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Inducible and Transmissible Genetic Events and Pediatric Tumors of the Nervous System

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Transplacental carcinogenesis/Brain tumors/Tumor suppressor genes/Genetically engineered mice/ Gene-environment interactions.

Tumors of the nervous system most often occur in both children and adults as sporadic events with no family history of the disease, but they are also among the clinical manifestations of a significant number of familial cancer syndromes, including familial retinoblastoma, neurofibromatosis 1 and 2, tuberous sclerosis, and Cowden, Turcot, Li-Fraumeni and nevoid basal cell carcinoma (Gorlin) syndromes. All of these syndromes involve transmissible genetic risk resulting from loss of a functional allele, or inheritance of a structurally defective allele, of a specific gene. These genes include RB1, NF1, NF2, TSC1, TSC2, TP53, PTEN, APC, hMLH1, hPSM2, and PTCH, most of which function as tumor suppressor genes. The same genes are also observed in mutated and inactive forms, or are deleted, in tumor cells in sporadic cases of the same tumors. The nature of the mutational events that give rise to these inactivated alleles suggests a possible role of environmental mutagens in their causation. However, only external ionizing radiation at high doses is clearly established as an environmental cause of brain, nerve and meningeal tumors in humans. Transplacental carcinogenesis studies in rodents and other species emphasize the extraordinary susceptibility of the developing mammalian nervous system to carcinogenesis, but the inverse relationship of latency to dose suggests that low transplacental exposures to genotoxicants are more likely to result in brain tumors late in life, rather than in childhood. While not all neurogenic tumorrelated genes in humans have similar effects in experimental rodents, genetically engineered mice (GEM) increasingly provide useful insights into the combined effects of multiple tumor suppressor genes and of gene-environment interactions in the genesis of brain tumors, especially pediatric brain tumors such as medulloblastoma.

INTRODUCTION

Neoplasms of the brain and other parts of the nervous system occur in both children and adults, but different neoplasms and different tumor sites characterize the two age groups. Tumors of the nervous system, especially the brain, the eye, and the sympathetic nervous system, are the second most common category of childhood cancers, after leukemia and lymphoma. More than 7500 cases of cancer occur annually among children in the United States, of which approximately 19 per cent are tumors of the central nervous system (CNS) and 18 per cent are neuroblastomas arising in the

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adrenal medulla and sympathetic ganglia. Cancer remains the second leading cause of death, after accidents, among American children.¹⁾ Pediatric brain tumors are the subject of a recent on-line summary of clinical progress and challenges by a study group convened by the U.S. National Cancer Institute (http://prg.nci.nih.gov/brain/pediatrics.html, April 2005).

Some pediatric neoplasms and some adult-onset neurogenic tumors occur as aspects of the clinical manifestations of certain familial cancer syndromes, and can now be traced to inherited defective alleles of specific tumor-related genes.²⁾ During the last 40 years, 1965–2005, the causes of tumors of the nervous system have been the focus of numerous studies in both the laboratory and the clinic. These studies were driven first by experimental findings that the mammalian brain is highly susceptible to carcinogenic exposures during early life, and later by the discovery of specific genes that, in mutated forms, are involved in the pathogenesis of some of these neoplasms. Despite intensive experimental and epidemiologic investigations, and despite numerous observations that suggest a role of environmental factors in

Georgetown University Medical Center, Washington, DC, USA. In this paper, genes of non-human species are written in lower-case italic font (e.g., p53) and human genes in upper-case italic (e.g., TP53). Names of homologous genes in human and non-human species are not necessarily identical.

the causation of neurogenic tumors, no chemical agent or mixture has yet been identified as a carcinogen for the human nervous system.

The brief latencies of brain tumors and other nervous system tumors in children have often been cited as support for the hypothesis that these tumors may result from encounters with environmental carcinogens during prenatal or early postnatal life. Transplacental and perinatal chemical carcinogenesis has been the subject of several international conferences and symposia with published proceedings^{3–5)} in which reviews and summaries of this still-evolving field of research are available. This paper is taken in part from a recent review of the roles of conventional and genetically modified mice in clarifying the etiology of neurogenic tumors of childhood.⁶⁾

NEUROTROPIC CARCINOGENESIS IN EXPERIMENTAL ANIMALS

Neurogenic tumors are rarely seen in conventional bioassays for carcinogenicity, in which rats and mice are usually exposed systemically to the suspect agent by repeated injection, ingestion or inhalation during their entire adult lives. Only a few chemicals have caused significant incidences of brain tumors in adult rats in long-term bioassays. These include acrylonitrile, acrylamide, 1,3-butadiene, ethylene oxide, glycidol, and isoprene; the alkylating agents 1,3-propane sultone and 2-methylaziridine; and a number of hydrazine derivatives including 1,2-diethylhydrazine.⁷⁾ No neurogenic tumors of any kind have been significant endpoints of bioassays for carcinogenicity in mice.

When brain tumors have occurred in bioassays in rats, a few meningeal tumors of various kinds have often been seen, together with tumors of glial origin. These include granular cell tumors, a kind of meningeal tumor peculiar to the rat. Meningeal tumors have also been seen at low frequency in transplacental carcinogenicity studies in rats and in other species. However, in long-term bioassays "neurogenic" tumors are frequently induced only in the CNS and the meninges, and only in rats.

Nerve tumors have seldom occurred in bioassays in adult rats, and peripheral neuroblastic tumors have been only incidental findings, not statistically significantly associated with exposure to the chemical under test. Neuroendocrine tumors, especially of the stomach, do occur in rats and other rodent species under certain conditions,⁸⁾ but these are beyond the scope of this discussion.

Because of their rarity, when brain, nerve, or meningeal tumors occur in bioassays they tend to attract special notice. It is often inferred that these experimental tumors predict brain tumor risk in humans who are exposed to the same substances. In fact, as of this writing it has not been possible to establish such a causal association by epidemiologic studies of occupationally exposed individuals. No chemical compound or mixture has been unequivocally identified as a cause of brain tumors in humans.

Transplacental carcinogenesis by alkylating agents in rats and mice

The landmark discoveries of Hermann Druckrey and his coworkers in the 1960s revealed the extraordinary vulnerability of the rat fetus to chemical induction of neurogenic tumors.⁹⁾ Since then, many carcinogens have been identified that can cause tumors of the nervous system in experimental animals when given systemically to the mother animals during pregnancy. The most-studied of these carcinogens is the potent direct-acting monofunctional alkylating agent, *N*-nitrosoethylurea (ENU). The evolution of this body of knowledge has been summarized.^{10–13)}

The most common CNS tumors induced transplacentally in rats and mice are not embryonal tumors, but rather gliomas of various kinds, most commonly either oligodendrogliomas or mixed gliomas with an oligodendroglial component. In humans, such tumors occur in adults but very rarely in children. In rats they occur in both the brain and the spinal cord; in mice, which are much less susceptible than rats to transplacental carcinogenesis in the nervous system, the spinal cord is spared and CNS tumors develop only in the brain.

In offspring of both rats and mice exposed to ENU during gestation, tumors arise preferentially in the peripheral nervous system including the cranial and spinal nerve ganglia. These neoplasms have the classic histologic features of human schwannomas, but are aggressively invasive.¹⁴⁾ They have been described as malignant neurinomas in the European literature and as malignant schwannomas in American studies. They are not exactly comparable to malignant peripheral nerve sheath tumors (MPNSTs) in humans.

The stage of prenatal development at which susceptibility to transplacental carcinogenesis begins in experimental animals varies with the characteristics of the carcinogen. Direct-acting alkylating agents such as ENU freely cross the placenta and do not require metabolic activation by maternal, placental or fetal tissues. When given as single doses to the dams before approximately post-conception day 10, when organogenesis begins in the developing rat fetuses, these substances cause malformations but do not cause tumors. Tumors develop after birth in offspring exposed at approximately day 12 of gestation or later. The incidence and multiplicity of brain and nerve tumors in the offspring increase rapidly thereafter, becoming maximal in animals exposed to ENU during the final week of gestation.¹⁵⁾ Most direct-acting alkylating agents other than ENU are also transplacental carcinogens. These compounds also induce neurogenic tumors in the offspring of rats that have been exposed during pregnancy, and often cause various kinds of non-neurogenic tumors as well.

In contrast, many metabolism-dependent carcinogens are carcinogenic to the fetal rat only when given to the dam at

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the very end of gestation, when fetal metabolic competence has presumably evolved to the stage that the carcinogen can be biotransformed by fetal tissues. Some, but not all, metabolism-dependent carcinogens also cause neurogenic tumors when given transplacentally to rats, but most of these substances cause tumors chiefly in other organ systems. It must be emphasized that not all transplacental carcinogens cause tumors of the nervous system, even in the exquisitely sensitive rat species.

Transplacental and perinatal carcinogenesis in nonrodent species

Transplacental carcinogenicity experiments with ENU have been conducted in conventional rats, mice, Syrian hamsters, rabbits, dogs, and patas and cynomolgus monkeys.¹⁶⁾ In Old World monkeys, ENU is a transplacental carcinogen when given in repeated doses throughout pregnancy, beginning during the first trimester.¹⁶⁾ In all these species, the fetal nervous system is a target of the carcinogen, and is the major target organ in the rat. The rat fetus at day 15 of gestation is at least 50 times more susceptible than the adult.¹⁵⁾ and welltolerated single doses of ENU can cause multiple primary brain tumors in 100 per cent of exposed offspring. In rodents and rabbits (but not in non-human primates) neurogenic tumors occur in the offspring in both the central and the peripheral nervous systems, although not in the eye or the adrenal medulla, features that are at variance with the spectrum of human pediatric tumors. The spectrum of transplacentally induced neurogenic tumors in non-human primates more closely resembles the pattern of human pediatric tumors, in that neurogenic tumors induced by ENU have thus far been found only in the brain, and not the peripheral nervous system.

Dose-effect relationships and latency

The incidence and multiplicity of neurogenic tumors in the offspring increase with increasing dose of carcinogen administered to the mothers, but tumor latency is inversely related to the dose.¹⁷⁾ However, even at the highest tolerable doses of carcinogen, few of the resulting cancers in the offspring arise early in postnatal life, which can be considered the period preceding onset of sexual maturity, approximately 5–6 weeks of age in mice and 6–10 weeks of age in rats. Most transplacentally induced neurogenic tumors in rats and mice only become clinically evident during adult life, or even in old age. The majority of the induced tumors are more closely comparable to tumors of middle and late adult life in humans, with respect to both cell type and site of origin, than to human tumors of childhood. Only a very small percentage of these tumors have an embryonal histologic pattern.

Dose-effect relationships for causation of neurogenic plus renal tumors by single injections of ENU to newborn rats are given in Table 1. These data show *increasing* incidence and multiplicity of tumors of the kidney and nervous system with increasing dose, and monotonically *decreasing* latency with increasing dose. These data do not extend to very low doses, but at the lowest dose tested, the mean latency for tumors in rats given a single injection of this carcinogen as newborns was 500 days, or more than half the lifespan of untreated animals.¹⁷

Dose-effect relationships for prenatal exposures follow the same patterns as in neonates and adults, but the slopes of the curves are generally different. For some carcinogens and target tissues, the rodent fetus may be two decimal orders of magnitude more susceptible than the dams throughout the range of tolerated doses.

Molecular pathogenesis

It is now well recognized that many kinds of tumors may arise by several different pathways, and that these may vary from species to species. Interspecies differences in tumor spectrum resulting from exposure to the same carcinogen are now at least partially understandable as a result of progress in identification of the different genetic pathways that are involved in pathogenesis of various tumors in different species. For example, peripheral nervous system tumors

Table 1. Dose-response relationships for induction of tumors of brain, spinal cord, nerves and renal tumors combined, by directly-acting *N*-nitrosoethylurea in newborn rats.¹⁷⁾ Total tumor incidence and multiplicity increase monotonically with increasing dose, while latency is inversely proportional to increasing dose.

Single injection (mg/kg)	Total number of injections × injections/week	Number of animals	Per cent with tumor	Tumors/animal ^a	Mean latency (days)
5	1	28	32	0.3	500
10	1	16	69	1.3	310
20	1	16	100	2.2	240
40	1	15	100	2.3	205
80	1	16	100	2.9	183

^a Total tumors observed in the group divided by the number of animals at risk at the time that the first tumor appeared.

induced transplacentally in rats by ENU contain a potent transforming gene, *neu*, which is readily detectable by transfection of NIH 3T3 indicator cells in monolayer culture. *Neu* in these tumors is activated by a specific point mutation in the transmembrane domain of this gene.¹⁸⁾ The same pathway has been shown to operate in neurofibromas and schwannomas induced transplacentally by ENU in Syrian hamsters¹⁹⁾ and in transplacentally induced schwannomas in mice.²⁰⁾

This pathway cannot operate in humans because the gene sequence of the human homologue of *neu*, *HER2*, does not allow the same amino acid substitution from a single point mutation (Fig. 1).²¹⁾ Activating mutations of protein kinases of the epidermal growth factor receptor family do occur in certain human carcinomas and contribute to their development,²²⁾ but the activating mutations of *HER2* in non-small cell lung cancers occur in the kinase domain of the oncogene, rather than the transmembrane domain.²³⁾



Fig. 1. Diagram of the extracellular, intracellular, and transmembrane domains of the oncogene *neu* and its activation by point mutation in the transmembrane domain in malignant schwannomas in rats, mice and hamsters. The base sequence of the human homolog of *neu*, *HER2*, precludes an equivalent change in base sequence in a single mutational event. Reproduced from Perantoni and Rice²¹⁾.

In contrast, DNA from the gliomas induced transplacentally by ENU in F344 rats in the same experiment did not transform NIH 3T3 cells *in vitro*.¹⁸⁾ No transforming gene (oncogene) has yet been shown to play a significant etiologic role in chemically induced brain tumors in rodents or in humans, although mutationally activated *ras* genes have been observed in small subsets of human brain tumors.²⁴⁾

BRAIN, NERVE AND MENINGEAL TUMORS IN HUMANS

The current World Health Organization Classification of

Tumors, the so-called "blue books," devote an entire volume to the pathology and genetics of tumors of the nervous system²⁾ (with the exception of retinoblastoma). It has become essential to combine genetics and morphology for the proper classification of a significant fraction of these neoplasms.

Most neurogenic tumors of childhood are clinically and histologically distinctive neoplasms that occur uniquely or at least predominantly in this age group. Some are composed of primitive or undifferentiated cells, a fact that is intuitively consistent with their origin during early life when the nervous system is still developing. These include the primitive neuroectodermal tumors (PNETs) of the brain, notably medulloblastoma of the cerebellum; retinoblastoma; and neuroblastoma. Other pediatric brain tumors include various kinds of gliomas. Many pediatric glial tumors such as pilocytic astrocytomas of the brain stem (astrocytoma grade I), are well differentiated and clinically much more benign (lower histologic grade) than the more common high-grade astrocytic tumors of adults (e.g., glioblastoma, or astrocytoma grade IV). In further contrast to adults, gliomas that arise in children include a high proportion of ependymomas and papillomas of the choroid plexus, while adult gliomas are most commonly of astrocytic (and less commonly oligodendroglial) origin.

Causation and latency

Environmental causes of human neurogenic tumors remain obscure. High-intensity external ionizing radiation during childhood, delivered to the head as therapy for leukemia or other malignancies, is the only "environmental" carcinogen that has been clearly linked to human cancers of the nervous system. Cerebral PNETs (not medulloblastomas) have developed in children who had received a total radiation dose to the CNS of 18-24 Gy 7-9 years before, at 3-8 years of age.²⁴⁾ Prophylactic irradiation of the CNS for ALL can also cause low-grade diffuse and anaplastic astrocytomas and even glioblastoma in children. After a standard therapeutic dose of 24.4 Gy at a mean age of 4.8 years, the interval between irradiation and appearance of clinical signs of brain tumor was 7.6 \pm 2.3 years.²⁵⁾ In a retrospective cohort study of 9720 children treated for ALL, there was a 22-fold excess risk of subsequently developing a CNS tumor, and the risk was even higher in children aged 5 years or less at the time of ALL diagnosis.²⁶⁾ The much lower estimated doses (mean, 1.5 Gy) of radiation directed at the scalp of older children to treat *tinea capitis* resulted in a relative risk of 2.6 for gliomas, which was less than that for meningiomas (relative risk, 9.5) or for nerve-sheath tumors (relative risk, 18.8) or for all neural tumors of the head and neck combined (relative risk, 8.4). Many of these tumors had a prolonged latency (up to 30 years) and presented clinically well after childhood, but at these comparatively low doses a steep dose-effect relationship was observed, with relative risk approaching 20 after estimated doses of 2.5 Gy.²⁷⁾

For meningiomas a clear dose-effect relationship has been recognized, with higher radiation dose leading to increased risk. Irradiation to treat tinea capitis at a retrospectively estimated mean X-ray dose per patient of 1.5 Gy was associated with a significantly increased relative risk of 8.4 (95 per cent confidence interval, 4.8-14.8) for neurogenic tumors of the head and neck.²⁷⁾ This is a relatively low therapeutic dose. compared to what is used for anti-tumor therapy. Meningiomas typically occur in middle-aged individuals, but also occur in children and in the very old. Meningiomas that result from radiation exposures during childhood can arise in late adult life, after latencies of 30 years or more, and can have extraordinarily long latencies; a case with a 63-year latency period has been reported.²⁸⁾ As with chemical carcinogenesis in experimental animals, tumor latency is inversely proportional to intensity of exposure: average latencies of 35, 26, and 19-24 years have been reported for meningiomas induced by low-, moderate-, and high-dose radiation, respectively.^{28,29)}

Maternal and paternal exposures to carcinogenic chemicals and chemical mixtures before conception, and maternal exposures during pregnancy, have not been conclusively shown to increase the risk of neurogenic tumors in children. Even maternal cigarette smoking, perhaps the most potent and most widespread exposure to any chemical carcinogenic mixture during pregnancy, has been associated with only a very small elevated relative risk for all kinds of cancer in childhood (RR = 1.10; 95% confidence interval [CI], 1.03– 1.19) in a meta-analysis of more than 30 individual studies.³⁰⁾ For central nervous system tumors in offspring of mothers who smoke the risk was even smaller, and statistically non-significant (RR = 1.04; CI, 0.92–1.18). Interestingly, exposure to paternal cigarette smoke was more strongly associated with increased risk of brain tumors in children than exposure to maternal tobacco smoke (RR = 1.22; CI, 1.05-1.40).³⁰

Tumor suppressor genes and somatic cell mutations

Homozygous deletion or missense mutation of the retinoblastoma tumor suppressor gene, RB1, gives rise specifically to retinoblastoma and contributes to the pathogenesis of certain mesenchymal tumors in children.³¹⁾ Patterns of retinoblastoma inheritance led Knudson to develop the two-hit theory of carcinogenesis^{32,33)} and ultimately led to discovery of tumor suppressor genes, of which RB1 was the first to be characterized.³⁴⁾ Subsequently, many other tumor suppressor genes and a number of genes that behave somewhat differently from tumor suppressors have been discovered, some of which are important in the pathogenesis of various human tumors. These include NF2 in meningiomas,³⁵⁾ schwannomas,³⁶⁾ and spinal ependymomas;³⁶⁾ PTEN in high-grade gliomas;³⁷⁾ TP53 in diffusely infiltrating astrocytomas³⁸⁾ and in a variety of other tumors; and PTCH³⁹⁾ and APC or β *catenin*⁴⁰⁾ in different subsets of medulloblastomas (Table 2).

Familial cancer syndromes and germ-line mutations in tumor-related genes

Children of parents who are carriers of a defective allele of certain tumor-associated genes are at risk for the familial cancer syndromes listed in Table 3. These particular familial cancer syndromes include certain neurogenic tumors among

 Table 2.
 Somatic cell mutations in tumor suppressor genes and other tumor-related genes in common sporadic (non-familial) tumors of the human nervous system. Chromosomal alterations have also been noted in oligodendrogliomas and in cerebral ependymomas, but no specific genetic loci have yet been identified that are commonly altered in these tumors.

Tumor	Gene	Structural defect/mutation	Reference
Retinoblastoma	RB1	Loss of functional gene sequence	Smith and O'Brien ³⁴⁾
Meningioma	NF2	Small insertion or deletion/nonsense mutations in 60% of sporadic cases	Louis et al.35)
Schwannoma	NF2	Inactivating loss-of-function point mutations, mostly frameshifts, throughout the coding sequence, with loss of wild-type allele	Louis <i>et al</i> . ³⁶⁾
Spinal ependymoma	NF2	Inactivating mutations	Louis et al. ³⁶⁾
High-grade gliomas	PTEN	Loss-of-function mutations	Tohma <i>et al.</i> ³⁷⁾
Diffusely infiltrating astrocytomas	TP53	Point mutations (transitions and transversions) clustered in exons 5–8, especially "hot spot" codons 175, 196, 213, 245, 248, 273 and 282 (CpG sites)	Kleihues et al. ³⁸⁾
Medulloblastoma	TP53	Mutations in 5-10 % of tumors	
	PTCH	Inactivating mutations (truncated protein product) in 8% of sporadic cases, especially desmoplastic medulloblastomas	Wolter <i>et al.</i> ³⁹⁾
	APC or β-catenin	Wnt signaling pathway mutations in 13% of sporadic cases	Huang <i>et al</i> . ⁴⁰⁾
	?	17p Deletions, including deletions distal to TP53 at 17p13.1	

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Table 3. Familial cancer syndromes and germ-line mutations in tumor-associated genes. Modified from.²⁾

Syndrome	Gene	Chromosome	Neurogenic tumors ^a	Reference
Familial retinoblastoma	RB1	13q14	Retinoblastoma	Smith and O'Brien ³⁴⁾
Li-Fraumeni	TP53	17p13	Astrocytomas, PNET	Ohgaki et al. ⁴¹⁾
Neurofibromatosis 1	NF1	17q11	Neurofibromas, MPNST, optic nerve gliomas, astrocytomas	von Deimling et al.42)
Neurofibromatosis 2	NF2	22q12	Bilateral vestibular schwannomas, meningiomas, peripheral schwannomas, spinal ependymomas, astrocytomas	Louis et al. ³⁶⁾
Nevoid basal cell carcinoma syndrome (Gorlin)	PTCH	9q31	Medulloblastoma	Reifenberger <i>et al.</i> ⁴³⁾
Turcot	APC	5q21	Medulloblastoma	Cavenee et al.44)
	hMLH1 hPSM2	3p21 7p22	Glioblastoma	
Tuberous sclerosis	TSC1 TSC2	9q34 16p13	Subependymal giant cell astrocytomas	Wiestler <i>et al.</i> ⁴⁵⁾
Cowden	PTEN	10q23	Dysplastic cerebellar gangliocytoma	Wiestler <i>et al.</i> ⁴⁶⁾

^a MPNST, malignant peripheral nerve sheath tumors; PNET, primitive neuroectodermal tumors.

their manifestations,²⁾ including retinoblastoma (familial retinoblastoma syndrome)34); astrocytomas and PNETs (Li-Fraumeni syndrome)⁴¹; neurofibromas, optic nerve gliomas, astrocytomas, and malignant peripheral nerve sheath tumors (MPNSTs; neurofibromatosis type 1)⁴²; bilateral vestibular schwannomas, meningiomas, peripheral schwannomas, spinal ependymomas and astrocytomas (neurofibromatosis type 2)³⁶; medulloblastomas (nevoid basal cell carcinoma [Gorlin] syndrome)43); medulloblastomas and glioblastomas (Turcot syndrome)⁴⁴); subependymal giant cell astrocytomas (tuberous sclerosis)⁴⁵; and dysplastic cerebellar gangliocytoma (Cowden syndrome).46) The germline mutations in parental carriers may reflect a continuing transmission of a mutation that arose generations ago, as with germline TP53 mutations in Li-Fraumeni syndrome,⁴¹⁾ or may have newly arisen in a parent of a syndrome patient, most often in the paternal germline. Newly arising germline mutations have been well documented for neurofibromatosis types 1⁴²⁾ and $2^{36)}$ and for nevoid basal cell carcinoma (Gorlin) syndrome.43)

It has been postulated that carriers of a defective allele of *TP53* may be especially susceptible to environmental carcinogens, but no specific example has been documented in human experience. Laboratory exploration of the roles of gene-gene and gene-environment interactions in the genesis of neurogenic tumors, both pediatric and adult, has recently been made possible by the creation of genetically engineered mouse (GEM) models of many familial cancer syndromes. Genetic engineering approaches have also made it possible to study the consequences of loss of one or both alleles of two or more suppressor genes in the same individual.

GENETICALLY ENGINEERED MOUSE (GEM) MODELS

Genetically engineered mouse models of human neurogenic tumors have recently been reviewed and evaluated in the context of clues to the etiology of pediatric brain tumors. The recommendations of a panel convened for this purpose by the NCI Mouse Models of Cancer Consortium have recently been published.⁴⁷⁾ GEM models have been established for many, but not all, major kinds of neurogenic tumors. For example, there is as yet no GEM model for oligodendroglioma, mixed glioma (oligodendroglioma-astrocytoma), or ependymoma.

Neurogenic tumors in GEM models

A large number of genetically engineered mice predisposed to specific tumors of the central and peripheral nervous system have been described, but relatively few GEM tumors have been studied as *etiologic* models for the corresponding human neoplasms. Animals heterozygous for specific defective cancer-related genes have enormous potential for investigations of possible gene-environment interactions in the etiology of childhood brain tumors, but their use for this purpose has yet to be fully explored. However, promising findings have been reported from experiments on the mouse p53 and ptc1 genes.

Gene-gene interactions

The mouse gene *ptc1* appears to function in a very similar manner to the homologous human gene, *PTCH*, which is known to act as a tumor suppressor gene and is inactivated by mutation in a subset of human medulloblastomas. *Sonic hedgehog*, a homologue of the Drosophila segment polarity

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Group	Tumor-bearing animals (%)	Medulloblastomas	Other tumors
Controls, $ptc^{+/+}$	0	0	0
	1(3)	0	1
Controls, <i>ptc</i> ^{+/-}	15(50)	2	13
	5(19)	2	3
3 Gy at age 3 months, $ptc^{+/+}$	6(14)	0	6
3 Gy at age 3 months, $ptc^{+/-}$	21(51)	0	22
3 Gy at age 4 days, $ptc^{+/+}$	14(30)	0	14
3 Gy at age 4 days, $ptc^{+/-}$	38(75)	26	17

Table 4. Medulloblastomas and other tumors in *ptc* knockout mice subjected to 3 Gy X-irradiation at age 4 days and at age 3 months.⁵⁵⁾

gene *hedgehog*, is expressed in vertebrate tissues that are known to be inductive centers for embryonal patterning.⁴⁸⁾ Its protein product acts through a receptor that is encoded by the gene *patched* (human homolog, *PTCH*; mouse homolog, *ptc1*).⁴⁹⁾ Inactivation of one allele of *ptc1* in mice by genetic methods results in a 7–14% incidence of GEM medulloblastomas in the *ptc*^{+/-} heterozygous mice.

GEM medulloblastoma incidence is dramatically increased in $ptc1^{+/-}$ mice that have been genetically modified to lack functional p53. Such $ptc1^{+/-}$ p53^{-/-} mice suffer a GEM medulloblastoma incidence of more than 95 per cent, and the tumors occur very early in life, when the animals are less than 12 weeks of age.⁵⁰⁾ The importance of p53 for GEM medulloblastoma induction in mice is emphasized by other genetic constructs in p53-null mice that also develop GEM medulloblastomas. For example, somatic inactivation of Rb by complex genetic constructs in p53-null mice produced genetically engineered mice which developed GEM medulloblastomas as early as 7 weeks of age.⁵¹⁾ Also, null mutation of poly(ADP-ribose) polymerase, or PARP, causes a high incidence (49 per cent) of aggressive GEM medullo-blastomas in $p53^{-/-}$ mice. In these double knock-out mice, lesions on the outer surface of the cerebellum that eventually develop into overt GEM medulloblastomas are distinguishable as early as 8 weeks of age.52)

Likewise, mice in which one allele of the *Nf1* locus and one allele of the linked p53 gene have been inactivated develop both central and peripheral nervous system tumors that are very similar to the nerve and brain tumors that occur in human neurofibromatosis type 1 disease.^{53,54)}

Gene-environment interactions

Neonatal X-irradiation (3 Gy) significantly increased the incidence of medulloblastomas in $ptc^{+/-}$ heterozygous mice to approximately 50%, but irradiation of $ptc1^{+/-}$ adults had no effect on GEM medulloblastoma incidence⁵⁵⁾ (Table 4).

The dramatically higher susceptibility of newborn $ptc1^{+/-}$ mice to X-rays is consistent with earlier findings of increased early-life susceptibility to direct-acting carcinogens in conventional mice⁵⁷⁾ and rats.¹⁵⁾

Transplacental carcinogenesis in the nervous system has been studied in the offspring of $p53^{+/-}$ mice given ENU during pregnancy. This treatment resulted in the rapid development of primary brain and nerve tumors (11 cerebral glioblastomas, 1 cerebellar medulloblastoma, and 1 malignant trigeminal schwannoma) in 70 per cent of the $p53^{-/-}$ offspring (n = 17), and the first of these tumors killed the host at only 8 weeks of age. Brain and nerve tumors (2 cerebral glioblastomas, 10 malignant schwannomas) also developed later in life in 12/55 of the $p53^{+/-}$ offspring, but the wild-type allele had been lost in these tumors. No neurogenic tumors developed in 37 ENU - exposed $p53^{+/+}$ offspring, or in untreated controls (total, 59) of any of the three genotypes.⁵⁶⁾ In contrast, in a large series of transplacental carcinogenicity studies in conventional mice of various strains, 27 tumors of the brain and meninges were observed in 1,652 mouse offspring, an incidence of only 1.6 per cent; 98 malignant schwannomas were also observed, originating mostly from cranial nerves.⁵⁷⁾ It can be inferred from these studies that the tumor suppressor gene p53 is important in controlling expression of the neoplastic phenotype in mouse brain cells, and that loss of p53 increases incidence and reduces latency of brain tumors induced transplacentally by ENU in mice.

CONCLUSIONS

The various neurogenic tumors in humans in general and in children in particular are characterized by different genetic abnormalities. There is no such thing as a "brain tumor oncogene" or "brain tumor suppressor gene," as different genes are increasingly being shown to play crucial roles in the development of specific neoplasms. The human genes that have been found to be crucial to development of specific pediatric neurogenic tumor types all appear to have tumor suppressor functions, including *RB1*, *TP53*, *PTCH*, *NF1*, *NF2*, *TSC1* and *TSC2*, and are inactivated in tumors by deletion, truncation, or point mutations.

Genetic engineering procedures have been used to investigate the consequences of deletion or mutation of the murine homologues of a number of these genes. Genetically engineered mice carrying some of these altered genes develop genetically engineered murine (GEM) tumors that strikingly resemble the corresponding human cancer, histologically, anatomically, and with respect to occurrence before sexual maturity. These GEM tumors sometimes arise with extraordinarily short latencies, in contrast to those that occur in conventional animals exposed transplacentally to chemical carcinogens. For medulloblastoma there is a satisfactory mouse model of the tumor, created by genetically engineering the inactivation of two medulloblastoma-associated tumor suppressor genes. The $ptc1^{+/-} p53^{-/-}$ mouse provides perhaps the most convincing and satisfactory genetically engineered animal model of a human pediatric nervous system tumor. GEM medulloblastomas in $ptc1^{+/-} p53^{-/-}$ double knockout mice closely resemble the corresponding human neoplasms in morphology, anatomic location, and latency. Moreover the synergistic effect of knockouts in both ptc1 and p53 is consistent with human experience in that medulloblastoma is one of the many kinds of human brain tumor in which germ-line TP53 mutations play a role in pathogenesis.⁴¹⁾

The tumor suppressor gene TP53 is the most frequently mutated gene in human tumors, including many neurogenic tumors, and it is reasonable to predict a significant role for this gene in other species also. The best evidence in mice is indirect and comes from carcinogenicity studies in p53 knockout mice, which are more highly susceptible to DNAreactive carcinogens than conventional mice with wild-type p53.56) Identification of mutant TP53 sequences in human tumors is relatively straightforward, as no TP53 pseudogenes exist in the human genome. In contrast, characterization of p53 in naturally occurring and chemically induced tumors in conventional mice and rats has been difficult because of the presence of pseudogenes in rat and mouse genomes, one in the mouse⁵⁸⁾ and several in the rat.⁵⁹⁾ Pseudogenes must be avoided during PCR amplification to avoid mistaking pseudogene sequences for genuine mutations. The earlier experimental literature is flawed, and much confusion has resulted from failure to recognize the existence and significance of the rodent p53 pseudogenes. Reports of p53 mutations in rodent tumors-especially multiple mutations and the same mutation in several tumorsmust be interpreted with caution.

In marked contrast to the successes with p53 and ptc1, retinoblastoma not only does not occur naturally in experimental rodent species but deletion or inactivation of the mouse

Rb gene by genetic engineering does not cause retinoblastomas in mice. Homozygous deletions or missense mutations of the retinoblastoma tumor suppressor gene, RB1, give rise specifically to retinoblastoma and contribute to the pathogenesis of certain mesenchymal tumors in children³¹⁾ but this same genetic defect in genetically modified mice causes an entirely different spectrum of congenital defects and does not lead to GEM retinoblastoma or any other kind of tumor of the eve.^{60,61} Similarly, germline mutations in the *TSC*² locus predispose to subependymal giant cell astrocytomas in humans, but the Eker rat which has an abnormality in the homologous tsc2 gene is predisposed to renal epithelial tumors and does not develop brain tumors. Germline mutations in Nf2 likewise predispose mice to tumor development, but the tumors are sarcomas, lymphomas, and epithelial tumors of the lung and liver, not schwannomas, meningiomas and ependymomas. It appears not really possible to have a "true" mouse model of tumors such as the RB1 retinoblastoma, or of the neurofibromatosis type 2 or tuberous sclerosis syndromes, as the natural genetic pathways to these conditions in humans simply do not operate in mice. Such interspecies differences in genetic pathways to the various kinds of neurogenic tumors complicate the design of etiologic rodent models for human neurogenic tumors of childhood.

Transforming genes, or oncogenes, of either human or viral origin have not been shown to play a major role in neurogenic tumor causation in either children or adults. Conversely, the only cellular oncogene that has been found to control neoplastic transformation in rodent nervous tissues in vivo appears to play no role in human neurogenic tumor development. Malignant schwannomas of the peripheral nervous system are readily induced in rodents by transplacental administration of carcinogenic alkylating agents, and arise by a molecular pathway which is unique to this kind of tumor. This pathway involves a specific point mutation in the transforming gene, neu.¹⁸⁻²¹) This mutation can not occur as a single point mutation in humans because the DNA base sequence of the homologous human gene HER2 is different from the rodent sequences, and two adjacent single-base mutations would be required to effect the same mutation in HER2.²¹⁾

The sources of most mutations in suppressor genes in adult and pediatric neurogenic tumors are not known. The one known agent that induces brain tumors, nerve sheath tumors, and meningiomas in humans is ionizing radiation,²⁷⁾ which is a potent mutagenic and clastogenic agent. As neonatal X-radiation is synergistic with the genotype in causation of medulloblastomas in neonatal $ptc 1^{+/-}$ knockout mice,⁵⁵⁾ an even more marked synergism might be predicted in these knockout mice following prenatal exposures to X-ray or chemical carcinogens, and perhaps in other knockout genotypes as well. Double knockout $p53^{-/-}$ offspring of $p53^{+/-}$ mice given ENU during pregnancy develop primary brain

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tumors in high incidence and with remarkably short latencies. Double knockouts with $p53^{+/-}$ would be especially interesting for further studies with perinatally administered carcinogens in view of the dramatically increased susceptibility to GEM medulloblastomas and other GEM brain tumors that results from loss of p53 and a second suppressor gene. No prenatal X-irradiation or transplacental chemical carcinogenesis studies have yet been reported on $ptc1^{+/-}$, $ptc1^{+/-}p53^{+/-}$ or $ptc1^{+/-}p53^{-/-}$ mice, or in single-gene knockout mice lacking genes other than ptc1 and p53. Gene-environment interactions urgently need to be studied in all the available knockout mice.

Defective alleles of tumor-related genes clearly are inherited, and do pose a genetic risk to the future of offspring of carriers of these alleles. The roles of these genes in causation of pediatric brain tumors, and the consequences of genegene and gene-environment interactions, are rapidly becoming better understood through experimental studies in genetically engineered mice.

REFERENCES

- Chow, W. H., Linet, M. S., Liff, J. M., Greenberg, R. S. (1996) Cancers in children. In Cancer Epidemiology and Prevention, 2nd Edition, Eds. D. Schottenfeld and J. F. Fraumeni, Jr., pp. 1331–1369 Oxford University, Press, New York.
- 2. Kleihues, P., Cavenee, W. K., Eds. (2000) Pathology and Genetics of Tumours of the Nervous System, World Health Organization Classification of Tumours, International Agency for Research on Cancer, Lyon.
- Tomatis, L., Mohr, U., Eds. (1973) Transplacental Carcinogenesis, IARC Scientific Publications No. 4, International Agency for Research on Cancer, Lyon.
- Rice, J. M., Ed. (1979) Perinatal Carcinogenesis. NCI Monograph No. 51, National Cancer Institute, U. S. Government Printing Office, Washington, DC.
- Napalkov, N. P., Rice, J. M., Tomatis, L., Yamasaki, H., Eds. (1989) Perinatal and Multigeneration Carcinogenesis. IARC Scientific Publications No. 96. International Agency for Research on Cancer, Lyon.
- 6. Rice, J. M. (2004) Causation of nervous system tumors in children: insights from traditional and genetically engineered animal models. Toxicol. Appl. Pharmacol. **199**: 175–191.
- Rice, J. M., Wilbourn, J. D. (2000) Tumors of the nervous system in carcinogenic hazard identification. Toxicol. Pathol. 28: 202–214.
- 8. IARC (2003) Predictive value of Rodent Forestomach and Gastric Neuroendocrine Tumours in Evaluating Carcinogenic Risks to Humans. International Agency for Research on Cancer, Lyon.
- Druckrey, H., Ivankovic, S., Preussmann, R. (1966) Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. Nature 210: 1378– 1379.
- 10. Rice, J. M. (1973) An overview of transplacental chemical carcinogenesis. Teratology 8: 113–126.

- 11. Rice, J. M. (1979) Perinatal period and pregnancy: intervals of high risk for chemical carcinogens. Environ. Health Perspect. **29**: 23–27.
- Rice, J. M. (1981) Effects of prenatal exposure to chemical carcinogens and methods for their detection. In Developmental Toxicology, Eds. C. A. Kimmel and J. Buelke-Sam, Chapter 11, pp191–212 Raven Press, New York.
- Anderson, L. M., Diwan, B. A., Fear, N. T., Roman, E. (2000) Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ. Health Perspect. 108, Suppl. 3: 573–594.
- Rice, J. M., Ward, J. M. (1988) Schwannomas (induced), cranial, spinal, and peripheral nerves, rat. In ILSI Monographs on Pathology of Laboratory Animals. Nervous System, Eds. T. C. Jones, U. Mohr and R. D. Hunt, pp154–160 Springer-Verlag, Berlin, Heidelberg.
- Ivankovic, S., Druckrey, H. (1968) Transplacental induction of malignant tumors of the nervous system. I. Ethylnitrosourea in BD-IX rats [in German]. Z. Krebsforsch. 71: 320– 360.
- 16. Rice, J. M., Rehm, S., Donovan, P. J., Perantoni, A. O. (1989) Comparative transplacental carcinogenesis by directly acting and metabolism dependent alkylating agents in rodents and nonhuman primates. In Perinatal and Multigeneration Carcinogenesis, Eds. N. P. Napalkov, J. M. Rice, L. Tomatis and H. Yamasaki, IARC Scientific Publications No. 96, pp17–34 International Agency for Research on Cancer, Lyon.
- Druckrey, H., Schagen, B., Ivankovic, S. (1970) Induction of neurogenic malignancies by single administration of ethylnitrosourea (ENU) to newborn and young BD IX rats [in German]. Z. Krebsforsch. 74: 141–161.
- Perantoni, A. O., Rice, J. M., Reed, C. D., Watatani, M., Wenk, M. L. (1987) Activated *neu* oncogene sequences in primary tumors of the peripheral nervous system induced in rats by transplacental exposure to ethylnitrosourea. Proc. Natl. Acad. Sci. USA 84: 6317–6321.
- Buzard, G. S., Enomoto, T., Hongyo, T., Perantoni, A. P., Diwan, B. A., Devor, D. E., Reed, C. D., Dove, L. F., Rice, J. M. (1999) *neu* Mutation in schwannomas induced transplacentally in Syrian golden hamsters by *N*-nitrosoethylurea: high incidence but low allelic representation. J. Cancer Res. Clin. Oncol. **125**: 529–540.
- Buzard, G. S., Enomoto, T., Anderson, L. M., Perantoni, A. O., Devor, D. E., Rice, J. M. (1999) Activation of *neu* by missense point mutation in the transmembrane domain in schwannomas induced in C3H/HeNCr mice by transplacental exposure to *N*-nitrosoethylurea. J. Cancer Res. Clin. Oncol. 125: 653–659.
- Perantoni, A. O., Rice, J. M. (1999) Mutation patterns in nonras oncogenes and tumour suppressor genes in experimentally induced tumours. In The Use of Short- and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evaluation, IARC Scientific Publications No. 146, Eds. D. B. McGregor, J. M. Rice and S. Venitt, pp88– 122 International Agency for Research on Cancer, Lyon.
- 22. Dibb, N. J., Dilworth, S. M., Mol, C. D. (2004) Switching on kinases: oncogenic activation of BRAF and the PDGFR fam-

ily. Nat. Rev. Cancer 4: 718–727.

- Shigematsu, H., Takahashi, T., Nomura, M., Majmudar, K., Suzuki, M., Lee, H., Wistuba, I. I., Fong, K. M., Toyooka, S., Shimizu, N., Fujisawa, T., Minna, J. D., Gazdar, A. F. (2005) Somatic mutations of the *HER2* kinase domain in lung adenocarcinoma. Cancer Res. 65: 1642–1646.
- Brustle, O., Ohgaki, H., Schmitt, H. P., Walter, G. F., Ostertag, H., Kleihues, P. (1992) Primitive neuroectodermal tumors after prophylactic central nervous system irradiation in children. Association with an activated K-ras gene. Cancer 69: 2385–2392.
- Cavenee, W. K., Furnari, F. B., Nagane, M., Huang, H. J. S., Newcomb, E. W., Bigner, D. D., Weller, M., Berens, M. E., Plate, K. H., Israel, M. A., Noble, M. D., Kleihues, P. (2000) Diffusely infiltrating astrocytomas. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp10–21 IARC Press, International Agency for Research on Cancer, Lyon.
- Neglia, J. P., Meadows, A. T., Robison, L. L., Kim, T. H., Newton, W. A., Ruymann, F. B., Sather, H. N., Hammond, J. D. (1991) Second neoplasms after acute lymphoblastic leukemia in childhood. New Engl. J. Med. **325**: 1330–1336.
- Ron, E., Modan, B., Boice, J. D. Jr, Alfandary, E., Stovall, M., Chetrit, A., Katz, L. (1988) Tumors of the brain and nervous system after radiotherapy in childhood. N. Engl. J. Med. 319: 1033–1039.
- Kleinschmidt, D., Lillehei, K. O. (1995) Radiation-induced meningioma with a 63 year latency period. Case report. J. Neurosurg. 82: 487–488.
- Harrison, M. J., Wolfe, D. E., Lau, T. S., Mitnick, R. J., Sachdev, D. P. (1991) Radiation-induced meningiomas: experience at the Mount Sinai Hospital and review of the literature. J. Neurosurg. **75**: 564–574.
- Boffetta, P., Tredaniel, J., Greco, A. (2000) Risk of childhood cancer and adult lung cancer after childhood exposure to passive smoke: A meta-analysis. Environ. Health Perspect. 108: 73–82.
- Wong, F. L., Boice, J. D. Jr., Abramson, D. H., Tarone, R. E., Kleinerman, R. A., Stovall, M., Goldman, M. B., Seddon, J. M., Tarbell, N., Fraumeni, J. F. Jr., Li, F. P. (1997) Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. J. Am. Med. Assoc. 278: 1262–1267.
- Knudson, A. G. (1971) Mutation and cancer: Statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA 68: 820–823.
- Knudson, A. G., Hethcote, H. W., Brown, B. W. (1975) Mutation and childhood cancer: A probabilistic model for the incidence of retinoblastoma. Proc. Natl. Acad. Sci. USA 72: 5116–5120.
- Smith, B. J., O'Brien, J. M. (1996) The genetics of retinoblastoma and current diagnostic testing. J. Pediatr. Ophthalmol. Strabismus 33: 120–123.
- Louis, D. N., Scheithauer, B. W., Budka, H., von Deimling, A., Kepes, J. J. (2000) Meningiomas. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp176–184 IARC Press, Lyon.
- Louis, D. N., Stemmer-Rachamimov, A. O., Wiestler, O. D. (2000) Neurofibromatosis type II. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W.

K. Cavenee, pp219-222 IARC Press, Lyon.

- Tohma, Y., Gratas, C., Biernat, W., Peraud, A., Fukuda, M., Yonekawa, Y., Kleihues, P. and Ohgaki, H. (1998) PTEN (MMAC1) mutations are frequent in primary glioblastomas (de novo) but not in secondary glioblastomas. J. Neuropathol. Exptl. Neurol. 57: 684–689.
- Kleihues, P., Davis, R. L., Ohgaki, H., Burges, P. C., Westphal, M. M., Cavenee, W. K. (2000) Diffuse astrocytoma. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp22–26 IARC Press, Lyon.
- Wolter, M., Reifenberger, J., Sommer, C., Ruzicka, T., Reifenberger, G. (1997) Mutations in the human homologue of the Drosophila segment polarity gene patched (*PTCH*) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res. 57: 2581–2585.
- Huang, H., Mahler-Araujo, B. M., Sankila, A., Chimelli, L., Yonekawa, Y., Kleihues, P., Ohgaki, H. (2000) APC mutations in sporadic medulloblastomas. Am. J. Pathol. 156: 433– 437.
- Ohgaki, H., Vital, A., Kleihues, P., Hainaut, P. (2000) Li-Fraumeni syndrome and *TP53* germline mutations. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp231–234 IARC Press, Lyon.
- von Deimling, A., Foster, R., Krone, W. (2000) Neurofibromatosis type I. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp 216–218 IARC Press, Lyon.
- Reifenberger, G., Wiestler, O. D., Chenevix-Trench, G. (2000) Naevoid basal cell carcinoma syndrome. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp240–241 IARC Press, Lyon.
- Cavenee, W. K., Burger, P. C., van Meier, E. G. (2000) Turcot syndrome. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp238– 239 IARC Press, Lyon.
- Wiestler, O. D., Lopes, B. S., Green, A. J., Vinters, H. V. (2000) Tuberous sclerosis complex and subependymal giant cell astrocytoma. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp227–230 IARC Press, Lyon.
- 46. Wiestler, O. D., Padberg, G. W., Steck, P. A. (2000) Cowden disease and dysplastic gangliocytoma of the cerebellum / Lhermitte-Duclos disease. In Pathology and Genetics of Tumours of the Nervous System, Eds. P. Kleihues and W. K. Cavenee, pp235–237 IARC Press, Lyon.
- Weiss, W. A., Israel, M., Cobbs, C., Holland, E., James, C. D., Louis, D. N., Marks, C., McClatchey, A. I., Roberts, T., Van Dyke, T., Wetmore, C., Chiu, I. M., Giovannini, M., Guha, A., Higgins, R. J., Marino, S., Radovanovic, I., Reilly, K., Aldape, K. (2002) Neuropathology of genetically engineered mice: consensus report and recommendations from an international forum. Oncogene 21: 7453–7463.
- Marigo, V., Laufer, E., Nelson, C. E., Riddle, R. D., Johnson, R. L., Tabin, C. (1996) Sonic hedgehog regulates patterning in early embryos. Biochem. Soc. Symp. 62: 51–60.
- 49. Stone, D. M., Hynes, M., Armanini, M., Swanson, T. A., Gu,

Q., Johnson, R. L., Scott, M. P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J. E., de Sauvage, F., Rosenthal, A. (1996) The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. Nature **384**: 129–134.

- Wetmore, C., Eberhart, D. E., Curran, T. (2001) Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. Cancer Res. 61: 513–516.
- Marino, S., Vooijs, M., van Der Gulden, H., Jonkers, J., Berns, A. (2000) Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. Genes Dev. 14: 994– 1004.
- Tong, W. M., Ohgaki, H., Huang, H., Granier, C., Kleihues, P., Wang, Z. Q. (2003) Null mutation of DNA strand breakbinding molecule poly(ADP-ribose) polymerase causes meduloblastomas in p53(–/–) mice. Am. J. Pathol. 162: 343–352.
- Reilly, K. M., Loisel, D. A., Bronson, R. T., McLaughlin, M. E., Jacks, T. (2000) Nf1; Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat. Genet. 26: 109–113.
- Cichowski, K., Shih, T. S., Schmitt, E., Santiago, S., Reilly, K., McLaughlin, M. E., Bronson, R. T., Jacks, T. (1999) Mouse models of tumor development in neurofibromatosis type 1. Science 286: 2172–2176.
- Pazzaglia, S., Mancuso, M., Atkinson, M. J., Tanori, M., Rebessi, S., Majo, V. D., Covelli, V., Hahn, H., Saran, A. (2002) High incidence of medulloblastoma following X-ray–

irradiation of newborn Ptc1 heterozygous mice. Oncogene **21**: 7580–7584.

- Oda, H., Zhang, S., Tsurutani, N., Shimizu, S., Nakatsuru, Y., Aizawa, S., Ishikawa, T. (1997) Loss of p53 is an early event in induction of brain tumors in mice by transplacental carcinogen exposure. Cancer Res. 57: 646–650.
- Wechsler, W., Rice, J. M., Vesselinovitch, S. D. (1979) Transplacental and neonatal induction of neurogenic tumors in mice: Comparison with related species and with human pediatric neoplasms. Natl. Cancer Inst. Monogr. 51: 219–226.
- Zakut-Houri, R., Oren, M., Bienz, B., Lavie, V., Hazum, S., Givol, D. (1983) A single gene and a pseudogene for the cellular tumour antigen *p53*. Nature **306**: 594–597.
- 59. Weghorst, C. M., Buzard, G. S., Calvert, R. J., Hulla, J. E., Rice, J. M. (1995) Cloning and sequence of a processed *p53* pseudogene from rat: a potential source of false 'mutations' in PCR fragments of tumor DNA. Gene **166**: 317–322.
- Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A., Weinberg, R. A. (1992) Effects of an Rb mutation in the mouse. Nature **359**: 295–300.
- Lee, E. Y., Chang, C. Y., Hu, N., Wang, Y. C., Lai, C. C., Herrup, K., Lee, W. H., Bradley, A. (1992) Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature **359**: 288–294.

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