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14-3-3σ down-modulation: a ubiquitous marker for breast cancer?

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Keywords

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Introduction

A number of promising molecular markers of breast tumor progression have been recently elucidated, most of which are altered in only a subset of breast cancers. Previous reports identified 14-3-3σ (σ), a molecule normally activated at the G2 cell cycle checkpoint in response to DNA damaging agents, as a putative molecular marker that is downregulated in breast tumor cells. Because these initial studies were performed in only a few breast carcinoma cell lines, it was not clear to what extent σ serves as a general marker of breast tumor progression.

Aims

To monitor the expression patterns of σ in an extensive panel of breast epithelial cell lines, both nonmalignant and tumorigenic, and in primary breast tumors. To explore the molecular mechanism of σ gene silencing and to examine how σ downmodulation contributes to tumor progression in the breast.

Comments

Aberrant promoter methylation is proving to be a critical epigenetic mechanism contributing to tumorigenic progression. This report provides compelling evidence that the DNA damage response gene 14-3-3σ is inactivated in breast tumors by a methylation-dependent gene silencing mechanism and that this negative regulation may be causal to tumorigenic progression. While the data indicate that 14-3-3σ is the consistent invasive breast cancer marker identified to date, it is not yet clear whether this protein will serve as a useful indicator for early detection.

Methods

Northern blots were performed to evaluate the expression of σ in nontumorigenic human mammary epithelial cells (both primary and immortalized), in two breast carcinoma cell lines and 48 primary breast tumors (invasive ductal carcinomas). Genetic causes of downmodulation were explored in σ-negative samples using PCR-based assays of loss of heterozygosity (LOH) and by direct gene sequencing. Methylation status of σ promoter sequences were evaluated in both malignant and nonmalignant cell types using sodium bisulfite DNA sequencing and methylation-specific PCR. Finally, the efficacy of DNA damage repair at G1- and G2-specific checkpoints was assayed by scoring the frequency of asymmetric chromosomal segregation during metaphase in both σ-positive and σ-negative cells exposed to stage-specific ionizing radiation.

Results

The expression of σ, while clearly detectable in nonmalignant human mammary epithelial cells (ie six non-immortalized strains and five immortalized strains), was found to be absent in three breast carcinoma cell lines and in 45 of 48 (94%) primary breast tumors. Examination of 45 paired sets of normal and tumor patient DNAs showed only one LOH. Direct sequence of sigma coding region cDNAs from σ-negative cells (two cell lines and seven tumor tissues) showed no destabilizing genetic mutations that could account for the reduced expression levels. A striking correlation, however, was demonstrated between σ downmodulation and σ promoter hypermethylation: four σ-positive cell lines displayed unmethylated σ promoter CpG island sequences, and σ-negative cells (two cell lines and 10 primary breast tumors) showed complete or partial methylation of the σCpG island. Moreover, treatment of two σ-negative breast carcinoma cell lines with the DNA methyl transferase inhibitor, 5-aza-dC, led to demethylation of the σCpG region and reactivation of σ gene expression. Finally, while σ-positive and σ-negative cells lines both displayed similar G1 cell cycle checkpoint control responses, they exhibited different G2 checkpoint functions with σ-negative cells showing up to two-fold more G2-type chromosomal aberrations than observed with σ-positive cells.

Discussion

The demonstration that 14-3-3σ is reproducibly downmodulated in a large selection of breast carcinoma cell lines and tumor samples suggests that it may be a good candidate for a general marker for invasive breast carcinoma. Loss of σ expression appears not to be due to genetic changes such as LOH or more subtle sequence mutations, but rather is regulated.

References

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