

*Advances in Brief***Tissue Microarrays for Gene Amplification Surveys in Many Different Tumor Types**

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Abstract

Gene amplifications are common in many different tumor types and may confer diagnostic, prognostic, or therapeutic information for patient management. Tedious experiments are often required to determine which tumor types have amplifications of a specific oncogene. To facilitate rapid screening for molecular alterations in many different malignancies, a tissue microarray consisting of samples from 17 different tumor types was generated. Altogether, 397 individual tumors were arrayed in a single paraffin block. To determine whether results from the literature can be reproduced on minute tissue samples (diameter, 0.6 mm), amplification of three extensively studied oncogenes (*CCND1*, *CMYC*, and *ERBB2*) was analyzed in three fluorescence *in situ* hybridization experiments from consecutive sections cut from the tissue microarray. Amplification of *CCND1* was found in breast, lung, head and neck, and bladder cancer, as well as in melanoma. *ERBB2* was amplified in bladder, breast, colon, stomach, testis, and lung cancer. *CMYC* was amplified in breast, colon, kidney, lung, ovary, bladder, head and neck, and endometrial cancer. These results confirm and even extend existing data in the literature on such amplifications. In summary, we applied three fluorescence *in situ* hybridization experiments to analyze amplifications of three oncogenes in three × 397 tumors within a week. This demonstrates the power of using minute arrayed tissue specimens for tumor screening.

Introduction

Development and progression of tumors is driven by a cascade of genetic alterations. Increased gene dosage by gene

amplification is a common mechanism for oncogene overexpression in many tumors. Amplification occurs at >40 different regions of the human genome (reviewed in Ref. 1). The critical genes giving amplified tumor cells a growth advantage are still unknown at many amplification sites. However, there are several regions of the genome where amplification is regularly associated with overexpression of known oncogenes. This applies, for example, to *ERBB2* at 17q21, *CMYC* at 8q24, and *CCND1* at 11q13.

Most oncogenes display a very broad tumor spectrum, whereas others tend to be activated primarily in certain cancer types. Amplification or overexpression of a particular oncogene has traditionally first been described in one tumor type. Subsequently, other tumor types have been evaluated, mostly in the order of their perceived importance, with rare tumors sometimes neglected. Therefore, it may take several years from the discovery of a potentially important molecular alteration to the definition of primary tumor types where this specific alteration may play a role. Our recently developed tissue microarray technology has the potential to greatly facilitate analysis of alterations in multiple tumor types (2). In this technique, 0.6-mm diameter tumor biopsies are retrieved from selected regions of archival tissue blocks, and hundreds of such cylindrical samples are subsequently precisely arrayed in a new paraffin block. Sections containing hundreds of tumor samples permit a high throughput analysis of multiple targets at the DNA, RNA, or protein level. To address the question of whether the analysis of small arrayed samples collected from potentially heterogeneous tumors can provide meaningful information about distribution and frequency of gene amplification in different tumor types, we analyzed three previously well-studied oncogenes (*ERBB2*, *CMYC*, and *CCND1*) on a multitumor array composed of 397 samples derived from 17 different tumor types. The comparison of array results and existing data from the literature suggests that tumor arrays are a powerful tool to rapidly screen different tumor types for gene copy number alterations (“tumor screening”).

Materials and Methods

Material and Microarray Construction. A total of 417 tissue samples consisting of 397 primary tumors from 17 different tumor types and 20 normal tissues were snap-frozen and stored at -70°C . Specimens were fixed in cold ethanol (4°C) for 16 h and then embedded in paraffin. A H&E-stained section was made from each block to define representative tumor regions. Tissue cylinders with a diameter of 0.6-mm were then punched from tumor areas of each “donor” tissue block and brought into a recipient paraffin block using a custom-made precision instrument as described (2). Five- μm sections of the resulting multitumor tissue microarray block were transferred to glass slides using the paraffin sectioning aid system [adhesive-coated slides (PSA-CS4x), adhesive tape, and UV lamp; Instru-

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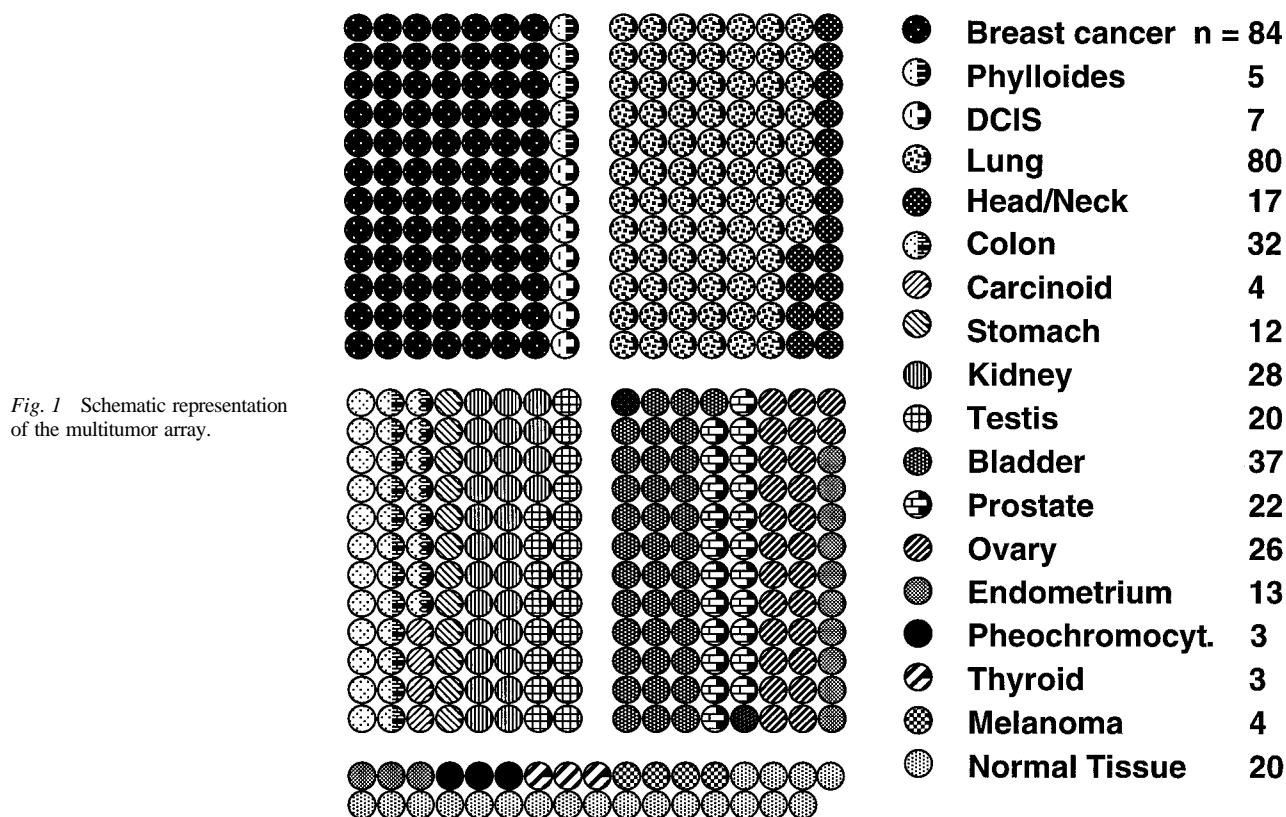


Fig. 1 Schematic representation of the multitumor array.

medics, Inc., Hackensack, NJ], supporting the cohesion of 0.6-mm array elements.

The primary tumors consisted of 96 breast tumors (41 ductal, 28 lobular, 6 medullar, 5 mucinous, and 4 tubular carcinomas; 7 DCIS²; and 5 phylloides tumors), 80 carcinomas of the lung (31 squamous, 11 large cell, 2 small cell, 31 adenocarcinoma, and 5 bronchioloalveolar carcinomas), 17 head and neck tumors (12 squamous cell carcinomas of the oral cavity and 5 of the larynx), 32 adenocarcinomas of the colon, 4 carcinoids (3 from the lung and 1 from the small intestine), 12 adenocarcinomas from the stomach, 28 clear cell renal cell carcinomas, 20 testicular tumors (10 seminomas and 10 teratocarcinomas), 37 transitional cell carcinomas of the urinary bladder [33 invasive (pT₁₋₄) and 4 noninvasive tumors], 22 prostate cancers, 26 carcinomas of the ovary (12 serous, 12 endometrioid, and 2 mucinous tumors), 13 carcinomas of the endometrium, 3 carcinomas of the thyroid gland, 3 pheochromocytomas, and 4 melanomas. Normal tissue from breast, prostate, pancreas, small bowel, stomach, salivary gland, colon, and kidney was used as control. A schematic illustration of the multitumor microarray and the arrangement of the array elements is shown in Fig. 1. Fig. 2A shows a H&E section from the array together with representative examples of four individual tumors (Fig. 2, B–E).

Each section from most of these tumor samples contained between 600 and 1300 tumor cells.

FISH. The tissue microarray sections were treated according to the Paraffin Pretreatment Reagent kit protocol (Vysis, Downers Grove, IL) before hybridization. FISH was performed with Spectrum Orange-labeled *CCND1*, *ERBB2*, and *CMYC* probes. Spectrum Green-labeled centromeric probes CEP11 and CEP17 were used as a reference (Vysis). Hybridization and post-hybridization washes were according to the “LSI procedure” (Vysis). Slides were then counterstained with 125 ng/ml 4',6-diamino-2-phenylindole in antifade solution. FISH signals were scored with a Zeiss fluorescence microscope equipped with double-band pass filters for simultaneous visualization of Spectrum Green and Spectrum Orange signals (Vysis). Amplification was defined as presence (in $\geq 5\%$ of tumor cells) of either >10 gene signals or more than three times as many gene signals than centromere signals of the respective chromosome, or tight clusters of at least five gene signals. The latter definition was selected because previous studies have shown that amplified DNA sequences are typically arranged in a nonrandom “clustered” fashion in interphase nuclei if the amplicon is located intrachromosomally (“homogeneously staining regions”; Ref. 3; Fig. 3).

Results

Technical Considerations. Between 75 and 85% of the tumor samples were interpretable after FISH analysis. FISH-related problems (weak hybridization, background, and tissue

² The abbreviations used are: DCIS, ductal carcinoma *in situ*; FISH, fluorescence *in situ* hybridization.

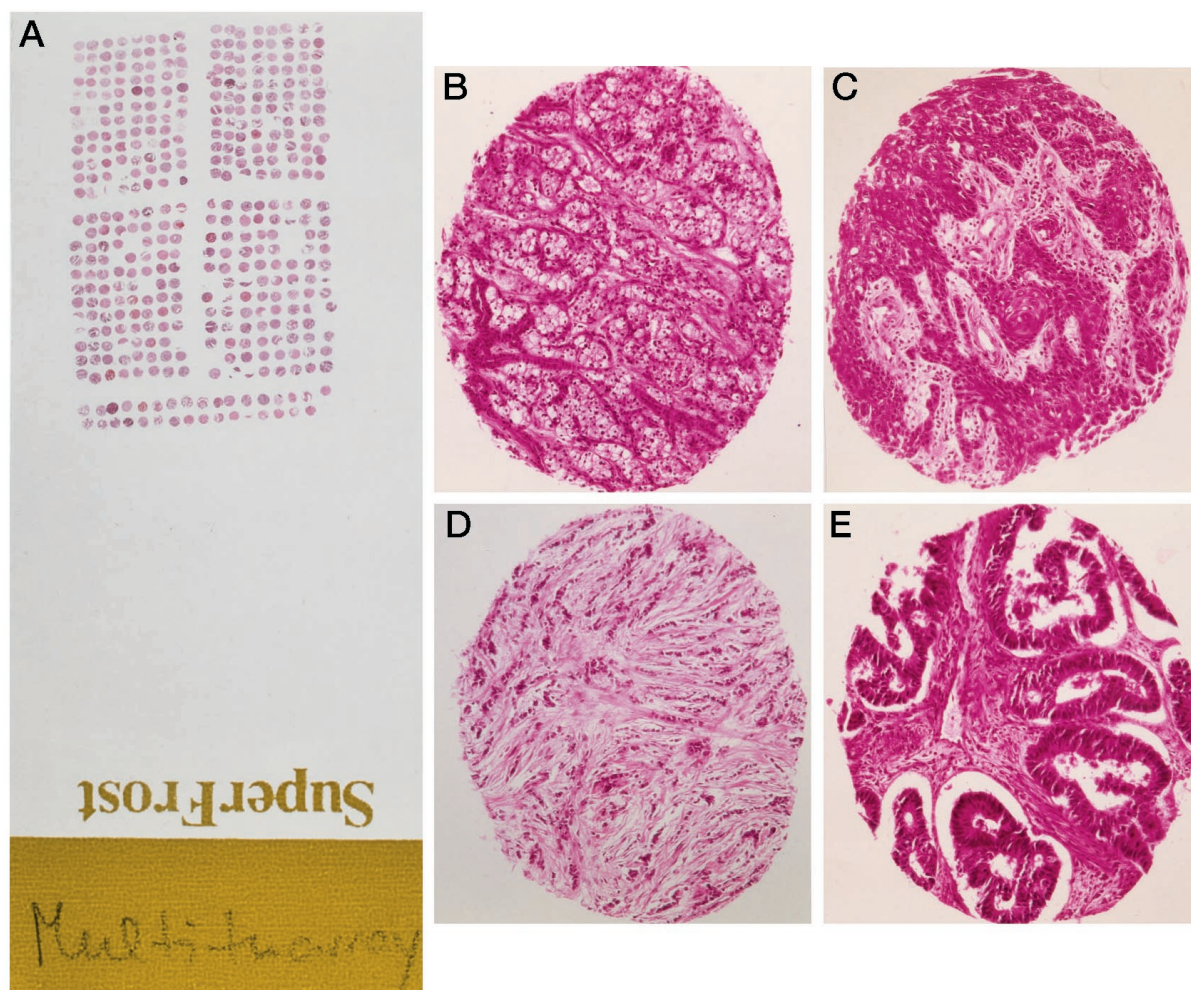


Fig. 2 H&E-stained section from an ethanol-fixed multitumor array (A) and four array elements: kidney cancer (B), squamous cell carcinoma of the lung (C), lobular invasive breast cancer (D), and colon carcinoma (E). B–E, $\times 400$.

damage) were responsible for about one-third of the noninformative cases. Other reasons for analysis failure were linked to the array technology, such as missing samples or too few tumor cells in some samples. The ratio between informative and not informative tumors differed slightly but not significantly between the different tumor types and the FISH probes. A detailed analysis of the reasons for noninformative results is shown in Table 1. Except for pheochromocytoma, which was not interpretable for *CCND1* and *ERBB2* analysis, results were obtained from all tumor types with all three FISH probes.

Gene Amplification. Seventy-two amplifications were found in 968 successfully hybridized tumor samples, whereas none of the normal tissues showed amplifications. Amplification usually involved almost all tumor cells within an array element. The presence of occasional tumor cells without detectable amplification may be attributed to truncated cells that had lost genetic material during sectioning or tissue pretreatment before hybridization. Amplifications were found for *CCND1*, *MYC*, and *ERBB2* in different tumor types. The amplification frequencies are shown for all tumor types in Tables 2 (*CCND1*), 3

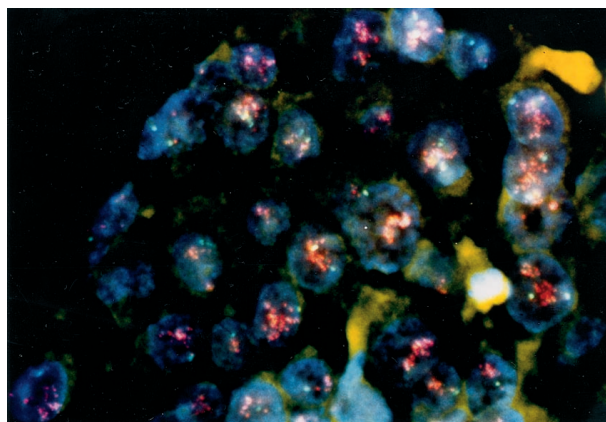


Fig. 3 Cyclin D1 amplification on an arrayed breast cancer sample. Tumor cells have a marked increase of red cyclin D1 signals as compared with green centromere 11 signals. $\times 600$.

Table 1 Reasons for noninformative tumor samples on the multitumor microarray

Gene	n	Informative samples	Samples not informative ^a						Total
			A	B	C	D	E	F	
CCND1	397	297 (75%)	15	22	11	13	13	26	100 (25%)
MYC	397	336 (85%)	15	22	12	11	1	0	61 (15%)
ERBB2	397	335 (84%)	15	22	1	12	4	8	62 (16%)

^a A–C: tumor tissue not interpretable due to problems linked to the array technology. A, samples not present on the glass slide. B, no tumor tissue present in samples. C, samples floated off during sectioning or FISH procedure. D–F: tumor tissue not interpretable due to problems related to the FISH procedure. D, no signals in intact cells. E, cells destroyed. F, high background.

Table 2 CCND1 amplification^a

Tumor	Amplification (%)	Cases studied	Method	Reference
Bladder	11	46	Southern	Bringuer <i>et al.</i> (20)
	11	137	FISH	Sauter <i>et al.</i> (21)
	14	28	FISH/Array	Schraml <i>et al.</i>
	21	97	Southern	Proctor <i>et al.</i> (22)
Breast	13	1171	Southern	Courjal <i>et al.</i> (13)
	23	22	Southern	Zukerberg <i>et al.</i> (23)
	23	62	FISH/Array	Schraml <i>et al.</i>
	24	64	Southern	Barbaresci <i>et al.</i> (24)
Carcinoid ^b	0	3	FISH/Array	Schraml <i>et al.</i>
Colon	0	47 cell lines	Southern	Leach <i>et al.</i> (25)
	0	10 + 6 cell lines	Southern	Sutter <i>et al.</i> (26)
	0	24	FISH/Array	Schraml <i>et al.</i>
DCIS	17	6	FISH/Array	Schraml <i>et al.</i>
	18	88	FISH	Simpson <i>et al.</i> (27)
Endometrium ^b	0	4	FISH/Array	Schraml <i>et al.</i>
Head/Neck	22	45	diff. PCR	Kyomoto <i>et al.</i> (28)
	23	57	Southern	Davidson <i>et al.</i> (29)
	26	23	SlotBlot	Akervall <i>et al.</i> (30)
	34	32	Southern	Callender <i>et al.</i> (31)
	34	32	Southern	el-Naggar <i>et al.</i> (32)
	34	56	Southern	Noguiera <i>et al.</i> (33)
	37	46	Southern	Jares <i>et al.</i> (34)
	38	16 cell lines	Southern	Xu <i>et al.</i> (35)
	38	16	FISH/Array	Schraml <i>et al.</i>
	57	89	Southern/SlotBlot	Muller <i>et al.</i> (36)
	Kidney ^b	0	21	FISH/Array
Lung	9	76	FISH/Array	Schraml <i>et al.</i>
	15	53	Southern/PCR	Betticher <i>et al.</i> (37)
	32	57	Southern	Marchetti <i>et al.</i> (38)
	0	61	Southern	Maelandsmo <i>et al.</i> (39)
Melanoma	3	43 cell lines	Southern	Walker <i>et al.</i> (40)
	25	4	FISH/Array	Schraml <i>et al.</i>
	0	20	FISH/Array	Schraml <i>et al.</i>
Ovary	3	237	Southern	Courjal <i>et al.</i> (13)
	0	4	FISH/Array	Schraml <i>et al.</i>
Phylloides ^b	0	4	FISH/Array	Schraml <i>et al.</i>
Prostate	0	6 cell lines	Southern	Han <i>et al.</i> (41)
	0	14	FISH/Array	Schraml <i>et al.</i>
Stomach	0	45	Southern	Akama <i>et al.</i> (42)
	0	9	FISH/Array	Schraml <i>et al.</i>
Testis ^b	0	16	FISH/Array	Schraml <i>et al.</i>
Thyroid	0	24	Southern	Zou <i>et al.</i> (43)
	0	1	FISH/Array	Schraml <i>et al.</i>

^a Studies on metastatic samples or tumors from treated patients were excluded.

^b No data in the literature were found for carcinoids, pheochromocytoma, phylloides tumors, or tumors of the endometrium, kidney, or testis.

(*CMYC*), and 4 (*ERBB2*), together with a summary of the previous literature. Coamplifications of all three genes were seen in two breast carcinomas. Coamplifications of two genes were found in two breast carcinomas (*CCND1/CMYC* and *CCND1/ERBB2*) and in one teratocarcinoma of the testis (*CMYC* and *ERBB2*).

Discussion

We recently described a tissue microarray technology where hundreds of individual tissue specimens are arrayed to a single tumor array block (2). Consecutive sections cut from the block provide starting material for the simultaneous *in situ* detection of

Table 3 *CMYC* amplification^a

Tumor	Amplification (%)	Cases studied	Method	Reference
Bladder	3	87	FISH	Sauter <i>et al.</i> (44)
Breast	3	33	FISH/Array	Schraml <i>et al.</i>
	1	100	Southern	Ottestad <i>et al.</i> (45)
	4	100	Southern	Lizard-Nacol <i>et al.</i> (46)
	6	54	Southern	Contegiacomo <i>et al.</i> (47)
	6	154	Southern	Watson <i>et al.</i> (48)
	8	311	Southern	Borg <i>et al.</i> (49)
	12	74	FISH/Array	Schraml <i>et al.</i>
	17	1052	Southern	Berns <i>et al.</i> (50)
	18	101	DotBlot	Chen <i>et al.</i> (51)
	21	52	Southern	Tavassoli <i>et al.</i> (52)
	25	65	Southern	Roux-Dosseto <i>et al.</i> (53)
Carcinoid ^b	0	2	FISH/Array	Schraml <i>et al.</i>
	3	29	FISH/Array	Schraml <i>et al.</i>
Colon	4	23	PCR	Rochlitz <i>et al.</i> (54)
	32	22	SlotBlot	Wang <i>et al.</i> (55)
	1	5	FISH/Array	Schraml <i>et al.</i>
DCIS ^b	18	11	FISH/Array	Schraml <i>et al.</i>
	66	15	Southern	Borst <i>et al.</i> (56)
Endometrium	0	21	Southern	Somers <i>et al.</i> (57)
	6	17	FISH/Array	Schraml <i>et al.</i>
	9	66	Southern	Leonard <i>et al.</i> (58)
	10	21	Southern	Merritt <i>et al.</i> (59)
	15	40	Southern	Volling <i>et al.</i> (60)
	25	8	Southern	Haughey <i>et al.</i> (61)
Kidney	5	22	FISH/Array	Schraml <i>et al.</i>
	8	36	DotBlot	Kozma <i>et al.</i> (62)
Lung	0	23	Southern	Sekine <i>et al.</i> (63)
	2	47 (SCLC)	DotBlot	Noguchi <i>et al.</i> (64)
	9	78	FISH/Array	Schraml <i>et al.</i>
	11	45 (SCLC)	Southern	Wong <i>et al.</i> (12)
	33	6 cell lines	Southern	Saksela <i>et al.</i> (10)
Melanoma ^b	0	3	FISH/Array	Schraml <i>et al.</i>
Ovary	8	24	FISH/Array	Schraml <i>et al.</i>
	28	25	Southern	Berns <i>et al.</i> (65)
	50	12	Southern	Sasano <i>et al.</i> (66)
Pheochromocytoma	0	2	FISH/Array	Schraml <i>et al.</i>
	0	6	Southern	Liu <i>et al.</i> (67)
Phylloides ^b	0	4	FISH/Array	Schraml <i>et al.</i>
Prostate	0	15	Southern	Fournier <i>et al.</i> (16)
	0	21	Southern	Latil <i>et al.</i> (18)
	0	19	FISH/Array	Schraml <i>et al.</i>
	8	25	FISH	Jenkins <i>et al.</i> (68)
Stomach	0	10	FISH/Array	Schraml <i>et al.</i>
	4	50	Southern	Ranzani <i>et al.</i> (69)
	7	14	Southern	Koda <i>et al.</i> (7)
	13	51	DotBlot	Nakata <i>et al.</i> (70)
	18	11	Southern	Nakasato <i>et al.</i> (8)
	26	23	Southern	Hajdu <i>et al.</i> (71)
	0	21	Southern	Schmidt <i>et al.</i> (72)
Testis	6	17	FISH/Array	Schraml <i>et al.</i>
	0	2	FISH/Array	Schraml <i>et al.</i>
Thyroid	0	45	Southern	Terrier <i>et al.</i> (73)
	7	15	Southern	Yang <i>et al.</i> (74)

^a Studies on metastatic samples or tumors from treated patients were excluded.

^b No data in the literature were found for phylloides tumors, carcinoids, melanoma, or DCIS.

DNA, RNA, or protein targets in a very high number of tissue samples. The tissue microarray technology is substantially different from the traditional multitissue ("sausage") blocks, which have often been used in pathology laboratories for antibody testing and which was first described 13 years ago (4). The most important advantages of the tissue array technology include increased capacity, negligible damage caused to the original tissue blocks, the

precise positioning of tissue specimens, and the possibility for automated construction and analysis of arrays. It is possible to retrieve dozens of punched samples from each donor block without significantly damaging it. This enables the generation of multiple replicate array blocks, each having samples from the same tumor specimens at identical coordinates. Depending on the thickness of the original tissue, between 100 and 200 sections can be cut from

Table 4 ERBB2 amplification^a

Tumor	Amplification (%)	Cases studied	Method	Reference	
Bladder	3	31	FISH/Array	Schraml <i>et al.</i>	
	4	24	Southern	Mellon <i>et al.</i> (75)	
	7	141	FISH	Sauter <i>et al.</i> (76)	
	11	35	PCR	Gorgoulis <i>et al.</i> (77)	
	26	92	PCR	Orlando <i>et al.</i> (78)	
Breast	6	71	FISH/Array	Schraml <i>et al.</i>	
	17	178	Southern	Prost <i>et al.</i> (79)	
	19	539	Southern	Borg <i>et al.</i> (80)	
	22	63	Southern	Knyazev <i>et al.</i> (81)	
	23	44	FISH	Kallioniemi <i>et al.</i> (82)	
	24	70	PCR	An <i>et al.</i> (83)	
	26	195	PCR	An <i>et al.</i> (84)	
	35	101	DotBlot	Chen <i>et al.</i> (85)	
	Carcinoid ^b	0	3	FISH/Array	Schraml <i>et al.</i>
		3	30	FISH/Array	Schraml <i>et al.</i>
Colon	5	19	Southern	Knyazev <i>et al.</i> (81)	
	30	23	PCR	Wang <i>et al.</i> (86)	
	27	15	FISH	Murphy <i>et al.</i> (87)	
DCIS	67	6	FISH/Array	Schraml <i>et al.</i>	
	0	11	FISH/Array	Schraml <i>et al.</i>	
Endometrium	4	50	Southern	Esteller <i>et al.</i> (14)	
	63	72	PCR	Czerwenka <i>et al.</i> (15)	
	0	0	FISH/Array	Schraml <i>et al.</i>	
Head/Neck	0	66	Southern	Leonard <i>et al.</i> (58)	
	0	21	Southern	Merritt <i>et al.</i> (59)	
	18	11 cell lines	Southern	Beckhardt <i>et al.</i> (88)	
	0	13	Southern	Freeman <i>et al.</i> (89)	
Kidney	0	15	Southern	Sato <i>et al.</i> (90)	
	0	34	Southern	Stumm <i>et al.</i> (91)	
	0	23	FISH/Array	Schraml <i>et al.</i>	
	17	70	PCR	Zhang <i>et al.</i> (92)	
	0	34	Southern	Knyazev <i>et al.</i> (81)	
Lung	1	75	FISH/Array	Schraml <i>et al.</i>	
	2	51	Southern	Shiraishi <i>et al.</i> (93)	
	3	29	Southern	Bongiorno <i>et al.</i> (94)	
	0	3	Southern	Knyazev <i>et al.</i> (81)	
Melanoma	0	4	FISH/Array	Schraml <i>et al.</i>	
	0	25	FISH/Array	Schraml <i>et al.</i>	
Ovary	4	23	Southern	Knyazev <i>et al.</i> (81)	
	14	65	Southern	Fajac <i>et al.</i> (95)	
	31	26	Southern	Fan <i>et al.</i> (96)	
	64	47	ISH	Hou <i>et al.</i> (97)	
	0	4	FISH/Array	Schraml <i>et al.</i>	
Phylloides ^b	0	15	Southern	Fournier <i>et al.</i> (16)	
	0	21	Southern	Latil <i>et al.</i> (18)	
	0	18	FISH/Array	Schraml <i>et al.</i>	
Prostate	44	62	FISH	Ross <i>et al.</i> (19)	
	6	34	Southern	Kameda <i>et al.</i> (98)	
Stomach	6	50	Southern	Ranzani <i>et al.</i> (69)	
	6	69	Southern	Tsujino <i>et al.</i> (99)	
	18	11	FISH/Array	Schraml <i>et al.</i>	
	0	17	FISH/Array	Schraml <i>et al.</i>	
Testis ^b	0	3	FISH/Array	Schraml <i>et al.</i>	
Thyroid	0	3	FISH/Array	Schraml <i>et al.</i>	
	4	27	Southern	Knyazev <i>et al.</i> (81)	

^a Studies on metastatic samples or tumors from treated patients were excluded.

^b No data in the literature were found for phylloides tumors, carcinoids, pheochromocytomas, or tumors of the testis.

each array block. This technology enables extensive analyses of even small primary tumors, thereby preserving often unique and precious tumor specimens for a large number of analyses that may be of interest in future investigations. We have calculated that >10,000 analyses may be performed from a tumor having a diameter of 10 mm. The application of a precision instrument to deposit the samples in a predefined format allowed not only the ability to

analyze a large number of different tumor samples but also facilitated the development of an automated analysis of arrayed tumors.

The question of whether minute tissue samples of potentially heterogeneous tumors are representative enough of their donor tumors to allow meaningful studies is critical to the tumor array concept. Here, we addressed this question by examining well-known molecular alterations in multiple different tumor

types. The analysis of three oncogenes in 397 specimens representing 17 different tumor types resulted in a total of 51 evaluations with respect to the question of whether a gene amplification occurs in a given tumor type. The absence of previously published studies in 14 instances illustrates the fact that some, especially rare, tumor types are often neglected in molecular studies. This is even more surprising because some of the most commonly known oncogenes whose amplification was originally discovered >10 years ago were analyzed in this study (5–12). For example, this study revealed for the first time an *ERBB2* amplification in an embryonal carcinoma of the testis. A comparison with previous results could be made for 37 evaluations. Our array data agreed with the previous literature on the presence or absence of gene amplification in 73% (Tables 2–4). Obviously, the number of samples per tumor type was too small for a comprehensive analysis of some tumor types in this pilot study. Previously described amplifications were not detected on the array in 9 of 25 tumor types from which <25 samples were examined. In contrast, when at least 25 cases were analyzed per tumor type, 92% of the known amplifications (11 of 12) were detected. Given the small size of our samples and the known heterogeneity in many tumors, sampling error is a possible reason for some of the discrepant cases. For example, this may account for the lack of *CCND1* amplification in arrayed samples from 20 informative ovarian carcinomas where *CCND1* amplification is known to be infrequent (13).

However, it is very likely that sampling problems are not the only reason for discrepancies between our study and previous results. If one studies the previous literature, there are sometimes substantial differences between the amplification frequencies observed in the individual studies of the same tumor type. Most of these inconsistencies may relate to the various methods used for detecting amplification as well as differences in the definitions of amplification. For example, Southern analysis detected amplification in only 2 of 50 endometrial tumors (14), whereas 45 of 72 samples were scored amplified by another study using quantitative PCR (15). Definitions of amplification by FISH have also been quite variable. For example, most investigators have not seen *ERBB2* amplifications in prostate cancer by FISH (16–18). However, Ross *et al.* (19) defined amplification as the presence of at least five *ERBB2* signals in tumor cells and reported amplification in 44% of prostate cancers. The definition of amplification in this study was such that aneuploidy, especially if associated with a high proliferation rate (presence of G₂-M and S-phase cells), would have been classified as DNA amplification. In our tissue microarray study, we used a very conservative cutoff to define amplification. In the light of the substantially inconsistent literature, our tissue microarray study is the first one where uniform methodologies and interpretation criteria were used for analysis of amplifications of several genes across a large number of different tumor types. Therefore, our results illustrate the advantage of multitumor tissue array analysis in providing an internally much more consistent picture of gene amplifications than is possible by literature comparisons.

In this study, frozen tumor tissues were fixed in cold ethanol because this procedure allows the retention of good quality nucleic acids from fixed tissue samples. However, it is also possible to use arrayed formalin-fixed tumor tissue samples

to investigate DNA copy number alterations by FISH. In a recent study, we analyzed one arrayed, formalin-fixed prostate cancer samples from biopsies and autopsies for amplifications of the androgen receptor gene *MYC*, *NMYC*, *CCND1*, and *ERBB2*. In these patients, between 78 and 92% of the biopsy samples and between 144% and 58% of the autopsy samples were interpretable with slight differences according to the quality of the probes (17). Nevertheless, the cold ethanol fixation is advantageous for FISH, because the samples require fewer pretreatments than samples fixed in 4% buffered formalin. The disadvantage of cold ethanol fixation seems to be that RNA slowly degrades in paraffin blocks after a few months of storage at room temperature.³ We, therefore, do not advise fixing large series of precious tissues in cold ethanol, unless RNase inhibitors are added and blocks are stored at –20°C to avoid degradation.

In summary, our data suggest that tissue microarrays may be very powerful for rapid identification of those tumor types where a particular molecular alteration is important. In this study, a considerable fraction of the knowledge collected in >100 previous investigations involving more than 8000 experiments was reproduced by three FISH experiments to a multitumor array. All of these experiments were executed and analyzed during a 1-week period. Thus, the tissue microarray approach provides a method for rapid screening of multiple genes and multiple tumor types with the same methodology, thereby leading to a more unbiased analysis of gene amplifications in various cancer types.

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