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MGMT and PTEN as potential prognostic markers in breast cancer

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ABSTRACT

Aim: To evaluate the prognostic importance of MGMT and PTEN concerning their correlation with other prognostic factors evaluated by immunohistochemistry (IHC) and the molecular phenotype of breast cancers.
Methods: IHC for estrogen and progesterone receptors, HER2, Ki67, p53, p63, e-cadherin, EGFR, CK5, CK14, MGMT and PTEN was performed on 200 breast tumors. Basal-like and luminal breast carcinomas were defined by the IHC evaluation of these markers. Fluorescent in situ hybridization (FISH) was performed for *PTEN* and *HER2* analysis using the Vysis PTEN and HER2 DNA probe kits (Abbott™). RT-PCR was performed to evaluate gene expressions of *MGMT* and *PTEN* in frozen tissue of 59/200 cases.
Results: 147/200 cases were triple-negative (73.5%), 47/147 were basal-like carcinomas (31.9%). 53 cases (26.5%) were luminal-like type A or B. 56 (93.3%) and 46 samples (76.6%) expressed lower levels of MGMT and PTEN mRNA, respectively, compared with normal breast ($p<0.001$). There was a positive correlation between the IHC results and the RT-PCR values for MGMT and PTEN. Tumors with homozygotic deletion of *PTEN* expressed little or no mRNA or protein. Positive p53, high Ki67, and basal-like tumors expressed significant lower MGMT and PTEN.
Conclusions: We hypothesize that MGMT and PTEN expressions have prognostic significance in breast cancer. Also, based on their predictive value of response to therapy, evaluating MGMT and PTEN and learning to interpret their patterns of immunoexpression will undoubtedly lead to a greater understanding of breast cancer and its treatment.

Introduction

O6-methyguanine-DNA methyltransferase (*MGMT*) lies on chromosome 10, and MGMT mediates DNA repair by removing mutagenic and cytotoxic adducts from the *O*6-guanine in DNA (Jacinto and Esteller, 2007; Pegg, 1990; Pegg et al., 1995). It prevents crosslinking by transferring an alkyl group to an internal cysteine residue, restoring guanine in the DNA and inactivating itself in the process. Loss of *MGMT* expression is rarely due to deletion, mutation, or rearrangement of *MGMT* but is associated with methylation of a specific region of a CpG island in *MGMT* (Danam et al., 1999; Munot et al., 2006). MGMT expression correlates inversely with p53 levels (Osanai et al., 2005) in hepatocellular, gastric, and breast cancers (Matsukura et al., 2001). However, no studies have linked MGMT expression or other important molecular prognostic factors and tumor prognosis in breast cancer.

Phosphatase and tensin homolog (PTEN), which also resides chromosome 10, is a tumor suppressor gene. PTEN has also been suggested to regulate stem cell self-renewal (Perez-Tenorio and Stal, 2002). PTEN is a lipid phosphatase that dephosphorylates phosphatidylinositol (3–5)-trisphosphate (PIP3), antagonizing the PI3-K/Akt pathway. Deletion of PTEN results in increased activation of the PI3-K/Akt pathway, which correlates with poor prognosis in breast cancer patients (Shoman et al., 2004). Furthermore, deletion or reduced expression of PTEN in many human tumors, including breast cancer, is associated with resistance to conventional therapeutic agents and relapse following initial treatment (Shoman et al., 2004).

PTEN mutations are not common in breast cancer, occurring in less than 5% of patients. Nevertheless, 30% to 50% of breast cancer patients have reduced PTEN expression, which, in turn, is associated with poor clinical outcome (Tsutsui et al., 2005). Approximately 50% of patients with breast cancer harbor a mutation in *PTEN* or have lost at least 1 copy of *PTEN*. Also, the complete loss of PTEN protein is more frequent in metastatic breast cancer than in primary tumors (Depowski et al., 2001).

The loss of 1 copy of *PTEN* increases the risk of tumor progression, and PTEN levels influence the initiation and progression of tumors in mice (Depowski et al., 2001). Low PTEN expression correlates with unresponsiveness to breast cancer therapies, such as trastuzumab (Herceptin™), tamoxifen, and gefitinib. Thus, we must understand how PTEN levels and its subcellular localization are regulated to determine the

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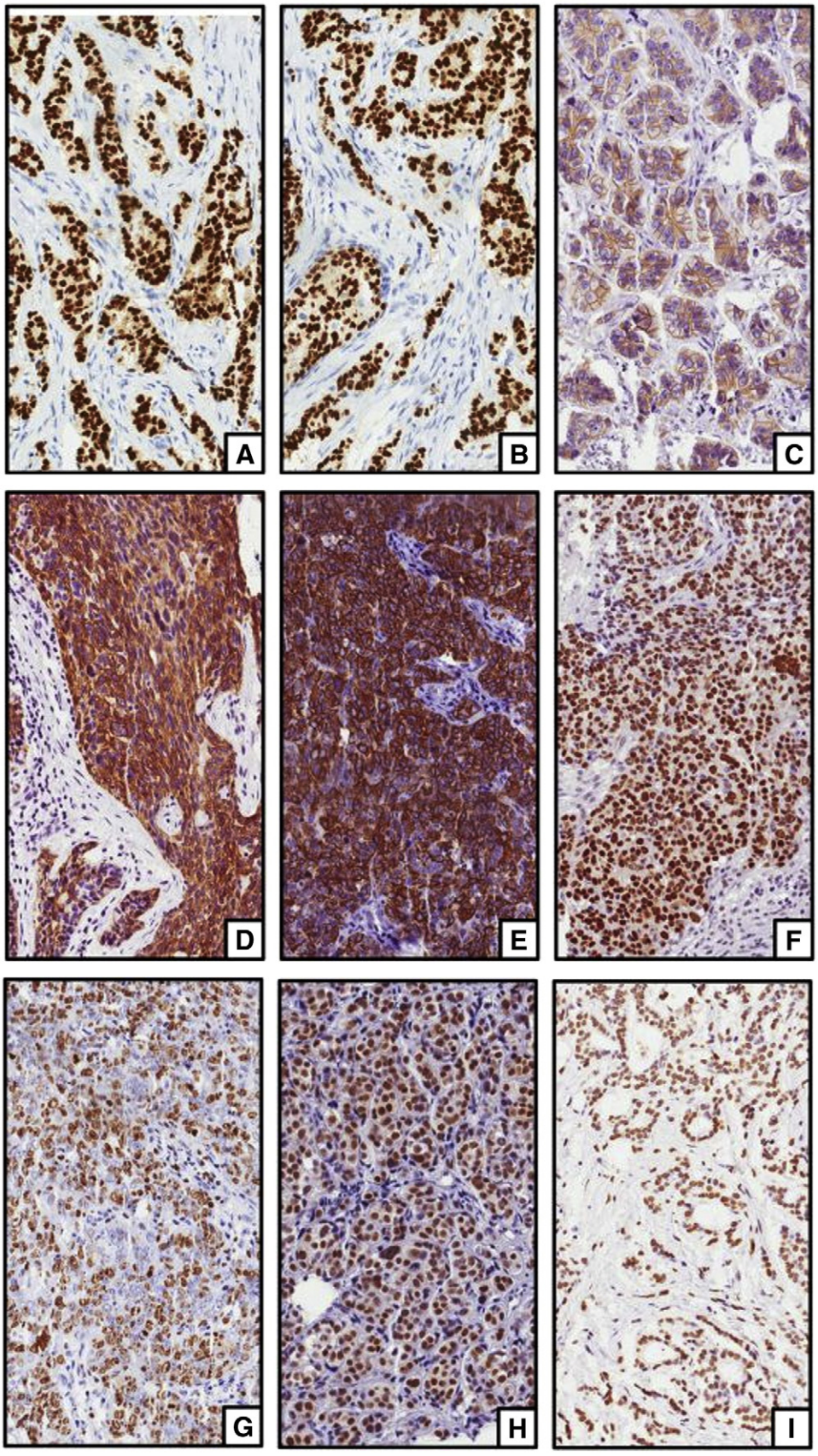

Fig. 1. Immunohistochemical staining pattern of the main markers used. A) estrogen receptor; B) progesterone receptor; C) HER2; D) CK5; E) CK14; F) p53; G) Ki67; H) MGMT; I) PTEN.