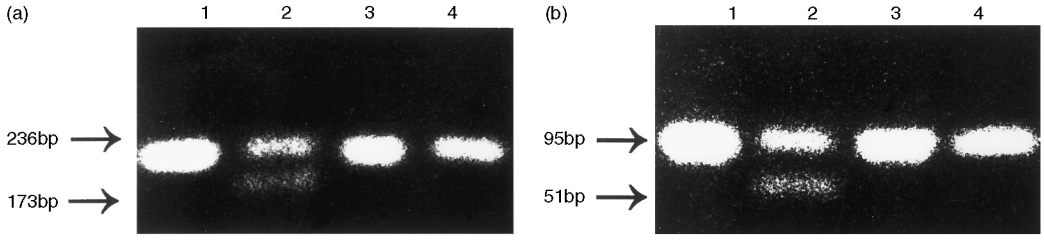

Fig. 1. (a) Allele-specific expression pattern of the *IGF2* in human meningiomas. In lanes 1 and 2, a case of meningioma that lacks imprinting for *IGF2*. In lanes 3 and 4, a case that shows monoallelic expression. Lane 1: PCR product of the cDNA of case 8 (see Table 1); lane 2: restriction analysis of the PCR product of lane 1: the presence of a fragment of 236 bp and one with 173 bp indicates that this case shows the presence of both alleles. Lane 3: PCR of cDNA of case 4; lane 4: restriction analysis of the fragment in lane 3 showing only the 236 bp fragment indicating monoallelic expression of the *IGF2* gene. (b) Allele-specific expression pattern of the *H19* gene in human meningiomas. In lanes 1 and 2, a sample of LOI of the *H19* gene is shown; in lanes 3 and 4 a sample of imprinting. Lane 1: PCR product of the cDNA of case 3 showing a 95 bp product; lane 2: restriction analysis with AluI of the product showing in lane 1. Two fragments (95 bp and 51 bp) are shown meaning LOI of the *H19* gene in case 3. Lane 3: PCR product of the cDNA of case 17; lane 4: restriction analysis with AluI of the product showing in lane 3: only one fragment detectable which means that in case 17 the *H19* gene is imprinted.

(Fig. 1a, b; Table 1). RFLP of genomic DNA-PCR and cDNA-PCR analysis was performed. For *IGF2*, an analysis using an ApaI/RFLP in the 3′-untranslated region of the *IGF2* gene was performed (Fig. 1a). For *H19*, an AluI/RFLP was used in order to identify the presence of the polymorphism in this gene (Fig. 1b).

15 (63%) of the 24 cases were informative at DNA level and thus, showed heterozygosity of the alleles of the *IGF2* gene by using the ApaI polymorphism in exon 9 of the *IGF2* gene [7]. In four meningiomas, 292 bp and 231 bp fragments were observed in the amplified cDNA samples. The other 11 informative cases showed monoallelic expression for *IGF2* as either the 292 bp or 231 bp band was detectable.

Ten (42%) of 24 meningiomas were heterozygous for the *H19* gene by using the AluI polymorphism in exon 5 of the *H19* gene [24]. Biallelic expression was found in 3 (30%) of 10 cases as evidenced by the presence of a 228 bp and 128 bp band after digestion of the genomic DNA-derived PCR product.

Table 1
DNA heterozygosity and allelic expression of *IGF2* and *H19* in human meningiomas

			IGF2/ApaI			*H19*/AluI		
Case	Age (years)/sex	Histology	gDNA	cDNA	Allelic usage	gDNA	cDNA	Allelic usage
1	52/f	II	a	–	–	a	–	–
2	60/f	II	a	–	–	a	–	–
3	64/m	II	a	–	–	a/b	a/b	BAE
4	94/f	II	a/b	a/–	MAE	a	–	–
5	60/f	II	a/b	a/–	MAE	a	–	–
6	53/f	I	a/b	a/–	MAE	a	–	–
7	49/m	II	a/b	a/–	MAE	a	–	–
8	58/f	II	a/b	a/b	BAE	a/b	a/b	BAE
9	71/f	II	a/b	a/–	MAE	a/b	–/b	MAE
10	37/f	II	a/b	a/–	MAE	a/b	a/b	BAE
11	71/f	I	a	–	–	a/b	a/–	MAE
12	53/m	I	a	–	–	a/b	a/–	MAE
13	51/f	I	a/b	a/b	BAE	a	–	–
14	56/m	I	a	–	–	a/b	a/–	MAE
15	57/f	II	a	–	–	a	–	–
16	53/f	II	a	–	–	a	–	–
17	33/f	III	a/b	a/–	MAE	a/b	a/–	MAE
18	74/f	II	a/b	a/b	BAE	a/b	–/b	MAE
19	63/f	II	a/b	a/–	MAE	a	–	–
20	79/f	II	a	a/–	–	a	–	–
21	62/f	II	a/b	a/–	MAE	a	–	–
22	41/f	I–II	a/b	a/–	MAE	a	–	–
23	22/f	I	a/b	a/b	BAE	a/b	a/–	MAE
24	3/f	I	a/b	a/–	MAE	a	–	–

BAE, biallelic expression; MAE, monoallelic expression; a, uninformative not a/a homozygote; a/b, heterozygote; a/–, monoallelic expression of a allele; –/b, monoallelic expression of b allele.

4. Discussion

Numerous studies have revealed abnormal imprinting of *IGF2* and *H19* in a wide range of tumours. In addition, overexpression of *IGF2* gene has also been reported in meningiomas [21–23]. This study shows that monoallelic expression for IGF2 as well as loss of imprinting for *H19* occurs in a number of human meningiomas which further underlines their pivotal role in meningioma tumorigenesis.

Relaxation of imprinting of the *IGF2* gene concomitantly with activation of the maternal allele has been found in Wilms' tumour, rhabdomyosarcoma and lung carcinoma [27]. Moreover, the resulting increase in the number of active *IGF2* genes may constitute a favourable step towards tumour progression [5,28–31]. In contrast, monoallelic expression of the *IGF2* gene was seen in hepatoblastoma, indicating that in this case normal imprinting of this locus is maintained [32]. Abnormally imprinted cells are susceptible to epigenetic modification when treated with 5-aza-2′-deoxycytidine, a specific inhibitor of cytosine DNA methyl-transferase [33].

H19 is paternally imprinted and the maternal allele is expressed transiently during embryogenesis whilst it is repressed in the adult [34]. The inactive *H19* allele is methylated within the promoter sequences, possibly resulting in an altered chromatin structure and thereby preventing the binding of specific transcription factors. In contrast, *IGF2* is preferentially methylated on the active allele [35]. *H19* may be involved in cellular differentiation as it is preferentially expressed in differentiating fetal tissues [12] and was also found to have a potential tumour suppressor activity in embryonal carcinoma cell lines [18]. Furthermore, decreased H19 mRNA expression has been reported in Wilms' tumours [6,19]. The similar expression pattern and reciprocal imprinting of *IGF2* and *H19* suggests that they are coordinately regulated by an enhancer which activates either *IGF2* or *H19* [36].

Loss of imprinting of *IGF2* is tightly linked to downregulation of *H19* in Wilms' tumours [6,19]. However, hepatoblastoma with loss of imprinting showed *H19* expression at levels comparable to those of hepatoblastomas with normal imprinting [37]. Consequently, expression of the maternal allele of *IGF2* in hepatoblastoma does not necessarily involve downregulation of *H19*.

We have previously demonstrated the presence of IGF2 mRNA transcripts and synthesis in glioma cell lines [38]. IGFs are probable survival factors that block a common late intracellular apoptosis pathway and, at the same time, act as progression factors to upregulate growth in gliomas [39]. Overexpression of IGF2 mRNA in meningiomas may, in some cases, be the result of a lack of imprinting for *IGF2*. It is likely that IGF2 as a survival factor blocks apoptosis and as a progression factor stimulates growth in meningiomas. However, most meningiomas present monoallelic expression for *IGF2* which could be due to mosaicism (MAE and LOI) within cells. Ohlsson and colleagues [40] showed that parental *IGF2* alleles can be differentially expressed in tumours indicating epigenetic heterogeneity. Loss of alleles and/or heterozygosity as a possible mechanism is excluded as the DNA is derived directly from the tumour. Recently it was shown that LOI of *IGF2* was region-specific in the adult human brain [41]. Finally, the possibility that some individual leptomeninges show imprinting for *IGF2* (e.g. polymorphism) has not yet been proven.

In conclusion, abnormal imprinting for *IGF2* and *H19* may play an important role in meningioma tumorigenesis. Therapeutic modulation of aberrant imprinting status may constitute a novel approach for the treatment of meningiomas.

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