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Progression

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12b. DISTRIBUTION CODE**13. ABSTRACT (Maximum 200 Words)**

Breast carcinoma is a major cause of cancer mortality in women in the United States. At the advanced stages, breast carcinoma is characterized by genetic instability, loss of cellular architecture and glandular disorganization. However there is a lack of information about the mechanism that generates these cellular changes. Centrosomes are subcellular organelles regulate the mitotic spindle and control the fidelity of chromosomal segregation. Centrosome abnormalities may result in missegregation of chromosomes and genetic instability, a common feature of cancer cells. Centrosome defects have been described in invasive ductal breast carcinoma. However, it is not known how early these abnormalities occur in breast cancer. To address this the study was conducted to determine whether centrosomes are abnormal in ductal carcinoma of the breast (DCIS). We analyzed semiquantitatively, the number, size and shape of centrosomes in paired normal lobules and DCIS lesions. Our study shows that centrosomes are abnormal in a significant fraction of DCIS lesions and suggest that centrosome dysfunction may be the cause of the chromosomal instability observed in DCIS. Our observations have important implications for the biology of breast cancer, that centrosome dysfunction are an early event in breast carcinogenesis and may be critical for cancer progression.

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Introduction.

Title: Centrosome defects as a contributor to genetic instability and glandular disorganization in breast tumor progression.

Breast carcinoma is a major cause of cancer mortality in women in the United States. At the advanced stages, breast carcinoma is characterized by genetic instability, loss of cellular architecture and glandular disorganization. However there is a lack of information about the mechanism that generates these cellular changes or how they contribute to breast tumor development and progression. Centrosomes are subcellular organelles regulate the mitotic spindle and control the fidelity of chromosomal segregation. Centrosome abnormalities may result in missegregation of chromosomes and genetic instability, a common feature of cancer cells. Centrosome defects have been described in invasive ductal breast carcinoma and also they occurred concomitant with genetic instability (Pihan et al., 1998; Pihan et al., 2000). To test the role of centrosomes in breast cancer, I induced centrosome defects in normal cells by overexpressing the centrosome protein pericentrin. Pericentrin-expressing cell lines exhibited several tumor-like features including abnormal spindles that missegregated chromosomes, genetic instability, cellular disorganization, growth in soft agar and loss of mitotic checkpoint control (Purohit et al., 1999). Based on these observations we propose a model in which centrosome defects and elevated pericentrin levels induce chromosome missegregation and loss of cellular architecture thus contributing to genetic instability, glandular disorganization and breast tumor progression. The specific aim is to determine whether centrosome defects are present in precancerous lesions of the breast and if they can predict breast tumor progression. To address this aim the study was conducted to determine whether centrosomes are abnormal in in situ ductal carcinoma of the breast (DCIS).

Body:

Methods:

Forty two breast biopsies containing ductal carcinoma in-situ (DCIS) were obtained from the surgical pathology files of U.mass. Memorial Health Care, University Campus. Of these, 31 cases had co-existing infiltrating ductal carcinoma while 11 contained DCIS alone. All slides were reviewed and appropriate formalin fixed, paraffin embedded blocks were selected for immunostaining.

Immunostaining with centrosome specific antibodies (Purohit et al., 2001).

Histologic sections were cut at 5 microns from the paraffin blocks, picked up on glass slides, heated at 60 ° C for 30 minutes, then deparaffinized and rehydrated through a series of xylene, alcohol and water. The slides were next heat-treated in a 0.2 mM solution of EDTA for 12 min in a microwave pressure cooker. Slides were then transferred to a TechMate 1000 (Ventana Medical System, Tuscon, AZ) automated immunostainer and treated with antibodies to pericentrin (rabbit polyclonal antibody used at 1:500 dilution), gamma tubulin (mouse monoclonal 1:3000, Sigma

Immunochemicals), Centrin (mouse monoclonal, 1:500, a gift from Jeffrey Salisbury). Slides were initially subjected to hydrogen peroxide and serum blocking steps and then incubated with primary antibody solutions for 24 hrs in a humid chamber to prevent desiccation. Next day slides were given several brief buffer washes and then reattached to the automatic immunostainer. Primary antibodies were detected by the standard ABC method using appropriate secondary antibodies and Horseradish peroxidase/DAB. Sections were lightly counterstained with hematoxyline. Optimum pretreatment and dilutions were determined by testing with both known positive and negative control tissue sections.

In situ hybridization with chromosome specific centromeric probes:

For in situ hybridization studies, tissue sections parallel to those stained by immunoperoxidase were deparaffinized and heated in a microwave pressure cooker for twenty min in a solution containing 0.01M sodium citrate (pH 6.0). After cooling to room temperature sections were treated with a solution of pepsin (40ug/ml) in 0.1 N HCl for 10 min. Pepsin digestion was stopped by washing the sections several times in 2XSSC at room temperature. Slides were then dehydrated in a series of alcohols and air-dried. Biotinylated probes to the centromeric regions of chromosomes 1 or 8 were added in hybridization buffer and slides were mounted sealing coverslips with rubber cement. Target DNA and probes were codenatured in a Hybrite oven (Vysis, Downers Grove, IL). Hybridized probe was detected using a modified ABC method using the TechMate 1000 and biotinyltyramine amplification. Sections were lightly counterstained with hematoxylin to reveal nuclei.

Results:

Analysis of sections immunostained for centrosome antigens:

In all cases we analyzed both centrosome number and structure in parallel sections immunostained for four different centrosome antigens. We used pericentrin and gamma tubulin immunostained sections to determine centrosome size, since pericentrin and gamma tubulin both distribute in pericentriolar material (PCM). To determine centrosome numbers we used centrin immunostained sections since centrin is present mainly in close association with centrioles and its smaller distribution volume facilitated the enumeration of centrosomes.

Analysis of sections hybridized with centromere specific probes.

A total of 100-120 nuclei in tumor and non-tumor areas of the section (identified by hematoxylin counterstain) were scored for centromeric signals. Chromosomal instability (CIN) was determined by computing the fraction of cells with signals greater than two. This cutoff is known to underestimate the true CIN level. Computing gains in chromosome number only however decreases the compounding effect of nuclear truncation artifact inherent to tissue sections. Cells in the G2 phase of the cell cycle

which have four copies of each chromosome can easily be distinguished from cells with supernumerary chromosomes because chromosomes occur as paired sister chromatids.

Immunoperoxidase studies with antibodies to pericentriolar material (PCM) and centrioles reveal that 52 (22/42), 61 (26/42) and 28 (12/42) percent of DCIS lesions has abnormal centrosome number, size and structure, respectively. In many of the lesions two or more centrosome abnormality coexisted. No centrosome abnormality could be detected in 28 (12/42) percent of the cases. Abnormal centrosome numbers correlated significantly with histologic grade, nuclear grade and the presence or absence of a neighboring invasive tumor component (Table 1). Increase in centrosome size but not the presence of structurally abnormal centrosomes also correlated significantly with the histologic grade of the lesion.

There was a good correlation between centrosome defects and chromosome instability (CIN) as the number of samples with more than 2 copies of chromosome # 1 was significantly higher in cases harboring centrosome defects than in those without. Lastly, there also was a significant association between the extent of chromosome instability and the histologic and nuclear grade of DCIS lesions (fig.1).

A. Centrosome number vs Histologic Grade

	DCIS-H	DCIS-L	Normal	Totals
Normal	12	9	7	28
Supernumerary	18	3	0	21
Totals	30	12	7	49

P Value 0.05 0.22

B. Centrosome number vs. Nuclear Grade

	0	1	2	3	Totals
Normal	7	8	9	4	28
Supernumerary	0	0	7	14	21
Totals	7	8	16	18	49

P Value 0.1 0.5 0.05

C. Centrosome size vs. Histologic Grade

	DCIS-H	DCIS-L	Normal	Totals
Normal	7	9	7	23
Abnormal	23	3	0	26
Totals	30	12	7	49

P Value 0.001 0.03

D. Centrosome shape vs. Histologic Grade

	DCIS-H	DCIS-L	Normal	Totals
Normal	21	9	7	37
Abnormal	9	3	0	12
Totals	30	12	7	49

P Value 0.25 0.22

E. Centrosome number vs. Invasive Component

	Non-Inv	Invasive	Totals
Normal	14	14	28
Supernumerary	4	17	21
Totals	18	31	49

P Value 0.02

Table 1. Correlation between centrosome defects and histologic features:

A. Centrosome numbers are displayed against the histologic grades such as DCIS-H, DCIS-L and Normal lobules. B. Centrosome numbers are shown against the nuclear grades as 0,1,2,3. C. Centrosome size versus histologic grades. D. Centrosome shape is quantitated against the histologic grade.

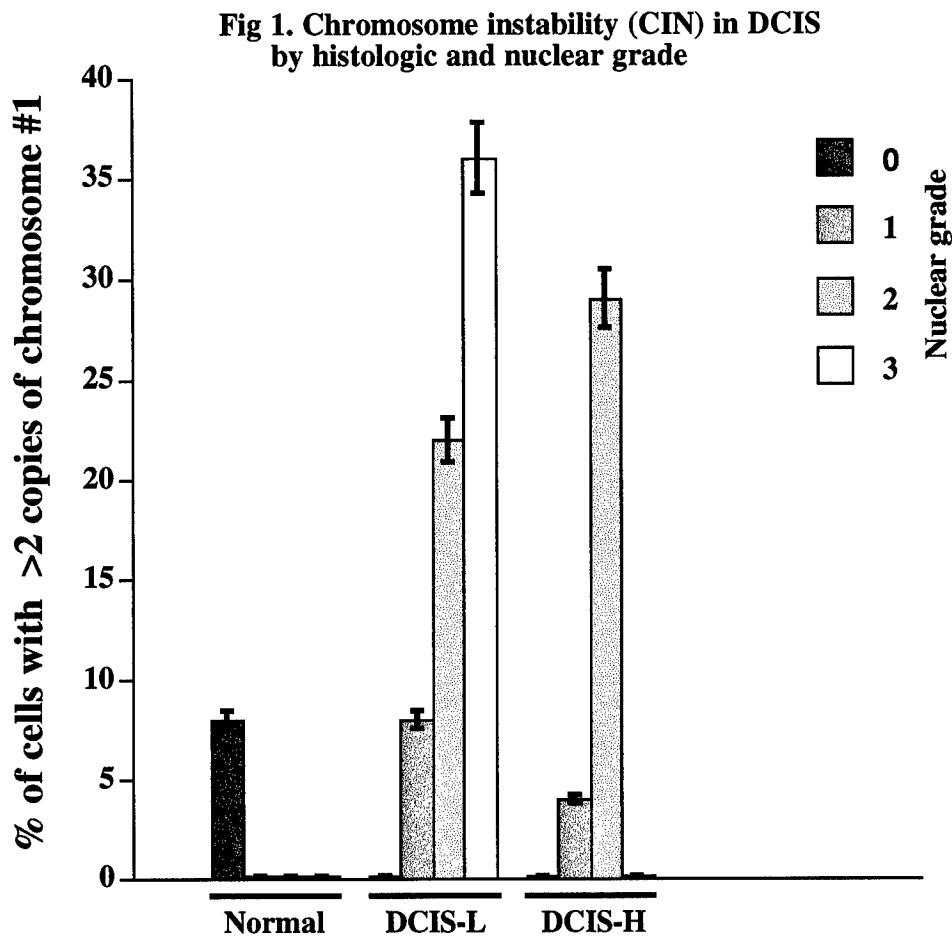


Figure 1. Chromosome instability (CIN) in DCIS by histologic and nuclear grade: Percentage of cells with > 2 copies of the chromosome #1 are quantitated in different nuclear grades in normal lobules, DCIS-L and DCIS-H histologic grades.

Key Research Accomplishments:

- German Pihan, Yening Zhou, Aruna Purohit and Stephen Doxey. (2001). Centrosome defects and genetic instability occur together in precancerous lesions of the breast, cervix and prostate. Amer . Soc. Cell Biol. Washington D.C. 12/2001.

Reportable outcomes:

United States Patent : Cancer detection by centrosome abnormality.

University of Massachusetts, Boston, MA.
Patent number: 5,972,626.

German Pihan, Yening Zhou, Aruna Purohit and Stephen Doxsey. (2001). Centrosome defects and genetic instability occur together in precancerous lesions of the breast, cervix and prostate. Amer. Soc. Cell Biol. Washington D.C. 12/2001.

Purohit, A., Pihan G. and Doxsey, S.J. Methods for the study of pericentrin in centrosome assembly and function (2001). Meth. Cell Biol, 67, 53-69.

Conclusion:

Centrosomes are abnormal in a significant number of DCIS lesions and correlate with higher histologic and nuclear grades and with the presence of an invasive tumor component. Our findings suggest that centrosome dysfunction may be involved in the histologic progression of DCIS lesions towards invasive cancer. Our observations have important implications for the biology of breast cancer as they suggest that centrosome dysfunction is an early event in breast carcinogenesis and may be critical for cancer progression.

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Pihan, G., Purohit, A., Knecht, H., Woda, B., Quesenberry, P. and Doxsey, S.J. 1998. Centrosome defects and genetic instability in malignant tumors. *Cancer Research*. 58:3974-3985.

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Purohit, A., Tynan, S.H., Vallee, R. and Doxsey, S.J. 1999. Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *J. Cell Biol.* 147:481-491.

Appendices

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Centrosome defects and genetic instability occur together in precancerous lesions of the breast, cervix and prostate.

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Centrosomes contribute to spindle organization, mitotic chromosome segregation and cellular architecture. We and others first demonstrated that centrosomes are abnormal in nearly all human malignant tumors¹⁻³. We also showed that the centrosome protein pericentrin is elevated in these tumors. These studies did not address whether centrosome defects were a secondary result of tumorigenesis or if they contributed to the process. If they are contributing factors, centrosome defects should occur in precancerous lesions and they should cause tumor features when induced artificially. We show that artificial elevation of pericentrin levels induced centrosome defects and caused or exacerbated cancer-like features including cell proliferation, growth in soft agar, genetic instability, and changes in cell and nuclear morphology^{2,4}. We also examined paraffin sections containing precancerous lesions of the breast (breast ductal carcinoma in situ, DCIS), cervix (squamous intraepithelial lesions of the uterine cervical, SIL) and prostate (prostate intraepithelial neoplasia, PIN) for centrosome defects by immunostaining for pericentrin, γ tubulin, centrin and for genetic instability by FISH. The percentage of SIL lesions that showed abnormal centrosome #, size and structure were 50%, 52% and 23%, respectively (n = 48). Centrosome defects were also identified in fresh cervical tissues (PAP smears) where numerous mitotic defects were also detected. The percentage of DCIS lesions that showed abnormal centrosome #, size and structure were 52%, 61% and 28%, respectively (n = 41). The percentage of PIN lesions that showed abnormal centrosomes (combined) was 24% (N = 17). No control samples in any tissue showed such defects. In all tissues examined, there was a significant correlation between centrosome abnormality and chromosome instability. These results are consistent with a model in which centrosome defects contribute to tumorigenesis.

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