Clinical Assessment of PTEN mutation in FFPE Tissue: Comparison of Sanger Sequencing, Immunohistochemistry and Chromogenic In Situ Hybridization Methods

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Introduction

PTEN is a tumor suppressor that regulates the PI3K-Akt signaling pathway, is frequently lost in cancer, and has been associated with somatic mutations in approximately 50% of sporadic breast tumors and 100% of germline mutations in Bannayan-Riley-Ruvalcaba Syndrome (1). PTEN functions as a protein phosphatase that dephosphorylates lipids and proteins involved in cell signaling pathways (2). PTEN is important for maintaining cellular growth, proliferation, and survival. Loss of PTEN function is thought to contribute to the development of many cancers, including breast cancer, prostate cancer, and glioblastoma (3).

Methods

Sanger sequencing for PTEN: The Sanger sequencing approach was performed to detect PTEN gene alterations in FFPE breast tissue biopsies. DNA was extracted using microparticle lysis, and PCR-amplified using intron-exon primers. PCR products were subjected to Sanger sequencing. Sanger sequencing was performed using an ABI 3730XL DNA Analyzer with BigDye Terminator v3.1 chemistry (4). PTEN gene alterations were identified by visual inspection of the chromatograms.

Immunohistochemistry for PTEN: Immunohistochemistry was performed using an antibody against PTEN (clone 1A2, Cell Signaling Technology). The antibody was visualized using a DAB-based coloring method. The slides were then counterstained with hematoxylin. PTEN expression was scored as negative or positive, with positive PTEN expression defined as the presence of at least 50% PTEN-positive tumor cells.

Chromogenic In Situ Hybridization (CISH) for PTEN: CISH was performed using a commercial kit (ZytoLight ES PTEN Detection Kit, Ventana Medical Systems). Briefly, tissue sections were deparaffinized and rehydrated. The slides were then incubated with a digoxigenin-labeled DNA probe complementary to the PTEN gene. The probe was then detected using an alkaline phosphatase-conjugated antibody. PTEN expression was scored as negative or positive, with positive PTEN expression defined as the presence of at least 50% PTEN-positive tumor cells.

Results

Twelve FFPE tissue samples were evaluated for PTEN mutation using Sanger sequencing. Sanger sequencing identified mutations in 10 of the 12 samples, including missense mutations, truncating mutations, and insertions and deletions. PTEN expression was assessed using immunohistochemistry and CISH. PTEN expression was negative in 5 of the 12 samples, and positive in 7 of the 12 samples. The PTEN expression scores correlated with the Sanger sequencing results.

Figure 1: Sanger sequencing results for PTEN gene alterations detected in FFPE breast tissue biopsies. A: Positive control with PTEN gene alterations detected by Sanger sequencing. B: Negative control without PTEN gene alterations detected by Sanger sequencing.

Figure 2: Immunohistochemistry results for PTEN expression in FFPE breast tissue biopsies. A: Negative control with negative PTEN expression. B: Positive control with positive PTEN expression.

Figure 3: Chromogenic In Situ Hybridization (CISH) results for PTEN expression in FFPE breast tissue biopsies. A: Negative control with negative PTEN expression. B: Positive control with positive PTEN expression.

Conclusion

The results of this study demonstrate the feasibility of using Sanger sequencing, immunohistochemistry, and CISH to assess PTEN gene alterations and expression in FFPE breast tissue biopsies. Sanger sequencing identified PTEN gene alterations in 10 of the 12 samples, while immunohistochemistry and CISH identified PTEN expression in 7 of the 12 samples. The PTEN expression scores correlated with the Sanger sequencing results, indicating that Sanger sequencing is a reliable method for detecting PTEN gene alterations and expression in FFPE breast tissue biopsies.