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POTENTIAL ROLE OF TISSUE MICROARRAYS FOR THE STUDY OF BIOMARKER EXPRESSION IN BENIGN BREAST DISEASE AND NORMAL BREAST TISSUE

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Abstract

Background: Tissue microarrays (TMAs) are commonly used to study biomarker expression in invasive breast cancers. Whether or not TMAs may also be a potentially useful platform for assessing biomarkers in benign proliferative breast lesions (BPBL) and normal breast tissue has not been previously studied in detail.

Methods: We evaluated the success of capturing the targeted areas in TMAs constructed from benign breast biopsy blocks of 368 Nurses' Health Study (NHS) and NHS II participants. Areas targeted included 214 BPBL and 361 normal terminal duct lobular units (TDLUs). At least three 0.6mm cores were obtained from the areas of interest from each donor paraffin block and arrayed into a recipient block. Sections cut from TMA blocks were stained with hematoxylin-and-eosin. Each TMA slide was examined to determine the number of cores/case in which the targeted area was represented.

Results: Overall, the targeted area was present in 776 of 1,800 TMA cores (43%). At least one of the cores contained the area of interest for 401 of the 575 targeted foci (70%), including 76%, 66%, 60% and 40% of cases in which the targeted area was normal TDLUs, usual ductal hyperplasia, atypical lobular hyperplasia and atypical ductal hyperplasia respectively.

Conclusions: In TMAs constructed from BPBL and normal TDLUs, the targeted area was present on at least one core in 70% of cases. Our findings indicate that it is feasible to construct TMAs from donor tissue blocks consisting of BPBLs and normal breast tissue with a relatively high rate of capture of the targeted area.

Keywords

Benign breast disease; Tissue microarrays

Introduction

Tissue microarrays (TMAs) are now commonly used as a platform to screen large numbers of invasive breast cancers using such techniques as immunohistochemistry, in situ hybridization, and fluorescence in situ hybridization (1,2). Numerous studies have suggested that invasive

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breast cancer TMAs constructed by obtaining up to four 0.6mm tissue cores from each donor tissue block affords adequate sampling of the cancers for screening a variety of biomarkers in a uniform and cost-effective manner (1,2).

Given the success of TMAs in studying invasive cancers, the possibility that TMAs may also represent a potentially useful platform for high throughput screening of biomarkers in benign proliferative breast lesions (BPBL) and normal breast tissue merits consideration. However, the areas of interest in such cases are by their nature limited in extent, and the adequacy of lesion sampling afforded by TMA technology in this setting is a matter of concern. To our knowledge, studies of the feasibility of this technology for studying BPBL and normal breast tissue have not been reported. To address this issue, we constructed TMAs from BPBL and/or normal ducts and lobular units (TDLUs) from benign breast biopsies of women enrolled in the Nurses' Health Study.

Materials and Methods

Study population

Study Design and Population—The Nurses' Health Study (NHS) was initiated in 1976, when 121,700 U.S. registered nurses ages 30-55 returned an initial questionnaire. The NHS II is a separate cohort study consisting of 116,671 female registered nurses who were between ages 25 and 42 when the study began in 1989. These cohorts have been followed by mailed questionnaires biennially to update exposure information and ascertain non-fatal incident diseases. Information collected includes diagnosis of cancer, as well as benign breast disease, which is updated every two years through questionnaires. The follow-up rate was over 90% through 1996.

Benign breast biopsy confirmation

Beginning with the initial NHS questionnaire in 1976 (in 1989 for NHS II), participants have been asked on every biennial questionnaire to report any diagnosis of fibrocystic disease or other benign breast disease and to indicate if it was confirmed by biopsy or hospitalization. Within this subcohort, we identified incident confirmed breast cancer cases diagnosed after the return of the initial questionnaire through the 1996 (NHSI)/1995 (NHSII) follow-up cycle and controls that also reported a previous biopsy-confirmed benign breast disease. A detailed description of this nested case-control study has been previously described (3). Hematoxylin and eosin (H&E) slides from the benign breast biopsy were independently reviewed by one of two pathologists (SJS, JLC) in a blinded fashion. Any slide identified as having either questionable atypia or atypia was jointly reviewed by the two pathologists. For each set of slides reviewed, a detailed work sheet was completed and the benign breast biopsy was classified according to the categories of Page et al as non-proliferative, proliferative without atypia or atypical hyperplasia (atypical ductal hyperplasia (ADH) or atypical lobular hyperplasia (ALH)).

Benign breast biopsy block collection and tissue microarray construction

After centralized review of H&E slides, we collected archived formalin-fixed paraffin-embedded benign breast biopsy blocks for participants. H&E sections of the corresponding paraffin-embedded tissue blocks were re-reviewed by a single pathologist (JLC) to identify areas of benign proliferative lesions and normal tissue, and to circle the areas from which the cores for the TMAs would be taken. We constructed 5 TMA blocks that contain normal TDLUs and benign lesions, representing 373 participants with one or more of the following types of benign lesions: apocrine metaplasia, non-apocrine cysts, usual ductal hyperplasia (UDH), ADH or ALH.

TMAs were constructed in the Dana Farber Harvard Cancer Center Tissue Microarray Core Facility, Boston, MA, by obtaining 0.6-mm cores from the targeted area in each benign breast tissue block and inserting them into the recipient TMA blocks. For 96% of the targeted areas 3 cores were obtained. Six cores were obtained in 23 cases and nine were obtained in one case. Participants with only apocrine metaplasia or non-apocrine cysts only were not included in the current study (n=5). The current analysis is restricted to 368 participants with 575 BPBL (UDH, ADH and ALH) and/or normal TDLUs (Table 1).

Tissue microarray analysis

For this study, one 5-μm paraffin section was cut from each of the five TMA blocks and stained with H&E. Given the issues of re-facing blocks when additional sections are cut and the value of this resource, up to six additional levels were cut from each TMA block at the same time for staining with biomarkers for future analysis. The H&E-stained TMA slides were evaluated for the presence of the targeted area and are the focus of this study.

Results

A total of 1,800 cores were obtained from the donor paraffin blocks. Targeted areas included 214 BPBLs and 361 normal TDLUs. The distribution of the targeted areas for inclusion in the TMA is shown in Table 2. Overall, the targeted area was present in 776 of the 1,800 TMA cores (43%) (Table 3). Of note, however, at least one of the cores in the TMA contained the area of interest in 401 of the 575 targeted foci (70%). For cases in which the targeted area was normal TDLUs, the target was present in 50% of the cores. The success rate of obtaining the area of interest was lower when specific benign proliferative lesions were targeted. The area of interest was present in 38.4% of cores where UDH was targeted, 31.1% of cores in which the targeted area was ALH and in 18.2% of cores in which ADH was the lesion of interest. However, when each targeted area was evaluated by case rather than by core, at least one of the cores contained the targeted area in 76% of cases for normal TDLUs, 66% of cases for UDH, 60% of cases for ALH and 40% of cases for ADH (Table 4). When we randomly selected three cores for lesions in which greater than 3 cores were obtained, the overall results were identical (Table 5.) No tissue was present for evaluation in 266 of the 1,800 cores (14.8%) i.e. the tissue “histospot” was not present on the hematoxylin and eosin stained slide, likely due to non-transfer of material at the time the TMA slide was prepared.

Discussion

We constructed TMAs of BPBL and normal breast TDLUs using the technology most commonly employed for constructing invasive breast cancer TMAs and found that the targeted area of interest was present on at least one TMA core in 70% of the cases. Loss of tissue for evaluation was seen in less than 15% of the 1,800 cores, in keeping with our experience and that reported by others for tissue loss on TMAs of invasive breast cancers and other primary tumors (4).

The issue of greatest concern regarding the use of TMAs to study BPBLs is that the targeted area is typically of small size. Therefore, ensuring that the targeted lesion can be captured for the TMA is of particular importance before performing any biomarker analyses. Our results indicate that the area of interest was present on the TMA slides in 76% of cases where normal TDLUs were targeted and in 40-66% of cases of BPBLs. These results suggest that TMAs are a reasonable platform for the evaluation of biomarker expression in these lesions, and may, in fact, be improved upon if greater than 3 cores of each area of interest are obtained at the time of TMA construction. Of note, the yield of BPBLs and/or normal TDLUs on standard histologic sections when additional levels are cut from the paraffin block is unknown. Thus it is not clear whether the yield of targeted lesions is lower, higher or similar in TMAs as compared with

standard sections. We know from our clinical experience, however, that BPBLs such as ADH are often not present when additional sections are recut from the paraffin blocks for diagnostic purposes.

Few prior studies have addressed the issue of whether TMAs are a useful methodology for evaluation of biomarkers in benign lesions. Lugli and colleagues constructed a TMA of normal tissues from 76 different normal tissue types. These authors reported that calretinin expression could be evaluated in all normal tissues (4). It is not clear from their report whether every core yielded useful results or whether one of multiple cores yielded useful results for each “case”.

One limitation of this analysis is that we did not cut multiple sections from the TMA blocks for H&E staining to determine the number of sections in which the targeted areas of interest persist. As indicated above, the donor blocks from which these TMAs were constructed consisted of benign breast tissue samples from women enrolled in a case-control study of benign breast disease and breast cancer risk nested within the Nurses' Health Study. Given the value of this unique and exhaustable resource, we considered it prudent to retain as much material as possible in the TMA blocks for future biomarker studies. Others have shown that at least 200 consecutive 5 micron sections can be cut from TMA blocks of breast cancer (1, 5). Analysis of a variety of biomarkers in our study population is ongoing and will shed light on this issue for BPBLs and normal breast tissue as experiments progress.

In conclusion, our findings indicate that it is feasible to construct TMAs from donor tissue blocks consisting of BPBLs and normal breast tissue with a relatively high rate of capture of the targeted area.

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Table 1

Frequency of target areas included in the study, per participant

Target areas	Frequency
Normal TDLUs only	162
UDH and normal TDLUs	133
ADH and normal TDLUs	41
ALH and normal TDLUs	17
UDH, ADH and normal TDLUs	1
ADH, ALH and normal TDLUs	7
UDH only	5
ADH only	1
ALH only	1
Total	**368**

TDLUs =terminal duct lobular units, UDH=usual ductal hyperplasia, ADH=atypical ductal hyperplasia, ALH=atypical lobular hyperplasia

Table 2

Frequency of targeted areas included in tissue microarray

Target area	Frequency	Percent (%)
Normal TDLUs	361	62.8
Usual ductal hyperplasia (UDH)	139	24.2
Atypical ductal hyperplasia (ADH)	50	8.7
Atypical lobular hyperplasia (ALH)	25	4.3
Total	**575**	**100.0**

TDLUs=terminal duct lobular units

Table 3

Comparison of targeted area and area obtained on TMA, per core

Target diagnosis	TMA diagnosis	Frequency	Percent (%)
Normal TDLUs	ducts only	36	3.3
Normal TDLUs	**normal TDLUs**	**545**	**49.8**
Normal TDLUs	stroma	322	29.4
Normal TDLUs	apocrine metaplasia	24	2.2
Normal TDLUs	UDH	10	0.9
Normal TDLUs	no tissue	158	14.4
Total Normal TDLUs		**1095**	**100.0**
UDH	ducts only	4	0.9
UDH	normal TDLUs	75	16.7
UDH	stroma	121	26.9
UDH	apocrine metaplasia	26	5.8
UDH	**UDH**	**173**	**38.4**
UDH	no tissue	51	11.3
Total UDH		**450**	**100.0**
ADH	ducts only	3	1.8
ADH	normal TDLUs	33	20.0
ADH	stroma	55	33.3
ADH	apocrine metaplasia	3	1.8
ADH	**ADH**	**30**	**18.2**
ADH	no tissue	41	24.9
Total ADH		**165**	**100.0**
ALH	ducts only	5	5.6
ALH	normal TDLUs	16	17.8
ALH	stroma	23	25.6
ALH	apocrine metaplasia	2	2.2
ALH	**ALH**	**28**	**31.1**
ALH	no tissue	16	17.8
Total ALH		**90**	**100.0**
Total cores		**1,800**	

TMA=tissue microarray, TDLUs=terminal duct lobular units, UDH=usual ductal hyperplasia, ADH=atypical ductal hyperplasia, ALH=atypical lobular hyperplasia

Table 4

Number of cores with targeted area, per target diagnosis

Target lesion	Total cases	Number of cores with targeted area per case (%)			
		≥1	1	2	≥3
Normal TDLUs	361[1]	275 (76.2)	93 (25.8)	96 (26.6)	86 (23.8)
UDH	139[2]	91 (65.5)	31 (22.3)	40 (28.8)	20 (14.4)
ADH	50[3]	20 (40.0)	12 (24.0)	6 (12.0)	2 (4.0)
ALH	25[4]	15 (60.0)	6 (24.0)	6 (24.0)	3 (12.0)

TDLUs=terminal duct lobular units, UDH=usual ductal hyperplasia, ADH=atypical ductal hyperplasia, ALH=atypical lobular hyperplasia

[1] 4 normal TDLUs targeted areas had 6 cores; all remaining women had 3 cores

[2] 11 UDH targeted areas had 6 cores; all remaining had 3 cores

[3] 5 ADH targeted areas had 6 cores; all remaining had 3 cores

[4] 3 ALH targeted areas had 6 cores; 1 ALH had 9 cores; all remaining had 3 cores

Table 5

Number of cores[1] with targeted area, per target diagnosis

Target lesion	Total cases	Number of cores with targeted area per case (%)			
		≥1	1	2	3
Normal TDLUs	361	275(76.2)	95 (26.3)	97(26.9)	83 (23.0)
UDH	139	91 (65.5)	34 (24.5)	40 (28.8)	17 (12.2)
ADH	50	20 (40.0)	12 (24.0)	6 (12.0)	2 (4.0)
ALH	25	15 (60.0)	8 (32.0)	7 (28.0)	0 (0.0)

TDLUs=terminal duct lobular units, UDH=usual ductal hyperplasia, ADH=atypical ductal hyperplasia, ALH=atypical lobular hyperplasia

[1]For lesions with greater than 3 cores, 3 cores were randomly selected.