Parra-Medina and Ramírez-Clavijo
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Why not to use punch biopsies in formalin-fixed paraffin-embedded samples of prostate cancer tissue for DNA and RNA extraction?

Rafael Parra-Medina[1,2,3*] and Sandra Ramírez-Clavijo[1]

Abstract

Extraction of DNA and RNA from formalin-fixed paraffin-embedded (FFPE) tissue blocks is a critical process in molecular oncology testing. Using FFPE, it is possible to choose the portion of tissue to study, taking into account the cell morphology, storage stability and storage conditions at room temperature, and make retrospective studies with clinical and pathological information. In prostate cancer tissue, in contrast with macroscopic tumors, it is not easy to identify the tumor; therefore, it is very important to make a microscopic diagnosis. We do not recommend punching this tissue because it can choose normal tissue for molecular analysis. In the present article we review the differences between punch biopsy and microdissection.

Keywords: Prostate cancer; FFPE, Punch, Microdissection, Molecular

1 Introduction

One of the aims of molecular biology is the search for new biomarkers to determine the prognosis and response to treatment of patients in the era of personalized medicine. Tissue is a fundamental source of information in molecular biology. The tissue may be stored in fresh or in formalin-fixed paraffin-embedded (FFPE) tissue blocks. FFPE tissue may be stored in pathology laboratories or biobanks together with its clinical and pathological information, which allows retrospective molecular studies to be made.

Extraction of DNA and RNA from FFPE tissue is a critical process in molecular oncology testing. Several events to consider in the preanalytical phases are fundamental in order to get nucleic acids with high quality. These include tumor percentage in the tissue sample [1], age of the FFPE block [2], the method used for nucleic acid extraction [3] and quantity [4].

The results of prostate tumor tissue studies can be very different depending on the characteristics of the piece of tissue from which the nucleic acids were extracted. Procuring inadequate tissue may give wrong data. Therefore, the aim of this article is to review those aspects to consider in prostate tissue sampling and why we do not recommend punching FFPE blocks in those cases.

1.1 Advantages and disadvantages of FFPE use in molecular studies

The ideal tissue to get the best quantity and quality of nucleic acids is in fresh tissue. However, it is much more logistically difficult and expensive to collect fresh tissue than FFPE samples. Fresh tissue requires liquid nitrogen or dry ice for the freezing process and ultralow temperature freezers for storage [5].

FFPE is a very good option for molecular studies (genomics, proteomics, glycomics, metabolomics and lipidomics) [7]. Among the advantages that FFPE has are

*Correspondence: Rafa.parram@gmail.com
[1] Faculty of Natural Sciences, Universidad del Rosario, Bogotá, Colombia
Full list of author information is available at the end of the article

the possibility to choose the portion of tissue to study, taking into account the cell morphology, storage stability and conditions at room temperature, and make retrospective studies with clinical (clinical history, diagnosis, treatment history and response and outcome) and pathological (morphology, immunohistochemistry and immunofluorescence results) information. Fortunately, only small fragments of DNA and RNA are required to reconstruct a sequence of interest [7]. DNA isolated from FFPE blocks is usable for PCR amplification even in products up to 250 bp in length [6]. RNA is less stable than DNA; however, it has been observed that FFPE may be successfully used in gene expression analysis even in tissue more than 10 years old [8].

The disadvantages of FFPE are related to failures during the preanalytical phase related to issues such as cold ischemia, fixation, type of paraffin, high temperatures, the protocol used and archival times. The fixation process may cause modifications of biomolecules such as crosslinkage of nucleic acids with proteins, covalent modifications of both DNA and RNA, and fragmentation of RNA, making it challenging to extract nucleic acids of high quality from FFPE tissues [9]. Fixation should be carried out in 10% neutral buffered formalin and it is important that the formalin penetrates the whole specimen. The fixation time should be 24 to 72 h. Over fixation may have an impact on the molecular results [10, 11]. The storage time and conditions (temperature and humidity) of FFPE blocks may affect the nucleic acids. Studies observed that 3 years of storage cause changes in the DNA quality. However, it has been observed than 18 years of storage may have only a minor influence on sequence quality [5, 10, 11].

1.2 Why not to punch FFPE blocks of prostate cancer tissue

Two techniques have been proposed for obtaining tissue in order to extract nucleic acids to carry out molecular tests such as PCR, qPCR and next-generation sequencing (NGS), among others. They are punch biopsy and the microdissection method (manual or laser capture) [12, 13]. The goal of microdissection is to extract the previously captured area of interest at the discretion of the researcher.

The integrity of nucleic acids is fundamental to get the best results. Morlote et al [12] compared the DNA integrity following punch biopsy and a type of microdissection (Pinpoint Slide DNA Isolation System). They found less degradation in DNA from punched samples that those taken by the Pinpoint technique ($P < 0.0001$). They included samples with a high number of tumor cells such as colorectal adenocarcinoma, lung adenocarcinoma, melanoma and glioblastoma. In contrast with these samples, prostate cancer develops multifocally in anywhere from 60 to 90% of patients [14]. Unlike other tumors, prostate cancer does not cause a macroscopic tumor and the changes may be subtle (color and texture); therefore, the prostate is cut by serial section at 2- to 3-mm intervals from apex to base. Each serial section is put in a cassette [15]. Under microscopy, the foci may have different sizes, shapes and distributions within the prostate. The lack of a macroscopic tumor means that there is no guarantee that there will be tumor cells in the fresh frozen section; therefore FFPE is the best option.

In prostate cancer and samples with small target cells, we recommend the use of the microdissection method (manual or laser capture). Laser capture microdissection has an advantage in small samples such as prostate cancer [16]; however, special equipment is required that may cause limitations in terms of the special training, cost and space required [12, 17]. We do not recommend punching FFPE samples of prostate cancer because, as the foci of glands can be small and variable in size, they may be difficult to identify in the middle of the paraffin block. The punch method may also cause contamination of tumor cells with surrounding necrosis, mucin pools, inflammatory cells and non-tumor tissue. The presence of non-tumor tissue may cause wrong data (Fig. 1). It has been observed that the molecular profile of tissue adjacent to the tumor presents an intermediate state between normal and tumor tissue [18].

In the microdissection method (manual or laser capture), the paraffin blocks are cut (5–10 microns in thickness) to get unstained slides (five to ten slides). These slides are deparaffinized with xylene and ethanol. Afterward, the slides have to be stained with hematoxylin and eosin (H&E) for a few seconds and then the pathologist has to identify the tissue target (Table 1). In manual capture microdissection, the pathologist has to extract the tissue with a blade under microscopy (Fig. 2). Previous studies observed that H&E staining does not have effects on the DNA yield nor molecular oncology test results [19].

For best results, it is very important to use extraction kits with high performance to obtain enough quantity and good quality of nucleic acids. Studies published by Carlsson et al [9] and Patel et al [3] compared commercial nucleic acid extraction kits on prostate biopsies. The results showed that the extraction kit affects the quantity and quality of extracted products. Carlsson et al [9] found that nucleic acids extracted with RNeasy® FFPE and QIAamp® DNA FFPE Tissue kits had the highest quantity and good quality. Also, they found a strong association between good nucleic acid extraction and the amount of tumor taken from the tissue sample. Patel et al [3] observed that the AllPrep kit by Qiagen is the most suitable for FFPE.

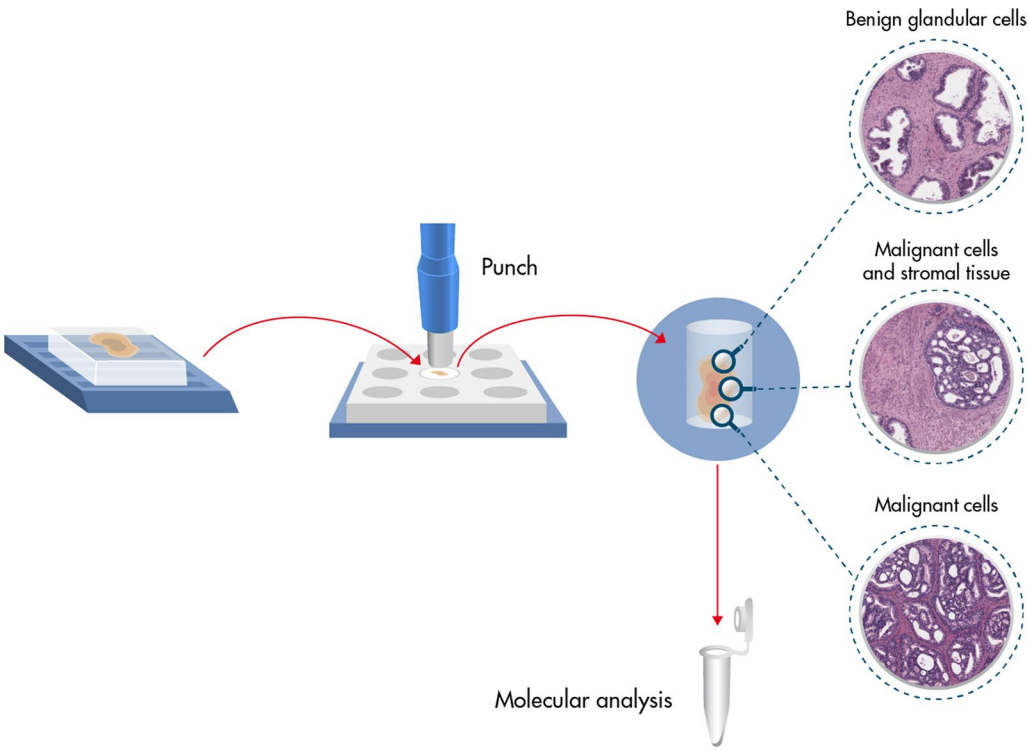

Fig. 1 Tissue obtained by punch biopsy. The tissue is on the FFPE blocks and is extracted by punch biopsy. The sample is sent to molecular analysis. The tissue may have malignant cells in the external part but inside, the paraffin may have benign glandular cells

Table 1 Protocol to deparaffinize the sections of FFPE tissue

1. Fresh xylenes (to deparaffinize the sections)-5 min	1
2. Fresh xylenes-5 min	2
3. 100% ethanol-30 sec	
4. 95% ethanol-30 sec	
5. 70% ethanol-30 sec	
6. Deionized water-30 sec	
7. Mayer's Hematoxylin (10%)- 30 sec	
8. Deionized water-rinse 15 sec (x 2)	
9. 70% ethanol-30 sec	
10. Eosin Y (10%) - 15 sec	
11. Deionized water-30 sec (x 2)	
12. 3% glycerol in deionized water-30 sec	
13. After removing the slide from the 3% glycerol step, shake the slide in the air to remove the layer of glycerol/water.	
14. The next 5–10 mins are the optimal time for microdissection. The tissue is dry, but retains a soft consistency. If the dissection takes more than a few minutes, the tissue will become increasingly brittle and the dissected fragments may be repelled as the needle is brought in proximity to the tissue. If the tissue becomes overly dry, re-soak in the 3% glycerol/water solution for 1–2 minutes.	

Table adapted from http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=2411

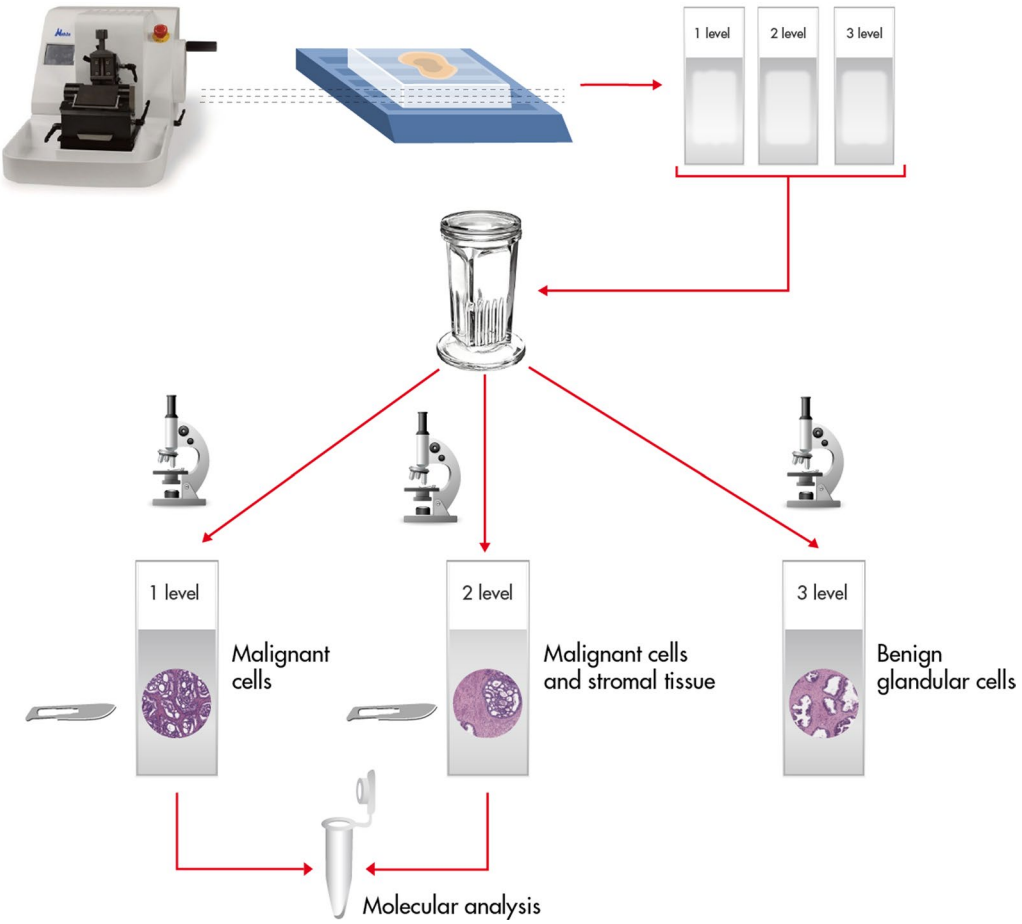

Fig. 2 Tissue obtained from microdissection. The tissue is cut with a microtome and unstained slides are obtained. These slides are deparaffinized (Table 1). The pathologist can choose the malignant cells and extracts the tissue with a blade under microscopy. For example, the whole slide in the first level, only glandular malignant cells in the second level. The third level has benign glandular cells. Therefore, only the tissue from first and second levels are sent for molecular analysis

Abbreviations
FFPE: Formalin-fixed paraffin embedded.

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Author details
[1]Faculty of Natural Sciences, Universidad del Rosario, Bogotá, Colombia. [2]Department of Pathology, Research Institute, Fundación Universitaria de Ciencias de la Salud, Bogotá, Colombia. [3]Department of Pathology, Instituto Nacional de Cancerología, Bogotá, Colombia.

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