Human Pathology (2017) **64**, 1–6

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Perspectives in pathology

Multisite tumor sampling: a new tumor selection method to enhance intratumor heterogeneity detection☆

José I. López MD, PhD[a,b,c,*], Jesús M. Cortés PhD[d,e,f]

[a]*Department of Pathology, Cruces University Hospital, 48903 Barakaldo, Spain*
[b]*Biomarkers in Cancer Unit, Biocruces Research Institute, 48903 Barakaldo, Spain*
[c]*University of the Basque Country (UPV/EHU), 48940 Leioa, Spain*
[d]*Quantitative Biomedicine Unit, Biocruces Research Institute, 48903 Barakaldo, Spain*
[e]*Ikerbasque: The Basque Foundation for Science, 48013 Bilbao, Spain*
[f]*Department of Cell Biology and Histology, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain*

Received 13 December 2016; revised 14 January 2017; accepted 9 February 2017

Keywords:
Tumor sampling;
Intratumor heterogeneity;
Targeted therapies;
Carcinogenesis;
Pathology;
Laboratory cost

Summary Intratumor heterogeneity (ITH) is increasingly being recognized as a highly complex process with high clinical impact that deserves special attention from practicing pathologists. The value of the ITH detection depends on the correctness of the pathologist's sampling. The goal of this review is 2-fold. On the one hand, we provide a basic scientific context for the practical pathologist's perspective. On the other hand, we encourage pathologists to adopt a more scientific and up-to-date approach to a key component of their daily work, namely, how to sample a tumor for reliable histological and molecular analysis. In particular, we review the consecutive steps of an efficient alternative to traditional approaches for detecting ITH: multisite tumor sampling. Notably, this type of sampling, based on a divide-and-conquer algorithm, is supported by scientific evidence showing its clinical applicability and practical advantages at no extra cost.

1. Introduction

Cancer is the final result of multiple complex changes in cell metabolism [1,2]. Although the use of highly sophisticated technology such as high-throughput DNA sequencing has improved our knowledge of the molecular mechanisms underlying carcinogenesis, intratumor heterogeneity (ITH) is not yet well understood [3,4]. ITH, the fact that a tumor is different at different sites, is of crucial importance in cancer research. ITH occurs in a nondeterministic manner so that the resulting heterogeneity patterns are unique and completely unpredictable for each tumor. Pathologists today have the challenge of identifying ITH efficiently, helping basic researchers to identify mutational signatures and oncologists to select better personalized therapies.

Kidney cancer is a very common type of cancer in Western countries. More than 62 000 new cases are expected in the United States in 2016 [5]. This type of cancer is a complex disease with multiple histological variations and uncertain prognosis [6,7]. Clear cell renal cell carcinoma (CCRCC) is by far the most common histological type of kidney cancer, accounting for 70%-80% of cases of kidney cancer in adults

☆ Disclosures: The authors declare no conflict of interest and no funding/support.

* Corresponding author. Department of Pathology, Hospital Universitario Cruces, Plaza de Cruces s/n, 48903 Barakaldo, Bizkaia, Spain.
E-mail address: joseignacio.lopez@osakidetza.eus (J. I. López).

http://dx.doi.org/10.1016/j.humpath.2017.02.010
0046-8177/

[8]. From the clinical point of view, it is an aggressive disease in which only radical surgery has been found to improve survival [9]. Chemotherapy and radiotherapy are not effective, and modern personalized therapies have so far had limited success [10]. For these reasons, CCRCC has attracted great attention in cancer research. Efforts are being made across the world, supported by significant financial investments, to improve our understanding of this type of cancer to develop better treatments.

CCRCC is the paradigm of a heterogeneous cancer from various points of view [10-17] and, hence, a very valuable test bed for ITH research. To the naked eye, heterogeneity may be obvious or subtle. Although some types of CCRCC seem homogenous to the naked eye, they may be very heterogeneous under the microscope. Furthermore, types of CCRCC that may seem homogenous under the microscope may be very heterogeneous at the molecular level, with different mutation profiles in different parts of the tumor.

This has clear clinical implications: mutations in the *BAP1* gene are associated with aggressive tumor behavior; mutations in the genes involved in the mTOR pathway make tumors more sensitive to targeted therapies; and mutations in the *PBRM1* gene are associated with a lower risk of biological aggressiveness [14,16,17]. On the other hand, some mutations are common (trunk mutations) to all the parts of the CCRCC. An example is the mutation in the *VHL* gene [13], making it a potential target for the development of new therapies, but all attempts to date have had disappointing results [17]. All the treatment failures have been associated with ITH, in particular, with the different branching patterns of CCRCC cells in different regions within the tumor [13]. The regional variability in CCRCC is unpredictable, varying between tumors, and it is not currently possible to develop an effective treatment strategy. This explains the high 5-year mortality rate in this type of cancer, which remains at around 40% [9].

In this review, we describe a simple efficient method that markedly improves routine detection of ITH in CCRCC without increasing costs [18]. The method is based on the divide-and-conquer (DAC) algorithm [19], which consists of recursively dividing a complex problem into simpler parts until these are sufficiently simple to be solved directly. Then, the partial solutions are combined to provide the solution to the overall problem.

DAC strategies have been used to solve complex problems in the field of biomedical sciences, for example, in biology and oncology, for selecting the most appropriate cells for biological experiments [20] or helping to interpret heterogeneity in breast cancer [21]. In this work, we consider the application of a DAC method to improving the performance of sampling in CCRCC given that these tumors are generally large and, hence, tend not to be completely sampled. Nevertheless, this method could be applied to any other type of tumor. By applying this algorithm, we achieve multisite tumor sampling (MSTS), and we propose this approach to optimize the detection of ITH.

2. The current paradox

Pathologists are the clinical specialists who handle surgical specimens and decide what parts of tumors are to be analyzed. In the case of small tumors (≤3 cm in diameter), it is affordable for pathologists to analyze the entire tumor. Some CCRCCs, however, are much larger, sometimes reaching 10 to 15 cm in diameter or even more, and this means that analyzing the entire tumor is not cost-effective. For this reason, pathologists take samples from large tumors following internationally accepted protocols [22-24]. This sampling involves selecting some parts of the tumor for analysis, with the goal of these parts being representative of the entire tumor. In particular, the consensus for CCRCC is to obtain a 1-cm^2 sample of tumor tissue for every centimeter of diameter of the tumor plus a sample of any areas that look suspicious to the naked eye. Unfortunately, ITH often occurs in areas that look identical to the naked eye, hindering the detection of ITH, and this is an important limitation of currently used protocols. Another limitation is the low overall percentage of the tumor analyzed in the case of large tumors. In routine clinical practice, more than 95% of the tissue of 10-cm–diameter tumors is not analyzed when following current official sampling protocols. In these cases, the information contained in some nonsampled areas of the tumor is lost forever.

Some studies have indicated that this loss of information is critical for patients [11,25]. It is not acceptable in modern clinical practice to fail to detect, for instance, areas of high-grade disease in CCRCC. The limitations of current protocols for detecting ITH have led researchers to call for urgent solutions [26]. To date, however, pathologists have not found a solution to this problem, and the latest versions of sampling protocols seem not to have taken this issue into account. To overcome these limitations, various authors have recently developed algorithms to assess ITH when there is very little material to analyze [27-30].

Pathologists are fully responsible for appropriately selecting tumor samples for analysis. Poor or incomplete sampling of a tumor can lead to poor or incomplete morphological and molecular studies, and this may have very negative implications for patients. The current paradox is that information that is crucial for patients and which is obtained from very sophisticated high-throughput sequencing technology completely depends on a tumor sampling method based on nonscientific arguments that were established before the era of molecular biology.

Incomplete tumor sampling is undoubtedly the central problem for pathologists today. Even simple morphological analysis can provide a lot of information on ITH when performed well. In relation to this, Andor et al [31] have recently demonstrated that traditional histopathological findings are well correlated with ITH in various types of cancer, including kidney cancer.

3. A step ahead: MSTS

When viewing the slices of a large tumor in the gross room, the pathologist is not able to detect ITH. At most, he/she might