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REVIEW ARTICLE

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Genetic alterations associated with the evolution and progression of astrocytic brain tumours

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Abstract Diffusely infiltrating low-grade astrocytomas (WHO grade II) have an intrinsic tendency for progression to anaplastic astrocytoma (WHO grade III) and glioblastoma (WHO grade IV). This change is due to the sequential acquisition of genetic alterations, several of which have recently been identified. In low-grade astrocytomas, *p53* mutations with or without loss of heterozygosity on chromosome 17p are the principal detectable change. Anaplastic astrocytomas contain *p53* mutations at an overall incidence of 34% and, in addition, loss of heterozygosity on chromosome 19q and frequent homozygous deletion of the *p16* tumor suppressor (MTS-1) gene. The most malignant astrocytic neoplasms, the glioblastoma, further shows loss of chromosome 10 and amplification of the epidermal growth factor receptor (EGF-R) gene at overall incidences of 66% and 34%, respectively. The type and distribution of *p53* mutations in astrocytic brain tumours are not suggestive of specific environmental carcinogens operative in their aetiology. Analysis of 91 families with *p53*germline mutations reported to date show that tumours of the nervous system account to 12% of all neoplasms. Of a total of 57 brain tumours reported, 30 were classified histologically and of these, 73% were of astrocytic origin. The observation that somatic *p53* mutations in sporadic brain tumours are largely restricted to those of astrocytic origin and that astrocytomas also prevail among CNS neoplasms associated with *p53* germline mutation strongly suggests, that *p53* mutations are capable of initiating neoplastic transformation in astrocytes of the human nervous system.

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Introduction

Considerable advances have been made in the elucidation of genetic alterations associated with neoplastic transformation in the human nervous system [1, 2, 13, 14, 22, 26]. A primary objective of these studies was the analysis of genetic alterations that accumulate during glioma progression. Astrocytic brain tumours (with the exception of the pilocytic astrocytoma) have an intrinsic tendency to progress towards a more malignant phenotype and ultimately to acquire the histopathological and clinical characteristics of a glioblastoma. There is increasing evidence that astrocytoma progression reflects the sequential accumulation of genetic alterations. The identification of these events and the assessment of their roles in malignant transformation are essential for our understanding of the evolution of astrocytomas and are prerequisite for therapeutic approaches at the molecular level.

The aetiology of human brain tumours is still largely unknown. Analytical epidemiological studies have revealed an increased risk of brain tumour development in association with certain occupations, including farming, dentistry, fire fighting, metalworking, and the rubber industry, but all attempts to identify a specific exposure or causative environmental agent have been unsuccessful [32, 34]. Some genotoxic carcinogens cause typical or even specific mutations in transformation-associated genes and thereby allow conclusions regarding the causative agent operative in the initiation of the respective neoplasms [9, 10]. We have, therefore, analysed *p53* mutations as a possible potential source of information on the aetiology of human CNS neoplasms. Further, a comparison was made between somatic *p53* mutations in sporadic brain tumours and CNS neoplasms associated with *p53* germline mutations. This led to the hypothesis that inactivation of the *p53* gene is capable of initiating

malignant transformation in astrocytes of the human CNS.

Genetic alterations in sporadic astrocytic brain tumours

In the histopathological grading of astrocytomas, it is mandatory to make a clear distinction between diffusely infiltrating astrocytomas (WHO grades II–IV) and the pilocytic (juvenile) astrocytoma (WHO grade I), which has a different location, age distribution, biological behaviour and genetic basis.

Pilocytic astrocytoma

The pilocytic (juvenile) astrocytoma (WHO grade I) is a benign, slow-growing tumour that occurs mainly in children and young adults with a peak incidence at age 10–13 years. Typical localizations are midline structures (medial temporal lobe, optic nerve, thalamus, brain stem and cerebellum). Progression from pilocytic astrocytoma to more malignant histological types is rare [3, 16, 17].

No *p53* mutations have been detected in 12 cases reported by us [26] and in 8 cases reported by Hunter et al. [11]. In a series of 7 pilocytic astrocytomas analysed by Lang et al. [20], 1 tumour had a *p53* mutation, but this lesion was atypically located in the cerebral hemispheres. Using immunohistochemistry, these authors also observed elevated levels of the p53 protein in 5 of their 7 pilocytic astrocytomas [20]. Pilocytic astrocytomas may show a loss of chromosome 17q, including the region encoding the NF-1 gene [40]. It is noteworthy that patients with neurofibromatosis type I often develop pilocytic astrocytomas of the optic nerve.

Low-grade astrocytoma

Low grade astrocytomas (WHO grade II) may be located in any region of the CNS but their preferential sites are cerebral hemispheres. This well-differentiated, diffusely infiltrating tumour typically manifests in young adults, with a peak in the third decade of life. Low-grade astrocytomas are macroscopically and histologically ill-defined and infiltrate surrounding brain structures with gradual incorporation of preexisting cells. They show nuclear atypia, but mitotic activity is absent. Low-grade astrocytomas have an intrinsic tendency to progress, usually over period of several years, to anaplastic astrocytoma or glioblastoma multiforme [3, 16, 17].

Several independent studies have shown that in low-grade astrocytomas *p53* mutations with or without loss of heterozygosity on chromosome 17p are the principle detectable change [6, 19, 20, 24, 26, 27, 33, 35, 37], although isolated cases with loss of heterozygosity on chromosomes 13 and 22 have been reported [14]. The incidence of *p53* mutations detected varies in the different studies from zero to 50%, the overall incidence being 24% [6, 20, 24, 26, 27, 33, 37]. By immunohistochemical analyses, P53 protein accumulation was observed at an overall incidence of 42% [20, 23, 27].

Anaplastic astrocytoma

Anaplastic astrocytomas (WHO grade III) often develop over a course of several years from low-grade astrocytomas but may also arise de novo, without clinical or histopathological evidence of a preceding benign glioma. The transition from low-grade to anaplastic astrocytoma is characterized histopathologically by a focal or diffuse increase in anaplasia, cellularity, nuclear atypia and by a marked increase in mitotic activity [3, 16, 17].

Several independent studies have demonstrated that anaplastic astrocytomas contain *p53* mutations at an overall incidence of 34% [6, 20, 24, 27, 37]. Immunohistochemically, P53 protein accumulation was observed at an overall incidence of 53% [20, 23, 27]. In 8% of anaplastic astrocytomas, inactivation of the P53 protein appears to be mediated by complex formation with the amplified MDM2 gene [27, 28]. Heterozygous or homozygous deletion of *p16* (multiple tumour suppressor 1; MTS-1) encoding an inhibitor of cyclin-dependent kinase 4 (CDK4) and amplification of CDK4 were observed at overall incidences of 44% and 27%, respectively [31, 42]. In addition, loss of heterozygosity on chromosome 19q has been reported in 44% of anaplastic astrocytomas, but the respective gene has not yet been identified [39].

Glioblastoma multiforme

Glioblastoma multiforme (WHO grade IV) is the most malignant neoplasm of the human CNS. It typically occurs in adults, with a peak incidence at 45–60 years, and usually causes the patient's death within 1 year after diagnosis. Glioblastoma multiforme is the end point of the progression from low-grade or anaplastic astrocytoma (secondary glioblastoma). However, glioblastomas may also develop rapidly without clinical or histopathological evidence of a less malignant precursor lesion (primary glioblastoma). The cerebral hemispheres are the preferential site, in particular the fronto-temporal region. Histopathologically, glioblastomas are characterized by a high degree of anaplasia and marked mitotic activity but the most characteristic features separating this lesion from low-grade and anaplastic astrocytoma are vascular endothelial proliferations and areas of necrosis [3, 16, 17].

In several independent studies, *p53* mutations were identified in 13–39% of the cases, the overall frequency being 25% [5, 6, 22, 24, 27, 33]. Accumulation of p53 protein was found in 40% of the glioblastomas [22, 25, 27]. In 5% of glioblastomas, amplification of MDM2 gene has been reported [25, 27, 28]. Heterozygous or homozygous loss of *p16* and amplification of CDK4 were

observed at overall incidences of 63% and 76%, respectively [7, 8, 15, 29, 31, 36, 42]. Schmidt et al. [31] reported that CDK4 amplification was common in tumours not showing loss of *p16*, and CDK4 and MDM2 were shown to be coamplified in many cases. In addition, more than 60% of glioblastomas have loss of chromosome 10 [6, 19, 27, 35, 38, 43]. Moreover, they contain, in more than one third of cases, an amplification and overexpression of the EGF-R gene, sometimes in a truncated and rearranged form, which has structual and functional similarlity to v-*erb*B [4, 12, 19, 27, 35, 38, 44]. All the glioblastomas with EGF-R amplification showed simultaneous loss of chromosome 10 [38]. Reduced expression of tumour suppressor gene DCC has been reported in 7 of 8 glioblastomas (88%) [30].

Model of glioma progression

There is increasing evidence that the progression from low-grade to anaplastic astrocytoma and glioblastoma is associated with a cumulative acquisition of multiple genetic alterations (Table 1). The observation that *p53* mutations are present at a similar incidence in low-grade astrocytoma, anaplastic astrocytoma and glioblastoma multiforme may indicate that loss of *p53* function is involved during a rather early stage of neoplastic transformation. The incidence of P53 immunoreactive astrocytomas is generally higher than that of *p53* mutations (Table 1). This may be due to (1) the presence of *p53* mutations outside the regions analysed, including regulatory sequences, (2) accumulation of wild-type P53 protein, or (3) stabilization of P53 protein by the formation of complexes with other cellular (MDM2) or viral oncoproteins [22, 25]. Loss of heterozygosity of chromosome 9p21 has been observed in anaplastic astrocytomas and glioblastomas. This region is the location of both *p16*,which codes for the cyclin-dependent kinase 4 inhibitor, and the *p15*gene. Homozygous or heterozygous loss of *p16* is frequent in anaplastic astrocytomas and glioblastomas, whereas *p16* mutations are rare in these tumours (Table 1). The possibility that another tumour suppressor gene located on 9p21 plays a part in the evolution of astrocytomas cannot be ruled out. Amplification of the CDK4 gene (cyclin-dependent kinase 4) has also been found in anaplastic astrocytomas and glioblastomas (Table1). Although the available data may not be complete, it is clear that abnormalities of several genes involved in cell cycle

Table 1 Genetic alterations in astrocytic brain tumours

Histological typing	Genetic alterations	Frequency	%	References
Pilocytic astrocytoma (WHO Grade I)	P53 accumulation	5/7	71	[20]
	p53 mutation	1/27	4	[11, 20, 26]
	LOH 17p	0/7	0	[19]
	LOH 17q (NF1)	4/20	20	[40]
	LOH 19q	0/6	0	[39]
	LOH 10	0/7	0	[19]
	EGF-R amplification	0/15	0	[19, 38]
Low grade astrocytoma (WHO Grade II)	P53 accumulation	10/24	42	[20, 23, 27]
	p53 mutation	12/49	24	[6, 20, 24, 26, 27, 33, 37]
	LOH 17p	8/33	24	[6, 19, 27, 35, 37]
	LOH 19q	0/6	0	[39]
	LOH 10	1/27	4	[6, 19, 27, 35]
	EGF-R amplification	3/43	7	[12, 19, 27, 35, 38]
	MDM2 amplification	0/8	0	[27]
	Loss of *p16*	0/8	0	[31]
	CDK4 amplification	0/8	0	[31]
Anaplastic astrocytoma (WHO Grade III)	P53 accumulation	19/36	53	[20, 23, 27]
	p53 mutation	19/56	34	[6, 20, 24, 27, 37]
	LOH 17p	23/59	39	[5, 6, 19, 27, 35, 37]
	LOH 19q	4/9	44	[39]
	LOH 10	9/57	16	[6, 19, 27, 35, 38]
	EGF-R amplification	6/50	12	[12, 19, 27, 35, 38]
	MDM2 amplification	3/36	8	[27, 28]
	Loss of *p16*	18/41	44	[31, 42]
	CDK4 amplification	3/11	27	[31]
Glioblastoma multiforme (WHO Grade IV)	P53 accumulation	36/90	40	[22, 25, 27]
	p53 mutation	34/134	25	[5, 6, 22, 24, 27, 33]
	LOH 17p	28/113	25	[5, 6, 27, 35]
	LOH 19q	11/46	24	[40]
	LOH 10	123/187	66	[6, 19, 27, 35, 38, 43]
	EGF-R amplification	92/267	34	[4, 12, 19, 27, 35, 38, 44]
	MDM2 amplification	7/137	5	[25, 27, 28]
	Loss of *p16*	121/192	63	[7, 15, 31, 36, 42]
	p16 mutation	1/74	1	[7, 15, 36]
	CDK4 amplification	25/33	76	[29, 31]
	DCC reduced expression	7/8	88	[30]

regulation are operative in glioma progression, including *p53*, cyclin-dependent kinases and their inhibitors. Since loss of heterozygosity of chromosome 10 and amplification of EGF-R gene are found almost exclusively in glioblastomas, these genetic alterations are considered late events in glioma progression.

The presence of subsets of glioblastomas has been postulated on the basis of the different combinations of *p53* mutations, loss of heterozygosity on chromosomes 17p and 10, and EGF-R amplification [19, 41]. According to von Deimling et al. [41], EGF-R amplification and LOH of chromosome 10 occur significantly more often in patients without LOH on chromosome 17p. In addition, these apparently manifest in patients significantly younger than those with glioblastomas characterized by EGF-R amplification [41]. Based on the analysis of genetic alterations in 65 astrocytic tumours, Lang et al. [19] suggested that the genetic pathways for the development of glioblastoma multiforme progressing from lower grade astrocytoma (secondary glioblastoma) are different from those for de novo glioblastoma (primary glioblastoma). Secondary glioblastomas were characterized as tumours with *p53* mutation and LOH of chromosome 17p. Primary glioblastomas were characterized as tumours without *p53* mutations but with amplification of EGF-R and LOH of chromosome 10 [19].

Type and distribution of p53 mutations in astrocytic brain tumours

In sporadic astrocytic brain tumours, *p53* mutations are mainly located in the highly conserved region of the gene, with clusters at codons 175, 248 and 273 [22, 26]. These codons are among the six hot spots found in a variety of human tumours [9, 10, 21]. Among *p53* mutations identified in astrocytic brain tumours, G:C–>A:T transitions are most frequent and they are located predominantly at CpG sites (Table 2). These patterns of *p53* mutations are similar to those in colon cancer, sarcomas and lymphomas [9] but different from those in non-small-cell lung cancer and liver cancer, which are considered to be associated with tobacco-related carcinogens and aflatoxin B_1, respectively. The transition mutations

Table 2 Nature of *p53* mutations in human neoplasms

Cancer	Mutations at G:C			G:C→A:T Transition atN CpG sites	Mutation at A:T			References
	→A:T	→T:A	→C:G		→T:A	→G:C	→C:G	
Brain	73%	5%	3%	52%	0%	16%	3%	[5, 6, 20, 22, 24, 26, 27, 33, 37]
Colon	79%	0%	3%	67%	3%	15%	0%	[9]
Sarcoma	66%	17%	7%	53%	0%	0%	0%	[9]
Lymphoma	57%	4%	4%	47%	6%	19%	11%	[9]
Germline mutations	64%	13%	2%	49%	10%	0%	2%	[18]
Lung: non SCLC	20%	57%	13%	10%	7%	3%	0%	[9]
Liver	16%	74%	5%	0%	0%	5%	0%	[9]

Table 3 Tumours associated with *p53* germline mutations

Organ	Tumour type[a]	Incidence[b]
Breast	Carcinoma	24%
Bone	Osteosarcoma	13%
Soft tissue	Rhabdomyosarcoma (55%) Malignant fibrous histiocytoma (10%) Fibrosarcoma (10%) Spindle cell sarcoma (5%) Liposarcoma (5%) Leiomyosarcoma (5%) Chondrosarcoma (5%) Neurofibrosarcoma (3%)	12%
Nervous system	Astrocytoma (73%) Medulloblastoma/PNET (17%) Ependymoma (3%) Choroid plexus papilloma (3%) Schwannoma (3%)	12%
Gastrointestinal	Carcinoma	7%
Female reproductive organs	Carcinoma	6%
Lung	Carcinoma	5%
Haematopoietic system	Leukaemia and lymphoma	4%
Adrenal gland	Carcinoma	4%
Other organs	Various histologies	13%

[a] Values in parenthesis indicate percentage of tumours with histopathological typing.
[b] Data based on 475 tumours from 91 reported families [18]

at CpG sites can best be explained as being due to the deamination of 5-methylcytosine residues and considered to be endogenous (not caused by genotoxic environmental carcinogens). In summary, no specific mutations or mutational hot spots that could be suggestive of environmental carcinogens operative in aetiology were found in human brain tumours.

Brain tumours associated with *p53* germline mutations

Up to now, 91 families with germline *p53* mutations have been reported, with a total of 475 tumours in affected family members [18]. Breast cancer developed most frequently, followed by sarcomas, brain tumours, and neoplasms of the gastrointestinal tract. Brain tumours occurred in 35 out of 75 pedigrees (47%). Of the 57 brain tumours recorded, 27 (47%) were not specified histologically. Of the remaining brain tumours 22 (73%) were of astrocytic origin (Table 3). This, together with the observation of frequent *p53* mutations in sporadic astrocytomas [26], strongly supports the view that loss of *p53* function carries a risk of malignant transformation for astrocytes more than any other cell type of the human nervous system.

Among *p53* germline mutations in 91 families, point mutations causing amino acid substitutions were the most frequent (81%). Among these, G:C–>A:T transition mutations prevailed and 77% of them were located at CpG sites (Table 2). Thus, the type of *p53* germline mutations was similar to that found in sporadic astrocytic brain tumours (Table 2), suggesting that these germline *p53* mutations may also have evolved endogenously rather than as a consequence of interaction with environmental carcinogens.

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