Morphological studies of rat brain tumors induced by N-nitrosomethylurea

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Experimental glial tumors were induced by weekly intravenous injections of N-nitrosomethylurea (NNMU) in rats. The tumors included low- and high-grade gliomas of the astrocytic series and mixed gliomas. The histology of the tumors did not vary significantly with serial passage through tissue culture, subcutaneous implantation, or freezing. These neoplasms provide reliable animal models of brain tumors common to man.

Key Words: brain tumors, experimental · N-nitrosomethylurea · astrocytoma · glioma · rat tumors

Up to the present, the most commonly used experimental glial brain tumor has been the ependymoblastoma. However, this is a rare tumor in man constituting under 1% of intracranial tumors. For purposes of studying the biological behavior of brain tumors and evaluating various therapeutic protocols, it would be useful to have stable glial tumor models that are common to man.

Experimental primary intracranial tumors have been induced in animals with a variety of agents including carcinogenic hydrocarbon pellets, adenovirus, Elaionycin, and bisacetylamino-fluorine. Particularly high yields of brain tumors have been reported by Druckrey, et al., using intravenous injections of N-nitrosomethylurea into either pregnant or nonpregnant adult rats. He found that the time-dose relationship was an important factor in the induction of tumors while strains of rat or sex were less important factors. In this study, we report further work begun in this laboratory by Benda, et al., on the successful passage of glial tumors induced according to Druckrey’s method in both tissue culture and serial animal passage. The tumors induced include both low- and high-grade glial malignancies of the astrocytic series.

Method

A total of 108 male, 12-week-old rats weighing 100 to 125 gm (60 Wistar rats, 48 CD Fisher rats) were confined two in a cage and fed Purina Lab Rat Chow and water ad libitum. A solution was made up of 125 mg of crystalline N-nitrosomethylurea (NNMU)* dissolved in 25 ml of distilled sterile water at room temperature, and each rat was given 5 mg/kg weekly for 36 weeks by tail vein injection under ether anesthesia. Dosages were based on weekly weighings prior to administration. After the 36-week period, the animals were sacrificed either when neurological signs (paralysis, circling movements, ataxia, convulsions) or poor

* Supplied by Columbia Organic Chemicals Company, Columbia, South Carolina.
general health supervened (lethargy, cachexia). At autopsy, a search was made for other primary tumors. Brains were examined grossly and by light microscopy. Histological stains used were hematoxylin and eosin (H & E) on all sections, phosphotungstic acid hematoxylin (PTAH) to demonstrate glial fibrils, and Van Giesson and Mallory trichrome stains for collagen. Further steps in this experiment are summarized in Fig. 1.

Brain tumors were placed in primary culture according to the method of Benda, et al. Cells that grew out were implanted intracerebrally or subcutaneously into the flank of either newborn Wistar or CDF rats (1 to 7 days old) of the same strain as the parent rat. When a tumor mass became palpable, it was excised and divided for histological study, tissue culture, and reimplantation subcutaneously in other newborn or young rats, or frozen for later use (Fig. 1). The tissue to be frozen was prepared by homogenization in a 10 Broeck model Pyrex glass tissue grinder in a mixture of equal parts by weight of tumor and 20% solution of glycerine in normal saline. Homogenization was continued until a fine suspension was obtained, usually within 1 to 2 min. The homogenate was removed with a sterile syringe and 22-gauge needle and placed in a Will scientific apparatus glass ampoule, which was then heat-sealed. The ampoule was then wrapped in absorbent cotton to prevent quick freezing and then placed into a low-temperature deep freeze set at -70°C.

Frozen cells in ampoules were thawed rapidly in an agitated water bath at 37°C. Thawed cells were rapidly implanted via needle and syringe into subcutaneous tissue of newborn or young rats.

Direct subcutaneous transplantation of tumor to other rats was done by excising the tumor, weighing it, homogenizing it, and re-injecting 0.2 ml of a suspension with a concentration of 5 cc normal saline per gram of tissue. Within 2 to 4 weeks, 90% of the subcutaneous implants yielded palpable tumors.

Results

Of a total of 108 Wistar and CD Fisher (CDF) male rats, 58 survived the 36 weekly injections. Autopsy showed primary brain tumors in 22 of the animals. Only 11 of these tumors, 4 Wistar and 7 CDF, were successfully propagated in both subcutaneous tissue and tissue culture and maintained over at least two generations.

These 11 tumors developed between 17 and 43 weeks after termination of NNMU injections, with a mean of 24 weeks (Table 1). They were: three low-grade astrocytomas, stable over at least six passages; four high-grade astrocytomas, stable over a number of generations varying from 2 to 14; two spindle cell tumors, stable for 2 to 4 passages; and two mixed gliomas one of which evolved into a high-grade astrocytoma, which was then stable. A plasmacytoma arose at the site where the other mixed glioma (Wistar Rat 2) was injected into the animal. The plasmacytoma has been stable for 19 generations.

Histopathology

Low-Grade Astrocytoma (Tumors 13, 15, 16). Two primary tumors (Tumors 13 and 16) showed a similar histological pattern. The neoplasms were cellular, uniform, and with indistinct cytoplasmic borders. The nuclei were round to oval, with little variation
Rat brain tumors induced by N-nitrosomethylurea

TABLE 1
Data on 11 primary tumors grown in rats

<table>
<thead>
<tr>
<th>Tumor No.</th>
<th>Tumor Type</th>
<th>Time From End of Injections to Tumor (wks)</th>
<th>Final Type of Tumor Cell Lines</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>astrocytoma grade 3-4</td>
<td>31</td>
<td>same lines 1 A–D</td>
</tr>
<tr>
<td>2</td>
<td>mixed glioma</td>
<td>12</td>
<td>line 2A: plasmacytoma</td>
</tr>
<tr>
<td>3</td>
<td>astrocytoma grade 3-4</td>
<td>17</td>
<td>line 2B: malignant spindle-cell tumor same</td>
</tr>
<tr>
<td>6</td>
<td>astrocytoma grade 3-4</td>
<td>17</td>
<td>same astrocytoma grade 3-4</td>
</tr>
<tr>
<td>9</td>
<td>astrocytoma grade 3-4</td>
<td>17</td>
<td>same</td>
</tr>
<tr>
<td>12</td>
<td>astrocytoma grade 3-4</td>
<td>17</td>
<td>same</td>
</tr>
<tr>
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<td>same</td>
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<td>same</td>
</tr>
<tr>
<td>16</td>
<td>astrocytoma grade 3-4</td>
<td>17</td>
<td>same</td>
</tr>
<tr>
<td>17</td>
<td>astrocytoma grade 3-4</td>
<td>17</td>
<td>same</td>
</tr>
<tr>
<td></td>
<td>Av. 24 wks</td>
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in size or shape, with homogeneous diffuse chromatin. Mitoses were rare. The cells were arranged in sheets in most parts of the tumor but in some areas tended to line up in a fascicular pattern giving the appearance of interdigitating bundles of spindle cells. The interstitium was finely fibrillar with only an occasional PTAH-positive fibril, and lacked collagen histochemically as well as by polarization (Fig. 2 left). Endothelial proliferation was not prominent, and necrosis was not seen. Three cell lines were established from these two tumors, and they all remained stable histologically for the six generations studied. The third tumor (Tumor 15) had sheets of round to ovoid cells with uniform nuclei set in a fibrillar background. Mitotic figures were rare. The most striking feature

Fig. 2. Low-grade astrocytomas. Left: Tumor 13, showing uniform spindle cells and fine fibrillar background. H & E, ×290. Right: Tumor 15, showing palisading about vessel. H & E, ×390.
of this tumor was the marked tendency of the cells to palisade about vessels (Fig. 2 right). No rosettes, ependymal-lined canals or tubules, or blepharoplasts were seen. This tumor remained stable for the two generations studied.

High-Grade Astrocytoma (Tumors 1, 3, 9, 17). These tumors were extremely cellular with moderate pleomorphism and nuclei varying in shape from oval to round with a diffuse chromatin pattern and rare prominent basophilic nucleoli. The interstitium was finely fibrillar and composed of glial fibrils. Numerous mitoses were present, a few of which were atypical. Stellate areas of necrosis were bordered by palisading tumor cells (Fig. 3). There was also prominent palisading of cells about vessels. Increased vascularity and marked endothelial proliferation were also present.

Four cell lines (1 A, B, C, D) were established from Tumor 1. Two of these (1 A, B) remained stable over four and five passages respectively; the third (1 C) became necrotic in subcutaneous tissue and grew poorly; the fourth (1 D), after the sixth passage, developed into a tumor characterized by the predominance of very large (50 μ) tumor giant cells with bizarre nuclear patterns typical of a glioblastoma multiforme (Fig. 4). Tumors 3, 9, and 17 gave rise to a total of eight cell lines, each of which remained stable over several (up to 14) passages. However, none showed the remarkable development of giant cells exhibited by Tumor 1.

Mixed Glioma (Tumors 2 and 6). Tumor 6 had two coexisting, different-blending histological patterns. The predominant pattern was that of numerous elongated-to-ovoid cells with spindle-shaped nuclei and indistinct cytoplasm, with a glial background. Mitoses were infrequent, and endothelial proliferation was not seen. Palisading of cells about the vessels was noted. No collagen was present. The other pattern was that of closely packed, uniform round cells with distinct cytoplasmic borders, clear to eosinophilic cytoplasm, and round nuclei. Mitoses were infrequent. No necrosis was evident. The vessels were prominent, but there was no endothelial proliferation. This represented a mixed glioma, predominantly a Grade 2 astrocytoma, with areas suggestive of an oligodendroglioma (Fig. 5).

Two histologically similar cell lines were established from Tumor 6 on passage through tissue culture. By the second genera-

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Fig. 3. High-grade astrocytomas. Left: Tumor 17, showing atypical mitoses, nuclear pleomorphism, and pyknosis. H & E, ×740. Right: Tumor 6, showing palisading about necrotic focus. H & E, ×390.
Rat brain tumors induced by N-nitrosomethylurea

Fig. 4. High-grade astrocytoma. Tumor 1, showing malignant giant cells and extreme cellular pleomorphism. H & E, ×180.

Fig. 5. Mixed glioma. Tumor 6, showing border between astrocytic elements above and oligodendrocytic below. There is slight overlap between these two cellular components. H & E, ×390.

tion, there was loss of the oligodendroglioma pattern and retention of the astrocytic pattern with moderate cellular pleomorphism, increased mitotic activity, and conspicuous palisading of tumor cells around areas of necrosis. These features remained the same with subsequent passages.

Tumor 2 was also a mixed glioma and had two cell lines, which showed features characteristic of a Grade 3 or 4 astrocytoma for three passages in subcutaneous tissue. During the fourth passage of one cell line, a transplanted necrotic tumor was implanted subcutaneously, and, following this, a plasmacytoma developed at the site of implantation, stable for 19 generations. The other cell line developed into a pleomorphic spindle-cell tumor with abundant collagen.

Spindle-Cell Tumor (Tumors 12 and 14). Tumors 12 and 14 were distinctly nonglial. The cells were pleomorphic spindle-shaped and with frequent atypical mitoses. The background was collagenous, without a whorled pattern. No psammoma bodies or calcification was seen. Small vessels were prominent without endothelial proliferation. Tumor 12 was confined to the meninges and superficial cortex, and may represent a meningiosarcoma. The three cell lines from these two tumors remained stable for the four generations studied.

Conclusions

A spectrum of glial tumors has, therefore, resulted from this work, including low- and high-grade astrocytomas and mixed gliomas. The interval between the termination of injection and the appearance of the tumor did not appear causally related to the type of tumor. With the exception of the mixed gliomas, the histology of the tumors did not vary significantly during serial passage through tissue culture, subcutaneous implantation, or freezing. Both mixed gliomas gave rise to high-grade astrocytomas. We cannot explain the giant-cell change occurring in Tumor 1 since this tumor was handled in the same manner as the others.

These cell lines provide reliable models of a low- and high-grade astrocytoma. Further characterizations of these tumors are being studied in this laboratory by S-100 protein, electron microscopy, intracellular impedance recording, and tissue culture kinetics.
Summary

Primary intracerebral brain tumors were induced in rats using N-nitrosomethylurea. These tumors have been maintained in tissue culture and serial subcutaneous transplants so that stable cell lines of low- and high-grade astrocytomas are now available as experimental models.

Acknowledgments

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References


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