Research Article

Validation of Tumor Morphology in Sections Developed Using Tissue Microarray Technology in Astrocytomas and Oligodendrogliomas

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Abstract

Background: In the era of technological advancement, tissue microarray technology (TMA) plays a promising role in supplementing high-throughput molecular analytical studies. Fewer studies in the field of tumor pathology in brain biopsies demand for more analysis using this technique.

Aims/ Objectives: The main objective of this study is to validate whether the tumor morphology is conserved in TMA slides in comparison with the conventional H&E slides.

Materials and Methods: We analyzed 40 cases of malignant glial tumors (astrocytoma/ oligodendroglioma) using this technology where TMA blocks were constructed using 40 conventionally constructed formalin-fixed paraffin embedded blocks. H&E staining was carried out for all the TMA slides.

Results: Out of the 40 cases analyzed, 36 cases (90%) showed positive for tumor. The other 4 cases (10%) showed no evidence of tumor. Out of 36 cases, 31 cases (86.1%) matched the diagnosis done with both the methods.

Five cases (13.8%) showed non-concordant diagnosis when compared with the diagnosis given based on conventional H&E slides.

Conclusion: Tissue microarray technology is definitely a promising tool which can be used for further molecular analysis thereby reducing the costs of reagents. Tumor morphology is conserved on TMA slides which are representative of the original blocks and slides constructed using conventional method. This technique can be useful in the cases in which the tissue is limited and if further molecular analysis is required on such tissues.

Keywords: Brain tumors, Conventional diagnosis, H&E staining, Molecular techniques, FFPE sections.

Introduction

Tissue microarray technology is the most valuable tool for conducting high-throughput molecular analysis at both gene and protein levels on tissue sections in a clinical pathologic research setting.[1] Using this technique, hundreds of tissue samples containing tiny cores can be viewed on a single microscopic glass slide.[2] This technology can be used to study immunohistochemical markers, fluorescence in-situ hybridization at lower costs compared to the conventional methods.[3] The technology has been used to study many cancers like breast carcinoma,[5,6] prostate cancer,[7,8] colorectal cancer,[9] renal cell carcinoma,[10] malignant melanoma,[11] etc. This technique proves to be economically cheaper with regard to the reagent costs.[4] However, validation of preserved morphology on the tissue microarray section with that of the original H&E section, which is stained using the conventional method, is necessary.

Aim

In this study, we intended to validate the morphology of tissue microarray section with that of the conventional H&E section in brain tumor tissues, thereby proving that this technology is a reliable technique to conduct the required molecular analysis as tumor morphology is conserved, which is representative of the originally constructed block on which the diagnosis was made earlier.

Methodology

Cases

In our study, we selected 40 blocks of brain tissue biopsies diagnosed as grade II (n=8), III (n=12), IV (n=20) astrocytoma/ oligodendroglioma which were reported using the conventional H&E sections, based on histological characteristics. These were regarded as donor blocks to construct tissue microarray. The cases were operated at the Department of Neurosurgery, JIPMER, Puducherry, by obtaining informed consent.

Construction of TMA Blocks

The tissue microarray blocks were constructed using Beecher's Instruments, USA. Manual method of construction was used. Three sections of representative tumor areas were marked on the conventional H&E slide for each case by two experienced neuro-pathologists. Each recipient block consisted of 10 cases with 3 cores each. One block had 30 tiny cores and the last row had one extra tissue core which served as reference core for interpretation and indexing purposes. Four such blocks were constructed. Every tiny core measured 1 mm in diameter. These blocks were then subjected to routine sectioning of 4 µm thickness for H&E staining. The TMA slides were then examined by the pathologist who was blinded against the routine diagnosis made on the conventional H&E slide of the respective cases.

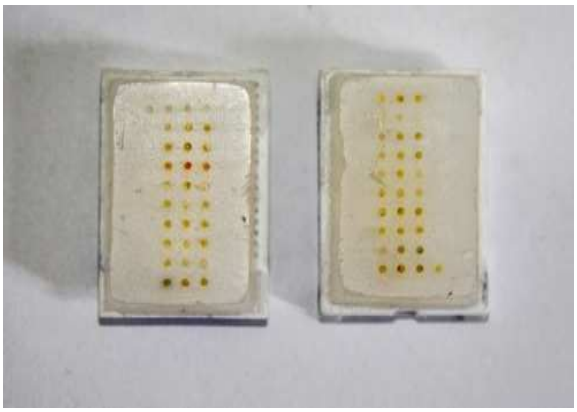

Figure 1.Tissue Microarray Blocks

Figure 2.Tissue Microarray Slides

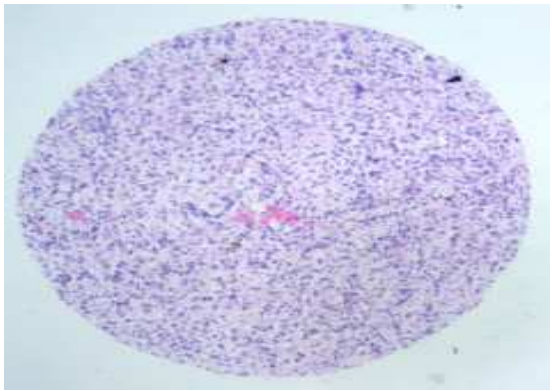

Figure 3.Oligodendroglioma Grade 2- TMA section (H&E X 100)

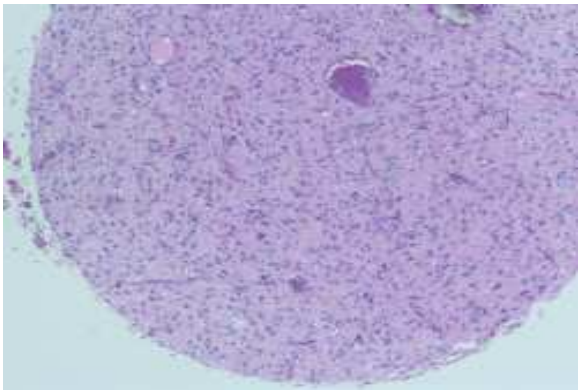

Figure 4.Glioblastoma Grade 4- TMA Section (H&E X 200)

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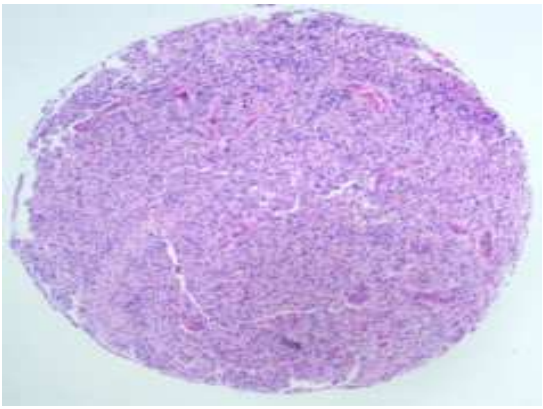

Figure 5.Glioblastoma Grade 4-TMA Section (H&E X 100)

Statistical Analysis

The weighted kappa statistic was carried out to assess the agreement between the diagnoses made using conventional and tissue microarray method.

Results

Out of 40 cases analyzed for morphology on both conventional and tissue microarray H&E sections, 4 cases showed no tumor evidence, 31 cases showed concordant results, 5 cases showed non-concordant results. Amongst them, 2 cases were reported as Grade 2 in conventional method, whereas in TMA method they were reported as Grade 3. Also, 3 cases were reported as Grade 4 using conventional method, whereas they were reported as Grade 3 using TMA method. Rest of the cases matched the diagnoses with both the methods.

Statistical Analysis

We found a strong agreement between the diagnosis made on the conventional and tissue micro-array method with a kappa value of 0.86 which is found to be statistically significant at 5% level of significance ($p < 0.001$).

Discussion

There are various studies available in literature regarding the usage of this technology for high-throughput molecular analysis in various tumor types. However, in brain tissue biopsies it is very limited. There are a few studies available using this technology to analyze immunohistochemical markers in human gliomas[12] and oligodendrogliomas.[13] We conducted this study to see whether the representative tumor morphology is preserved in the constructed TMA block from the originally biopsied block for further molecular analysis.

However, we found 4 cases out of 40 cases that did not show evidence of tumor tissue on the TMA slide. This variation could be due to either technical error when punching the donor block or sampling error while marking the area of interest on the original H&E section or loss of tissue when floated in a water bath before slide preparation.

Out of the 5 cases which showed non-concordant results, 2 cases which were reported as Grade II in conventional method were reported as Grade III in TMA method. This discrepancy in grading may be due to marking of representative tumor area on conventional H&E slide and may cater single mitotic figures and the same may be sampled on a TMA slide, thus leading to a higher-grade diagnosis.

The other 3 cases were under-graded from Grade IV on conventional method to Grade III in TMA method. This may be due to lack of microvascular proliferation and palisading necrosis in the TMA slides. This may not be representative of the whole tumor tissue. The heterogeneity of tumor is a major disadvantage of this technology wherein the cores on the TMA section may not be representative of the whole tumor.[14,15]

However, the other 31 cases conserved tumor morphology and were diagnosed accurately on the TMA similar to conventional H&E section. This proves that TMA sections are reliable to conduct further molecular testing ranging from IHC studies for diagnostic or prognostic markers and fluorescent in-situ hybridization on these slides.

While understanding the development and progression of cancer demands innovative research to design more appropriate treatment regimens, tissue microarray technology supplements it by allowing to conduct such high molecular level research economically and easily.[4]

Conclusion

The construction or preparation of representative TMA blocks is necessary to preserve the accurate tumor tissue where its availability is limited. Formalin-fixed paraffin-embedded tissues can be used for downstream molecular analysis even after a decade. However, construction of such blocks depends on accurate marking of the tumor area by a skilled pathologist. And also analyzing the TMA slides to validate the representative tumor morphology is very essential. This technique definitely has proved as one of the reliable, faster and inexpensive in today's cancer biology research which includes basic research, oncology-prognostic and diagnostic.

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Conflict of Interest: None

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