Review

Genetic basis of glioma progression

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Abstract: The most frequent and malignant brain tumor is the glioblastoma, which may develop *de novo* (primary glioblastoma) or through progression from low-grade or anaplastic astrocytoma (secondary glioblastoma). These glioblastoma subtypes constitute distinct disease entities that affect patients at different age, and evolve through different genetic pathways. Primary glioblastomas develop in older patients (mean age, 55 years) and typically show *EGFR* amplification / overexpression, LOH on the entire chromosome 10, *PTEN* mutations and, occasionally, *MDM2* amplification. Secondary glioblastomas develop in younger patients (mean age, 40 years) and typically contain *TP53* mutations and/or $p14^{ARF}$ promoter methylation as earliest detectable alterations. Additional changes in the pathway leading to secondary glioblastomas include LOH on 19q and 10q, and *RB1* promoter methylation. Common to both primary and secondary glioblastoma is LOH on 10q, distal to the *PTEN* locus; a putative suppressor gene at 10q25-qter may be largely responsible for the glioblastoma phenotype.

The etiology of human gliomas is largely unknown. Hereditary diseases predisposing to the development of gliomas e.g. Li-Fraumeni syndrome, Turcot syndrome, NF1, and NF2 syndromes are rare and cannot explain the development of most of human gliomas. The presence of SV40 large T sequence has been observed in a variety of human brain tumors including gliomas, and they are likely be originated from the contamination of SV40 in poliovaccine between 1955-1962. However, there is no direct evidence that SV40 infection is associated with pathogenesis of human brain tumors. There is recent evidence that G:C→A:T transition mutations at CpG sites in the *TP53* gene are significantly more frequent in astrocytic tumors with promoter methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) than in those without methylation. This may suggest that endogenous alkylating agents that produce O^6-methylguanine or related adducts recognized by MGMT may be involved in the development of astrocytic brain tumors.

Key words: Glioblastoma; genetic pathway; TP53; EGFR; SV40; MGMT.

Genetic pathways to primary and secondary glioblastoma. The majority of glioblastomas develops very rapidly without clinical, radiological or morphologic evidence of a less malignant precursor lesion. They are termed primary or *de novo* glioblastomas. Patients with primary glioblastoma have a short clinical history (<3 months in the majority of cases), and typically present with large tumors which on MRI show central necrosis, ring enhancement and perifocal edema. Secondary glioblastomas develop slowly through progression from low-grade diffuse astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III).

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In a series of studies from our laboratory,[1)-9)] genetic analyses were carried out on glioblastomas that were selected on the basis of stringent criteria. Primary glioblastomas were included if the clinical history was less than 3 months and histopathological features of glioblastoma were present at the first biopsy. The possibility exists that primary glioblastomas may have a longer preoperative history but in order to clearly distinguish between both subsets, the window of eligibility was deliberately kept small. The diagnosis of secondary glioblastoma required at least two biopsies from the same patient, taken at an interval of <6 months to avoid a sampling error and clinical as well as histopathological evidence of progression from low-grade or anaplastic

astrocytoma.

TP53 / MDM2 / p14^{ARF} pathway. The p53 pathway is an important mechanism controlling the G_1/S phases of the cell cycle. Following DNA damage induced by chemicals, irradiation or other causes, p53 is activated and induces transcription of genes such as *p21$^{Waf1/Cip1}$*.[10),11)] *p14^{ARF}* binds to MDM2, resulting in the stabilization of both p53 and MDM2.[11),12)] Secondary glioblastomas have a high incidence of *TP53* mutations (>65%), of which approximately 90% are already present in the first biopsy,[1),13)] while *TP53* mutations are rare in primary glioblastomas (<10%). Amplification of *MDM2* is present in <10% of glioblastomas,[14)] and these all appear to be primary glioblastomas that lack a *TP53* mutation.[2),14)] Overexpression of *MDM2* was observed immunohistochemically in >50% of primary glioblastomas.[2)] Loss of p14^{ARF} expression was observed in the majority of glioblastomas (76%), and this correlated with the gene status, i.e. homozygous deletion or promoter methylation.[7)] There was no significant difference in the overall frequency of *p14^{ARF}* alterations between primary and secondary glioblastomas, but *p14^{ARF}* methylation was more frequent in secondary glioblastomas than primary glioblastomas.[7)] The analysis of multiple biopsies from the same patients revealed *p14^{ARF}* methylation already in one-third of low-grade astrocytomas.[7)]

Epidermal growth factor receptor (EGFR). *EGFR* amplification is present in ~40% of primary glioblastomas but in none of secondary glioblastomas analyzed.[4)] *EGFR* overexpression also prevailed in primary glioblastomas (>60%) *vs.* secondary glioblastomas (<10%).[1)] All primary glioblastomas with *EGFR* amplification showed *EGFR* overexpression and 11 of 15 (73%) of those with *EGFR* overexpression showed *EGFR* amplification.[4)] *EGFR* amplification is often associated with structural alterations. To date, at least seven mutated variants of *EGFR* have been identified,[15)] the most common being variant III (EGFRvIII, also called de2-7EGFR or delta EGFR),[16)] which occurs in 20-50% of primary glioblastomas with *EGFR* amplification.[17)-20)]

p16^{INK4a} / RB1 pathway. The RB1 protein controls progression through G_1 into S phase of the cell cycle. The CDK4/cyclin D1 complex phosphorylates the RB1 protein, thereby inducing release of the E2F transcript factor that activates genes involved in the $G_1 \rightarrow S$ transition.[10)] p16^{INK4a} binds to CDK4, inhibits the CDK4/cyclin D1 complex, and thus inhibits the $G_1 \rightarrow S$ transition.[10)] Homozygous *p16^{INK4a}* deletions were more frequent in primary than in secondary glioblastomas.[3),7)] However, there was no significant difference in the overall frequency of *p16^{INK4a}* alterations (homozygous deletion and promoter methylation) between these glioblastoma subtypes.[7)] Promoter methylation of the *RB1* gene was found significantly more frequently in secondary (43%) than in primary glioblastomas (14%). Methylation of the *RB1* promoter was not detectable in low-grade and anaplastic astrocytoma, indicating that *RB1* promoter methylation is a late event during astrocytoma progression.[8)] There was a significant correlation between loss of *RB1* expression and promoter methylation of the *RB1* gene, suggesting that promoter methylation is the major mechanism underlying loss of RB1 expression.[8)]

LOH on 1p, 13q, 19q. There are several loci frequently deleted in glioblastomas. LOH on 1p was detected at a similar frequency in primary (12%) and in secondary glioblastomas (15%).[6)] LOH on 13q was detected in 12% of primary and 38% of secondary glioblastomas, and typically included the *RB1* locus.[6)] LOH on chromosome 19q was significantly more frequent in secondary glioblastomas (54%) than primary glioblastomas (6%).[6)] The putative suppressor genes on 1p and 19q have not yet been identified.

LOH on chromosome 10 and PTEN mutations. Loss of heterozygosity (LOH) on chromosome 10 is the most frequent genetic alteration in glioblastomas and occurs in up to 80% of cases.[21)-26)] The majority of glioblastomas appear to have lost an entire copy of chromosome 10.[21)-25)] In cases with partial LOH on chromosome 10, at least three common deletions have been identified, i.e., 10p14-pter, 10q23-24 and 10q25-qter,[21),22),26)-28)] suggesting the presence of several tumor suppressor genes. Only one of these, the *PTEN* suppressor gene at 10q23.3, has been identified.[29),30)] It encodes a protein with homology to the catalytic domain of tyrosine phosphatase and to the cytoskeletal proteins tensin and auxilin.[29),30)]

LOH on chromosome 10 was detected at similar frequencies in primary (47%) and secondary glioblastomas (54%).[5)] The majority of primary glioblastomas showed LOH at all informative markers, suggesting loss of the entire chromosome 10, while secondary glioblastomas with LOH showed partial or complete loss of chromosome 10q but no loss of 10p.[5)] *PTEN* mutations occur almost exclusively in primary glioblastomas (32%) and rarely (4%) in secondary glioblastomas.[4)]

Since primary and secondary glioblastomas are usually histologically indistinguishable, at least one genetic alteration should be common, if the phenotype of these lesions is a reflection of genetic alterations.

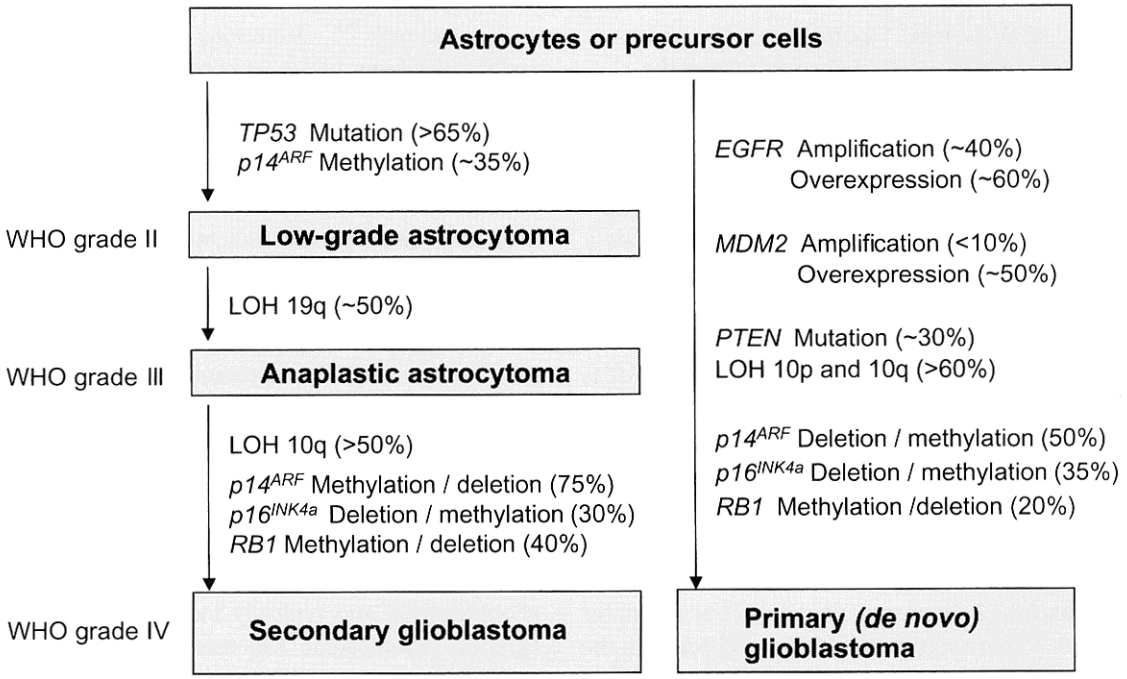

Fig. 1. Genetic pathways leading to primary and secondary glioblastomas.

Neuropathologists occasionally observe an abrupt transition from low-grade or anaplastic astrocytoma to glioblastoma, suggestive of the emergence of a new tumor clone. Such glioblastoma foci were microdissected and compared the chromosome 10 status with that of the respective low-grade or anaplastic astrocytoma areas of the same biopsy. In glioblastoma foci, deletions were typically detected distal from *PTEN* at 10q25-qter, covering the *DMBT1* and *FGFR2* loci,[31] suggesting that the acquisition of a highly malignant glioblastoma phenotype is associated with loss of a putative tumor suppressor gene on 10q25-qter.

The genetic pathways leading to the evolution of primary and secondary glioblastoma are summarized in Fig. 1. The most frequent and characteristic genetic alterations in primary glioblastomas are *EGFR* amplification / overexpression, LOH on 10p and 10q, and *PTEN* mutations. *TP53* mutations and $p14^{ARF}$ methylation are the earliest detectable alterations in the genetic pathway leading to secondary glioblastomas, while LOH on 19q, 10q, and loss of RB1 expression appear to be later events.

Genetic and gene expression profiles. Using cDNA expression arrays, significant gene expression changes in comparison to normal brain tissues have been observed in already low-grade diffuse astrocytomas.[32)-34)] In unselected glioblastomas, up- or down-regulation was found in >200 genes[33)] and in >3000 genes[34)] in comparison to normal brain tissues. A study using array based comparative genomic hybridization revealed significant amplifications of *CDK4*, *GLI*, *MYCN*, *MYC*, *MDM2*, and *PDGFRA* genes in glioblastomas.[35)] Frequently amplified genes in glioblastomas included PIK3CA (64%), EGFR (57%), CSE1L (57%), N-*ras* (50%), FGR (36%), ESR (36%), and PGY1 (36%).[35)] It remains to be shown which of these are causally related to malignant transformation of glial cells and which of these are associated with glioma progression.

Etiology of human gliomas. The etiology of sporadic brain tumors is still largely unknown. Epidemiological studies failed to detect an unequivocal causative link with environmental and lifestyle factors, with the exception of therapeutic irradiation.[36)]

Genetic susceptibility. The Li-Fraumeni syndrome is a familial cancer syndrome with an autosomal dominant trait of multiple primary neoplasms in children and young adults, with a predominance of soft tissue sarcomas, osteosarcomas and breast cancer and an excess of brain tumors, leukaemia and adrenocortical carcinoma.[37)-39)] In most kindreds with Li-Fraumeni syndrome, affected family members carry a germline mutation of one allele of the *TP53* gene.[40),41)] Approximately 50% of affected families develop at least one nervous system

tumors,[42] and majority of brain tumors (<70%) were of astrocytic origin, including low-grade astrocytoma, anaplastic astrocytoma, and glioblastoma.[42] It is notable that there are several families with a remarkable familial clustering of three or more brain tumors.[39),42] This raises the question of whether some *TP53* mutations carry an organ- or cell-specific component, but genetic analysis did not reveal mutational hotspots for specific tumor types or target tissues.[43] It is more likely that familial clustering of brain tumors is influenced by the genetic background of the affected families.[44),43] Up to date, 263 families with Li-Fraumeni syndrome have been reported (http://www.iarc.fr/p531/).

Turcot syndrome is an autosomal dominant disorder characterized by adenomatous colorectal polyps or colon carcinoma and by development of medulloblastoma or glioblastoma. Most Turcot syndrome occurs in the setting of the familial adenomatous polyposis (FAP) or hereditary non-polyposis colorectal carcinoma (HNPCC) syndrome. Gliomas (astrocytomas and glioblastomas) develop in mismatch repair associated Turcot syndrome type 1, which is characterized by an inherited DNA replication error defect that leads to genomic instability. Genes known to encode proteins involved in this process include hMLH1 at 3p21, hMSH2 at 2p16, and hPMS2 at 7p22.[45] Approximately 160 cases have been reported since 1949.[45]

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder predisposing to multiple neurofibromas, malignant peripheral nerve sheath tumors, pilocytic astrocytomas in the optic nerve, astrocytomas, and glioblastomas. The prevalence in most population is estimated to be 1:4,000.[46] Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder predisposing to schwannomas and gliomas (pilocytic astrocytoma and diffuse astrocytoma). The prevalence is 1:40,000 newborns.[47]

In summary, gliomas may be associated with hereditary syndromes, but these are very rare diseases, and cannot explain the development of majority of human gliomas.

Viral infection. Several viral oncogenes have the capacity to induce tumors of the central nervous system in experimental animals, including v-*src* large T, polyoma middle T, and SV40 large T antigen.[48)-50] Among these, SV40 is the only virus that has been repeatedly implicated in the etiology of human brain tumors.[51)-53] SV40 is a small double strand DNA virus, and the SV40 large T antigen is the primary viral gene product responsible for SV40 replication and SV40 mediated cell transformation.[54] SV40 is highly oncogenic in hamsters, inducing a variety of tumors including brain tumors. SV40 large T antigen binds to and inactivates several tumor suppressor proteins, including p53 and Rb, and is capable of transforming human cells *in vitro*.[52),55] SV40 can transform human cells[54] and cause chromosomal aberrations, aneuploidy, and point mutations in human cells *in vitro*.[56]

SV40 sequences have been detected at an overall frequency of approximately 35% of human brain tumors including gliomas,[52),55),57),58] while surrounding normal brain tissue rarely contained SV40.[58),57] An infectious SV40 wild-type strain has been rescued by transfection of DNA from a choroid plexus carcinoma into permissive monkey kidney cells.[52] The natural host of SV40 is the Macaque monkey and infection of SV40 to humans does not usually occur unless there is close contact with infected monkeys or their tissues.[59)-61] However, it is well known that through SV40-contaminated poliovaccine and adenovirus 3 and 7 vaccines which were prepared using monkey kidneys between 1955 and 1963, SV40 may have been accidentally introduced in large scale into human populations in the United States, Canada, and Europe.[52),61)-63] Some children treated with SV40-contaminated oral poliovaccine excreted SV40 for several weeks,[64] indicating that SV40 has the capacity to replicate in humans. SV40 sequences were not detected in any brain tumor from Finland, a country where SV40-contaminated polio vaccine was not used, while 25-56% of brain tumors from Switzerland contained SV40 sequences.[65] This corroborates a study on the absence of SV40 in mesotheliomas from Finnish patients[66] and strongly suggests that SV40 in human brain tumors originates from SV40-contaminated polio vaccine.

Because of the large number of people involved, the etiological role of SV40 in human cancers needs to be carefully investigated. However, no selective increase in the incidence of brain tumors has been reported in populations that received SV40-contaminated polio vaccine,[52] and incidence rates for brain tumors are similar in countries that did (U. S. A., Switzerland) or did not (Finland) use SV40-contaminated vaccine.[65] Thus, the available evidence does not support a causative role of SV40 in the development of human brain tumors. Instead, its presence may reflect a bystander infection due to an intra-tumoral microenvironment that favors viral replication in humans with latent SV40 infection.

Endogenous factors. O^6-Methylguanine-DNA methyltransferase (MGMT) is a repair protein that

specifically removes promutagenic alkyl groups from the O^6 position of guanine in DNA. MGMT therefore protects cells against carcinogenesis induced by alkylating agents, and it has been reported that MGMT activity is inversely correlated to tissue-specific tumorigenesis induced by alkylating agents in rats.[67),68)] In tumor cells, repair of O^6-alkylguanine adducts by tumor cells has been implicated in drug resistance, since it reduces the cytotoxicity of alkylating chemotherapeutic agents. Loss of MGMT expression may be caused by methylation of promoter CpG islands[69),70)] and has been observed in a variety of human cancers, including gliomas.[71)]

MGMT promoter methylation was detected in 75% of secondary glioblastomas, significantly more frequently than in primary glioblastomas (36%).[9)] Approximately 50% of low-grade astrocytomas, oligodendrogliomas, and anaplastic oligodendrogliomas also showed *MGMT* methylation.[9),72)] The majority of low-grade astrocytomas with *MGMT* methylation (92%) contained a *TP53* mutation, whereas only 39% of cases without *MGMT* methylation carried a *TP53* mutation. Furthermore, G:C→A:T transition mutations at CpG sites were significantly more frequent in low-grade astrocytomas with *MGMT* methylation (58%) than in those without (11%).[9)] These results suggest that loss of MGMT expression due to promoter methylation frequently occurs at an early stage in the pathway leading to secondary glioblastomas and appears to be associated with increased frequency of *TP53* mutations, in particular G:C→A:T transitions.

The best-characterized underlying mechanism of G:C→A:T transitions at CpG sites is the deamination of 5-methylcytosine which is clustered at CpG sites, resulting in a substitution of 5-methylcytosine by thymine. This occurs spontaneously or factor-mediated, e.g. through the action of oxygen radicals or by nitric oxide produced by nitric oxide synthase in conditions of chronic inflammation.[73)] However, it has been shown that O^6-methylation of the guanine moiety at CpG islands is not efficiently repaired by MGMT if normal 5-methylcytosine is present in the *TP53* sequence.[74)] This raises the possibility that *TP53* mutations at CpG sites may not be due to deamination of 5-methylcytosine alone. They may, in addition, result from endogenous factors that produce DNA adducts at the O^6 position of guanine. A great variety of adducts at this position have been shown to be substrates for repair by MGMT.[75)] Such adducts typically result from exposure to *N*-nitrosamides and related alkylating agents that cause brain tumors in rats,[76)] but it remains to be shown whether these carcinogens are involved in the etiology of human brain tumors.

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