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TITLE: Interphase Cytogenetics in Breast Tumors P53 Gene Alterations

PRINCIPAL INVESTIGATOR: Isabell A. Sesterhenn, M.D.

CONTRACTING ORGANIZATION: Armed Forces Institute of Pathology
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13. ABSTRACT *(Maximum 200 words)*

PURPOSE: To compare numerical chromosome aberrations and p53 gene alterations in 250 node-negative breast cancers to 250 non-positive breast cancers and their survival. SCOPE: Interphase cytogenetics will be performed in paraffin sections utilizing biotinylated probes specific for centromeric regions of chromosomes 1, 8, 11, 13, 17, 18, and X. Chromosome profiles based on 300 tumor cells will be generated for each patient. Serial sections of the same paraffin blocks will be utilized to assess p53 alterations immunohistochemically. If present, further amplification and sequencing of p53 gene will be done utilizing PCR denaturing gel gradient electrophoresis and coupled amplification and sequencing protocols. MAJOR FINDINGS: Preliminary analysis showed there was a statistically significant increase in the copy numbers of chromosome 1 with nuclear grade. Similarly, increasing numbers of chromosome 18 were observed with increase in nuclear grade. However, there was no significant increase in the copy numbers of chromosomes 8, 11, 13/21, 17 and X when correlated with nuclear grade. All of them showed extra copies in nuclear grade I, II, and III tumors. Comparison of copy numbers of the different chromosomes in tumor to normal epithelial cells showed a gain for chromosomes 8, 11, 13/21, 17, and X. However, in individual cases, loss of chromosome 17 and X chromosome was encountered.

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D. Schwan 13 Mar 97

PI - Signature Date

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MID-TERM REPORT - 93MM3506

TITLE OF PROPOSAL: Interphase Cytogenetics in Breast Tumors p53 Gene Alterations.

PRINCIPAL INVESTIGATOR: Isabell A. Sesterhenn, M.D.

Ref.: DD Form 448, Amendment received [dated 12/10/96] extending proposal period until 30 JUNE 1998.

PURPOSE: To compare numerical chromosome aberrations and p53 gene alterations in 250 node-negative breast cancers to 250 non-positive breast cancers and their survival. Although conventional cytogenetic analysis has been successful only in about 50 breast cancers, it has been helpful in identification of subtypes of ductal carcinomas. Since cytogenetic equivalents for gene amplification are net gain of chromosomal material and for loss of heterozygosity are net loss of chromosomal material or structural aberrations, we believe that interphase cytogenetics may be helpful in subclassification of ductal carcinomas. P53 gene alterations may prove to be an early event in breast cancer progression.

SCOPE: Interphase cytogenetics will be performed in paraffin sections utilizing biotinylated probes specific for centromeric regions of chromosomes 1, 8, 11, 13, 17, 18, and X. Chromosome profiles based on 300 tumor cells will be generated for each patient. Serial sections of the same paraffin blocks will be utilized by Dr. O'Leary to assess p53 alterations immunohistochemically. If present, further amplification and sequencing of p53 gene will be done utilizing PCR denaturing gel gradient electrophoresis and coupled amplification and sequencing protocols. The data will be analyzed by the Kaplan-Meyer and Cox proportional hazards model, comparing the two groups of patients. Pathology Data Division is follow-up in progress.

MAJOR FINDINGS: Two hundred ninety-two cases have been completed. An additional 78 cases are close to completion. Pathology Data Division follow-up is in progress.

Preliminary analysis of 37 node-negative tumors, statistical analysis was performed. The Jonckheere-Terpstra test (Hollander & Wolfe, 1973) was used to determine if the chromosomal spot counts were identically distributed in each grade considered. In addition, the standardized Jonckheere-Terpstra test statistics allow one to determine the relationship between chromosomal spot counts and grade. Two-sided asymptotic p-values were determined. Integer values were used for each chromosomal spot count. The Jonchkheere-Terpstra test was also used to compare normal tissue chromosomal spot counts to tumor chromosomal spot counts, with both tissues coming from the same biopsy.

Comparison of the chromosomal profiles to the tumor grade as determined by the Bloom and Richardson methods modified by Elston

did not show any correlation. However, if the tumors were grouped according to the nuclear grade, there was a statistically significant increase in the copy numbers of chromosome 1 with nuclear grade. Similarly, increasing numbers of chromosome 18 were observed with increase in nuclear grade. However, there was no significant increase in the copy numbers of chromosomes 8, 11, 13/21, 17 and X when correlated with nuclear grade. All of them showed extra copies in nuclear grade I, II, and III tumors. Comparison of copy numbers of the different chromosomes in tumor to normal epithelial cells showed a gain for chromosomes 8, 11, 13/21, 17, and X. However, in individual cases, loss of chromosome 17 and X chromosome was encountered.

p53 analysis is in progress.