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Cell Cycle Control in Pediatric Neuro-Oncology

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Introduction

Brain tumors are the most common solid tumor of childhood, second in frequency only to leukemia [1]. Astrocytomas represent the most common primary brain tumor of childhood comprising almost 50% of all brain tumors in this age group [2]. Of these, 20% are high-grade, or anaplastic lesions. Unlike adults with low-grade astrocytomas who frequently progress to higher grade lesions over time, the malignant transformation of a low-grade astrocytoma to high-grade one in a child is a rare event [3]. Medulloblastoma is another common brain tumor in childhood accounting for about 25% of pediatric brain tumors. This tumor is highly proliferative with a propensity to invade the fourth ventricle as well as the overlying subarachnoid spaces. As such, medulloblastoma is characterized by widespread dissemination along cerebrospinal fluid pathways. Unfortunately, many children who suffer from malignant astrocytoma and medulloblastoma will die from their tumors despite recent advances in surgery, radiation, and chemotherapy [4]. Improving the prognosis for these children will involve not only the identification of better treatment modalities, but a greater understanding of the pathogenetic basis for these tumors. Recently, several studies have shown that a class of genes which control cell cycle regulation play an important role in the pathogenesis of several different human tumors, including brain tumors.

Uncontrolled cellular proliferation is a hallmark of neoplasia. Experimental studies on the mechanisms of cell proliferation have begun to elucidate a basic tenet in cell cycle dysregulation in tumors, namely that uncontrolled proliferation may be caused by altered expression of positive growth regulators such as cyclins and cyclin-dependent kinases (CDKs), or the loss of negative regulators, including CDK inhibitors (CDKIs), and the retinoblastoma protein (pRB). As a result, a cell is no longer able to respond to important internal or external cues that check its growth.

In this review, we will discuss the impact of markers of tumor proliferation on our understanding of tumor growth and response to therapy; we will examine the alterations in several cell cycle regulatory genes as they have been described for human brain tumors with an emphasis on those described in pediatric brain tumors; finally, we will provide data which argue cogently for the targeting of cell cycle genes as a novel means by which the process of cell proliferation in pediatric brain tumors can be directly inhibited.

Cell Proliferation Markers

The human brain is a unique organ from a cell kinetic standpoint. Neurons become incapable of cell division in the early postnatal period. Although glial cells retain their proliferative potential, as is demonstrated in the process of reactive gliosis, it is still unclear what the initiating events are that transform a normal cell into a highly proliferative malignant brain tumor cell. However, a number of studies have now been performed which have characterized the proliferative indices of human brain tumors, including pediatric brain tumors. The original cell kinetic studies conducted by Hoshino et al. [5–9] on brain tumors

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established the importance of measuring the growth fraction, labeling index, and cell cycle time. Early studies on brain tumor cytokinetics were performed by pulsing intra-cranial tumors with ³H-thymidine, a marker of the S phase of the cell cycle. Then, using a technique that required the preoperative administration of bromodeoxyuridine (BUdR), a thymidine analogue that is incorporated into nuclear DNA during the S phase, Hoshino et al. [8, 9] showed elegantly that the tumor-labeling index is comparable by either BUdR immunohistochemistry or ³H-thymidine incorporation.

A recent advance in cell kinetic analysis of human brain tumors came from the identification of two proliferative markers, proliferating cell nuclear antigen (PCNA) and Ki-67. Antibodies to these proteins recognize a nuclear antigen expressed exclusively in proliferating cells. Ki-67 is found in cells that are cycling in late G1, S, G2, and M phases of the cell cycle, but is absent in quiescent G0 cells [10,11]. In general, labeling index values of brain tumors with Ki-67 antibodies are somewhat higher but parallel to those observed by BUdR immunohistochemistry [12]. Because G1, G2, and M phase cells are recognized by Ki-67 immunostains, it has been suggested to be an objective indicator of the biological behavior of cancer. Unlike BUdR, Ki-67 immunostaining can only be

performed on frozen sections. However, BUdR is a potential carcinogen and must be given intravenously to the patient preoperatively. Fortunately, an analogue antibody to Ki67 has been produced which enables identification of cycling cells in formalin-fixed, paraffin-embedded tissues. This antibody recognizes an antigen known as MIB-1. Today, PCNA, and MIB-1 immunohistochemistry can be performed routinely on paraffin-embedded material (fig. 1). Recent studies, however, have suggested that a certain level of PCNA immunostaining may be artifactual and not truly reflective of cell proliferation. Therefore, MIB-1 has become the favored proliferation marker which correlates well with in vitro BUdR uptake.

Cell Proliferation Marker Expression in Pediatric

Brain Tumors

Most of the literature on cell kinetic analyses generally pertains to adult brain tumors, although some studies have included pediatric tumors. Hoshino et al. [9] showed that the probability of survival among patients with a BUdR-labeling index under 1 % was much higher than in those with indices greater than 5%. Moreover, they reported on a cell kinetic study comparing four medullo-blastomas with malignant gliomas using ³H-thymidine incorporation [13]. The average labeling index was more

Fig. 1. Immunohistochemical stain of medulloblastoma with MIB-1 monoclonal antibody. This highly proliferative tumor shows a high labeling index (over 30%) of tumor cells as is demonstrated by the nuclei which stain darkly for the proliferative antigen. Original magnification, × 225.

than 10%, but the mean survival time was 6 years. In contrast, patients with malignant gliomas that had a labeling index of >5% died within 1 year of diagnosis. Therefore, a high labeling index was not necessarily associated with a poor prognosis in patients with medulloblastoma. This discrepancy in survival and labeling index levels can be attributed, no doubt, to the greater sensitivity of medulloblastoma to radiation therapy and chemotherapy. Ito et al. [14] reported on a BUdR-labeling analysis of 50 juvenile pilocytic astrocytomas. They found that the mean BUdR-labeling index was approximately 1%. Their results suggest that most juvenile pilocytic astrocytomas grow slowly as a result of these low BUdR-labeling indices. Some patients in their study had tumors with high labeling indices and some of their tumors recurred. However, in most cases, the clinical course was excellent regardless of the labeling index. Immunohistochemical analyses with Ki-67 have now been performed and reported for large series of human brain tumors including pediatric brain tumors [15–18]. In most studies, the Ki-67-labeling index, defined as the number of positive staining cells divided by the total number of tumor cells, has ranged from 0 to 50% and has correlated well with the histopathological grade of malignancy of the tumor. In pilocytic astrocytomas, this index was generally low with scores ranging from 0 to 1 %. In primitive neuroectodermal tumors and/or medulloblastomas, the index has ranged from 5 to 50%. The mean index

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^yS^ ^" *0% DNA synthesisCellgrowA Q i] 1 J
I~ G2 J

\ /m\ /

"f f Mitosis/cytokinesis

GO

Quiescence

Fig. 2. A proliferating cell passes through an orderly sequence of phases which comprise the cell cycle. Following mitosis, differentiated cells exit the cell cycle after mitosis to enter a state of quiescence called GO. If a cell is destined to continue to proliferate, it enters the first phase of interphase called G1, which is characterized by a period of cell growth and synthesis of components necessary for DNA synthesis. During this phase the cell is sensitive to conditions in its environment. If these conditions are unfavorable, the cell can arrest in G1. There is a point, called the restriction point (R), which is the decision-making point for cell cycle progression, and it precedes the onset of the S phase by 1–3 h. The S phase follows G1 and is the period in which chromosomal DNA is replicated. The next phase, called G2, is shorter than G1 and precedes the mitotic phase. During the G2 phase, DNA replication must be completed before the cell enters mitosis. In the mitotic phase, equal amounts of chromosomal material migrate to opposite poles of the cell. The subsequent division of the cytoplasm by cytokinesis results in two identical but separate daughter cells. The fundamental basis of many chemotherapeut-ic agents in use today for children with brain tumors is the blocking of tumor cell division in either a cell cycle phase-specific or nonspecific fashion.

for medulloblastoma is approximately 10%. In a series of 117 pediatric brain and spinal tumors from our center [17], the mean Ki-67 index was 0.81% in low-grade astrocytomas, 0.931% in pilocytic astrocytomas, 5.02% in anaplastic astrocytomas, 6.12% in glioblastomas, 2.83% in ependymomas, 6.45% in anaplastic ependymomas, 14.26% in choroid plexus carcinomas, and up to 22% in medulloblastomas. Bodey et al. [18] suggested that the highest indices were found among the poorly differentiated classic medulloblastomas. The inherent problem of regional variability must be considered in the interpretation of Ki-67 indices, especially in tumors with extensive necrosis or in tumors like anaplastic ependymoma which show well-differentiated and poorly differentiated areas. Interestingly, two groups, Schiffer et al. [19] and Iijima and Nakazato [20], showed that there is an inherent problem of regional variability in medulloblastoma. Light-looking areas (large cell areas) and dark areas (desmoplastic and classic areas) may have completely different proliferative indices (PCNA and MIB-1 staining). The light areas consistently showed lower staining indices than did the dark areas. It has been suggested that the light areas consist of cells with a more differentiated neuronal lineage with a relatively low proliferation potential. Pollack et al. [21] reported on the relationship between the MIB-1-labeling index and outcome in malignant gliomas of childhood. The median overall survival was more than 4 years in the low-MIB-1 index group compared with only 16 months in the high index group. Their results suggested the potential utility of the MIB-1-labeling index as a prognostic marker in children with malignant gliomas [21]. Because of the results from the studies listed above, application of these proliferation indices as an adjunct to the diagnosis of brain tumors has become widespread among neuropathologists.

The Cell Cycle

An understanding of the cell cycle is critical to understanding normal cell proliferation and the altered proliferation observed in various brain tumors, such as human gliomas. A proliferating cell passes through an orderly sequence of phases which comprise the cell cycle (fig. 2). Following mitosis, a cell enters interphase. Differentiated cells exit the cell cycle after mitosis to enter a state of quiescence called G0. The proliferating cell, on the other hand, progresses into the first phase of interphase called G1, which is characterized by a period of cell growth and synthesis of components necessary for DNA synthesis. S phase follows G1 and is the period in which chromosomal DNA is replicated. The next phase, called G2, is shorter than G1 and precedes the mitotic phase. During the G2 phase, DNA replication must be completed before the cell enters mitosis. In the mitotic phase, equal amounts of chromosomal material migrate to opposite poles of the cell. The subsequent division of the cytoplasm by cytokinesis results in two identical but separate daughter cells. The cell cycle phase which is most strategically positioned to control cell proliferation is G1. During the G1 phase, the critical decision (restriction point) to go on to DNA synthesis and mitosis is made [22]. This restriction point is the decision-making point for cell cycle progression, and it precedes the onset of S phase by 1–3 h. Extra-

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cellular factors such as growth factors may help to determine whether a cell in G0 phase will enter the cell cycle and proliferate (G0 to G1), or whether a proliferating cell continues to proliferate (G1 to S) or exits the cell cycle and becomes quiescent (G1 to G0). In short, before this checkpoint, growth factors are required to progress through the first phases of the cell cycle. On the other hand, once a cell has passed the restriction point it must undergo mitosis regardless of whether or not growth factor signals are present. Therefore, the molecular controls at the restriction point are the focus of intensive study. The decision to progress through the restriction point is determined by the activity of proteins which form the molecular cell cycle machinery, cyclins, CDKs and their inhibitors.

Cyclins and CDKs Act as Positive Growth Regulators

The major positive growth regulators of the cell cycle are a group of related proteins, the cyclins. Cyclins are a group of structurally similar proteins which share homology in a conserved 100-amino acid domain known as the cyclin box [23]. Cyclins are the positive regulatory sub-units of a class of related protein kinases, called CDKs. These cyclin-CDK complexes are the regulators of the major cell cycle transitions (fig. 3). When cells emerge from the G0 phase and enter G1, the expression of D- and E-type cyclins is induced. At the onset of the S phase, cyclin A is first detected followed by cyclin B during the interval between the S phase and mitosis (G2) followed by rapid degradation at the end of the M phase. There are two main families of cyclins, the mitotic cyclins and G1 cyclins. Mitotic cyclins consist of cyclin B and cyclin A. Cyclin A also has a second role in the S phase of the cell cycle. The G1 cyclins are cyclins C, D, and E. Cyclin can interact with different CDKs.

The CDKs are believed to phosphorylate key substrates which are required to facilitate the passage of the cell through each phase of the cell cycle. Substrates of CDKs can be structural proteins, which act as the actual effectors of a cell cycle phase transition, or regulatory molecules [24–26].

The best characterized targets of D-type cyclin kinase activity are the pRB family proteins. During the G1 phase, phosphorylation of pRB, by sequential activation of D-type cyclins and CDK4/CDK6 and cyclin E/CDK2 may be the single most important event in the cell cycle as it initiates the S phase [27–30].

CDKs Act as Negative Growth Regulators Cell proliferation consists of a balance between the expression of genes which stimulate growth and genes

Fig. 3. Cyclins are the positive regulatory subunits of a class of related protein kinases, called cyclin-dependent kinases (CDKs). These cyclin-CDK complexes are the regulators of the major cell cycle transitions. The CDKs are believed to phosphorylate key substrates which are required to facilitate the passage of the cell through each phase of the cell cycle. Substrates of CDKs can be structural proteins, which act as the actual effectors of a cell cycle phase transition, or regulatory molecules. Cyclin-CDK complexes have kinase activity, but if the complexes associate with CDK inhibitors (CDKIs), the complex is no longer active, which inhibit growth. Recently it has been shown that a family of CDKIs plays a major role in the negative regulation of cyclin-CDK activity [31, 32]. These G1 cyclin inhibitors are involved in the arrest in G1 of cells in response to antiproliferative signals. Therefore, all CDKIs are candidate tumor-suppressor genes. The CDKIs can be subdivided into two families, those which inhibit multiple cyclin-CDK complexes, such as p21CIP1, p27KIP1, p57KIP2, and those which inhibit the G1 cyclin-CDK4/6 complexes, p16INK4A, p15INK4B, p18INK4C, and p19INK4D (fig. 4). p21CIP1 plays an important role in p53-mediated G1-induced cell cycle arrest in response to DNA damage [33]. p27KIP1 is thought to mediate cell cycle arrest caused by cell contact inhibition or transforming growth factor β treatment [34, 35]. In addition CDKIs may be involved in such diverse processes as terminal differentiation, cellular senescence, repairment to DNA damage, and induction of apoptosis [36]. Loss of expression of a CDKI may have disastrous consequences for the cell. For example, loss of the p16INK4A has been linked to many human cancers. A simplified schema which enables visualization of the cyclins, CDKs, and CDKIs in practical terms in cell cycle control is shown in figure 5.

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 Cyclin A CDK2

Fig. 4. Cyclins are the regulatory sub-units of cyclin-dependent kinases (CDKs). Cyclin-CDK partners are shown for different cell cycle phases. Cyclin/CDK activity is inhibited by a number of specific inhibitors, in G1 of cells in response to antiproliferative signals. The multiple cyclin-CDK complexes, such as p21CIP1, p27KIP1, p57KIP2, and those which inhibit the G1 cyclin-CDK4/6 complexes, p16INK4A, p15INK4B, p18INK4C, and p19INK4D. p21CIP1 plays an important role in p53-mediated G1-induced cell cycle arrest in response to DNA damage. p27KIP1 is thought to mediate cell cycle arrest caused by cell contact inhibition or transforming growth factor β treatment.

TGF- β
 p53 cell contact
 ↓
 p21CIP1, p27KIP1, p57KIP2
 cyclinD CDK4/CDK6
 p16INK4A, p15INK4B, p18INK4C, p19INK4D
 cyclinA cyclinB
 CDK1
 p21CIP1, p27KIP1, p57KIP2
 p21CIP1, p27KIP1, p57KIP2
 p57KIP2

CELL CYCLE CONTROL

Fig. 5. Cell cycle control can be made analogous to the control of an automobile in that the drivers or accelerators of the cell cycle are the cyclins and cyclin-dependent kinases (CDKs), whereas the brakes are the CDK inhibitors (CDKIs), such as p15, p16, p21 and p27. Strategies to prevent tumor cell proliferation can be thought of in terms of decreasing the expression levels of the drivers (analogous to taking one's foot off the accelerator), or increasing the expression of the inhibitors (putting one's foot on the brakes). Indeed, several molecular biological strategies are being used today to accomplish these results with expression vector systems and anti-sense technology.

Dysregulation of the Cell Cycle in Brain Tumors

Proliferation of normal cells is controlled by multiple growth-regulatory pathways that act to ensure proper growth regulation. It is generally thought that cancer cells ignore many growth-regulatory signals due to mutation in genes (genetic 'hits') that control either the growth-promoting or growth-inhibitory pathways. Therefore, transformation of glial cells into malignant astrocytomas involves significant dysfunction of the cell cycle-control machinery. There have been a

number of recent studies which have shown alterations in cell cycle gene expression in human astrocytic tumors. Most of the studies on cell cycle gene expression in neurooncology generally pertain to adult brain tumors, although some studies include an analysis of pediatric tumors.

pl6INK4A (MTS1/CDKN2)

For human malignant gliomas, two genetic loci are particularly interesting: *MST1* and *CCND1* [37]. *CCND1* encodes cyclin D1 while the *MST1* locus controls expression of two independent but closely related tumor-suppressor genes, p16INK4A and p15INK4B. Interestingly, human cyclin D1 was cloned in a yeast complementation assay from a U18MG glioblastoma cDNA library [38]. p16INK4A is an important component of a cell cycle regulatory pathway involving cyclin D1, CDK4, and pRB. p16INK4A inhibits the kinase activity of the cyclin D1-CDK4 complex by binding to CDK4 and preventing its association with its catalytic subunit cyclin D1. Cyclin D1-CDK4 phosphorylates pRB during the G1 phase of the cell cycle to inhibit its growth-restraining activity. As a result, phosphorylated pRB is no longer able to bind to

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pl6WMA

Fig. 6. p16INK4A is an important component of a cell cycle regulatory pathway involving cyclin D1, CDK4, and retinoblastoma protein (pRB). p16INK4A inhibits the kinase activity of the cyclin D1-CDK4 complex by binding to CDK4 and preventing its association with its catalytic subunit cyclin D1. Cyclin D1-CDK4 phosphorylates pRB during the G1 phase of the cell cycle to inhibit its growth-restraining activity. As a result, phosphorylated pRB is no longer able to bind to and prevent the activity of the E2F transcription factors. Phosphorylation of pRB releases E2F transcription factors to enter into the S phase. This event is believed to be an important checkpoint regulating the transition from G1 into the S phase during cell cycle progression.

cyclin D1/CDK4
phosphorylate

and prevent the activity of the E2F transcription factors. This event is believed to be an important checkpoint regulating the transition from G1 into the S phase during cell cycle progression. Gene transfer of a full-length p16INK4A cDNA into U251MG or U87MG astrocytoma cell lines that did not express endogenous p16INK4A resulted in marked growth suppression [39]. Infection of the adenovirus vector carrying the full-length p16INK4A in U251MG or U87MG cell lines also arrested in the G0-G1 phases of the cell cycle and modified the transformed phenotype of cells including the ability to form colonies in soft agar [40]. It is clear, however, that deletion may not be the only mechanism for p16INK4A inactivation, as lack of expression of protein has been found in a significant percentage of grade-III and grade-IV malignant astrocytic tumors that retain wild-type p16INK4A [41, 42]. CpG island methylation of the p16INK4A promoter region may be responsible for decreased protein expression in the setting of an unaltered p16INK4A DNA sequence. This methylation-associated inactivation of p16INK4A expression may mechanistically result from structural changes in the chromatin containing the p16INK4A locus [43, 44]. Interestingly, p16INK4A homozygous deletion frequently involves deletion of the closely linked p15INK4B gene [45–49].

Derangements in both the positive cell cycle regulators, cyclin D1 and CDK4, and the negative regulators, p16INK4A and p15INK4B, have been found previously

in malignant astrocytomas [41–43, 45–62]. Interestingly, in glioma cells that do not have p16INK4A homozygous deletions, CDK4 overexpression and amplification are common, suggesting that a further mechanism for uncontrolled glioma cell growth is the overriding of a negative regulator by a positive regulator [41, 42, 48, 50, 55]. Indeed, it has recently been reported that loss of expression of p16INK4A or pRB, and overexpression of CDK4 occur in a mutually exclusive fashion in the majority of glioma cell lines or malignant glial tumors [42, 49, 50]. These results suggest that the p16INK4A-cyclin D1/CDK4-pRB axis is a critical growth regulatory pathway in malignant gliomas (fig. 6). In this context, it is conceivable that an alteration in any member of this pathway may play an important role in glial oncogenesis. p16INK4A point mutations are only rarely observed in malignant astrocytic tumors that retain a p16INK4A allele [46]. There is a correlation between the frequency of homozygous deletion and malignancy, since low-grade astrocytomas do not have homozygous p16INK4A deletion [46, 56]. The majority of these studies have detected a p16INK4A or p15INK4B mutation or loss of expression in the astrocytic tumors, while these molecular changes have not been detected in ependymomas or neuroectodermal tumors [45, 63]. In addition, loss of p16INK4A protein without p16INK4A gene deletion was reported in pituitary tumors [64].

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With regard to pediatric brain tumors, most investigators have found rare instances of p16INK4A homozygous deletions and no point mutations in medulloblastomas [45, 65, 66]. Petronio et al. [60] detected a single case of p16INK4A homozygous deletion among 20 primitive neuroectodermal tumors. In addition, p16INK4A homozygous deletions or CDK4 amplification were restricted to adult cases of gliomas, since neither was seen in pediatric high-grade gliomas [60]. Interestingly, Sure et al. [66] reported that loss of p16INK4A expression was observed in 61 % of pediatric glioblastomas using immunohisto-

chemistry which is within the range (40–60%) reported for homozygous deletions of the p16INK4A gene and loss of p16INK4A expression by other means in adult glioblastomas [41, 42, 45, 55]. Recently, overexpression of cyclin B1 and p16INK4 expression has been described in the SF-188 pediatric glioblastoma cell line [67].

p21CIP1 and Its Transcriptional Activator, p53 p21CIP1 plays an important role in p53-mediated G1-induced cell cycle arrest in response to DNA damage. For example, in response to DNA damage by drugs or radiation, internal signals lead to the increased expression of p53, which causes G1 cell cycle arrest mediated by activation of p21CIP1 [33, 68, 69]. We have recently shown that p21CIP1 expression is diminished in several astrocytic tumor cell lines with wild-type p53 status [67].

Infection of the p53-deficient astrocytoma cell line, U 373 MG with a replication-defective adenoviral vector containing p21WAF1/CIP1 led to G1 cell cycle arrest and inhibition of accumulation of cells with aneuploidy [70]. In addition, an alteration in the malignant phenotype of cells was evidenced by the loss of anchorage-independent growth in soft agar and the failure to induce tumorigenesis in both peripheral and intracranial xenograft models [70]. Another group has shown that infection of a pCL-based retroviral vector carrying p21CIP1 in rat glioblastoma cells showed a 91 % reduction in colony-forming efficiency and a 66% reduction in growth rate [71]. In addition, intracranial implantation of these infected cells showed complete disappearance of the p21CIP1-infected cells by day 10 and long-term survival compared to controls [71]. Interestingly, the ectopic expression of cyclin D1 in asynchronously growing cells (human glioma and rodent fibroblast cell lines) was accompanied by increased levels of the p53 protein and p21CIP1. Despite the induction of these cell cycle-inhibitory proteins, cyclin D1-associated CDK kinase remained activated and the cells grew essentially like that of the parental cell lines [72].

Jung et al. [73] performed a study which demonstrated altered expression of p21CIP1 in astrocytic tumor cells. In glioblastoma multiforme, there was marked elevation of p21CIP1 in samples harboring either wild-type or mutant p53. p21CIP1 expression was not elevated in samples harboring mutant type p53 in anaplastic astrocytomas. In addition, the level of p21 expression was very low in normal brain tissue [73]. Tsumanuma et al. [74] reported on the mutation of the p21CIP1 gene in 28 brain tumors. Since this mutation was detected in only two tumors (one astrocytic tumor and one medulloblastoma), they concluded that mutation of the p21CIP1 gene was infrequent in brain tumors [74].

There have been few studies describing the expression of p53 expression in gliomas of childhood [66, 75–79]. Mutations of the p53 gene in particular have been reported to be uncommon in childhood gliomas, a finding which contrasts with their frequent detection in adult gliomas. Litofsky et al. [76] did not find any p53 mutations in 12 anaplastic astrocytomas and 2 glioblastomas and concluded that p53 mutations are not of importance in the development of pediatric astrocytomas. On the other hand, Schiffer et al. [78] reported that p53 mutations were found in 2 of 8 glioblastomas and 1 of 9 anaplastic astrocytomas, whereas no mutations were found in 11 pilocytic astrocytomas. Sure et al. [66] also reported that mutations in the p53 gene were identified in 25% of glioblastomas. Indeed, Pollack et al. [79] reported on the relationship between mutations of the p53 gene and overexpression of p53 and prognosis. There was a significant association between p53 gene mutations and/or overexpression of p53 and poor prognosis. Sidransky et al. [80] showed that progression from low-to high-grade glioma in adults was associated with a clonal expansion of cells that had previously acquired a mutation in the p53 gene. In our center, we have observed consistent p53 immunopositivity in choroid plexus carcinomas, and a lack of positivity in most papillomas.

With respect to medulloblastomas, cytogenic analyses have shown a number of nonrandom chromosomal alterations, including deletions on chromosome 17 [81, 82]. Loss of 17p, the chromosomal arm on which the p53 gene is located, has been found in approximately one third of medulloblastomas. However, direct analysis of the p53 gene for mutations in medulloblastoma has revealed a low incidence equaling approximately 10% [83–86]. This suggests that other tumor-suppressor genes important in the pathogenesis of medulloblastoma may exist on chromosome 17p. Cogen and McDonald [87] have been investigating one candidate gene within the active-BCR-related locus which is on the distal portion of chromosome 17p.

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Other CDK Inhibitors

When the p27KIP1 gene was transfected with the human astrocytoma cell line, U373MG, using an adeno-viral vector system, there was cell cycle arrest in G1 and a marked decrease in the accumulation of aneuploid cells [88]. We have demonstrated that the p57KIP2-deficient human astrocytoma cell line, U343MG-A, is rapidly blocked in G1 following induced expression of p57KIP2 [89].

Studies in Transgenic Mice

Recently, molecular techniques have advanced remarkably so that the effect of deregulated overexpression or the absence of expression of a gene can be studied in the whole organism, such as a transgenic mouse. A number of important tumor-suppressor genes have been ‘knocked out’ in mice, and the phenotype of the mice has supported the candidate gene’s tumor-suppressive function. Homo-zygous knockout of p53 leads to spontaneous development of many different tumors, although none in the brain [90]. Double RB and p53 knockout mice develop endocrine tumors, including pituitary tumors and pineoblastomas [90, 91]. p16INK4A transgenic mice are susceptible to multiple tumors, although none in the brain [92]. p21CIP1 knockout has very little phenotypic effect without predisposition to tumor formation [93]. Homozygous p27KIP1 knockout results in a dramatic phenotype, with much larger sized mice with multiorgan hyperplasia and spontaneous development of intermediate lobe tumors of the pituitary gland [94–96]. p57KIP2 transgenic mice have delayed differentiation without tumor formation [97, 98].

Conclusion

The field of molecular biology has advanced remarkably, as has our understanding of the process of cell proliferation. A number of cell cycle-regulatory proteins play an important role in the regulation of cell proliferation. Cell proliferation consists of a balance between expression of genes which stimulate growth and genes which inhibit growth, such as Rb or p53 or CDKIs. We may now be in a position to design new biotherapeutic strategies to reduce the proliferation of brain tumor cells by blocking specific cell cycle proteins or by enhancing the function of CDKIs. For example, we have recently shown that transfection of U343 astrocytoma cells with a p16 retrovirus in addition to treatment with retinoic acid leads to signals which cause full differentiation of these malignant astrocytoma cells [99]. In the future, one can envision applying such combination therapy with cell cycle inhibitors and differentiation agents to

patients with malignant astrocytomas. Finally, with future advancements in targeting cells with gene therapy and with advances in molecular diagnosis, we believe that much of our knowledge about the cell cycle will be translated into new and effective therapies for children harboring intracranial neoplasms.

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