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Glioblastoma Pathology Sampling Method: Imprint After Seven-Point Sampling

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Abstract

Objective To standardize the pathological sampling method for glioblastoma (GBM) and provide more accurate pathological information for clinical practice. Methods We standardized the GBM pathological sampling method as "seven-site sampling", where the surgeon samples the GBM tumor mass at seven positions—center, anterior, posterior, medial, lateral, superior, and inferior—during surgery. This study included 40 GBM patients who underwent "seven-site sampling". We performed primary cell culture on tumor tissues sampled from each position and used immunohistochemistry to detect IDH1 protein and MGMT protein expression in tissues from the seven positions. Results Tumor tissues sampled from the tumor center successfully cultured tumor cells in 100% of cases, with cells showing elongated spindle-shaped morphology, rapid migration, and faster proliferation; the success rate of primary cell culture for tumor tissues sampled from positions beyond the T1-enhanced imaging area was 60%, with cells showing round and blunt-shaped morphology, slow migration, and slow proliferation. The two cell types exhibited significant differences in cellular morphology and cytological function. Differential IDH1 protein expression among the seven positions was observed in 10 cases, accounting for 25% of cases, with a statistically significant difference (P=0.001); differential MGMT protein expression among the seven positions was observed in 12 cases, accounting for 30% of cases, with a statistically significant difference (P=0.004). Conclusion Tumor cells could be cultured from tissues sampled at all seven positions, and differential expression of IDH1 and MGMT proteins was also observed. Although tumor tissues sampled from the center can accurately reflect the pathological grade of the tumor, considering the high heterogeneity of GBM, it is necessary to comprehensively consider the histopathological and molecular pathological characteristics of tissues sampled from other positions to more accurately reflect the biological characteristics of the tumor. Therefore, seven-site sampling is more instructive for the pathological diagnosis of GBM compared with traditional pathological sampling.

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Full Text

Preamble

Molecular and Histopathological Differences Depending on Different MRI Findings and Tumor Location in Glioblastoma

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Abstract

Objective: To standardize the pathological sampling method for glioblastoma (GBM) and provide more accurate pathological information for clinical practice.

Methods: We standardized the GBM pathological sampling procedure as the "Seven-Part Sampling Method," in which the surgeon obtains tissue samples from seven distinct locations within the GBM mass during surgery: center, anterior, posterior, medial, lateral, superior, and inferior. This study enrolled 40 GBM patients who underwent seven-part sampling. Primary cell culture was performed on tissues from each location, and immunohistochemistry was used to detect IDH1 and MGMT protein expression in the seven samples.

Results: Tumor cells were successfully cultured from 100% of center-sampled tissues, displaying spindle-shaped morphology, rapid migration, and high proliferation rates. In contrast, tissues sampled from other locations (outside the T1-enhanced region) showed a 60% success rate for primary cell culture, with cells appearing rounder, migrating slower, and proliferating more slowly. These two cell types exhibited significant differences in both morphology and biological function. IDH1 protein expression varied across the seven locations in 10 cases (25%, P=0.001), while MGMT protein expression differed in 12 cases (30%, P=0.004).

Conclusion: Tumor cells can be cultured from all seven sampling locations, and IDH1 and MGMT protein expression also varies among them. Although center-sampled tissue accurately reflects tumor histopathological grade, the extreme heterogeneity of GBM necessitates comprehensive analysis of pathological and molecular features from other locations to more accurately characterize the tumor' s biological properties. Therefore, the seven-part sampling method offers greater guidance for GBM pathological diagnosis compared to traditional sampling approaches.

Keywords: glioblastoma; seven-part sampling method; pathological diagnosis

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Introduction

Glioblastoma (GBM) exhibits extremely strong invasiveness and remarkable heterogeneity, which are primary reasons for the poor prognosis of GBM patients [1-2]. Due to its aggressive nature and lack of clear boundaries with surrounding normal brain tissue, combined with the fact that tumors are often located in or near functional areas, residual tumor cells after surgery are inevitable, leading to tumor recurrence [3-4]. As early as 2011, our center proposed surgical resection concepts for glioma management [5]. GBM is also a highly heterogeneous malignant tumor [6-7]. Consequently, traditional pathological sampling cannot represent the overall biological characteristics of the tumor, requiring surgeons to obtain multiple samples during surgery and mark the sampling locations on imaging (even with navigation assistance), rather than simply handing the specimen to pathologists for empirical sampling.

With the introduction of the new WHO classification of central nervous system tumors, molecular pathological diagnosis has become increasingly important. Protein expression profiles, including isocitrate dehydrogenase (IDH1), O6-methylguanine-DNA methyltransferase (MGMT), and EGFR, can indicate GBM patient prognosis and guide clinical treatment decisions (such as targeted drug selection) [8-9]. However, given the significant intratumoral heterogeneity of GBM, which part of the tumor tissue provides the most accurate pathological information? Are traditional pathological sampling results sufficiently comprehensive? To address this, we propose the "seven-part sampling method" performed by neurosurgeons during surgery to obtain more precise tumor pathological information and provide evidence and direction for clinical treatment.

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1. Materials and Methods

1.1 Seven-Part Sampling Method

During surgery, tumor tissue blocks were resected and sampled from the central region and peripheral regions of the tumor (Figure 1 [Figure 1: see original paper]). One sample was obtained from the tumor center, designated as (1) tumor center. Six samples were obtained from the peripheral region, designated as: (2) anterior; (3) posterior; (4) medial; (5) lateral; (6) superior; and (7) inferior. Sampling followed these principles: anterior/posterior and superior/inferior were defined according to the patient' s natural upright position; the side closer to the midline was defined as medial and the opposite as lateral. For centrally located bilateral lesions, the side with smaller volume was defined as medial and the side with larger volume as lateral. For the four different resection types of glioblastoma (Q, S, T, P) [10], center sampling generally targeted the T1-enhanced region (if the center contained necrotic tissue, sampling was performed at the necrotic periphery closest to the geometric center; if cystic degeneration was present, the solid component within the cyst was sampled). Peripheral sampling targeted areas near the edge of T2/FLAIR abnormalities

(for Q and S type resections) or near the edge of T1-enhanced regions (for T and P type resections). A total of 40 glioblastoma cases underwent seven-part sampling. Tissue from each location was divided into three portions: one for pathological diagnosis, one for primary cell culture, and one for frozen specimen storage. All patients provided informed consent for the use of pathological tissues in scientific research.

1.2 Primary Cell Culture

Primary cell culture was performed on tumor tissues from 40 GBM cases that underwent seven-part sampling. All procedures were conducted in a laminar flow hood. Tissues were minced into 1 mm × 1 mm × 1 mm pieces, digested with 0.25% trypsin for 5-10 minutes, and centrifuged at 1000 r/min for 3 minutes. The pellet was resuspended in complete medium containing 10% fetal bovine serum, plated in 10 cm culture dishes, and incubated at 37°C with 5% CO2. The medium was changed every 2-3 days.

1.3 Immunohistochemistry

Tumor tissues obtained through intraoperative seven-part sampling were washed with PBS to remove blood and cauterized necrotic tissue. Immunohistochemistry was used to detect IDH1 and MGMT protein expression in the seven tumor samples. Primary antibodies used were: IDH1 (ZM-0447, ZSGB-BIO, dilution 1:50) and MGMT (A0693, Abclonal, dilution 1:100). Secondary antibodies were purchased from Abcam. DAB chromogen was used to control staining intensity. Five high-power fields (400×) were randomly selected for observation. Expression was scored based on staining intensity and percentage of positive cells: (1) intensity (negative=0, light yellow=1, dark yellow=2, brown=3); (2) percentage (0%=0, 1-10%=1, 11-50%=2, 51-80%=3, >80%=4). The final score was the product of both parameters, with 4 considered positive expression [9]. Cases showing different immunohistochemistry results among the seven sampling locations were recorded as differential cases.

1.4 Statistical Analysis

SPSS 21.0 software was used for statistical analysis of immunohistochemistry data. Paired fourfold table consistency test was performed, with Kappa coefficient >0.75 indicating good consistency. $P<0.05$ was considered statistically significant.

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2. Results

2.1 Primary Cell Culture Results

Among the 40 GBM tissues subjected to seven-part sampling, primary culture was successful in all 40 cases (100%) from center-sampled tissues. For the six

peripheral locations, success was defined as cell growth from at least one of the six positions, with 24 cases meeting this criterion (60% success rate). For Q and S type resections, tumor cells cultured from the center showed spindle-shaped morphology with long pseudopodia, rapid migration from tissue blocks, strong migratory ability, and fast proliferation (Figure 2 [Figure 2: see original paper]A). In contrast, cells cultured from peripheral regions (sampled from T2/FLAIR abnormal areas) appeared rounder, lacked obvious pseudopodia, and proliferated significantly slower than center-derived cells (Figure 2B).

2.2 HE Staining Results

Tissues sampled from the tumor center showed typical glioblastoma features on HE staining, including prominent angiogenesis, high-density atypical tumor cells, and frequent mitotic figures. Tissues from the other six peripheral locations within T2/FLAIR abnormal areas showed scattered tumor cells and essentially no neovascularization, resembling low-grade glioma pathology (Figure 3 [Figure 3: see original paper]).

2.3 Immunohistochemistry Results

Immunohistochemistry for IDH1 and MGMT proteins was performed on all seven sampling locations from the 40 GBM cases. A case was recorded as differential if any of the six peripheral locations showed different results from the center. For IDH1 protein, 10 cases (25%) showed differential expression, with a Kappa coefficient of 0.5 and statistically significant difference (P=0.001, Figure 4 [Figure 4: see original paper]A, B). For MGMT protein, 12 cases (30%) showed differential expression, with a Kappa coefficient of 0.417 and statistically significant difference (P=0.004, Figure 4C, D).

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3. Discussion

GBM is a heterogeneous tumor composed of different clonal cell populations. A single tumor mass may contain tumor tissue components originating from multiple cell types and possesses extremely high invasiveness, enabling migration along white matter tracts and blood vessels to regions far from the tumor center [3,4,7,11-14]. Based on autopsy and historical surgical biopsies, tumor cells can appear far beyond the radiologically abnormal range. Genomic instability, cellular heterogeneity, and extensive infiltrative capacity make GBM treatment extremely challenging. Undeniably, the extent of surgical resection is a key factor affecting GBM patient prognosis. In our series of GBM patients who underwent "Q-type resection" followed by standard postoperative chemoradiotherapy at Nanfang Hospital, the median survival exceeded the internationally reported 14.6 months [15]. Unfortunately, despite the most aggressive treatment, although a small subset of patients can achieve long-term survival [16-18],

the overall prognosis for GBM patients remains poor. In fact, tumor heterogeneity has a decisive impact on the failure of comprehensive postoperative treatment. GBM exhibits not only temporal heterogeneity (genotypic differences may vary at different times) but also spatial heterogeneity. Therefore, targeted therapy directed at mutations in a specific region is inappropriate, yet conventional pathological sampling (often from only one specific region) cannot sample and verify mutations from all tumor locations. This necessitates exploration of a pathological sampling method that conforms to the principles of tumor heterogeneity.

In this study, we performed primary culture on tissues from different GBM regions. Tumor cells were successfully cultured from peripheral region tissues (at the junction between T2/FLAIR abnormal areas and normal MRI appearance) with a 60% success rate, validating the highly invasive nature of GBM cells. Moreover, tumor cells cultured from center and peripheral regions showed significant differences in both morphology and biological function, further demonstrating GBM intratumoral heterogeneity. Positive IDH1 mutant protein expression and negative MGMT protein expression are both favorable prognostic markers for GBM patients. However, immunohistochemistry for IDH1 and MGMT on tissues from different regions revealed differential expression in 10 and 12 of 40 GBM patients, respectively, further confirming the extreme heterogeneity within GBM masses.

Given this profound heterogeneity, which part of the tumor truly represents its biological characteristics? Based on literature analysis, we propose that tissues sampled from the peripheral region are more representative. Tumor cells can be cultured from regions that appear normal on imaging and histopathology [14], and these surgical and radiotherapy-resistant cells likely represent the true source of tumor recurrence and should be the target cells for GBM treatment [12]. Recent literature has emphasized the "Peritumoral Zone" [3]. Theoretically, the peripheral region is physically closer to residual tumor cells around the resection cavity and better represents the biological characteristics of these residual cells. This requires surgeons to emphasize en bloc resection and identification of important anatomical structures such as sulci and cisterns, with navigation-assisted sampling when necessary to ensure accurate spatial localization of tumor samples.

The rationale for selecting seven sampling locations is as follows: HE staining of center-sampled tissues revealed richer vasculature and higher tumor cell density and atypia compared to peripheral samples, making center sampling more reliable for determining histopathological grade and facilitating comparison with existing literature. Molecular pathological testing of peripheral samples better represents residual tumor cells after surgery and provides more scientific guidance for prognosis assessment and treatment selection. Considering sampling simplicity and feasibility, we selected six positions across three dimensions (anterior/posterior, superior/inferior, medial/lateral) plus the center. By integrating preoperative imaging, intraoperative navigation, and surgical tactile feedback,

neurosurgeons can precisely sample any tumor location—an advantage that traditional sampling methods lack. Therefore, we propose the "seven-part sampling method" for GBM, enabling neurosurgeons to sample various tumor regions during surgery to provide more comprehensive and accurate pathological information for clinical decision-making. Except for some WHO Grade I gliomas, all gliomas should follow the seven-part sampling principle, which will provide more histopathological and molecular information with significant implications for both clinical practice and research.

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