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Is Nuclear Structure Altered in Breast Cancer Cells?

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14. ABSTRACT To explore whether changes in nuclear architecture exist between normal and breast cancer cells, several nuclear markers were examined for their potential to reveal changes in nuclear architecture in normal human mammary epithelial cell (HMEC; prestasis 48R), altered but non-malignant HMEC (48RS, 184, and 184B5), and breast cancer cell lines (MCF-7 and MDA-MB-231). Immunofluorescence with anti-estrogen receptor α (ERα) antibodies detected the presence of ERα in MCF-7 cells but not MDA-MB-231 cells and low level of ERα is present in a pre-stasis HMEC line (48R) but not in other HMEC lines (48RS, 184, and 184B5). Examination of the distribution of RNA polymerase II "transcription factories" with an antibody directed at the hyperphosphorylated C-terminal tail of the largest subunit of RNA polymerase II showed greater nucleoplasmic staining over cytoplasmic staining in MCF-7 cells than in MDA-MB-231 cells, and qualitatively, our preliminary results suggest that a larger fraction of the large subunit of RNA pol II to be in transcription factories in the case of MCF-7 than MDA-MB-231 or HMEC lines (48R, 48RS, 184 and 184B5). Lastly, when bulk chromatin as well as specific chromosomes were assessed by staining with DAPI and chromosome paints (chromosome 1, 3, 18, and 19), respectively, the different cell lines failed to show any striking differences. Quantitative assessment of the transcription factories, chromosome locations, and DAPI stained nuclei needs to be performed to determine the degree to which nuclear structure is altered in breast cancer cells.

15. SUBJECT TERMS
Interphase nucleus, marker, nuclear structure, normal HMEC, RNA polymerase II, estrogen receptor α, chromosome territory, DAPI

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Table of Contents

Page

INTRODUCTION:

The process of transformation from normal breast to cancer cells leads not only to gene expression changes but also to changes in cellular architecture. Cytogenetic analysis of metaphase chromosomes shows that transformation and progression of breast cancer involves chromosomal alterations, aneuploidy, and genomic instability. For example, chromosome 1 is often associated with breast cancer aneusomy (Tsukamoto et al., 2001) and regions in chromosome 3p are often deleted in breast cancers (Yang et al., 2002). For the interphase nucleus, morphometric analysis of its size and shape serves as a critical component of a grading system for breast cancer and has prognostic value (Kamath et al., 2005). Since the size and shape of the interphase nucleus is altered in breast cancer cells, it is likely that the internal structures within the interphase nucleus are also altered. Two cases suggest that internal structures may be altered in the interphase nucleus of breast cancer cells. For the nucleolus, the number of silver-stained nucleolar organizer regions and perinucleolar compartments may correlate with breast cancer progression (Kamath et al., 2005). For the nucleoplasm, organization of estrogen receptor (ER) binding sites were different in different breast cancer cells, indicating underlying structural changes in the interphase nucleus (Htun et al., 1999); whether these differences in ER-binding site organization exist between normal and breast cancer cells is unknown.

To determine if the internal organization of interphase nucleus is altered in breast cancer cells, this study examined the distribution of bulk chromatin as well as specific components of the nucleoplasm in normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells. For the bulk chromatin distribution, normal breast cells, altered but non-malignant breast cells, and breast cancer cells, before and after hormone induction were stained with fluorescent DNA dyes. For specific components, the distribution of ER-binding sites, RNA polymerase transcription sites and specific chromosome territories between normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells were examined.

BODY:

We were able to examine the distribution of ER-binding sites and RNA polymerase II transcription factories in normal human breast cells and human breast cancer cells as specified as task 1 in the statement of work for the project. We obtained and cultured normal human breast cells (48R pre-stasis), altered but non-malignant human breast cells (48RS post-stasis, 184 post-stasis, and 184B5 immortalized), and human breast cancer cells (MCF-7 and MDA-MB-231) (task 1a). We transfected MCF-7 cells with a GFP-ERα fusion gene and then examined the effect of ligand-dependent distribution of GFP-ERα in the cells, by adding estradiol to the cells and examining the cells by fluorescence microscopy. We also performed immunofluorescence microscopy by using antibodies against ERα to determine ERα status of normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells that we had obtained and culture. ERα was not detected by an ERα monoclonal antibody in the altered but non-malignant human breast cells, 48RS post-stasis, 184 post-stasis, and 184B5 immortalized, and human breast cancer cells, MDA-MB-231. A low level of ERα was detected in the normal human breast cancer cells, 48R pre-stasis and a higher level of ERα was detected in human breast cancer cells, MCF-7. To look at the distribution of RNA polymerase II transcription factories in normal human breast cells and human breast cancer cells, we used antibodies against the

largest subunit of RNA polymerase II. Again the RNA polymerase II transcription factories were observed in normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells by immunofluorescence microscopy (task 1b).

We were also able to examine the subnuclear staining of chromatin and chromosomal territories in normal and breast cancer cells as specified as task 2 in the statement of work for the project. We obtained and cultured normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells (task 2a). We stained the bulk chromatin in normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells using DAPI stain and then observed the staining pattern by fluorescence microscopy. We also stained specific chromosomes (chromosomes 1, 3, 18 and 19) by using chromosome paints and observed the stained chromosomes using fluorescence microscopy in normal human breast cells, altered but non-malignant human breast cells, and human breast cancer cells (task 2b).

Our results showed that an ERα monoclonal antibody detected the presence of ERα in MCF-7 cells but not MDA-MB-231 cells; low level of ERα is present in a pre-stasis HMEC line (48R) but not in other HMEC lines (48RS, 184, and 184B5). Ligand-dependent ERα redistribution was readily observed with a GFP-ERα fusion protein but not by immunofluorescence. Qualitative assessment reveals a larger fraction of the large subunit of RNA pol II to be in transcription factories for the MCF-7 than MDA-MB-231 or HMEC lines (48R, 48RS, 184 and 184B5). Chromosome paints were used to identify chromosomes 1, 3, 18 and 19 in the interphase nucleus of different cell types but differences in their positions were not readily apparent by qualitative assessment.

KEY RESEARCH ACCOMPLISHMENTS:

•An ERα monoclonal antibody detected the presence of ERα in MCF-7 cells but not MDA-MB-231 cells; low level of ERα is present in a pre-stasis HMEC line (48R) but not in other HMEC lines (48RS, 184, and 184B5).

•Ligand-dependent ERα redistribution is readily observed with a GFP-ERα fusion protein but not by immunofluorescence.

•Qualitative assessment reveals a larger fraction of the large subunit of RNA pol II to be in transcription factories for MCF-7 than MDA-MB-231 or HMEC lines (48R, 48RS, 184 and 184B5).

REPORTABLE OUTCOMES:

Htun, H., R. S. Singh, and W. J. Dixon, Is Nuclear Structure Altered in Breast Cancer Cells? 5th Era of Hope, Breast Cancer Research Prog., Baltimore, MD. June 25-28, 2008.

CONCLUSIONS:

We were able to determine the ERα status for all the cell types that we obtained and cultured. Two of the cell types had levels of ERα that were detectable by immunofluorescence, a human breast cancer cell line, MCF-7, and a normal human breast cell type, 48R pre-stasis. The human breast cancer cell line was examined for ligand-dependent distribution of ERα binding sites. Since the 48R pre-stasis cells, a normal human breast cell type, has levels of ERα that are detectable by immunofluorescence, these cells can be examined for ligand-dependent distribution of ERα binding sites and the results can be compared with those for MCF-7 to see if a difference is observed between human breast cancer cells and normal human breast cells. As for the distribution of RNA polymerase II transcription factories, qualitative assessment did show some differences between different cell types. In particular, a larger fraction of the large subunit of RNA pol II was in transcription factories for the MCF-7 than MDA-MB-231 or HMEC lines (48R, 48RS, 184 and 184B5). The differences in position of chromosomes 1, 3, 18 and 19 were not readily apparent by qualitative assessment. Further quantitative assessment of the transcription factories, chromosome locations, and DAPI stained nuclei needs to be performed to accurately determine the degree to which the nuclear structure is altered in breast cancer cells.

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