Early Switch in Glial Protein and Fibronectin Markers on Cells during the Culture of Human Gliomas

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A switch between oligodendroglial and astrocytic markers is reported in a glioma cell line.[1] When interpreted collectively, studies of human gliomas *in situ* compared with serially passed and continuous cell lines from other gliomas suggest differences in abundance of glial protein and fibronectin.[2-6] The present study was designed to determine the stability of populations of cells with glial fibrillary acidic protein (GFAP) and cells with fibronectin (FN) after explantation into cell culture.

TABLE 1. Stability of Glial Fibrillary Acidic Protein after Explantation of Cells from Gliomas

Pair	Percentage of GFAP-Positive Cells		Difference in Positive Cells before and after Explantation[a]
	Before Explantation	After Explantation	
1	74	0	<0.001
2	36	11	<0.01
3	25	2	<0.001
4	79	2	<0.001
5	78	6	<0.001
6	69	16	<0.001
7	28	38	Not significant
8	38	13	<0.01
9	60	60	Not significant
10	75	0	<0.001

[a]Percentages of positive cells before and after explantation were compared by chi-square test and $p \leq 0.01$ was considered significant.

Frozen sections of 10 human gliomas and whole mounts of explants of the gliomas were fixed in methanol. They were rinsed in 0.02 M sodium phosphate buffer, pH 7.2 (PBS) and incubated with rabbit anti-GFAP (kindly provided by Dr. Lawrence Eng, Stanford University) diluted 1:100, rinsed in PBS, and incubated with rhodamine-conjugated goat anti-rabbit IgG (Cappel) diluted 1:50.[7] They were then rinsed in PBS

and incubated with fluorescein-conjugated anti-FN (Cappel) diluted 1:50 with PBS after reconstitution,[6] rinsed in PBS, incubated in 1 μg/ml 4',6-diamidino-2-phenylindole (DAPI),[8] rinsed in PBS, and mounted in Gelvatol. Sequential photographs of the same field under different illumination were taken on a Zeiss ICM 405 fluorescent and phase-contrast microscope. Cells positive for GFAP and for fibronectin were counted by hand and related to the total number of cells using fluorescence of nuclei labeled with DAPI and phase contrast as guides. Over 1000 cells were analyzed.

Certain patients varied substantially with respect to the percentage of GFAP-positive cells in their gliomas before and after explantation (TABLE 1). Variation in the percentage of fibronectin-positive cells was present in most of the same pairs (TABLE 2). Comparison of the mean percentage of positive cells in each group suggested two general tendencies. There appeared to be a tendency for the number of GFAP-positive cells to decrease after explantation, since the mean percentage of GFAP-positive cells was 56% before and 15% after explantation. There appeared to be an opposite tendency among fibronectin-positive cells. The mean percentage of fibronectin-positive cells was 18% before and 58% after explantation.

TABLE 2. Stability of Fibronectin after Explantation of Cells from Gliomas

Pair	Percentage of FN-Positive Cells		Difference in Positive Cells before and after Explantation[a]
	Before Explantation	After Explantation	
1	25	100	<0.001
2	18	65	<0.001
3	25	88	<0.001
4	10	48	<0.001
5	22	82	<0.001
6	21	18	Not significant
7	18	23	Not significant
8	2	53	<0.001
9	18	5	Not significant
10	25	95	<0.001

[a]Percentages of positive cells before and after explantation were compared by chi-square test and $p \leq 0.01$ was considered significant.

Individual cases were compared by chi-square test with respect to their percentage of positive cells before and after explantation. Seven pairs were significantly decreased in GFAP-positive cells and increased in fibronectin-positive cells after explantation (TABLES 1 and 2). Pairs 6, 7, and 9 did not show a significant change in one or both of these markers (TABLES 1 and 2).

Pairs 6, 7, and 9 were reexamined. Pair 6 decreased in GFAP-positive cells after explantation. However, it showed few fibronectin-positive cells after explantation. The majority of cells that grew into culture were negative for both GFAP and fibronectin markers. Pairs 7 and 9 showed both a relatively large percentage of GFAP-positive cells and small percentage of fibronectin-positive cells after explantation.

These observations provide evidence for an early switch in abundance of cells with glial fibrillary acidic protein and cells with fibronectin during early passage of human gliomas into cell culture. In the majority of cases in this study, the number of cells with GFAP decreased and the number of cells with fibronectin increased after explantation of cells from gliomas. Not all cases demonstrated this switch in the present study.

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