BRAIN TUMOR PATHOLOGY:
CURRENT DIAGNOSTIC HOTSPOTS AND PITFALLS
Brain Tumor Pathology: Current Diagnostic Hotspots and Pitfalls

by

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# TABLE OF CONTENTS

**Introduction** 1

Chapter 1  
*The Origin of Gliomas in Relation to the Histological Diagnosis* 3

Chapter 2  
*Molecular Genetics Outline of Brain Tumors* 19

Chapter 3  
*General Remarks* 25

Chapter 4  
*Astrocytic Tumors I* 27

Chapter 5  
*Astrocytic Tumors II* 59

Chapter 6  
*Oligodendroglial Tumors* 83

Chapter 7  
*Ependymal Tumors* 113

Chapter 8  
*Neuronal and Mixed Glio-Neural Tumors I* 123

Chapter 9  
*Neuronal and Mixed Glio-Neural Tumors II* 141

Chapter 10  
*Peculiar Tumors* 155

Chapter 11  
*Cell Migration and Invasion* 161

Chapter 12  
*Apoptosis* 171
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>The Ubiquitin-Proteasome System</td>
<td>183</td>
</tr>
<tr>
<td>14</td>
<td>Angiogenesis</td>
<td>189</td>
</tr>
<tr>
<td>15</td>
<td>Meningiomas</td>
<td>199</td>
</tr>
</tbody>
</table>

References  

Index
Since Bailey and Cushing (1926), all brain tumor classifications have been called histogenetic. The nosographic position that the tumor types progressively acquired in the classification systems derived from the resemblance of tumor cells to those of the cytogenesis, modified whenever new information became available from different biological research fields and especially from molecular genetics. Classically, on the basis of the rough correspondence between the mature/immature aspect of tumor cells and the benign/malignant biological behavior of the tumors, the histological labels contained a prognostic significance. The supposed origin of the tumors was thus a factor for prognosis. Later on, with the concept of anaplasia (Cox, 1933; Kernohan et al., 1949) new criteria were introduced for establishing the malignancy grades of tumors. Immunohistochemistry and later molecular genetics further refined the prognostic diagnoses, substantially increasing the opportunities to recognize the cell origin of tumors, beside revealing the pathogenetic mechanisms. Prognoses became more accurate, as required by the greater and more targeted possibilities of therapy.

Molecular genetics, on the one hand, gave us a deeper knowledge of tumorigenesis and tumor transformation, but on the other hand, it made things more complicated, demonstrating for example that phenotypically similar tumors may have different genetic assets and vice versa, with important implications for prognoses drawn from diagnoses. Recently, microarray gene profilings are demonstrating that genetically-based prognosis may be more reliable than histologically-based prognosis (Nutt et al., 2003). In the meantime, new variants and new tumor entities have been described (Cenacchi and Giangaspero, 2004) which should be added to the WHO book (Kleihues and Cavenee, 2000), showing the double relation to molecular genetics.

The prognosis drawn from histological diagnosis had to become more refined, also because new radiotherapeutic procedures and chemotherapeutic strategies became more demanding, as they were retailed on specific prognoses and diagnoses. Another requirement for more precise prognostic categorization of tumors derived from the introduction of the statistical-mathematical method, epitomized in multivariate analysis, used for the study of the outcome of tumors or of their time to progression (TTP), was assumed as a means for the evaluation of the efficacy of therapies.

In the last few years, the increased diagnostic-prognostic requirement was paralleled by a reduced quantity of tumor tissue available for examination. The clinical diagnostics by neuro-imaging techniques, with functional, diffusion and intraoperative MRI and spectroscopy (Rees, 2003), SPECT and PET procedures have been strongly facilitated to the point that often the tumor nature can be foreseen. This led to earlier discovery of tumors, at a stage when they are still of small dimensions. At the same time,
surgical procedures also improved, for example with the introduction of the neuro-
navigator, so that quite often a sheer diagnostic function is required from
neurosurgery. Moreover, the availability of different modalities of radiotherapy,
such as neutron-, proton- and ion-radiation with a better therapeutic planning and a
conformational-three-dimensional implementation, the possibility to reach deeply
located and irregularly shaped tumors, stereotactic radiotherapy and the γ knife, not to
mention chemotherapy, use of stem cells, immunotherapies, biodegradable
polymers, convection-enhanced drug delivery (Dunn and Black, 2003), oncolytic
viruses (Jiang et al., 2003), etc., contributed enormously to modifying the collaboration
between neurosurgeon and pathologist. The tumors as a consequence are currently
recognized earlier and therefore they are smaller and surgical specimens are of
reduced size, but at the same time a more precise prognosis from histology is being
required. The introduction of stereotactic biopsy procedures was the first step in
this direction. If the discovery of new variants and of new prognostic categories of
brain tumors is added, a greater possibility of pathology error becomes
comprehensible.

The advancement of neurobiological studies of the development of the nervous system
and the recent emphasis given to stem cells progressively modified our conception
of the cell composition of a tumor, since the morphological and antigenic aspect
of its cells oscillates between that of progenitors to that of mature specific cells with
the possibility of going in one direction or the other, the so-called de-differentiation
a Shih and Holland, 2004). This may lead us to evaluate the origin of the tumor,
which in turn may have an influence on the prognosis drawn from histology in
practice.

The aim of this work is to discuss the practical importance, at the moment of a
histological diagnosis, of some not yet resolved biological problems of brain tumors,
the components of which may have influence on the diagnosis, and to emphasize the
dilemmas that arise in attributing one significance or another to the different findings.
Chapter 1

THE ORIGIN OF GLIOMAS IN RELATION TO THE HISTOLOGICAL DIAGNOSIS

The old question of the origin of brain tumors becomes once again of great interest with the therapeutic application of the concept of stem cells. The parallelism between the morphological aspect of tumor cells and that of the cells during cytogenesis, on which Bailey and Cushing’s and the so-called histogenetic classifications were based (Figure 1), has recently been shaken by a number of in vivo and in vitro observations. These can influence both the diagnostics, when it has to be performed in small samples, where the histological patterns of the tumors are frequently lacking, and the experimental therapeutic strategies.

In humans, it is still impossible to identify the cells which give rise to tumors prior to their transformation (Holland, 2001), because the earliest stages of glial tumor development are not known; and the first visible lesions, i.e. for example astrocytomas, are already organized as tumors when they are recognized.

Theoretically, it is accepted that astrocytomas derive from astrocytes and oligodendrogliomas from oligodendrocytes; and, since adult glia does not proliferate, whereas basically the development of a tumor requires that the transforming events affect proliferating cell populations, their original cells must be precursor cells or neural stem cells. It is also very well known that the proliferation is slow in low-grade gliomas and quick in high-grade gliomas, and that a transition from the former to the latter through anaplasia can occur. Our working concepts on tumor formation are based on the old multistage model which establishes that tumors develop in the three stages of initiation, promotion and progression.

Years of experimental studies on the oncogenetic effects of nitrosourea derivatives in rats have made it clear that the induced tumors arise from primitive neuroepithelial cells of the ventricular zone (VZ) or from its derivative subventricular zone (SVZ) or from cells of the so-called “renewal” of the adult that occurs in the remnants of SVZ or sub-ependymal layer, hippocampus, cerebellum, first cortical layer (Figure 2).

Classically, neurons and glia derive from primitive neuroepithelial cells or neural stem cells of the VZ and SVZ, characterized by self-renewal and multipotency (Figure 3). They differentiate along different pathways under extrinsic and intrinsic stimulations. Neurogenesis occurs first through organizing centres which generate signals inducing the expression of patterning genes encoding
transcriptional factors and controlling neuronal subtypes in the adjacent neuroepithelial cells (Kobayashi et al., 2001).

Markers are in order nestin, vimentin, A2B5, GFAP and then O4, proteolipid protein, galactocerebroside, myelin basic protein and synaptophysin and neurofilaments for neurons. Growth factor signaling controls the passage from one stage to the other: PDGF and bFGF promote the passage from stem cells to precursors, the passage from precursors to O2A progenitors and to astrocyte

**Figure 1.** Cell differentiation in the course of cytogenesis

**Figure 2.** Cells of origin of gliomas

-Primitive neuroepithelial cells
  -Germinative zones
  -Proliferating-migrating cells

-Neural stem cells
  -Proliferation
  -Differentiation
  -Hippocampus
  -Sub-ependymal layer
  -I cortical layer
  -Cerebellum
Figure 3. Germinative zone in the rat, H&E, x 25 and x 200
precursors is promoted by PDGF and CNTF, EGF, respectively. The passage from O2A progenitors to oligodendrocytes and type 2 astrocytes is promoted by CNTF and CNTF, EGF, respectively and inhibited by PDGF and bFGF (Goldman, 2000; Holland, 2001). During migration, glia cells continue to proliferate. In time, the VZ disappears and the SVZ decreases in size persisting in the adult as a sub-ependymal cell layer (Figure 4).

Radial glia that derives from neuroepithelial stem cells at the onset of neurogenesis is particularly important. The soma borders on the ventricle and the processes extend to the pial surface as scaffolding to migrating neurons (Figures 5, 6).
Radial glia has astrocytic characteristics and most progenitors of VZ possess radial glia features. It expresses RC2, nestin, vimentin, GFAP, GLAST (glutamate-aspartate transporter) and it is neurogenetic (Ever and Gaiano, 2005). Radial glia state is maintained by Notch signaling through ligand Delta1, ErbB through Neuroregulin and FGFR through FGF (Ever and Gaiano, 2005). On the other hand, it is known that these are critical for glioma cell survival and proliferation (Purow et al., 2002) from neural stem cells occurring in the sub-granular zone of the dentate gyrus of the hippocampus and as astrocytes and ependymal cells under the anterior lateral ventricular wall (Clarke, 2003) (Figure 7). The existence of neuroectodermic stem cells not only in embryonal life, but also in the adult, changed our interpretation of many processes occurring in the brain and it became a basic working concept. Particularly important are the recent interpretations already mentioned that neurospheres formed from SVZ cells, both in embryos and in the adult, are ultimately astrocytes and that radial glia also may represent stem cells or progenitors during embryonal development (Doetsch et al., 2003), confirming the hypothesis that stem cells are found within the lineage neuroepithelium – radial glia – astrocytes (Alvarez-Buylla et al., 2001). To this hypothesis belongs also the concept that in the adult, glia cells or a
Tumors can arise from stem cells either of VZ or SVZ during embryonal life or induction in the rat by transplacental Ethylnitrosourea (ENU). The tumors were observed to originate from the VZ and from the derived SVZ (Schiffer et al., 1978-80; Lantos and Pilkington, 1979). The target of Ethylnitrosourea administered transplacentally was the germinative zone. Here the alkylation of O6 of guanine, O2 of cytosine and O2 and O4 of thymidine took place with consequent coupling errors during transcription (Kleihues and Rajewsky, 1984) and the defective repair of DNA (Goth and Rajewsky, 1974). Short-term, as stop to mitoses and nuclear deaths in the germinative zones (Bosch, 1977), and long-term phenotypic effects, such as early lesions, microtumors and tumors in the brain hemispheres, starting in the periventricular white matter (Schiffer, 1997, 78), represented the consequences of nitrosourea derivative action (Figures 8, 9, 10, 11).

The vulnerability of neuroepithelial cells to neoplastic transformation resulted from the interaction of several factors, among which were the number of replicating cells at risk at a particular time, the length of time during which a cell population remains in cycle, the state of differentiation etc. It explained the different incidence of the subset of them may represent latent stem cells throughout the brain and there is evidence that cells from non-neurogenetic regions, if cultured with bFGF/EGF, become neurons (Doetsch et al., 2003).

Figure 7. Scheme of cell migration from the sub-ependymal layer, EC = ependymal cells, SC = stem cells.
1. The Origin of Gliomas in Relation to the Histological Diagnosis

Figure 8. Transplacental ENU tumors in the rat. Early phase in the periventricular white matter, H&E, x 200. From Schiffer et al., 1978

Figure 9. Transplacental ENU tumors in the rat. Periventricular oligodendrogial micro-tumor, H&E, x 200. From Schiffer, 1997
various tumor types and also the phenotype of tumors. On these observations was based the concept of “window of vulnerability” for each precursor cell type (Rubinstein, 1985, 1987). Another concept emerged as very important, i.e., that experimentally more genetic alterations are needed for tumor transformation as more advanced the differentiation status of progenitor cells becomes (Shih and Holland, 2004). The origin of tumors from transplacental ENU has been recently studied again and it has been demonstrated that either SVZ cells or cells of early lesions in the white matter were nestin-positive and so were cells cultivated from SVZ of exposed rats, confirming definitely that tumors arise from stem or precursor cells (Recht et al., 2003).

Using as tumor inducer Methylnitrosourea, instead of Ethylnitrosourea, either intra-cerebrum or intra-peritoneum or subcutaneously, and administering it in the adult, when the germinative zone is no more present, it was demonstrated that tumors arose in the periventricular white matter, corpus callosum, hippocampus (Schiffer et al., 1970) and their origin could not have been different than that of the SVZ or the sub-ependymal layer (Figure 12).

The relationship between differentiation of neuroepithelial cells during cytogenesis and glioma formation points out firstly the existence of characteristics common to progenitor cells and malignant cells, represented by simplicity of the form, proliferation capacity, potentiality to differentiate and capacity of migration (Dai and Holland, 2003). Secondly, some of the genetic alterations that characterize malignant gliomas, such as those that activate signal transduction pathways and those disrupting the cell cycle arrest machinery and represent the molecular signature of these tumors, concern genes/proteins involved in the regulation of differentiation during cytogenesis and may have effects on the differentiation/de-differentiation status of the cells (Dai and Holland, 2003). In the differentiation process the main signals are: EGF, FGF, PDGF, CNTF, IGF, SHH,
1. The Origin of Gliomas in Relation to the Histological Diagnosis

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Figure 11. Transplacental ENU tumors. Scheme of developing tumors. From Schiffer et al., 1978

Signaling through receptor Notch regulates astrocytic and oligodendrocytic tracing. In developed human tumors, the morphology of their elements does not correspond entirely to that of mature cells of a certain line, but it is rather composed of a mixture of cells either resembling the stages of cytogenesis, or even expressing markers of immature cell types (Dai and Holland, 2003). As a consequence, the tumor cells will appear de-differentiated if compared with mature cells; or, alternatively, this means that they originate from progenitor-like cells in the tumor.

In humans, the possibility of tracing back the origin of the tumors from the aspect of the cells is impossible. Only in animals can this be achieved through the “lineage tracing”. In developed human tumors, the morphology of their elements does not correspond entirely to that of mature cells of a certain line, but it is rather composed of a mixture of cells either resembling the stages of cytogenesis, or even expressing markers of immature cell types (Dai and Holland, 2003). As a consequence, the tumor cells will appear de-differentiated if compared with mature cells; or, alternatively, this means that they originate from progenitor-like cells in the tumor.

The evidence that maximally makes the resemblance of tumor cells to cells of the cytogenesis less reliable for establishing the cell origin of the tumors shows that the differentiation of cells during cytogenesis may undergo environmental, epigenetic and genetic influences. This means that the histology of a tumor “would be more a reflection of the environment and time of initiation than the cell of origin” and this would decide whether a tumor ultimately becomes, for example, an astrocytoma or an oligoastrocytoma (Recht et al., 2003). This is demonstrated by some experiments. Introducing Akt and Kras in mouse brains by a retroviral technique it is easier to obtain tumors in nestin-expressing than in GFAP-expressing cells, especially if there is a loss of CDKN2A (Uhrbom et al., 2002), so that genetic deregulations would appear more important than the cell stage of origin. Epigenetic events could be responsible for dedifferentiation: in U 373 MG cell lines of glioblastoma, TGFα or other factors acting on TKRP can reduce GFAP mRNA and enhance nestin expression, whether or not affecting vimentin (Sultana et al., 1999; Zhou and Skalli, 2000). The transforming event could also block the differentiation of a neural stem cell (Pereira et al., 1998), according to the old concept of maturation arrest (Cairncross, 1987), and it could hit either a neural stem cell or a tumor cell which re-acquires properties of a neural stem cell. Other examples are available: in rat progenitor cells the over-expression of Akt or Ras produces tumors with the phenotype of human glioblastomas which, on the other hand, are known for showing an over-expression of them (Holland et al., 2000), whereas the over-expression of PDGFR.B gives tumors with the phenotype of oligodendrogliomas (Dai et al., 2001). It should also be taken into account that different phenotypes can be sustained by the same genetic basis (Kraus et al., 1995). In practice, the neoplastic transformation of glial precursors produces tumors with the phenotypes of astrocytoma and oligodendroglioma and this depends on the activation or inactivation of specific protein pathways. A deeper knowledge of the relationship between molecular pathways and tumor phenotype is very important for discovering the origins of gliomas that tentatively are at the moment only deduced from the phenotype of tumor cells.
The following properties are recognized in neural stem cells: undifferentiation, capacity to form neurospheres and to proliferate, self-maintenance and clonogenicity (Reynolds and Weiss, 1992). Neurospheres derive from one neural stem cell and can be recognizable because they express nestin and a new surface antigen, CD133, which is a 120 kDa five-transmembrane cell surface protein, once a haematopoietic stem cell marker (Uchida et al., 2000). In various experiments all these properties have been demonstrated in neurospheres formed from surgical specimens of glioblastoma. Therefore, the problem of the origin of gliomas needs to take on board not only the relationship of tumor cells to neural stem cells, but also the existence of tumor stem cells, since only a proportion of tumor cells are clonogenic when xenografted (Recht et al., 2003).

The hypothesis that in general cancer stem cells exist is based on the observation that tumor cells are heterogeneous and variably express differentiated antigens typical of the organ, but only a minority of them are self-renewing, multipotent, clonogenic and continuously replenishing mature cells (Reya et al., 2001). In vitro, cells from human gliomas generating clusters of clonally derived cells resembling neurospheres, self-renewing and proliferating, and capable of differentiation have been demonstrated (Singh et al., 2003), also in pediatric tumors (Hemmati et al., 2003). Brain tumors, therefore, besides arising from the transformation of neural stem cells, can contain tumor stem cells that once transplanted into mice reproduce tumors with the characteristics of glioblastoma (Galli et al., 2004). It has been shown that the CD133\(^+\) cell sub-population from human tumors exhibits stem cell properties in vitro and show a capacity for self-renewal and reproduce tumors which can be serially transplanted. On the contrary, CD133\(^-\) cells engrafted do not reproduce tumors (Singh et al., 2004). Whether these are real tumor stem cells deriving from the transformation of normal neural stem cells (Shih and Holland, 2004) or they represent the product of an extreme de-differentiation due to anaplasia, i.e. they correspond to the new clones developed by accumulation of mutations and are selected by competition, with a high proliferation rate and lacking any differentiation antigen, is really difficult to say, also because the latter may have acquired stem cell properties (Figure 13). Paraffin FISH studies on CD133\(^+\) xenografts from a GBM demonstrated that tumor cells showed amplification of the EGFR gene, so that both CD133\(^+\) and CD133\(^-\) cells bear the same cytogenetic alterations and therefore they are clonally derived (Singh et al., 2004). A peculiar hypothesis has been put forward for gliomas, i.e. that tumor stem cells divide asymmetrically. One daughter cell remains as a cancer stem cell in the germinative zone and the other migrates away and proliferates. This would have therapeutic consequences (Berger et al., 2004).

The demonstration of stem cell markers in the adult brain and in brain tumors and of the occurrence of neural stem cells in the adult brain and at variance with tumor stem cells in brain tumors is becoming a very complicated and challenging matter. Uchida et al. (2004) propose three explanations for the occurrence of stem cell markers in brain tumors: positive cells are transformed neural stem cells expressing nestin and Musashi-1; they are cells re-expressing nestin (Dahlstrand et al., 1992;
Neural and tumor stem cells

Figure 13. Neural and tumor stem cells

Tohyama et al., 1992) because de-differentiated; they are exogenous stem cells attracted to the tumor tissue (Park et al., 2002). In an infantile tumor the expression of nestin + GFAP and nestin + Tuj-1, similarly to what can be seen in the normal post-natal sub-ventricular zone, has been demonstrated (Uchida et al., 2004), even though this is not a proof that the former derives from the latter. An interesting observation was that the grafted tumor cells in the adult brain, but also in vitro after some time, became quiescent, similar to normal post-natal or adult neural stem cells in situ (Morshead et al., 1994).

In all this matter the role of nestin is not so clear-cut. Nestin is typical of neuroepithelial/progenitor cells in rats and humans and its expression precedes that of vimentin and GFAP, disappearing from the CNS in adult age with the exception of ependymal cells. This has been demonstrated in the progenitor cells during development and in the subependymal layer of adults (Hockfield and McKay, 1985; Lendhal et al., 1990) with no definite temporal relationship with vimentin and GFAP that should appear later in the development. There is a certain discrepancy among the various researchers concerning technical problems and specificity of the staining. Nestin expression, for example, is considered characteristic of progenitor cells, but since it characterizes reactive astrocytes in brain injury (Tohyama et al., 1992; Lin et al., 1995; Krum and Rosenstein, 1999), one wonders whether the latter cells de-differentiate in their cell reaction or whether nestin cannot be taken as specific of undifferentiation (Holland, 2001). In the first case, nestin could not be indicative of a stem cell status or there would be no possibility to distinguish in tumors between

Neural stem cell
Normal differentiated cell
Tumor stem cell
Differentiated tumor cell
neural stem cells and tumor stem cells, unless the latter are capable of re-acquiring nestin expression even if they represent a transformed and not an undifferentiated phenotype.

In reactive astrocytes (Figure 16A) the expression of nestin would represent the embryonal regression of cytoskeleton connected with their morphological plasticity. In hippocampus, it decreases with the age of the subject (Abdel-Rahman et al., 2004). There are alternative interpretations, very complicated, concerning the up-regulation of multiple embryonic proteins in adult astrocytes following injury for re-enacting a microenvironment reminiscent of that during the embryonic period (Clark et al., 1994; Nakamura et al., 2003).

In some findings nestin and vimentin are not markers of stem cells (Singh et al., 2004). In pediatric brain tumors, nestin has been found to be expressed in PNET, anaplastic astrocytomas and ependymomas, but almost never in low-grade astrocytomas in one series (Tohyama et al., 1992) and not expressed in another series of gliomas (Dahlstrand et al., 1992). Using two different antibodies (Tohyama et al., 1992 and Grigelioniené et al., 1996), after antigen retrieval nestin was demonstrated in ependymal cells, cells of the germinal matrix and radial fibres of a human foetus, co-expressed with vimentin, but not with GFAP, and in tumor cells of pediatric ependymoma, PNET, glioblastoma and pilocytic astrocytoma associated with vimentin, GFAP and S-100. In our experience nestin is expressed in ependymomas, pilocytic astrocytomas and much more in glioblastomas than in astrocytomas (Figure 14). It is not expressed in oligodendrogliomas, with the exception of GFOC (Figure 15). Also endothelial cells were found to be positive (Almqvist et al., 2002) and

![Figure 14. Glioblastoma. Nestin-positive cells, DAB, x 400](image-url)
Vimentin was shown to be expressed in all the three cell lines from a malignant astrocytoma, whereas nestin was variably positive in the most motile and invasive cells (Rutka et al., 1999). In U-373 MG cells, TGFα was observed to reduce GFAP mRNA; however it did not modify vimentin but increased nestin (Zhou and Skalli, 2000).

The discussion about the relationship between progenitor cells and tumor development has just started and a fundamental question, concerning not only transplacental ENU tumors, but also human tumors, is the timing of cell migration from the subependymal layer, considering that its cells are normally capable of migrating and providing a continual source of parenchymal cells (Levison and Goldman, 1997). One wonders whether an established tumor derives from recently migrated or from the first migrated cells.

New hypotheses on the glioma origin concern astrocytes and radial glia as possible multipotent stem cells, both in vitro and in vivo (Steindler and Laywell, 2003). Astroglial lineage, from radial glia to astrocytes, might act as stem cells either in embryos and in adulthood (Doetsch, 2003). SVZ astrocytes have been considered to be a marker of rapidly growing endothelial cells (Sugawara et al., 2002). In our experience, it is expressed in micro-vascular proliferations of glioblastomas and oligodendrogliomas (Figure 16).
mutations occurring in gliomas may influence the differentiation or the trans-differentiation state. Cultured p16 and p14/-/- astrocytes maintain a diploid status, differentiated status, but in glioblastomas they are inactivated (Holland, 2001). This brings us back to the distinction between the undifferentiated or the transformed phenotype of tumor stem cells. All these changes must be interpreted in terms of new clones with the new phenotype. Another important demonstration of how a differentiation can be influenced is that gliomas may originate because of the considered in vivo primary precursors and acting as stem cells in vitro (Doetsch et al., 1999) and forming neurospheres. These cells may undergo transformation.

There is also enough evidence, with the mechanism of the “lineage tracing” in the adult CNS, of a trans-differentiation from one cell type into another and that mutations occurring in gliomas may influence the differentiation or the trans-differentiation state. Cultured p16 and p14/-/- astrocytes maintain a diploid status, but shift to a rapidly proliferating status losing GFAP and acquiring nestin expression (Holland et al., 1998). This means that p16 and p14 keep astrocytes in a differentiated status, but in glioblastomas they are inactivated (Holland, 2001). This brings us back to the distinction between the undifferentiated or the transformed phenotype of tumor stem cells. All these changes must be interpreted in terms of new clones with the new phenotype.
inability of progenitor cells to differentiate, as happens with PDGF on precursors expressing GFAP which assume oligodendroglial morphologies (Dai et al., 2000).

There is no evidence that tumors can develop from the proliferating reactive glia; however, they might originate from radial glia that is capable of proliferation, into which differentiated astrocytes can regress under certain stimuli (Magavi et al., 2000). Today bone marrow stem cells must also be considered as a possible source of tumors, because of their capacity to differentiate along the neuroectodermal line (Mezey et al., 2000).
Theoretically, molecular genetics is well able to be of help at the moment of diagnosis, even of small samples, but actually its procedures are rather complicated and need time, so that they are feasible only in selected and specifically well-equipped laboratories. The use of molecular genetics for the identification of the tumor type, sub-type or grade is still at present a challenge, also because a complete histological validation of molecular genetics data is still lacking and it is not known how far the correlation found between molecular genetics data and survival, statistically studied, can be of use in single cases. Treatises of the last ten years (Schiffer, 1997; Kleihues and Cavenee, 2000, Ironside et al., 2002; Burger et al., 2002) represent a good basis for codifying brain tumor diagnoses, whereas molecular approaches in this direction only recently started to acquire a practical importance (Louis et al., 2001). For example, by multidimensional scaling analysis of gene expression profiles, a good distinction of glioblastomas, anaplastic astrocytomas, oligodendrogliomas and anaplastic oligodendrogliomas has been achieved, including a small group of glioblastomas with extended survival, and it parallels morphological classification showing a good correlation with survival (Fuller et al., 2002). The problem, however, is still how far all this can be used in the single case at the moment of diagnosis.

Brain tumors are supposed to originate as monoclonal and, for genetic instability, to undergo a genetic heterogeneity, accompanied with increased mutation rate and proliferation capacity and followed by a phenotypic heterogeneity. New clones arise that better adapt themselves to the environment, show a greater proliferation potential and compete with the predecessors in a kind of selection by competition, losing the differentiating capacity. Inactivation of tumor suppressor genes and accumulation of mutations are the main genetic characteristics. The loss of differentiation characteristics of a given cytogenetic stage, with regression to those of preceding stages, is called anaplasia (Russell and Rubinstein, 1989). It can be realized also by an accelerated growth of already differentiating cells or by maturation arrest (Cairncross, 1987). In the different phases of tumor progression by anaplasia, genotypic alterations are associated with pathologic phenotypes, according to an established scheme (Louis, 1997) (Figure 1).
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<th>Tumor stage</th>
<th>Associate pathology</th>
<th>Genetics</th>
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<tbody>
<tr>
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<td>Apoptosis</td>
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In the stage of astrocytoma grade II the most common finding is the absence of wt p53 and the existence of a non-functional p53 pathway. Roughly 60% of tumors show loss of alleles on 17p including TP53 locus, whereas the retained TP53 allele is mutated in most cases (Rasheed et al., 1994). Over-expression of PDGFR is another alteration of this stage where mechanisms allowing cells to evade apoptosis are supposed to occur (Louis et al., 1997) (Figure 2). In anaplastic astrocytomas, alterations of oncogenes/proteins regulating the cell cycle occur, in the so-called Rb-pathway.

![Figure 1. Progressing anaplasia and genetic alterations](image)

**Figure 1.** Progressing anaplasia and genetic alterations

**Figure 2.** Role of PDGFR and apoptosis in glioma development
Normally, Rb1 sequesters E2F transcription factor, but when phosphorylated or when RB1 is mutated, E2F initiates the entry into S phase. Rb1 phosphorylation is produced by the complex cyclin D1/CDK4-6 heterodimer. Normally p16 binds CDK4 inhibiting the formation of CDK4/Cyclin D1 heterodimer, but CDKN2A/p16 homozygous deletion and CDK4 over-expression can activate the cycle (Figure 3).

All the events leading to deregulation of the cell cycle are mutually exclusive. In glioblastomas, alterations of many oncogenes serving different cell functions are found. Beside those of the PDGF/PDGFR and EGF/EGFR pathways, mitogenic through Ras/MAPK pathway, p53 pathway modulated by Mdm2 and p14 (Figures 2, 4), pRb pathway with involvement of cell cycle regulation through cyclins and kinases, and PTEN pathway are active.

![Figure 3. pRb pathway](image)

The occurrence of highly amplified cells for EGFR at the tumor edges has a particular meaning (Okada et al., 2003), whereas of special interest is the frequent finding of truncated EGFR, ΔEGFR, with auto-phosphorylation of tyrosine kinase, continuous signaling and no internalization-degradation of the complex L/R (Figure 5). PTEN through its proteinphosphatase activity regulates cell migration and invasion and through its phophoinositolphosphatase via Akt/PKB regulates cell proliferation, survival and apoptosis (Figure 5) (Louis and Cavenee, 2001; Maher et al., 2001; Knobbe et al., 2002; Collins, 2004).

Particular importance is attributed today to the genetic changes of PI3K cascade. Its activation follows amplification, rearrangement or over-expression of EGFR (Collins, 2002), but one third of glioblastomas show mutations of PTEN and this gene inhibits PI3K activation of Akt (Knobbe et al., 2003). On the other hand, Akt is activated in the majority of glioblastomas (Choe et al., 2003) and it plays an important role in the development of these tumors (Holland et al., 2000; Sonoda et al., 2001). It has been found that carboxyl-terminal modulator protein (CTMP) inhibits Akt phosphorylation at threonine 308 and serine 473 (Maira et al., 2001), so...
that inactivation of this protein can abolish inactivation of Akt. In a series of glioblastomas and glioblastoma cell lines, CTMP did not show mutations or deletions. However, in 40% glioblastomas and 67% glioblastoma cell lines, reduced mRNA levels associated with hypermethylation of the CTMP promoter were found and became thus a common finding in glioblastoma (Knobbe et al., 2004).

![Diagram of the role of MDM2](image)

*Figure 4. The role of MDM2*

Many alterations accompany the formation and progression of gliomas, appearing also specific in some tumors, so that the clinician is provided with biological and pathogenetic information supplementary to those deriving from the biopathology of tumors and even more clarifying. In some tumors, prognostic subtypes, for example, can be defined only on their molecular features or better on these than on their sheer phenotype. In the same way, sensitivities to certain therapies can be discovered which could not be detected otherwise and this confers the new genetically integrated classification clinico-practical goals.

Gene expression profiling is substantially contributing to these new requirements (Louis et al., 2003). For example, the promoter hyper-methylation of MGMT in relation to TP53 mutations is emerging as an important factor for prognosis and therapy in astrocytic tumors. It is associated with a reduced period free survival (PFS) in diffuse astrocytomas to the point that it is considered as a
predictive factor in the clinical course, more than of malignant progression, and as indicating possible efficacy of chemotherapy (Komine et al., 2003).

There are special events that are considered as specific of malignancy and show very complicated phenotypic and genotypic characteristics which must be considered separately, i.e. cell migration and invasion, angiogenesis and apoptosis. The knowledge of their regulating molecular pathways and of their morphogenesis is believed to offer opportunities for identifying therapeutic approaches.