Virchows Arch (2001) 438:591–594
DOI 10.1007/s004280100393

ORIGINAL ARTICLE

P.L. Fernández · I. Nayach · E. Fernández · L. Fresno
A. Palacín · X. Farré · E. Campo · A. Cardesa

Tissue macroarrays ("microchops") for gene expression analysis

Received: 26 October 2000 / Accepted: 20 December 2000 / Published online: 1 March 2001

Abstract We describe a simple system of tissue arraying with multiple tissue fragments obtained with a biopsy punch from selected areas of paraffin blocks. The new blocks thus constructed allow multiple tissue sections in which the uniform shape of the fragments coupled with a geometrical display and a significant amount of tissue per case allows a dependable, cost-effective way to screen tumors or other kinds of tissues with techniques such as immunohistochemistry. This system avoids the disadvantages of previous laborious methods of tissue arraying, such as expensive equipment and scarce tissue sampling, and it can be implemented in any institution with minimal cost and elaboration.

Keywords Tissue array · Immunohistochemistry · Microchops

Introduction

The endpoint of the analysis of many genomic alterations requires evaluation of the expression of altered genes, thus providing critical information about the status of the messenger (m)RNA and, most importantly, the ultimate effector, the expressed proteins. Therefore, techniques, such as immunohistochemistry (IHC) and RNA in situ hybridization (ISH), among others, are required for a meaningful evaluation of gene expression. Samples subject to IHC and ISH usually consist of large (average 1 cm^2) formalin-fixed paraffin-embedded tissue sections of which, although varying with the type of tissue and tumor, only selected areas harbor the lesion of interest. The remaining tissue is non-tumor stroma or inflammatory. Most IHC or ISH studies select at least one slide/section per tumor case. Thus, the screening of a tumor type for the expression of genes usually requires a large number of slides processed for IHC or ISH, with the subsequent costs of time and money. To optimize the efficiency of these screening studies, intelligent cost-effective systems have been developed in which multiple samples corresponding to different kinds of tissues and subjects are arranged within a single paraffin block, which is handled as one sample. This technique was first described by Battifora [1], who stacked multiple, fixed tissue fragments in a "sausage" with which a single paraffin block was generated. This procedure was initially intended to serve as a source of control sections for IHC stainings with numerous antibodies and consisted of many different normal or tumor tissues easily recognizable upon microscopic examination. The diameter of the different fragments of the block averaged 1–2 mm and were usually not arranged in a precise manner. Later, the increasing number of tissues and their similarity when only one tumor type was selected for creating the block, lead to the design of some type of geometrical arrangement of these small pieces, requiring cumbersome block elaboration [2, 7]. A high-density microarraying of small samples, which follows a similar principle as that of complementary (c)DNA microchips [4], has recently been developed [5] and is now being implemented in some institutions. The blocks thus generated harbor a great number of samples (up to 1000), which may be quickly and cheaply processed for IHC or ISH. Despite the great progress that "tissue microarraying" (TMA) has provided, this system has some important drawbacks. First, and most important, when dealing with neoplasms, tumor heterogeneity must be taken into account. Indeed, most tumors, mainly malignant, have a variety of cell populations within their boundaries reflecting a range of differentiation and phenotypes. Therefore, samplings of less than 1 mm^2 from a tumor whose area within a regu-

P.L. Fernández (✉) · I. Nayach · E. Fernández · L. Fresno
A. Palacín · X. Farré · E. Campo · A. Cardesa
Department of Anatomical Pathology,
Hospital Clínic and Institut d'Investigacions Biomèdiques
"August Pi i Sunyer" (IDIBAPS), University of Barcelona,
Barcelona, Spain
e-mail: plfernan@clinic.ub.es
Tel.: +34-93-2275450, Fax: +34-93-2275717

P.L. Fernández
Department of Anatomical Pathology, Hospital Clínic,
Villarroel 170, Barcelona 08036, Spain

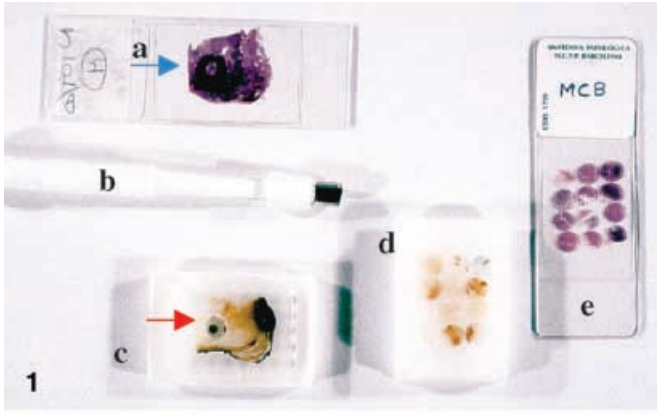
lar slide is, for instance, 1 cm^2, means that only 1/100 of the tissue of this particular section is studied. Moreover, the small size of the sample most likely precludes the existence of corresponding normal tissue or premalignant lesions within the same fragment with which to compare the results. The second disadvantage that might make this system unaffordable by some institutions is the need for specific equipment and training.

Based on the above philosophy of multiple tissue samples arranged in a single histolopathological block, we have developed a simple system of tissue arraying in which the uniform shape of the fragments coupled with a geometrical display and a significant amount of tissue per case permits a dependable, cost-effective way to screen tumors or other kinds of tissues with techniques, such as IHC and ISH. Given its antecedents and the size of the arrayed samples, we have decided to name this method "tissue microchops" (TMCs).

Materials and methods

The technique for manufacturing TMCs requires the participation of a pathologist and a surgical pathology technician who will collaborate after the initial production of routine tissue blocks and histopathological diagnosis. These blocks, which serve as a source for individual samples, can also be obtained from the archives of any surgical pathology department, provided that the original processing was adequate for the technique for which the TMC is intended (adequate fixation, paraffin embedding, and storage). The phases of TMC constructions are:

1. Selection of the area of interest by the pathologist. In order to save time for the pathologist, this can be frequently done during routine examination. Ideally, the selected area contains tumor, representative normal tissue of the organ, and/or premalignant lesions when possible (for instance, infiltrating carcinoma, intraductal carcinoma, and normal breast tissue). The diameter of the area varies according to the size of the punch (2–8 mm of diameter in our system; Fig. 1a). We recommend the use of the 6-mm instrument, since this allows a fair number of good size samples (maximum 20 per slide).
2. After locating the selected area by superimposing the slide on the paraffin block surface, a punch-extraction of the paraffined sample is obtained (Fig. 1b) and adequately labeled with the specimen number. For this, we use a skin-biopsy punch (Stiefel Laboratories Ltd, Sligo, Ireland; Fig. 1b). The extraction of the sample from the block, if made carefully, does not usually cause damage to the remainder of the block, allowing further obtaining of sections which, logically, lack the punched out area (Fig. 1c).
3. The construction of a single TMC block by the technician usually takes around 0.5–1 h and starts by immersing the punch in a regular hot paraffin bath to loosen the tissue cylinders that cannot be extracted intact in any other way. The cylinders are then placed onto a thin layer of semi-melted paraffin contained in a regular paraffin mold and easily arranged following a geometrical pattern, ideally rectangular for maximal efficiency (Fig. 1d), of which careful recording must be obtained. We usually generate a "map" for every TMC prior to embedding, which is filled before or during the process. This procedure is similar to routine sample preparation in any histopathology laboratory and does not require specific equipment or techniques.
4. Each TMC can then be cut as a regular paraffin block and mounted onto the appropriate glass slide (regular, silaned, etc; Fig. 1e).



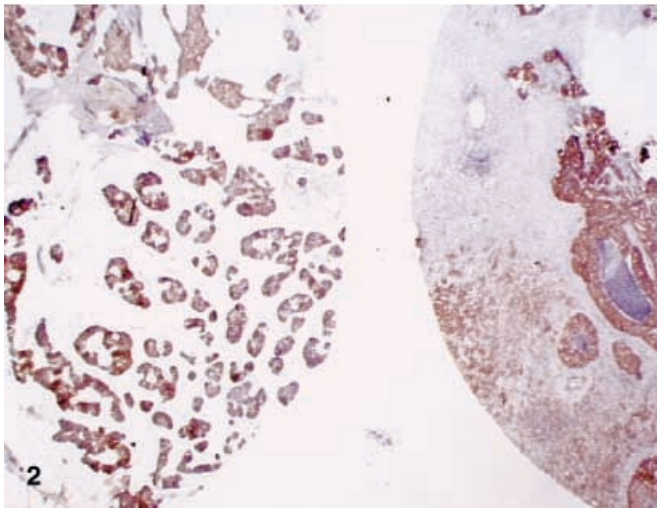


Fig. 1 Tissue microchop manufacture. The selected tissue area is labeled on a glass slide (**a**, *blue arrow*). The equivalent area is punched out from the paraffin block and remains within the punch (**b**). The paraffin block shows the hole but remains otherwise intact (**c**, *red arrow*). After melting in a hot paraffin bath, the tissue is arrayed on a new paraffin block (**d**) from which multiple sections can be obtained for different techniques (**e**)

Fig. 2 Low-power view of c-erb-B2 immunostaining in two adjacent breast carcinomas within a microchop. Both in situ carcinoma comedo-type and infiltrating carcinoma are seen in the sample on the right. ×10

Discussion

We consider the above a simple, rapid, cost-effective system for generating multi-tissue paraffin blocks suitable for IHC and ISH, which avoids some of the problems of using regular one slide per one case or tissue microarray blocks. This technique provides single paraffin slides with a fair number of different samples of a significant size, thus providing several advantages over the other methods:

1. The size of the samples is around 100 times greater than that of TMA (area 0.28 mm^2) [3], and the samples are around 10–20 times larger than those obtained with the Battifora and Petrosyan methods (area

Table 1 Comparative estimation of costs for the conventional, microchop, and microarray methods

	Samples/slide	Size of samples (mm^2)	Technical cost ($) of immuno--histochemistry[a]	Extra manufacturing cost ($) for technician[e]	Extra pathologist's cost[e] ($)	Total cost ($) per sample[f]	Extra equipment cost
Conventional	1	100	55	0[b]	0[b]	55	None
Microchop	20	28	2.75	0.79[c]	1.57 (1 h)	5.11	6-mm Punch ($2.50)
Microarray	1000	0.28	0.05	0.09[d]	1.57 (50 h)	1.71	Microarrayer ($7500)

[a] Estimation of immunohistochemical study with Herceptest (Dako, Glostrup, Denmark) per sample
[b] Included in routine processing or evaluation of samples
[c] 1 h divided by 20 samples
[d] 6 h for one block with 1000 samples divided by 1000 samples
[e] Estimated $15.70/h for technician and $31.50/h for pathologist. Estimated pathologist's time: 3 min per sample (selection of block and areas)
[f] Totals do not include the extra equipment

1–2 mm^2) [2, 7] when a 6 mm puncher is used (area 28.2 mm^2), allowing the inclusion of different tissue types in the test, such as a normal component and premalignant lesions when they are relatively close to the tumor (Fig. 2). Nevertheless, this can also be accomplished with TMA when including several areas of the original donor block in the same multisample construct, thus allowing good representation, which can even provide clinically relevant molecular findings [3, 6, 8].

2. The number of the cases per slide, although not by far as high as in TMA, is great enough to considerably decrease the time and cost of processing the samples, the number of which may vary from 9 to 20 per slide, depending on the size of the puncher used. Another interesting possibility is the combination of multiple areas of the same tumor within the same slide, thus allowing a thorough analysis of multiple phenotypes when dealing with highly heterogeneous neoplasms, such as sarcomas, germ-cell tumors, etc.
3. The simultaneous IHC staining or hybridization of many cases within one slide greatly decreases the inter-case variations derived from the technical processing of the slides, which is further improved when automated IHC systems are introduced. Nevertheless, a standardized processing of the initial paraffin blocks (type of fixative, time of fixation, embedding material, etc) is mandatory for dependable results.
4. A great number of slides per TMC can be obtained (usually more than 50, depending on the final thickness of the TMC block). Therefore, as in regular paraffin blocks, many different experiments can be performed with the same TMC.
5. This system is perfectly affordable by any histopathology laboratory, since it does not require expensive, specific equipment, as is the case for TMA, nor cumbersome manipulation using specific molds or plastowax grids [2, 7], and it can significantly decrease the cost in materials and time required for processing multiple samples. An estimation of the time and costs of the conventional, TMC, and TMA methods applied to IHC is presented in Table 1.

One of the originalities concerning the method proposed here is the use of a cheap punch readily available in most institutions, where dermatologists and otorhynolaryn-gologists make frequent use of it and which provides easy, reproducible, and minimally destructive extraction of selected areas of a given tissue. This punch can also be directly applied to fixed or fresh tissue for sample extraction. In this regard, we routinely use it for fresh prostate tissue extraction from prostatectomies after coronal sectioning, thereby avoiding surgical margin disturbance [9]. Finally, and although we are still in the process of testing it, we feel that this system can be most likely applied to other techniques requiring paraffin tissue sections, such as DNA ISH and laser microdissection. In summary, TMC may be regarded as a simple method for optimizing laboratory techniques requiring paraffin-embedded tissue sections and may be easily implemented in any institution with surgical pathology support.

Acknowledgements Grants: CICYT 97/0096 and CICYT 99/20 from Ministerio de Educación y Cultura; Fondo de Investigaciones de la Seguridad Social

References

1. Battifora H (1986) The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. Lab Invest 55:244–248
2. Battifora H, Mehta P (1990) The checkerboard tissue block. An improved multitissue control block. Lab Invest 63:722–724
3. Bubendorf L, Kolmer M, Kononen J, Koivisto P, Mousses S, Chen Y, Mahlamaki E, Schraml P, Moch H, Willi N, Elkahloun AG, Pretlow TG, Gasser TC, Mihatsch MJ, Sauter G, Kallioniemi OP (1999) Hormone therapy failure in human prostate cancer: analysis by complementary DNA and tissue microarrays. J Natl Cancer Ins 91:1758–1764

4. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM (1996) Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 14:457–460
5. Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schrami P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi O-P (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4:844–847
6. Moch H, Schraml P, Bubendorf L, Mirlacher M, Kononen J, Gasser T, Mihatsch MJ, Kallioniemi OP, Sauter G (1999) Identification of prognostic parameters for renal cell carcinoma by cDNA arrays and cell chips. Verh Dtsch Ges Pathol 83:225–232
7. Petrosyan K, Press MF (1997). Multispecimen tissue blocks in pathology: an improved technique of preparation. Lab Invest 77:541–542
8. Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, Mihatsch MJ, Kallioniemi OP, Sauter G (1999) Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res 5:1966–1975
9. Wheeler TM, Lebovitz RM (1994) Fresh tissue harvest for research from prostatectomy specimens. Prostate 25:274–279