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Determining the Optimal Numbers of Cores Based on Tissue Microarray Antibody Assessment in Non-Small Cell Lung Cancer

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Abstract

Background: Tissue microarrays (TMAs) have been commonly utilized in translational research to rapidly screen numerous biomarkers in large samples. One major concern has been the adequate assessment of biomarkers affected by within-tumor heterogeneity and by molecular targets.

Methods: Our study was designed to answer a fundamental question: How do researchers define the optimal cores to sample when designing a TMA study to minimize sampling bias and core artifact? We compared the staining results from a full-section tissue slide to the virtual TMA and from the actual TMA to the virtual TMA.

Results: Three cores were demonstrated as optimal for markers such as TTF-1 and p53, but no optimal core number could be determined for markers such as Ki-67 due to the poor TMA representation of the entire tissue.

Conclusion: We propose that before using TMAs to analyze large samples, particularly with significant within-sample heterogeneity, a preliminary investigation using a virtual TMA could help decide target markers to be tested for valid and valued results.

Keywords: Lung cancer; Tissue microarrays; Immunohistochemistry TT; F-1; p53; Ki-67

Introduction

Tissue microarrays (TMAs) are a technically effective and cost-efficient tool to assess multiple tissue samples from a large cohort of patients on a single microscope slide [1]. Although used mostly with immunohistochemistry (IHC), TMAs can be applied for other methodologies, which employ paraffin embedded tissue such as in-situ hybridization and fluorescence in-situ hybridization. Furthermore since only small cores are removed from the donor blocks, the remaining block can be sectioned further with traditional, single tissue section methods. Because of these strengths, TMAs are commonly utilized in translational research to rapidly screen numerous biomarkers in large samples while minimizing variability when multiple individual sections are stained. However, since only a fraction of the tumor is examined with TMAs, one of the major concerns for this technology has been the adequate assessment of biomarkers affected by within-tumor heterogeneity as well as by molecular targets. Several studies have looked at different tumor types and biomarkers to assess the correlation between cores and whole sections and to determine the minimum adequate number of cores [2-10]. The reported concordance between TMAs and whole sections has been variable, ranging from 84% to 98%, usually corresponding to the number of cores. Most studies recommend two cores of 0.6mm each; some studies demonstrate that three to five cores provide a higher concordance [3,5,10]; while others suggest that one core may be sufficient [11,12]. The recommended number of cores appears to vary with the tumor type as well as by the biomarkers under study. Most studies show a better concordance for biomarkers that are diffusely expressed in tumors, [2,3] with markers such as p53 and Ki-67 demonstrating more variation [3,13]. The type of scoring of the IHC also influences the concordance. Indeed, a dichotomous scoring system, such as positive versus negative, has better concordance than 3-tier systems or assessing the percentage of positive cells [2,14]. Finally, tumor subtypes may influence the interpretability of the TMAs [4].

The heterogeneity of lung cancer has long been recognized. Nearly all lung adenocarcinomas had more than one morphologic subtype, which was appreciated with extensive tumor sampling but seldom recognized on small biopsy specimens [15]. Roggli and colleagues reported that only 34% of tumors were considered homogeneous, with 43% characterized by at least one slide showing a major histologic type different from the remainder of the tumor. The level of heterogeneity can also be appreciated with the immunostaining of tumor markers [16]. For example within a single tumor, the staining index of Ki-67 varied anywhere between 0% to 30%; the results determined as negative or positive based on a 10% cut-off depended on the histologic section examined. To date, very few lung cancer studies that used TMAs have focused on tumor and biomarker heterogeneity. Only one study was found that compared whole section staining to TMAs [17], where the authors built TMAs comprised of three cores of 1.35mm each. Schmidt et al used a complex scoring system to obtain an immunoreactive score (IRS), which varied between 0 and 12; a score of nine or greater indicated a high positive expression. Multiple histologic subtypes of non-small cell carcinomas were included as well as some cases of small cell lung carcinomas. The concordance between the whole section and TMA for two biomarkers (TA-MUC1 and Lewis Y) was good (greater

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than 80%) with both sensitivity and specificity above 80%. The main challenge with this study is that the construction of the TMA was different from what is commonly built (2 to 3 cores of 0.6mm) and therefore consumed a much greater amount of tissue.

Lung adenocarcinoma and squamous cell carcinoma account for 80% of all primary lung cancer, representing a high level of within- and between- tissue heterogeneity. The goal of our study is to determine the optimum number of 0.6mm cores per lung tumor tissue sample needed to accurately assess the overall tissue expression using TMAs for the following biomarkers: TTF-1, p53, and Ki-67. Because of the issues related to tissue loss during sectioning and staining, and staining artifact at the edges of tissue inherent to IHC and more apparent on small specimens [1,13], a "virtual" TMA was built for comparison between the actual TMAs and the whole sections.

Materials and Methods

Subjects

Under approval from the Mayo Clinic Institutional Review Board, our lung cancer research program prospectively identifies all primary lung cancer cases evaluated at Mayo Clinic, Rochester, Minnesota [18]. For the current study, ten pathologically confirmed adenocarcinoma (n=5) and squamous cell carcinoma tumors (n=5) were chosen, the two most common histological types of primary lung cancer. The five adenocarcinoma samples (cases 1-5) were grade 2 or 3 and stage IA or IB. The five squamous samples (cases 6-10) were all grade 3 and stage IA, IB, or IIB. All ten patients were Caucasian, six were female, and the median age at diagnosis was 72.5 years.

Three Antibodies Selected for immunohistochemistry

The thyroid transcription factor -1 (TTF-1) gene was selected because its staining pattern is usually uniform despite histologic heterogeneity [19]. TTF-1 is a regulatory gene in lung development, which plays an important role in normal lung function and morphogenesis. TTF-1 is expressed normally in the terminal respiratory unit of the lung, comprised of the peripheral airway and small bronchioles; TTF-1 is also expressed in over 80% of pulmonary adenocarcinomas and is usually absent in squamous cell carcinomas. In contrast, p53 and Ki-67 were selected because of their inter- and intra-tumoral heterogeneity as biomarkers. *p53* is a tumor suppressor gene associated with apoptosis and is expressed in response to a variety of signals such as DNA damage. Abnormalities involving the *p53* gene are a common occurrence in lung cancer, and levels of the p53 protein correlate well with the missense mutation of the gene. Ki-67 is a nuclear protein expressed in all phases of the active cell cycle, although Ki-67 is quite low prior to the S phase and is not seen in quiescent cells.

Immunoreactivity was assessed according to the intensity of the stain (1+ for weak, 2+ for moderate, and 3+ for strong) and percentage of positive tumor cells, assessed from 0% to 100% in increments of five percent. The assessment was conducted for whole-mount sections as well as for each core in the TMA. The mid-point of this range for the whole-mount section was used as the targeted true value for all comparisons.

Tissue microarray

For each case, all hematoxylin and eosin (H&E) sections were reviewed and the most representative paraffin block of the tumor was chosen to build the TMA. Ten areas were selected to represent the morphologic heterogeneity of the tumor (Figure 1). The cores were biopsied from selected regions of the tissue samples, and the same cores were used for both the virtual and actual TMAs across antibodies (Figure 2). The results from the TMA slides were compared to reads from the standard full tissue section glass slides. Figure 3 presents the work flow for the process and construction of the virtual and actual TMAs. When the actual and virtual TMAs were scored, only cores that were at least 50% of entire and contained adenocarcinoma or squamous cell carcinoma were scored. Actual or virtual cores with folds, tears, over 50% loss, or other significant artifacts were not scored. Actual TMAs tend to have more missing data than virtual TMAs due to the inherent greater fragility of the 0.6mm core compared to a full-tissue section.

Statistical methods

The distribution of the TMA percentage of positive cells for all three antibodies was summarized via box plots; TMA intensity was summarized via bar charts. The targeted true value of percentage (TVP) and true value intensity (TVI) were read from the full-section tissue slide for each sample. The median virtual and actual TMA percentage from the core reads for each sample were compared with the targeted true value for the sample. An exact match was defined when the TVP equaled the median of the core reads, and a range match was defined when the TVP fell between the first and third quartiles of the core reads.

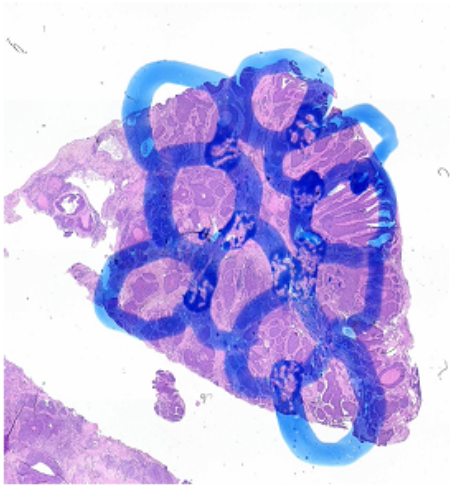

Figure 1: H&E stained slide with 10 areas marked for TMA construction.

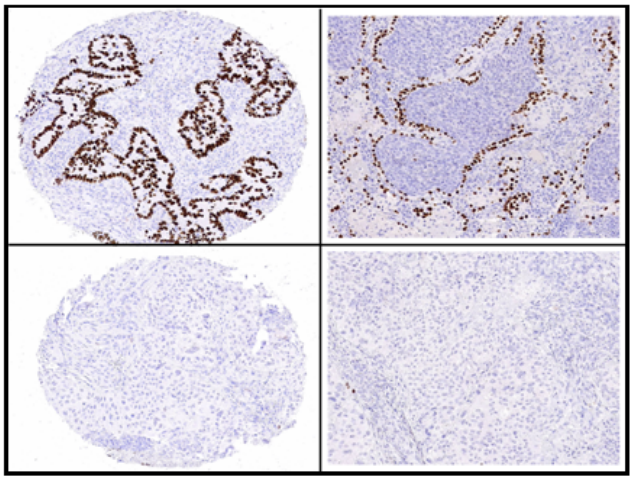

Figure 2: Immunostained actual (left panels) and virtual (righr panels) TMA cores showing positive (top panels) and negative (bottom panels) TTF- 1 staining.

1. Cut five 5µm sections.
    a. Stain the first section with H&E.
    b. Hold the 2nd-5th sections.
2. Mark 10 areas on the H&E slide.
3. Construct randomized TMA from 10 areas marked on the H&E slide.
4. Cut another five 5µm sections.
    a. Stain the first section with H&E.
    b. Hold the 2nd-5th sections.
5. Batch stain sections 2-4 from intact blocks and from the actual TMA.
    a. 2nd with p53
    b. 3rd with TTF-1
    c. 4th with Ki-67
6. Digitize full-section slides and TMA slides.
7. Construct the virtual TMA by cutting and pasting digital image areas from each immunostained slide to match the randomized actual TMA as indicated by the marks on the H&E slide.
8. Read and record scores of the virtual and actual TMAs and of the full-section slides using Bacus image viewing software.

Figure 3: Process now for the virtual and actual TMA construction and scoring.

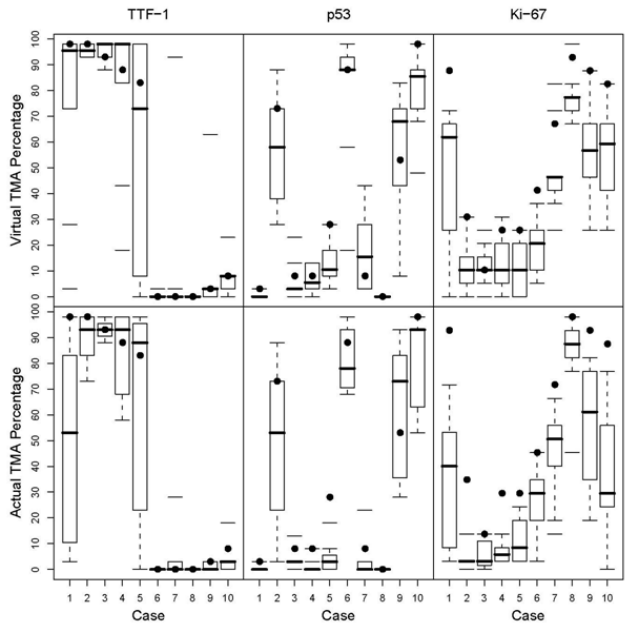

Figure 4: Case by case distribution of the virtual and actual TMA percentage of positive cells for the TTF-1, p53, and Ki-67 antibodies. True values are denoted by*.

Associations between the virtual and actual percentages were measured by the level of agreement in range matching.

A bootstrap resampling technique (without replacement) was used to estimate the variability associated with selecting a few cores (virtual and actual) per region for the TVP and TVI. Specifically, 1 through 10 cores were sampled; the mean and standard deviation of the 1,000 iterations were recorded for percentage of positive cells. The percentage of weak, moderate, and strong expressions of the 1,000 iterations was recorded for intensity. Of note, bootstrap analyses were not performed for stain-negative TMA cores (case 6 in actual TTF-1, case 8 in virtual and actual TTF-1, and case 8 in virtual and actual p53,); for cases where TMA results were not available on all 10 cores, the maximum number of cores with results was used in the bootstrap analyses.

Accuracy was defined as the TVP that was captured by the 95% bootstrap confidence interval (CI); precision was defined by the width of the 95% bootstrap CI. Accuracy and precision were evaluated for each of the three antibodies by the number of cores sampled for each case. A *sufficient* number of cores needed to capture the TVP was considered to be achieved when the sampled cores from both the virtual and actual TMAs provided accurate estimates (TVP captured by the 95% bootstrap CI) and the actual TMA 95% CI lower bound was greater than 0% if the TVP was greater than 0%. The *optimal* number of cores per sample was determined by the fewest number of cores where a *sufficient* estimate of the TVP was achieved. Finally, the median and mode of the *optimal* number of cores for each marker antibody was used to determine a recommended number of cores to sample.

Results

True value percentage

The TVP ranged from 0% to 100%, and the percentage did not seem to influence the probability of the sample to be read correctly by either virtual or actual TMA cores. The consistency of the true value results was most evident in the TTF-1 antibody (see Figure 4). All five adenocarcinoma cases had a TVP within 20% of each other (ranging from 81-100%), and the five squamous cases had a TVP within 10% of each other (ranging from 0-10%). Four of the five adenocarcinoma cases were relatively consistent in the p53 (1-30%) and Ki-67 (11-35%) antibodies while results for the squamous cases were scattered (p53, 0-100%; Ki-67, 41-95%).

True value intensity

Similar to the TVP, the TVI had little impact on the probability of the sample to be read correctly by either TMA method. TTF-1 had strong intensity for all five adenocarcinoma cases and moderate intensity for both squamous cases with greater than 0% positive staining. The TVI for p53 was very heterogeneous while Ki-67 was strong for all ten cases.

Virtual TMA and full-section tissue slides

Results from the TMA percentage of positive cells and TMA intensity are displayed in Figure 4 and Figure 5. Virtual TTF-1 was the best antibody at predicting TVP with its ten range matches, five of which were exact (all squamous samples). The p53 antibody was less accurate than TTF-1, and Ki-67 showed barely any accuracy.

Virtual and actual TMAs

Each antibody had 100 possible test results (10 cores from 10 cases), and a large difference between TMA methods was the total number of usable results. On average, the actual TMA returned 16 fewer results for

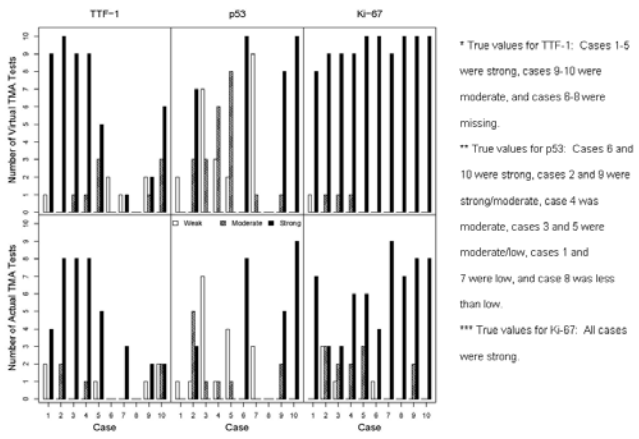

Figure 5: Case by case distribution of the virtual and actual TMA intensity for the TTF-1*, p53**, and Ki-67*** antibodies.

percentage than the virtual TMA; TTF-1 had the largest discrepancy, where the virtual TMA returned 99 results and actual TMA returned 80. TMA expressions of intensity also generated varying numbers of results. Despite the differences, virtual and actual TMA results were by and large consistent with each other. Specifically, all three antibodies had at least 80% agreement on TVP range matching. Additionally, eight virtual TTF-1 medians were within 5% of their respective actual TMA medians, and p53 and Ki-67 each had nine within 10% of each other. Notably, the median for all Ki-67 observations underestimated the TVP except for one virtual median that equaled the TVP. The trend was also evident in p53 and to a lesser extent in TTF-1.

Bootstrap results

Excluding cases 6 (negative actual TMA staining) and 8 (negative virtual and actual TMA staining), all eight TTF-1 observations provided sufficient results for sampling at least one core (Table 1 and

Case	Number of Cores (Virtual/Actual)	True Value	Number Sampled	Virtual TMA			Actual TMA		
				Mean	95% CI	% Dec[1]	Mean	95% CI	% Dec[1]
TTF-1									
3	10/8	**91-95**	1	96.0	89.4, 102.6	-	93.1	86.2, 100.0	-
			2	96.0	91.7, 100.3	34.4	93.0	88.5, 97.6	34.2
			3	96.0	92.7, 99.3	24.5	93.0	89.7, 96.3	26.7
			4	**96.0**	**93.4, 98.6**	19.8	**93.0**	**90.4, 95.6**	22.3
			5	96.0	93.8, 98.2	16.0	93.0	90.9, 95.0	20.6
5	10/7	**81-85**	1	60.2	-17.8, 138.1	-	60.6	-19.0, 140.2	-
			2	59.9	5.9, 113.9	30.7	59.4	5.9, 112.9	32.8
			3	**60.3**	**20.4, 100.3**	26.0	**60.7**	**23.2, 98.1**	30.0
			4	59.9	28.7, 91.1	21.9	60.5	32.5, 88.6	25.2
			5	60.7	34.3, 87.1	15.4	60.4	39.9, 80.9	26.9
9	9/10	**1-5**	1	8.4	-29.8, 46.5	-	0.9	-1.8, 3.6	-
			2	8.3	-16.8, 33.3	34.4	0.9	-0.9, 2.7	33.5
			3	8.5	-10.6, 27.6	23.7	0.9	-0.5, 2.3	23.3
			4	8.4	-6.6, 23.5	21.2	0.9	-0.2, 2.0	20.3
			5	**8.4**	**-3.7, 20.4**	20.1	**0.9**	**0.0, 1.8**	19.8
p53									
3	10/10	**6-10**	1	5.8	-7.4, 19.0	-	3.3	-3.3, 9.8	-
			2	5.7	-3.0, 14.4	34.1	3.4	-1.1, 7.9	32.0
			3	5.7	-0.8, 12.3	24.6	3.4	0.0, 6.8	22.7
			4	**5.6**	**0.4, 10.8**	20.6	**3.4**	**0.7, 6.1**	21.3
			5	5.7	1.4, 10.0	17.0	3.4	1.2, 5.6	17.4
5	10/8	**26-30**	1	12.5	-1.0, 26.0	-	4.4	-6.8, 15.6	-
			2	12.5	3.5, 21.6	33.3	4.5	-3.1, 12.0	32.5
			3	12.5	5.6, 19.4	23.7	4.4	-1.2, 9.9	27.2
			4	12.4	7.1, 17.8	22.4	4.3	0.1, 8.6	22.6
			5	12.6	8.1, 17.0	16.3	4.4	1.2, 7.7	22.9
9	9/7	**51-55**	1	57.4	13.8, 100.9	-	61.5	10.8, 112.2	-
			2	57.4	28.8, 86.1	34.3	61.6	28.9, 94.3	35.5
			3	**57.4**	**35.6, 79.2**	23.9	**61.5**	**37.5, 85.5**	26.6
			4	57.4	40.3, 74.6	21.3	61.6	43.2, 80.0	23.4
			5	57.6	43.7, 71.5	19.0	61.6	48.5, 74.7	28.6
Ki-67									
3	10/8	**11-15**	1	15.1	1.7, 23.4	-	5.3	-4.4, 15.1	-
			2	15.0	6.1, 23.8	33.8	5.4	-1.0, 11.7	34.8
			3	15.0	8.0, 21.9	21.4	5.4	0.6, 10.2	24.3
			4	15.0	9.5, 20.5	21.0	5.4	1.7, 9.1	22.9
			5	15.0	10.4, 19.5	16.7	5.4	2.5, 8.3	23.2
5	10/9	**26-30**	1	14.0	-2.9, 30.9	-	11.3	-4.0, 26.6	-
			2	14.0	3.4, 24.7	36.9	11.4	1.1, 21.7	32.7
			3	14.0	5.6, 22.4	21.1	11.4	3.8, 19.0	26.2
			4	14.1	6.9, 21.4	13.6	11.3	5.4, 17.3	21.9
			5	14.0	8.2, 19.8	20.1	11.4	6.5, 16.3	18.0
9	10/10	**86-90**	**1**	**58.0**	**21.7, 94.4**	-	**52.5**	**10.8, 94.2**	-
			2	58.2	34.0, 82.4	33.5	52.7	25.0, 80.3	33.8
			3	58.0	39.9, 76.1	25.2	52.2	31.1, 73.3	23.6
			4	57.9	43.1, 72.7	18.3	52.5	35.5, 69.6	19.2
			5	57.9	45.3, 70.5	15.0	52.5	39.0, 66.1	20.4

Table 1: Selected bootstrap resampling results for percentage of positive cells in all three antibodies (complete cores are provided in Supplementary Tables S1-S3).

Supplementary Table S1 online). The minimum number of selected cores able to provide a sufficient estimate was one for five cases, and the maximum number was eight for three cases. The optimal number of cores to select was two (case 1), three (cases 4, 5, and 7), four (cases 2 and 3), and five (cases 9 and 10), with a median of 3.5 and a mode of 3 cores.

In the p53 antibody, after excluding case 8 (negative virtual and actual TMA staining), six observations provided sufficient results for at least one level of core sampling (Table 1 and Supplementary Table S2 online). The minimum number of selected cores able to provide a sufficient estimate was one for three cases and the maximum was six for two cases. Due to the TMA results that did not accurately represent a TVP, sufficient and optimal numbers of cores to sample could not be determined for cases 1, 4, and 5. The optimal number of cores to select was one (case 10), three (cases 2, 6, and 9), four (case 3), and five (case 7), with a median and mode of 3 cores.

In the Ki-67 antibody, seven cases lacked accurate TMA results and sufficient and optimal numbers of cores could not be determined, leaving only three cases (cases 7, 8, and 9) having sufficient results and occurred when sampling one core (Table 1 and Supplementary Table S3 online). For the same three cases, no optimal core number could be determined, indicating the accuracy and precision of using one core will not be improved meaningfully even sampling up to 10 cores.

Discussion

In this study, virtual and actual TMAs were conducted with three marker antibodies (TTF-1, p53, and Ki-67), resulting in six sets of expression staining outcomes to compare with the staining results of the entire tissue slide. Despite the loss of cores with the construction of the actual TMA, similar expressions for all three antibodies were observed between the virtual and actual TMAs. We confirmed that immunostains known to have uniform staining in whole sections are likely to provide more consistent results from TMAs, while more heterogeneous biomarkers can lead to more unreliable results. Indeed, our TMA results showed that TTF-1 best represented the TVP and was the most consistent across methods. Both virtual and actual TMAs for p53 and Ki-67 tended to underestimate the true value and was most evident for Ki-67.

The ultimate goal of the study was to determine the optimal number of cores needed to build a TMA to objectively represent the whole tissue. Based on the law of diminishing returns, there is a point where the cost of increasing the number of cores will not be offset by the gain in reduced variance. To determine the ideal number of TMA cores to use, the percentage of positive cells was simulated from a varying number of core reads via bootstrap resampling analysis. We demonstrated that three or four cores are optimal for markers such as the TTF-1 and p53 antibodies, but no optimal core number could be determined for markers such as Ki-67 due to the poor ability of the Ki-67 TMA to represent the entire tissue. Our results suggest that, for markers such as Ki-67, TMAs may not be useful. Indeed in one study, the coefficient of variation within tumors for Ki-67 varied between 14.1% and 68.8% [16]. Furthermore, our results support findings that not all TMA constructs fit one tumor type and researchers need to be aware that different constructs may be needed for different tumor types [20]; the authors advise researchers that the same construct may not be the best choice for different markers and researchers have to have an idea about the immunostain variability in a tumor type.

Conclusion

Within- and between-tumor heterogeneity could lead to false interpretation of an immunostain; therefore, we propose that before using TMAs to analyze thousands of patient samples, a preliminary investigation using virtual TMAs could help discriminate between immunostains that would yield valid results on TMAs and immunostains that would not.

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Conflicts of Interest

None of the authors have any conflicts of interest.

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References

1. Horvath L and Henshall S (2001) The application of tissue microarrays to cancer research. Pathology 33: 125-129.
2. Fons G, van der Velden J, Burger M, Ten Kate F (2009) Validation of tissue microarray technology in vulvar cancer. Int J Gynecol Pathol 28: 76-82.
3. Rubin MA, Dunn R, Strawderman M, Pienta KJ (2002) Tissue microarray sampling strategy for prostate cancer biomarker analysis. American Journal of Surgical Pathology 26: 312-319.
4. Hecht JL, Kotsopoulos J, Gates MA, Hankinson SE, Tworoger SS (2008) Validation of tissue microarray technology in ovarian cancer: results from the Nurses' Health Study. Cancer Epidemiol Biomarkers Prev 17: 3043-3050.
5. Rosen DG, Huang X, Deavers MT, Malpica A, Silva EG, et al. (2004) Validation of tissue microarray technology in ovarian carcinoma. Mod Pathol 17: 790-797.
6. Simon R, Mirlacher M and Sauter G (2004) Tissue microarrays. Biotechniques 36: 98-105.
7. Kallioniemi OP, Wagner U, Kononen J, Sauter G (2001) Tissue microarray technology for high-throughput molecular profiling of cancer. Human Molecular Genetics 10: 657-662.
8. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, et al. (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4: 844-847.
9. Hoos A, Urist MJ, Stojadinovic A, Mastorides S, Dudas ME, et al. (2001) Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. Am J Pathol 158: 1245-1251.
10. Camp RL, Charette LA, Rimm DL (2000) Validation of tissue microarray technology in breast carcinoma. Lab Invest 80: 1943-1949.
11. Fukuoka J, Fujii T, Shih JH, Dracheva T, Meerzaman D, et al. (2004) Chromatin remodeling factors and BRM/BRG1 expression as prognostic indicators in non-small cell lung cancer. Clin Cancer Res 10: 4314-4324.
12. Fukuoka J, Dracheva T, Shih JH, Hewitt SM, Fujii T, et al. (2007) Desmoglein 3 as a prognostic factor in lung cancer. Hum Pathol 38: 276-283.
13. Hoos A, Cordon-Cardo C (2001) Tissue microarray profiling of cancer specimens and cell lines: opportunities and limitations. Lab Invest 81: 1331-1338.
14. Skacel M, Skilton B, Pettay JD and Tubbs RR (2002) Tissue microarrays: a powerful tool for high-throughput analysis of clinical specimens: a review of the method with validation data. Appl Immunohistochem Mol Morphol 10: 1-6.
15. Roggli VL, Vollmer RT, Greenberg SD, McGavran MH, Spjut HJ, et al. (1985) Lung cancer heterogeneity: a blinded and randomized study of 100 consecutive cases. Hum Pathol 16: 569-579.
16. Macdonald C, Michael A, Colston K, Mansi J (2004) Heterogeneity of immunostaining for tumour markers in non-small cell lung carcinoma. Eur J Cancer 40: 461-466.
17. Schmidt LH, Biesterfeld S, Kummel A, Faldum A, Sebastian M, et al. (2009) Tissue microarrays are reliable tools for the clinicopathological characterization of lung cancer tissue. Anticancer Res 29: 201-209.
18. Yang P, Allen MS, Aubry MC, Wampfler JA, Marks RS, et al. (2005) Clinical Features of 5,628 Primary Lung Cancer Patients: Experience at Mayo Clinic from 1997-2003. Chest 128: 452-462.
19. Yatabe Y, Mitsudomi T, Takahashi T (2002) TTF-1 expression in pulmonary adenocarcinomas. Am J Surg Pathol 26: 767-773.
20. Eckel-Passow JE, Lohse CM, Sheinin Y, Crispen PL, Krco CJ, et al. (2010) Tissue microarrays: one size does not fit all. Diagn Pathol 5: 48.