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Nuclear and Metabolic Quantification for Enhanced Ductal Carcinoma In Situ Risk Stratification

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Summer Undergraduate Research Program

Nuclear and Metabolic Quantification for Enhanced Ductal Carcinoma In Situ Risk Stratification

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Abstract

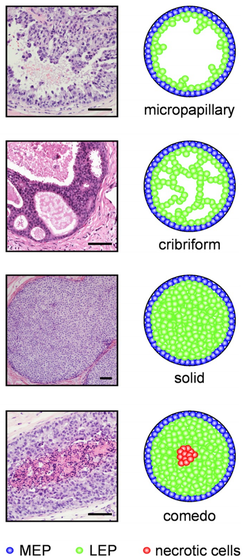
Ductal carcinoma *in situ* (DCIS) is currently considered an early and localized form of ductal breast cancer stemming from the epithelial ductal cells. These lesions are largely heterogenous, categorized by their morphologies, amount of necrosis, and stromal changes. Even though 10-year mortality rate for DCIS is 1-2.6% while that of early invasive breast cancer is 7-10%. Yet, current recommended treatment for DCIS is breast-conserving surgery and radiation or mastectomy – the same treatment regimen recommended for early invasive breast cancer. This assumes that all DCIS will progress to invasive breast cancer if left untreated. However, mounting evidence indicates that a significant number of DCIS would remain indolence and never progress to invasive cancer. Current risk stratification is based on grade and hormone receptor (estrogen and progesterone) status. While the underlying mechanisms for DCIS to invasive cancer progression are not well understood, an improvement in the quantification of cellular morphology, the extracellular matrix and the metabolism modification of the tumor microenvironment could provide a more accurate and objective prognostication and treatment recommendations. DCIS is currently graded manually by a surgical pathologist using a representative number of areas on the slide. This risks grading bias between different pathologists. By using an automated software to measure quantifiable attributes such as nuclear density, size, and degree of variation of all areas of DCIS on the slide, we can have a more uniform and objective scoring system that would have minimal bias and variation. In addition, we will quantify heterogeneity of collagen arrangement, collagen fiber profile in the stroma as well as the metabolic modifications in the tumor microenvironment of "low risk" vs "high risk" DCIS to determine factors that could provide us with a better prognostication system.

Introduction

DCIS comprises of approximately 25% of all breast cancer cases in the United States. Incidence of DCIS have increased over the past several decades due to the widespread use of mammographic screening. The standard treatment for DCIS is lumpectomy followed by radiation therapy, or mastectomy alone. However, clinical trials are ongoing to determine new risk stratification systems that could identify low risk DCIS cases that can omit or receive less invasive treatment.

One difficulty when assigning a standard treatment for DCIS is its large heterogeneity. DCIS is graded into 3 groups: low, intermediate, and high grade. These categories are judged by pathologists on morphology (Fig 1a), amount of necrosis, and tumor grade. Figure 1a demonstrates the four morphological architectures. Studies have suggested that micropapillary has been correlated to the lowest risk of recurrence and metastasizing, while a presence of necrosis indicates higher risk.

Other characteristics have been identified as potential indicators for high grade DCIS. Nuclear size and density are factors that change in most cancer cases. Tumor infiltrating lymphocytes (TILs) are the immune response to DCIS and are observed as a potential indicator of DCIS risk. Lastly, hormonal receptors, particularly HER2 receptor positivity, have been indicated as an early indicator for DCIS becoming high grade. All these factors likely play some role in DCIS grade, but for this study, we will look particularly at nuclear size, density, and optical redox ratio.



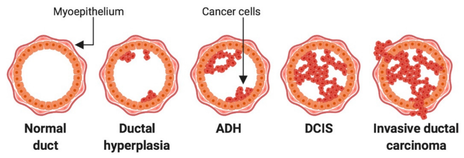


Figure 1: DCIS morphology with H&E- stained samples (above). Progression of breast duct from normal to invasive carcinoma (below).

Methods

1. DCIS cases were selected and stratified based on HER2 and estrogen receptor (ER) status:

   Group A = HER2-/ER+; least aggressive
   Group B = HER2+/ER+; slightly more aggressive than HER2+/ER-
   Group C = HER2+/ER-; more aggressive than HER2-/ER+
   Group D = HER2-/ER-; "triple negative", most aggressive receptor status

   A breast surgical pathologist, Dr. David Wagner, was consulted to score the DCIS slides.
2. Slides were cut, stained, digitally scanned, and assessed in QuPath (software for whole slide image analysis of H&E slides and automated nuclear quantification). Regions of interest (ROIs, individual ducts) were manually selected using the software's polygon tool (Fig 2a), followed by the automatic quantification of the nuclear data for the ROI (Fig 2b).
3. Data from QuPath was exported to Microsoft Excel. Single factor ANOVAs and *ad hoc* student t-tests were used for statistical analysis.
4. Endogenous fluorescence from the metabolic intermediates, NADH and FAD, were acquired at the MITI Research Core.
5. Using FIJI (NIH ImageJ), ductal ROIs were selected and the optical redox ratio (ORR, FAD/(NADH+FAD)) was calculated. (Fig 2c)

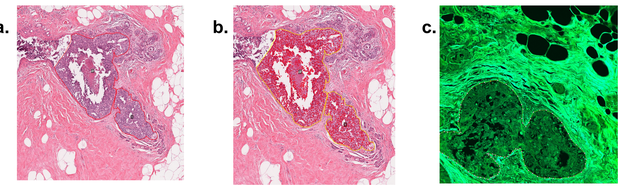


Figure 2: **(a)** ROI selected from H&E-stained slide from nuclear quantification. **(b)** QuPath runs nuclear recognition software and highlights identified regions. This is exported to Excel for further data analysis. **(c)** FIJI analysis quantifies NADH/FAD luminescence from selected ROI.

Results

HER2 and ER Status are Related to Differences in Cell Density and Nuclear Area

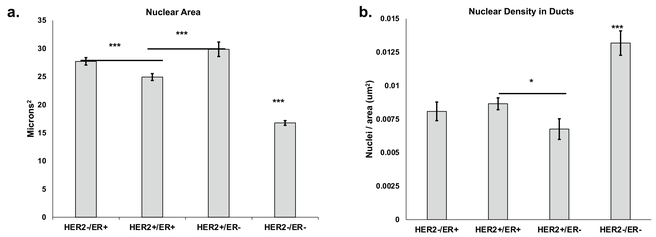


Figure 3: **(a)** We predicted area would increase with receptor status associated aggressiveness because of the rapid-reproducing nature of cancer. Nuclei in Group D (most aggressive receptor status) were significantly smaller than all other groups. Group B (more aggressive) also showed a smaller nuclei than Groups A and C nuclei. **(b)** Nuclear density in Group D (most aggressive receptor status) was significantly larger than all other groups. Group B (more aggressive) also showed a larger nuclear density than Groups A and C nuclear densities.

Results

HER2 and ER Status are Related to Ductal Cell Heterogeneity

Hayward et al., proposed that ducts could be accurately sorted into DCIS grade by the area of their nuclei. Following their criteria, we determined the percentage of each DCIS grade in each group. The criteria for each DCIS grade are as follows:

Normal Duct < 20 µm
DCIS Grade 1: 20 – 30 µm
DCIS Grade 2: 30 – 40 µm
DCIS Grade 3: > 40 µm

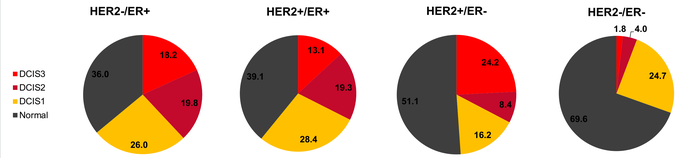


Figure 4: The more aggressive DCIS (Group B and D) are comprised of mostly normal and Grade 1 DCIS while the least aggressive DCIS (Group A) has the grades more evenly distributed.

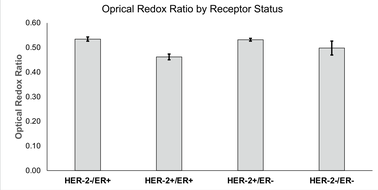


Figure 5: Groups A and C showed a statistically higher than Group B ($p < .01$) NADH intensity. There was no statistical difference between Groups A and C, or any group with Group D ($p > .05$).

Conclusion

- Nuclear density would be expected to increase with the progression of DCIS. Our data showed group D ("triple negative", most aggressive receptor status) has the highest density compared to the other three groups. In addition, Group B had significantly higher density than C which is expected since HER2+/ER+ cancer are known to be more aggressive than HER2+/ER-.
- Interestingly, our data showed a significant *decrease* in nuclear size associated with increasing degree of aggressiveness (Group D) of the DCIS with the most aggressive group has a distinct significantly lowest nuclear area.
- We predicted greater heterogeneity in the Group D ducts, the most aggressive receptor status. The greatest heterogeneity was observed in the least aggressive receptor status samples.
- There were no gross differences in cellular metabolism measured using ORR.
- Ongoing studies will increase sample sizes, stratify samples by recurrence, and included measurements of extracellular matrix changes (collagen density) to better resolve recurrence risk in individual with DCIS.

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2. Slides of breast tissues were prepared by UNMC's Tissue Sciences Facility
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