An Update on the Cellular and Molecular Biology of Brain Tumors

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ABSTRACT: Patients with tumors of the central nervous system (CNS) remain difficult to treat despite recent advances in surgical, chemo- and radiotherapeutic techniques. A better understanding of the molecular and cellular biology of neoplasia is providing neuroscientists with a framework on which to devise novel therapies for these patients. It thus becomes imperative that neurologists and neurosurgeons be aware of these advances in basic science that may eventually have a positive impact on patient management. This paper reviews our present knowledge of the process of CNS oncogenesis and the roles that chemicals, viruses, oncogenes, growth inhibitor genes, and growth factors play in the process.

RESUME: Mise à jour sur la biologie cellulaire et moléculaire des tumeurs cérébrales Les patients porteurs de tumeurs du système nerveux central (SNC) demeurent difficiles à traiter malgré les progrès récents réalisés dans le domaine de la chirurgie, de la chimiothérapie et de la radiothérapie. Une meilleure compréhension de la biologie moléculaire et cellulaire des néoplasies fournit aux chercheurs une structure logique pour élabore de nouvelles thérapies pour ces patients. Ainsi, il devient impératif que les neurologues et les neurochirurgiens prennent connaissance de ces progrès dans le domaine des sciences fondamentales parce qu’ils peuvent éventuellement avoir un impact positif sur le traitement de ces patients. Nous revoyons dans cet article les connaissances actuelles sur le processus d’oncogénèse dans le SNC et les rôles que jouent les produits chimiques, les virus, les oncogènes, les gènes inhibant la croissance et les facteurs de croissance dans ce processus.

This paper reviews the factors regulating the growth and differentiation of brain tumors and is based on the paradigm that oncogenesis is a multistep process. In this model, a carcinogenic substance is thought to act on a target cell and induce biochemical change in the deoxyribonucleic acid (DNA) of the host cell. It is hypothesized that to firmly establish an inheritable change in the altered host genome the cell must undergo a round of cellular proliferation. Once “initiated”, the cell evolves through a series of changes, aided by certain “promoting” agents, which eventually lead to the malignant phenotype. This evolution can be seen when an initially low grade astrocytoma is later, at reoperation or autopsy, found to be highly malignant or demonstrated within a single tumor which contains areas of different degrees of anaplasia merging imperceptibly with areas of gliosis.

I will discuss in this review how this process occurs, try to explain the role that oncogenes and their products play in the process and, finally, briefly speculate on some of the new possibilities of treatment that may result from this knowledge in cellular biology.

INITIATORS

Neoplasms of the central nervous system (CNS) account for approximately 2% of malignancies. However, up until the age of 20, they account for 1/4 of human cancers. The fact that cells must undergo a round of cellular proliferation prior to becoming initiated may in part explain why the developing central nervous system is so susceptible to carcinogenic influences. This susceptibility of the developing central nervous system to can-
Cinogenic influences has been used to advantage in experimental systems studying the carcinogetic process by various substances such as viruses and chemicals. Despite the wealth of animal studies demonstrating chemical and viral carcinogetic in the CNS, vinyl chloride has been the only substance shown to be related to human brain tumor development.

Viruses and CNS Tumors

The role of viruses in the induction of primary intracranial tumors has been a matter of discussion for many years. Although there exists extensive circumstantial evidence and reports of the recovery of human papovavirus from an intracerebral reticulum cell sarcoma and glioblastoma multiforme and adenoviruses from a pituitary adenoma and an astrocytoma, it remains to be proven that viruses play a direct and causative role in the pathogenesis of human brain tumors. Despite this, the evidence for viral induction of brain tumors in laboratory animals is quite extensive.

Broadly speaking, viruses can be classified into DNA or RNA viruses. The main groups of DNA viruses that have been implicated are the papovaviruses and the adenoviruses. These viruses have induced a variety of tumors ranging from ependymomas, choroid plexus, papillomas, medulloblastomas, pinoeytomas, glioblastomas and undifferentiated neuroectodermal tumors in a variety of animals ranging from hamsters to monkeys. The avian, murine, and simian sarcoma viruses are retroviruses of the RNA group that have been shown to induce mainly astrocytic tumors in various rodents, dogs, and monkeys.

Multiple theories exist on how viruses induce brain tumors but most describe an alteration of host genome expression. The retrovirus contains the enzyme, reverse transcriptase, and it is postulated that this enzyme gives the retrovirus its oncogenic potential. Once within a host cell, the reverse transcriptase is able to form a DNA copy of the viral RNA using transfer RNA as template structures. It is then able to construct a complementary strand of the viral DNA and insert this into the host genome. By doing so, the new viral DNA may now alter the expression of certain host cell genes regulating the mitogenic process and use host cell machinery to replicate its own viral proteins. This process may allow reexpression of host cell genes normally present for cell growth and differentiation (ie: “proto-oncogenes”). With the alteration of the normal control of such gene expression, the cell may then undergo oncogenesis. Another possibility is that the viral gene may itself be homologous to the normal cellular proto-oncogene, and because of its position, not be subject to the usual controls. This characteristic might allow the viral gene to be expressed continuously, leading to the malignant phenotype.

Chemical Carcinogenesis

Selzgman and Shear were the first to demonstrate that chemical substances could induce brain tumors. They placed cyclic aromatic hydrocarbons directly within the brain and noted that a variety of brain tumors could be induced. Since then, the two main groups of chemicals which have been shown to produce neoplasia in the CNS, by either implantation or administered systemically, have been the aliphatic alkylating agents and the poly cyclic hydrocarbons. Methyl and ethyl nitrosourea and several other methylating and ethylating agents have been shown to cause brain tumors in up to 90-100% of the offspring of pregnant rats injected with such material. It appears as though initiation of target cells requires a covalent interaction between the carcinoen or a metabolically activated carcinoen by-product and the DNA molecule. For example, the N-nitroso compounds have a high affinity for the CNS and cross the blood brain barrier easily. They have a half life of less than 10 minutes and are metabolized to alkyl cations which interact covalently with the nucleophilic groups of DNA to cause mispairing of nucleotide sequences and thus point mutation in the host genome. Such mutations may also be related to chromosomal deletions and duplications. These mutations may remove normal control mechanisms placed on genes regulating cell growth and differentiation and lead to malignancy. The diacylitrizenes and the hydrazines are both thought to act in a similar fashion.

One other factor that may be functional in brain tumor chemical carcinogetic may be an impaired ability of the damaged host cell to repair its DNA. Kleihues et al demonstrated the long term persistence of O6-methylguanine in rat brain DNA of adult female PV-1X rats given a single intravenous injection of N-[3H]-methyl-N-nitrosurea (10 mg. kg-1, 25 m Cl-mmol-1) and suggested that the repair capacity of neural tissue for O6-methylguanine was reduced. Such deficient DNA repair capacity may be related to CNS-specific chemical carcinogetic.

Promoters

It has never been shown conclusively that promoters are directly involved with human carcinogetic; however, various hormones, immunosuppressants and some viruses are thought to function as such. The most significant of these appears to be the association between secretion of messengers in the endocrine system and the link with cell proliferation. Lloyd et al have shown a direct correlation between the secretory activity of a prolactin cell and its proliferation. This correlation may also be seen in other hormonally regulated endocrine cells such as thyrotrophs, corticotrophs, somatotrophs, parathyroid cells and gastric parietal cells. Conversely, treatment of certain tumors such as prolactinomas with dopamine agonists which may act as “anti-promoters”, suppress secretory activity, decrease tumor growth and cause tumor involution.

Oncogenes, Growth Factors, and Multistage Carcinogenesis

Chemical carcinoen do not introduce new DNA into a host cell but rather affect genes necessary for normal cell growth and differentiation (termed “proto- oncogenes”). Activated proto-oncogenes become “oncogenes” capable of contributing to the carcinogetic process. Two hypotheses have been proposed to explain the mechanism of activation of proto-oncogenes. The first involves a retrovirus carrying a viral-one (v-onc) gene integrating into host DNA or the insertion of a viral promotor and/or enhancer turning on expression of a cellular-one (c-onc) gene. The second involves structural chromosomal abnormalities such as deletions, duplications and reciprocal translocations associated with aberrant expression of a proto-oncogene.
Signal Transduction

Exactly how oncogenes implement the transformation of the malignant phenotype is unknown but a major theme that has emerged is that the proto-oncogenes represent a subset of genes that normally code for components in the pathways of cellular signal transduction.

A multicellular organism must be able to coordinate the behaviour of individual cells. It can do this through endocrine, paracrine or autocrine communication. In the endocrine system, a chemical messenger is secreted into the blood stream to act as a distant tissue (for eg. insulin). The paracrine system acts at neighbouring cells within a short range and the autocrine system acts at the same cell secreting the messenger. In all of these systems, once the signal has been received, a cascade of signal transduction flows through the cytoplasm and eventually into the nucleus to alter the expression of certain genes. Since proto-oncogenes are a set of genes that normally code for components in these pathways of signal transduction, alterations or “activation” of proto-oncogenes will cause aberrations in signal transduction and thus disrupt normal growth, differentiation and intercellular communication.

Protein kinases play a central role in these processes. Activation of protein kinases can occur in 3 ways. First, occupancy at the Beta adrenergic receptor leads to activation of adenylate cyclase and through coupling of G regulatory proteins, increased in cyclic AMP (CAMP) activates protein kinase A, a serine and threonine kinase. Several agonists activate phospholipase C phospholipase via protein kinase C (PKC), also a serine and threonine kinase. The tumor promoter 12-0-tetradecanoylphorbol-acetate (TAP) apparently acts in place of DAG. The final step in this process is the activation of a single proto-oncogene is but a single step in the multistep process.

Oncogenes and Brain Tumors

The sis gene was first known as a viral transforming gene (v-sis) of the Simian Sarcoma Virus (SSV) and later found to be closely related to the cellular sis (C-sis) gene that produced PDGF. The protein encoded for by v-sis in SSV transformed cells called p28 is similar to PDGF and is secreted in a biologically active form capable of stimulating protein synthesis. This gene product appears to stimulate proliferation of the cell which produced it (i.e. autocrine mechanism). However, exogenously added PDGF antibodies do not completely suppress the growth of tumor cells, suggesting that the autocrine model in itself is insufficient to explain PDGF-related growth.

The second proto-oncogene of importance in gliomas is the c-erb-B gene encoding for 3 of the 4 domains of the EGF receptor. Calcium then activates several calmodulin-dependent enzymes (such as protein kinases, phosphatases, phosphodiesterases) and also affects the cytoskeleton. The tumor promoter 12-0-tetradecanoylphorbol-acetate (TAP) apparently acts in place of DAG. The final method of protein kinase activation is via stimulation of growth factor receptors by substances such as platelet derived growth factor (PDGF), epidermal growth factor (EGF), insulin and certain lymphokines. They activate a tyrosine kinase present on the cytoplasmic portion of the receptor.

Exactly how the proteins phosphorylated by all these kinases reach the nucleus and affect genomic expression is unknown but a hypothesis is that it may occur through phosphorylation of DNA methyltransferase that methylates DNA and affects gene expression. They may also alter expression of the myc and fos oncogenes which are genes thought to be important in cell proliferation and differentiation. Alternative explanations have been through activated forms of oxygen or alteration of levels of poly-ADP-ribose.

Shapiro et al performed cytometric studies on freshly excised glioblastoma and found that low grade areas contained cells with chromosomal number near diploid, whereas cytologically malignant area cells were mainly tri- and tetraploid. Karyotype analysis revealed multiple chromosomal anomalies including translocations, deletions and duplications; however, over-representation of chromosome 7 and under-representation of chromosome 22 and the sex chromosome were the most common patterns.
N-myc oncogene expression has been demonstrated in neuroblastoma, retinoblastoma and astrocytoma.\textsuperscript{15,83,84} Proliferation of neuroblastomatous cells leads to a 20-fold increased expression of this oncogene and differentiation of these cells lead to markedly decreased N-myc oncogene expression.\textsuperscript{15,84}

Finally, the N-neu oncogene has also been described in neuroblastomas and is felt to act in a similar fashion to the \(v\)-erb-B oncogene in that it encodes for a tyrosine kinase.\textsuperscript{85} Thus, one can appreciate that there exist abundant data associating the aberrant expression of genes responsible for cell growth and neoplasia within the CNS.

**Differentiation and Growth Inhibition**

Since the normal organism is able to control growth and induce differentiation of the cells once the growth period or stimulus for growth has ceased, it seems logical that nature has developed a set of genes not only for growth but also a set to induce cells to undergo "terminal" differentiation and terminate continued proliferation. These genes have been called "growth inhibitor genes" by Weinstein.\textsuperscript{52} One could easily postulate that activation of oncogenes or inactivation of "growth inhibitor genes" or "differentiation genes" could lead to growth and/or failure to differentiate. Such mutations would be recessive as it would be necessary to delete both alleles for tumors to occur. There is increasing evidence that such genes exist and are related to oncogenesis.\textsuperscript{86-90} For example, in Drosophila, flies homozygous for the mutation, "giant larvae" have an arrest of the development of the optic centres, eventual proliferation of ganglion cell precursors and development of neuroblastosmas.\textsuperscript{91} In humans, neuroblastosmas are known to spontaneously differentiate\textsuperscript{92} and this may represent a spontaneous expression of these growth inhibitor genes. Exactly how these genes work is unknown but Weinstein has suggested that it is possibly through protein phosphatases, protein kinases opposite to PKC, phospholipase inhibition, transcription or translation inhibitors of cell proliferation genes or through genes that induce differentiation.\textsuperscript{52} The products of such genes may be lipid related rather than polypeptides since certain prostaglandins have inhibited tumor cell growth or induced differentiation. There already exist several agents that mimic the action of such genes or induce their expression. These agents include: retinoids, vitamin D derivatives, glucocorticoids, dimethylsulphoxide (DMSO), cyclic AMP, butyrate, TPA, mezerin, TGF-beta and certain interferons.\textsuperscript{86,93,94}

Thus, there exists a set of genes which codes for the pathways that maintain a cell in cycle and a set that allow the cell to differentiate and terminate its proliferation. By altering the balance of growth-stimulating and growth-inhibiting gene expression, one may gain new venues for the treatment of brain tumors.

**Other Factors Involved in Carcinogenesis**

Undoubtedly, there are multiple other factors involved in the carcinogenic process. The immune responses to malignant cells play a major role here but are beyond the scope of this paper.\textsuperscript{95} Micro-environmental factors such as positional effects and polarity of a cell in relation to the rest of the developing cell mass are important controls governing cellular proliferation and differentiation. Cell to cell contact through recognition of surface antigens such as N-CAM, NG-CAM, and myelin-associated glycoprotein, is important in the developing nervous system and is thought to play a major role in CNS development.\textsuperscript{96} Gradients of oxygen, ions and nutrients, especially calcium, and the role of calcium in regulating calmodulin dependent enzymes such as phosphatases is an area of intense interest.\textsuperscript{97,98} The extracellular matrix undoubtedly influences cell to cell interactions necessary for the control of cell growth.\textsuperscript{99} Thus, when considering oncogenesis, one must remember that the cell does not live alone, but that the final expression of malignancy depends on interaction of the cell with its environment.

**A NEW FRAMEWORK FOR THERAPY**

This new knowledge of the molecular and cellular biology of brain tumors has provided a framework from which to develop new therapeutic modalities. One can hypothesize that initiated neoplastic cells with altered genetic controls secrete a group of growth promoting substances that induce proliferation in neighbouring cells via autocrine, paracrine and possibly endocrine mechanisms. Proliferating cells losing expression of differentiation genes might then develop the morphology of reactive or malignant cells. Phenomena such as glioma heterogeneity, mixed tumors or tumor angiogenesis might occur as a result of this tumor cell communication.

In the future, we may be using therapies based on knowledge of the cellular and molecular biology of brain tumors. By altering the balance of growth-stimulatory and growth-inhibitory gene expression one might induce differentiation in tumors. Utilization of growth factor antagonists, or agents designed to alter intracellular signal transduction, or tumor cell invasiveness might one day be therapeutic options for our patients afflicted by tumors of the central nervous system.

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