Successful laboratory growth and analysis of CUSA-obtained medulloblastoma samples

Technical note

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The development of the Cavitron ultrasonic surgical aspirator (CUSA) has facilitated neurosurgical intervention for removal of central or peripheral nervous system tumors adjacent to or within vital structures. However, laboratory studies defining the phenotypic and genotypic properties of these tumors, both in cell culture and as xenografts in immunoincompetent animals, require viable tumor fragments free of microbial or red blood cell contamination. This report describes the use of a readily available sterile trap with the CUSA which, in conjunction with centrifugation and ammonium chloride lysis of the bloody aspirate, allowed collection of concentrated viable human medulloblastoma tumor cells. These cells were successfully established in cell culture and as transplantable xenografts in athymic mice.

Key Words: ultrasonic surgical aspirator • medulloblastoma • cell culture • xenograft

The development of the Cavitron ultrasonic surgical aspirator (CUSA),* allowing tissue dissection by fragmentation, has provided a means of enhancing the safety of removing central and peripheral nervous system tumors. However, the consequences of CUSA removal on the viability necessary to allow the in vitro and in vivo growth, establishment in culture, and analysis of tumor cells removed with this technique are unclear. Previous reports have suggested that tumor cells collected in the irrigation fluid of the CUSA are viable and demonstrate short-term growth in cell culture; furthermore, Lewis lung carcinoma tumor cells obtained by CUSA were tumorigenic in C57B1/10 mice. Nevertheless, successful sustained growth and analysis of CUSA-obtained human brain-tumor samples have not been reported and the concern is raised that comprehensive study of these tumors may be adversely affected by use of this technique.

We now report simple modifications of the CUSA and handling of aspirated tumor allowing sterile collection of concentrated human medulloblastoma fragments. The subsequent growth and analysis of these fragments in long-term cell culture and transplantation of xenografts in athymic nude mice are described.

Materials and Methods

Tumor Collection

The sterile collection of tumor fragments was facilitated by interruption of the suction line of a CUSA with a sterile trap, most efficiently accomplished with a sputum collector. Following collection of the fragments, the trap was sealed, placed on ice, and immediately transported to the laboratory. This technique was carried out in four children with posterior fossa tumors while undergoing craniotomy and gross total resection of a medulloblastoma using a CUSA equipped with a sterile trap.

Tumor Fragment Handling

The bloody irrigation fluid containing the tumor fragments was centrifuged at 1000 rpm for 5 minutes, the contaminating red blood cells in the pellet were lysed with 0.83% ammonium chloride (0°C), and the centrifugation/lysis procedure was repeated until a pellet virtually free of red blood cells was obtained. The tissue fragments were mechanically disaggregated into
ally transplantable xenografts derived from human me-
erations, establishing conclusively that they are seri-
both intracranial and subcutaneous sites for 10 gen-
and D425 Med tumors have been serially passaged at
graft formation in the host animals. The D384 Med
medulloblastoma. The D384 Med, D407 Med, and D425
Med tumors grew as highly cellular and invasive neo-
plasms, largely located within the subarachnoid space; D384 Med and D425 Med grew subcutaneously as
highly cellular and invasive undifferentiated tumors.

Chromosomal Analysis
Chromosomal analysis, to be reported separately in
detail, confirmed the human origin of the cell lines in
vitro and of the xenografts in vivo. Furthermore, D384
Med and D425 Med demonstrated double minutes in
both the cell lines and xenografts.

Discussion
The development of the CUSA has greatly facilitated
neurosurgical intervention for removal of central or
peripheral nervous system tumors adjacent to or with-
in vital structures. However, laboratory studies defin-
ing the phenotypic and genotypic properties of these
tumors, both in cell culture and as xenografts in im-
munoincompetent animals, require viable tumor frag-
ments free of microbial or red blood cell contamination.
Our laboratory has successfully established both human
glioma and medulloblastoma continuous cell lines and
transplantable xenografts derived from large tumor
fragments taken from operative material in the routine
manner.3-7 Cell lines and transplantable xenografts
initiated with CUSA-derived human tumor fragments
have not been previously reported. Successful use of
these fragments requires sterile collection of viable tu-
mor cells with atraumatic removal of the blood char-
acteristically present in the CUSA aspirate.

Our use of a readily available sterile sputum trap,
coupled with serial centrifugation and ammonium chlo-
ride-mediated lysis of contaminating red blood cells,
provided a concentrated source of viable tumor cells,
facilitating initiation into cell lines and inoculation into
athymic mice. Studies in progress with these new cell
lines and xenografts, particularly with D384 Med and
D425 Med, are continuing in order to define the biology
and therapeutic profile of medulloblastoma. Application
of the technique reported here may allow labora-
tory growth and analysis of central and peripheral
nervous system tumors resected by the CUSA and is
particularly adaptable to many of the less frequently
occurring childhood brain tumors, for which there is a
great paucity of established lines. Furthermore, use of
this approach to collect viable tissue for subsequent
transplantation (such as into patients with Parkinson’s
disease) may bear investigation.

References
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Manuscript received August 8, 1989.
Accepted in final form November 13, 1989.
This work was supported by Grants NS20023, CA44640, NS00958, CA11898, CA43722, and CA36245 from the National Institutes of Health; Grant CH403 from the American Cancer Society; and Bristol-Myers Grant R18-100.
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