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Cell kinetic investigations in brain tumors studied by serial stereotactic biopsy

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Summary

Between July 1983 and July 1986, 109 consecutive patients affected by brain tumors and selected for stereotactic biopsy underwent *in vitro* investigation of cell kinetics. The potential proliferative activity of these different neoplasms, evaluated as *in vitro* 3H-thymidine labeling index (LI), has been determined in 46 mature astrocytomas, 19 anaplasic astrocytomas, 25 glioblastomas, 5 cystic craniopharyngiomas, 6 metastatic tumors, 4 primary C.N.S. lymphomas, 3 pineal germinomas and one choroid plexus papilloma. The relevance of LI on prognosis has been evaluated in 35 patients affected by glial tumors with adequate follow-up longer than 2 years. This retrospective study allowed us to demonstrate the prognostic value of LI in mature astrocytoma and anaplasic astrocytoma series. The feasibility of LI determination by *in vitro* procedure has been demonstrated also in non-neuroectodermal tumors. In craniopharyngioma the LI investigations allowed to demonstrate a peculiar topographic arrangement of cells in S phase.

Introduction

The technique of serial stereotactic biopsies is currently utilized to assess the histological nature of deep brain lesions detected by C.T. and N.M.R. examinations. The data obtained by this procedure are utilized to guide to different treatments of brain tumors [1].

In spite of this, the conventional histological examination provides poor prognostic information in patients affected by mature glial tumors and particularly in those planned for conservative strategy (*i.e.* chemotherapy, external radiotherapy, or clinical and neuroradiological surveillance) [2,3].

An approach to this controversial matter may concern the study of cell kinetics as previously suggested by Hoshino et al. which proposed the preoperative administration of intravenous 3H-thymidine in patients submitted to the surgical ablation of brain tumors [4–6]. The same authors recently developed another method for the *in vivo* determination of the potential proliferative activity of surgically resected tumors and obtained significant results by preoperative intravenous administration of bromodeoxyuridine (BudR) and flow cytometric analysis [7].

Aim of this report was to verify the 'in vitro' feasibility of cell kinetic investigations in small fragments of brain tumors obtained by serial stereotactic biopsies and to define the clinical relevance of cell kinetics. Similar investigations have been previously performed in other than C.N.S. tumors [8,9]. This study has been performed by *in vitro* 3H-Thymidine labeling and the fraction of S

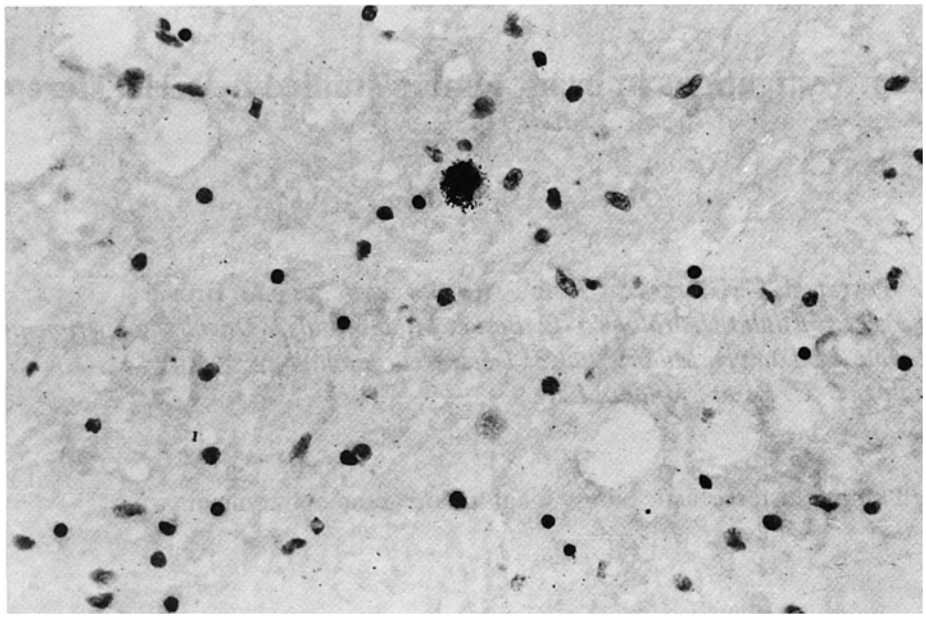

Fig. 1. Histological picture of mature astrocytoma in an autoradiographic image (× 400).

phase cells has been evaluated on serial stereotactic biopsies from neuroectodermal and non-ectodermal brain tumors.

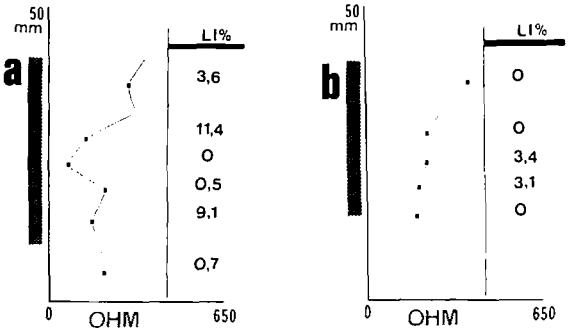

Fig. 2. Relationship between transtumoral impedance profiles [12] and labeling index (LI%) in two different glial tumors. Ordinates: depth from the cortex to the targets along the sterotactic trajectory; the hatched area represents the tumor location as estimated from preoperative neuroradiological assessment. Abscissa: impedance values in Ohms; the targets are indicated by squares on the impedance profile; the vertical line on the right represents the impedance value expected in normal sound nervous tissue. The LI% values obtained in each target are indicated on the right side. a: left frontal glioblastoma; b: left frontal mature astrocytoma. The LI% zero values detected in glioblastoma correspond to the histological finding of regressive intratumoral changes; the LI% zero values detected at the boundaries of mature astrocytoma correspond to edematous tissue free from neoplastic cells infiltration.

Patients and methods

Patients

Between July 1983 and July 1986, 109 consecutive patients affected by brain tumors and selected for stereotactic biopsy underwent *in vitro* investigation of cell kinetics by stereotactic procedure. The age ranged between 4 and 66 years (mean 42 years); 75 patients were males.

Ninety patients were affected by glial tumors at different morphological grading. In 55 patients of this series the glial tumors were located in the hemispheric white matter; in 27 cases the neoplasms were deeply seated within the thalamus and the basal ganglia and in 8 cases within the brainstem.

Of the extrinsic tumors 5 were craniopharyngiomas, 6 metastatic tumors, 4 primitive non-Hodgking lymphomas, 3 pineal germinomas and 1 choroid plexus papilloma of lateral ventricle.

In each tumor the LI was determined in all the tissue samples provided by the sterotactic serial biopsy and the highest LI value detected along the stereotactic transtumoral trajectory was assumed as representative of tumor cell kinetics. The tissue fragments provided by serial stereotactic biopsy include samples obtained in sound tissue, samples

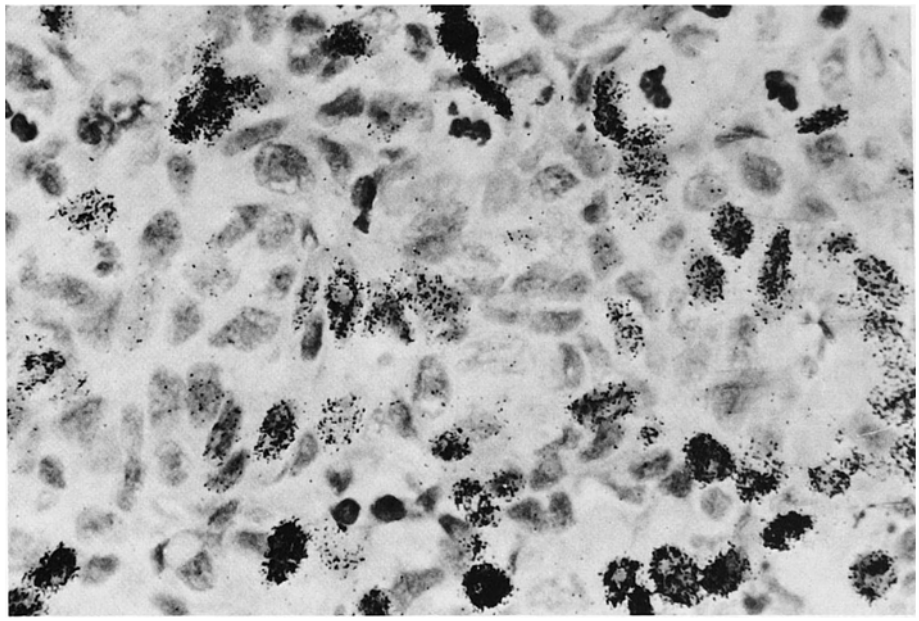

Fig. 3. Autoradiographic histological picture of a glioblastoma (× 640).

obtained in peritumoral potentially infiltrated areas, and samples obtained from true neoplastic tissue. In our experience the average or median LI values resulted misleading because dependent from the number of samples performed outside or at the periphery of the tumor.

LI has been determined in 100 patients, in 9 cases the evaluation was not feasible because major the tissue fragmentation during sampling.

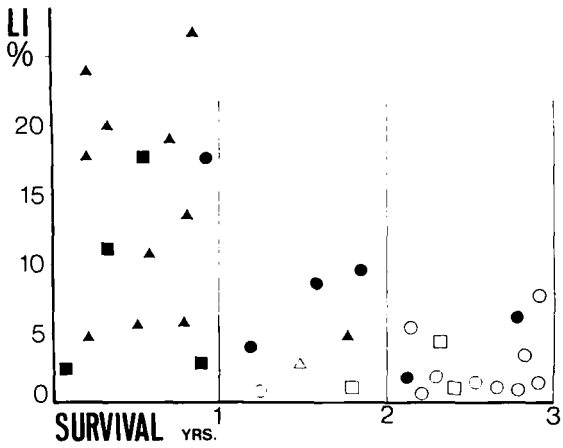

Fig. 4. Graphic representation of the relationship between LI% and histological diagnosis in a consecutive series of 35 patients which underwent only conservative therapy. Triangles: glioblastomas, squares: anaplastic glioma, circles: mature astrocytoma. The filled symbols mean the patient dead at the time of the retrospective study; the empty symbols mean the patients alive.

Stereotactic procedure

The Riechert frame and apparatus have been utilized. Ventriculography has been performed in stereotactic conditions and the C.T. and N.M.R. images of the lesions have been transposed in the stereotactic planes by mathematical method [10]. Stereotactic carotid angiography has been performed in the temporal lesions to avoid conflict with the Sylvian vessels [11]. The electrical impedance has been monitored along the full transtumoral trajectory to reveal necrotic areas, cysts and major peritumoral edema [12,13]. The depth EEG recording has been performed along the trajectory in presence of poorly defined lesions in order to localize the more lesional areas [14,15]. The Sedan bioptic instrument has been utilized to obtain tissue specimens from multiple different targets within the peritumoral areas and the estimated tumoral

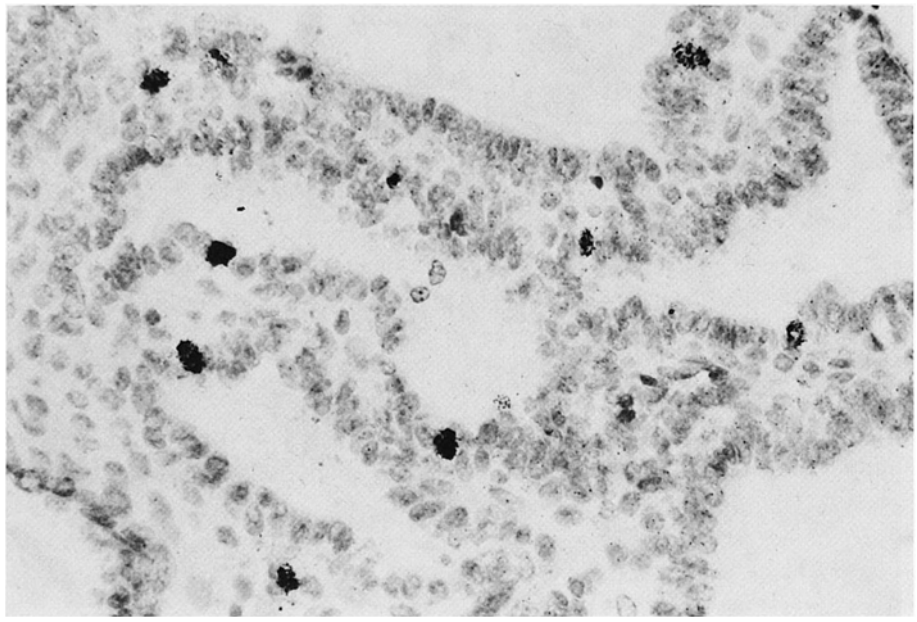

Fig. 5. Autoradiographic histological picture of cystic craniopharyngioma (× 360). This patient underwent intracavitary Bleomycin treatment after the sterotactic biopsy.

core [16]. In each patient have been obtained 2–7 tissue samples (average 4 samples). The obtained cylinders of tissue (8 mm long, 1.8 mm diameter) have been longitudinally split to provide mirror specimens for morphological and cell kinetic studies. Finally a small silver marker, N.M.R. compatible, has been placed at the deepest target to confirm postoperatively the site of biopsy.

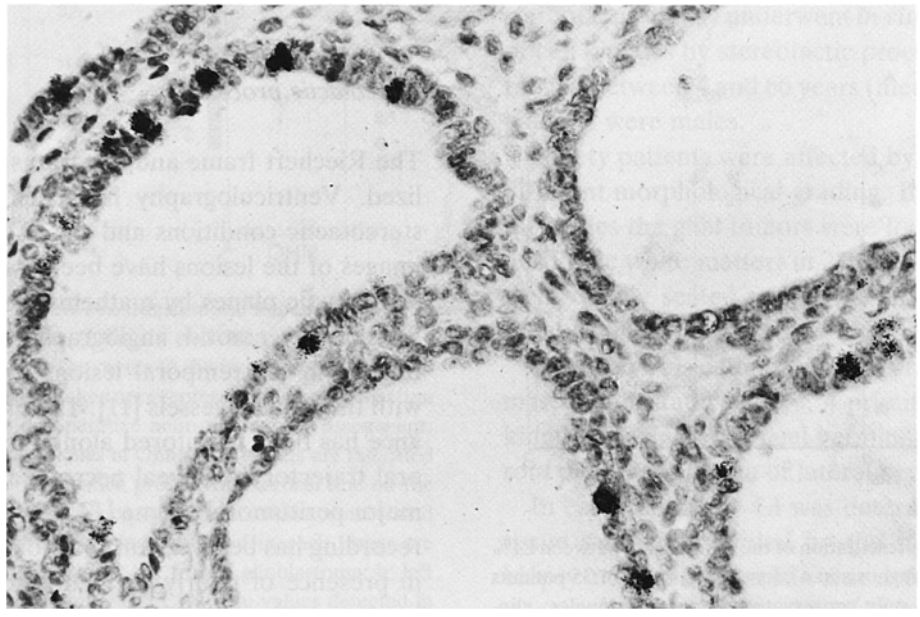

Fig. 6. Autoradiographic histological picture of a cystic craniopharyngioma (× 360). After the stereotactic biopsy this patient has been referred to the Service of Neurochirurgie, St. Anne Hospital in Paris for the intracavitary application of radiocolloids.

Laboratory procedure

Samples of tissues devoted to cell kinetic studies have been immediately placed in cold RPMI 1640 (GIBCO, Grand Island, NY) medium and processed within 1 hour. The specimens have been incubated in 2 ml of complete medium with 3H-Thymidine (RPMI 1640 with 20% FCS plus antibiotics – 6 Ci/ml; s.a. 5 Ci/mmole; Radiochemical Centre, Amersham, UK) at 37 C for 1 hour in a shaking water bath. After the incubation, the biopsy specimens have been fixed in Bouin's solution for 1 hour, embedded in paraffin, sectioned at 4 μm for autoradiographic procedure. Deparaffinized slides have been processed with the stripping film (Kodak AR10, Kodak, Rochester, NY) technique and exposed at 4 C for 10 days. The slides have been developed in Kodak D19b for 5 minutes at 18 C, fixed and stained with hematoxylin and eosin at 4 C (Fig. 1). Finally the slides have been examined by optical microscopy and 1000 to 10000 cells have been scored for each tumor. Grain number per nucleus was always superior to 10. The labeling index (LI) has been calculated as the ratio between percentage of labeled cells and total cells [17].

Results

No labeled cells were found in sound tissue and in reactive gliosis detected at the boundaries of extrinsic and non infiltrating tumors.

The highest mean LI value (13.0%) was detected in glioblastoma series (25 patients), but with a high intertumor variability (from 1.3% to 26.8%). Moreover a high intralesional heterogeneity was observed along the stereotactic transtumoral trajectory (Fig. 2a). In some areas a low LI value was detected in spite of the presence of monstruous neoplastic glial cells and degenerative changes typical of malignant gliomas. An area with neoplastic proliferating cells in glioblastoma is shown in Fig. 3.

Table 1. Cell kinetics features in glial tumors

	Mean	Range
Glioblastoma (25 cases)	13.0	1.3–26.8
Anaplastic astrocytoma (19 cases)	12.8	0.8–25.5
Mature astrocytoma (46 cases)	4.8	0.3–17.5
Cystic craniopharyngioma (5 cases)	1.9	1.17–3.5
Metastasis (6 cases)	16.6	12.7–19.8
Primitive non-Hodskin lymphoma (4 cases)	11.42	5.5–15.6

In anaplastic astrocytoma (19 patients) cell kinetics ranged between 0.8% and 24.5% and the mean LI value was 12.8%. In this series a slight intratumoral LI variability was found; areas with numerous viable neoplastic cells without regressive changes were sometimes scarsely labeled. In other words, the poorly labeled areas of anaplastic astrocytomas resulted morphologically different from the poorly labeled areas of glioblastomas characterized by regressive changes. These data suggest the existence in anaplastic astrocytomas of neoplastic cell populations which largerly appear in a resting phase. It has to be stressed that the diameter of the fragment tissue utilized for S phase investigation is 0.4 mm diameter and no diffusion problem occurred in our series as in other Authors reported series [9].

In mature astrocytoma (46 patients) the LI values along the stereotactic transtumoral trajectory were uniform in 78% of the neoplasms (Fig. 2b). Cell kinetics ranged between 0.3% and 17.5% and the mean LI value was 4.8%.

In a retrospective series of 35 patients monitored along three years by serial clinical, C.T. or N.M.R. examinations, the relationship between LI, histological grading and survival has been analyzed and the results are schematically represented in Fig. 4. It was observed that in this series, the patients harbouring mature astrocytomas with high LI values and/or with high intratumoral LI variability [18] has a neuroradiological evolution and clinical course similar to those observed for malignant tumors including increased volume of the tumor and appearing of contrast enhancement at CT scan controls.

In patients which underwent the stereotactic procedure for cystic craniopharyngiomas cell kinetics ranged between 1.17% and 3.5% and the mean LI value was 1.9%. In four cases the labeled

cells were more frequently observed in the epithelial layer of admantinous cells within the wall of microcystic structures (Figs. 5–6).

In metastatic lesions cell kinetics ranged between 12.7% and 19.8% with a mean LI value of 16.6%. No useful information is provided by these data to guide the treatment and the prognostic evaluation of these patients.

In primary non-Hodgking lymphomas cell kinetics ranged between 5.5% and 15.6% and the mean LI value was 11.42% (Table 1).

In germinomas (three cases) the LI values were 0.08%, 0.1%, 0.12%. In the single examined choroid plexus papilloma of lateral ventricle no labelled cells were observed.

Discussion and conclusion

The feasibility of *in vitro* cell kinetic determinations in tumors investigated by serial stereotactic biopsies has been assessed in neuroectodermal and non-neuroectodermal brain neoplasms. Cell kinetics appears remarkably relevant in mature astrocytomas: nevertheless in this series high LI values are indicative of patients at risk. In fact these 'active' astrocytomas behave as malignant tumors and patients must be candidate to the destruction of the lesion also when it is deeply seated and unresectable by conventional surgery or microsurgery. In our opinion, the indication to stereotactic radiosurgery [12,15], interstitial radiotherapy [22] and stereotactic laser resection [23] may be widely modulated according to LI value; moreover the reported methodology made easier the search of neoplastic cells in peritumoral areas and the assessment of the real volume of the lesion according to the spatial 3D configuration proposed by Daumas Deport *et al.* [24].

In glioblastomas cell kinetics seems more strictly related to the morphological patterns. The presence of an unexpected low LI value in glioblastomas, may be probably due to regressive intratumoral changes in the site of the stereotactic sample.

In cystic craniopharyngiomas the distribution of proliferating cells within the wall of the intratumoral microcysts may orientate the intracavitary treatments with antitumoral agents. The meaning of this peculiar localization of proliferating cells is now under investigation with particular regard to the responsiveness of these tumors to intracavitary treatments with Bleomycin [19] or radiocolloides [20], although this hypothesis require further confirmations on a larger series of patients.

In conclusion, the described methodology allows to compare the histological pattern to the potential cell proliferative activity. In long terms both these informations will be finally matched to the outcome of brain tumors treated by differentiated surgical and/or conservative strategies. Our data seems to be promising for the search of a prognostic index in glial brain tumors.

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