RESEARCH NOTE

A multi-site cutting device implements efficiently the divide-and-conquer strategy in tumor sampling [version 1; referees: 3 approved with reservations]

Jose I. Lopez[1-3], Jesus M. Cortes[4-6]

[1]Department of Pathology, Cruces University Hospital, Barakaldo, Spain
[2]Biomarkers in Cancer Unit, Biocruces Research Institute, Barakaldo, Spain
[3]University of the Basque Country, Leioa, Spain
[4]Quantitative Biomedicine Unit, Biocruces Research Institute, Barakaldo, Spain
[5]Ikerbasque: The Basque Foundation for Science, Bilbao, Spain
[6]Department of Cell Biology and Histology, University of the Basque Country, Leioa, Spain

v1 **First published:** 06 Jul 2016, **5**:1587 (doi: 10.12688/f1000research.9091.1)
Latest published: 06 Jul 2016, **5**:1587 (doi: 10.12688/f1000research.9091.1)

Abstract

We recently showed that in order to detect intra-tumor heterogeneity a Divide-and-Conquer (DAC) strategy of tumor sampling outperforms current routine protocols. This paper is a continuation of this work, but here we focus on DAC implementation in the Pathology Laboratory. In particular, we describe a new simple method that makes use of a cutting grid device and is applied to clear cell renal cell carcinomas for DAC implementation. This method assures a thorough sampling of large surgical specimens, facilitates the demonstration of intratumor heterogeneity, and saves time to pathologists in the daily practice. The method involves the following steps: 1. Thin slicing of the tumor (by hand or machine), 2. Application of a cutting grid to the slices (*e.g.*, a French fry cutter), resulting in multiple tissue cubes with fixed position within the slice, 3. Selection of tissue cubes for analysis, and finally, 4. Inclusion of selected cubes into a cassette for histological processing (with about eight tissue fragments within each cassette). Thus, using our approach in a 10 cm in-diameter-tumor we generate 80 tumor tissue fragments placed in 10 cassettes and, notably, in a tenth of time. Eighty samples obtained across all the regions of the tumor will assure a much higher performance in detecting intratumor heterogeneity, as proved recently with synthetic data.

Open Peer Review

Referee Status: ? ? ?

	Invited Referees 1	2	3
version 1 published 06 Jul 2016	? report	? report	? report

1 **Thomas V. Colby**, Mayo Clinic USA

2 **Christopher DM Fletcher**, Brigham and Women's Hospital USA

3 **Manuel Areias Sobrinho Simoes**, University of Porto Portugal, University of Porto Portugal

Discuss this article

Comments (0)

Corresponding authors: Jose I. Lopez (joseignacio.lopez@osakidetza.eus), Jesus M. Cortes (jesus.m.cortes@gmail.com)

How to cite this article: Lopez JI and Cortes JM. **A multi-site cutting device implements efficiently the divide-and-conquer strategy in tumor sampling [version 1; referees: 3 approved with reservations]** *F1000Research* 2016, **5**:1587 (doi: 10.12688/f1000research.9091.1)

Grant information: JMC is funded by Ikerbasque: The Basque Foundation for Science.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

First published: 06 Jul 2016, **5**:1587 (doi: 10.12688/f1000research.9091.1)

Introduction

In the light of current findings provided by numerous sequencing tools, it is known that practically all human neoplasms display some degree of intratumor heterogeneity (ITH)[1]. Characteristically, ITH is not uniformly distributed along the tumor; instead, it shows a regional distribution following a stochastic pattern, the final result being unique, unpredictable, and dynamically varying along the time[2]. The correct identification of ITH is mandatory now that targeted therapies are offering promising results to patients[3], but pathologists - the specialists in charge of tumor selection for analysis - seem to have not given, so far, an appropriate answer to this issue.

We have recently proposed a reliable, affordable and time-saving solution to this problem[4]. The goal is twofold; to improve ITH detection and to perform ITH at affordable laboratory costs. This simple solution is based on the divide-and-conquer algorithm (DAC). Noteworthy, tumor sampling following DAC outperforms the routine protocol sampling for identifying ITH and, it does it, at a similar cost[4]. However, pathologists could consider that DAC is a time-consuming method when grossing, which might make it difficult to introduce it in routine practice. In this brief report, we describe a simple procedure to overcome this problem.

Method and results

The so-called DAC algorithm[5] is based on recursively breaking down a problem in smaller parts (divide) until these are simple enough to be solved directly (conquer). Then, partial solutions are combined to solve the original problem. DAC strategies have been largely applied in science to solve complex problems, including several challenging issues in biomedical areas. As early as in 1967, DAC helped clinicians to correlate hypoglycemia with infantile convulsions[6]. In addition, DAC has been useful in cell biology and oncology, for instance in selecting the appropriate cells for biological experiments[7] and, more recently, in helping to decipher breast cancer heterogeneity[8].

Here DAC is applied to clear cell renal cell carcinomas (ccRCCs), since these tumors are frequently large and, for this reason, impossible to be totally sampled. Any other large tumor, however, can benefit from this method. The DAC strategy (Figure 1) requires the pathologist to select, instead of a few large fragments, a substantial number of small ones widely distributed along the entire tumor. However, pathologists under a daily routine pressure can perceive this method as laborious and time-consuming.

A simple device consisting of a cutting grid (here, a potato cutter) will overcome this inconvenience. When applied directly to the whole tumor surface previously thin-sliced, the grid will cut it into small cubes in one shot (Figure 2). Next, the pathologist's decision will consist simply in selecting the cubes that will be processed for analysis as previously reported[4]. The method can be applied (and has been tested) to both fresh and formalin-fixed tissue, saves time, and assures a uniform sampling distribution along the tumor. The objective for improving efficiency of targeted therapies is the discovery of the complete ITH spectrum, and not its exact location. Thus the selected cubes included in the cassettes (six to eight cubes per cassette) will provide much more thorough information of the tumor, both under the microscope as well as at the molecular level.

Discussion

The use of the DAC method to help sampling strategies is not new. Indeed some authors have applied the algorithm in particle physics to improve the diffusion sampling in generalized ensemble simulations[9]. This approach, or any other with scientific basis, has not been implemented for tissue selection in Pathology laboratories so far, since the pathologists did not consider tumor sampling a complex problem in the pre-molecular era.

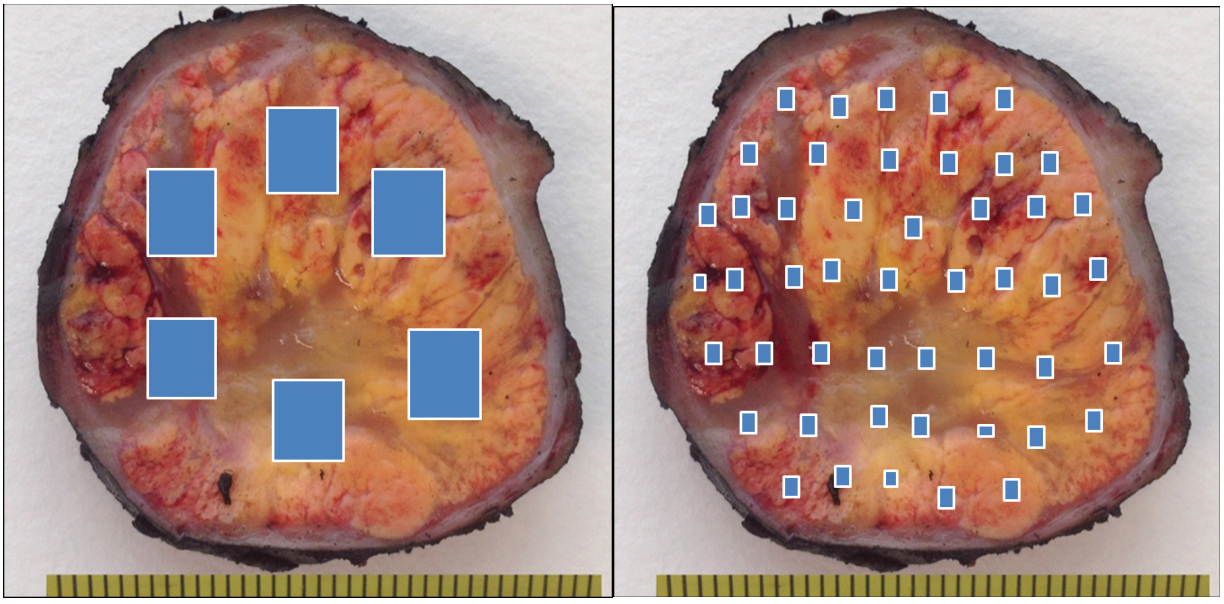

Figure 1. Schematic representation of routine (left) and divide-and-conquer (right) strategies in tumor sampling.

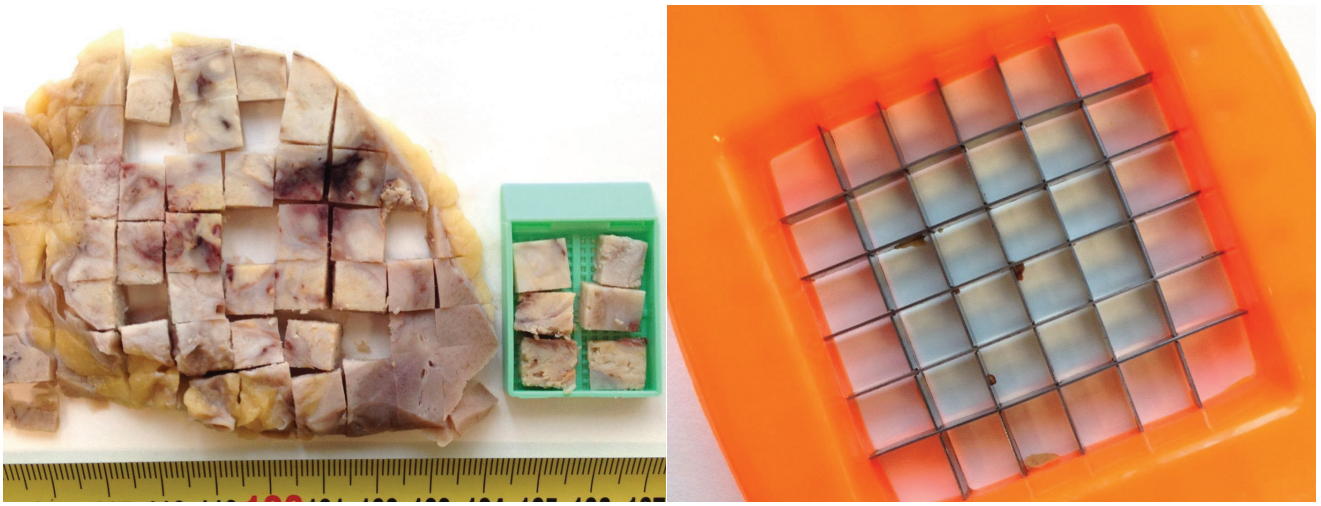

Figure 2. Tumor tissue sampling after being divided with a cutting grid.

An experience-based reasoning says that this option will save pathologist's time when handling large tumors, in a manner which is inexpensive and reliable at the same time. In combination with the changes proposed for the technician training in our previous report[4], this new alternative will make the pathologists' routine much faster and robust providing an integrated solution to fulfill basic researchers' expectations[10]. If the DAC strategy is adopted as a suitable method to increase the amount of information given to oncologists, pathologist's routine will move from the classic big-fragments-into-the-cassette routine to a sort of rudimentary tissue microarray building, as recently proposed[4].

Figures are demonstrative. For instance, the DAC strategy applied to a ccRCC of 10 cm in diameter - a quite common situation in routine pathology - will generate approximately 80 small samples (of about 4–5 mm in size) that would be included in 10 cassettes for a thorough tumor examination. Importantly to remark, the same 10 cm in diameter tumor would need also 10 cassettes for the analysis, with one tumor sample per cassette, in the case of routine sampling protocols[11].

Depending on the pathologist's skills, the time to collect 80 small samples in the grossing room is variable, but in any case, long. For this reason, any successful alternative must necessarily overcome this hurdle. A feasible choice would be an electric bacon slicer, but a long bladed knife will also work. To note, slicing electric machines are being increasingly used in pathology for handling radical prostatectomies[12] and other surgical specimens[13], and they are the first step in the whole-mounting processing for tumor mapping. In this case, the obtained ccRCC slices can be quickly cut in one shot by pressing on the entire tumor surface with a cutting grid. The procedure will generate many cubes ready to be included within a cassette. If we assume that tumor sampling following a DAC strategy is appropriate for improving ITH detection, the use of a cutting grid will shorten significantly the total process ensuring a uniform and widespread selection of the samples. A straightforward estimation with some practical cases indicates that the time for obtaining 80 samples with this method is reduced to a tenth as we cut 10 small tumor pieces at the same time.

Conclusions

The present paper describes a new method for tumor sampling in routine pathology inspired by the DAC algorithm[4]. Once DAC has been proved to be efficient for ITH detection, we expect that the use of a cutting grid will make affordable its widespread application. Objectives are twofold: ITH detection improvement and time optimization (cost) in Pathology laboratories.

Author contributions

JIL and JMC identified the problem and gave a realistic solution. JIL and JMC wrote this note.

Competing interests

No competing interests were disclosed.

Grant information

JMC is funded by Ikerbasque: The Basque Foundation for Science.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Gay L, Baker AM, Graham TA: **Tumour Cell Heterogeneity [version 1; referees: 5 approved].** *F1000Res.* 2016; **5**: pii: F1000 Faculty Rev-238.
PubMed Abstract | Publisher Full Text | Free Full Text
2. Gerlinger M, Rowan AJ, Horswell S, *et al.*: **Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.** *N Engl J Med.* 2012; **366**(10): 883–892.
PubMed Abstract | Publisher Full Text | Free Full Text
3. Hiley C, de Bruin EC, McGranahan N, *et al.*: **Deciphering intratumor heterogeneity and temporal acquisition of driver events to refine precision medicine.** *Genome Biol.* 2014; **15**(8): 453.
PubMed Abstract | Publisher Full Text | Free Full Text
4. López JI, Cortés JM: **A divide-and-conquer strategy in tumor sampling enhances detection of intratumor heterogeneity in routine pathology: A modeling approach in clear cell renal cell carcinoma [version 1; referees: 4 approved].** *F1000Res.* 2016; **5**: 385.
PubMed Abstract | Publisher Full Text | Free Full Text
5. Cormen TH, Leiserson CE, Rivest RL, *et al.*: **Introduction to Algorithms.** 2nd Edition, MIT Press, 2001.
Reference Source
6. **Divide and conquer.** *JAMA.* 1967; **202**(13): 1144.
PubMed Abstract | Publisher Full Text
7. Eisenstein M: **Cell sorting: Divide and conquer.** *Nature.* 2006; **441**(7097): 1179–1185.
PubMed Abstract | Publisher Full Text
8. Kristensen VN: **Divide and conquer: the genetic basis of molecular subclassification of breast cancer.** *EMBO Mol Med.* 2011; **3**(4): 183–185.
PubMed Abstract | Publisher Full Text | Free Full Text
9. Min D, Yang W: **A divide-and-conquer strategy to improve diffusion sampling in generalized ensemble simulations.** *J Chem Phys.* 2008; **128**(9): 094106.
PubMed Abstract | Publisher Full Text
10. Soultati A, Stares M, Swanton C, *et al.*: **How should clinicians address intratumour heterogeneity in clear cell renal cell carcinoma?** *Curr Opin Urol.* 2015; **25**(5): 358–366.
PubMed Abstract | Publisher Full Text
11. Trpkov K, Grignon DJ, Bonsib SM, *et al.*: **Handling and staging of renal cell carcinoma: the International Society of Urological Pathology Consensus (ISUP) conference recommendations.** *Am J Surg Pathol.* 2013; **37**(10): 1505–1517.
PubMed Abstract | Publisher Full Text
12. Egevad L: **Handling of radical prostatectomy specimens.** *Histopathology.* 2012; **60**(1): 118–124.
PubMed Abstract | Publisher Full Text
13. Helliwell TR: **ACP Best Practice No 157. Guidelines for the laboratory handling of laryngectomy specimens.** *J Clin Pathol.* 2000; **53**(3): 171–176.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Referee Status: ? ? ?

Version 1

Referee Report 21 July 2016

doi:10.5256/f1000research.9785.r15138

Manuel Areias Sobrinho Simoes[1,2]
[1] Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal
[2] Department of Pathology and Oncology, University of Porto, Porto, Portugal

The article “A multi-site cutting device implements efficiently the divide-and-conquer strategy in tumor sampling” represents an intelligent and practical evolution of the previous article of the same authors on the challenging issues of intratumor heterogeneity and tumor sampling.

Tumor heterogeneity is one of the most important features of any approach to oncobiology and/or oncology. Together with other factors with which it is related – topography and time (evolution) – tumor heterogeneity is a key issue of cancer diagnosis and cancer treatment.

López and Cortes addressed this issue adequately in their previous article demonstrating the importance of finding ways of evaluating tumor heterogeneity and topography via a clever and innovative strategy – Divide-and conquer (DAC) strategy – of tumor sampling.

I must confess I liked very much the previous article but I did not know how much effort one would need to implement a successful DAC strategy. It was therefore an agreeable surprise to see the continuation of the work having the real life of a Pathology Laboratory as the objective.

I think the authors describe reasonably well the method they have “invented” and I concur with them it looks a simple procedure that assures a rather complete sampling of large surgical specimens in a relatively short period of time. However, for the sake of utility, I think the authors should provide a more detailed description of the “technicalities” of the procedure.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Report 08 July 2016

doi:10.5256/f1000research.9785.r14804

Christopher DM Fletcher
Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA

This is valuable and innovative work, which provides a simple and practical approach to more efficient sampling of larger human tumors, with the goal of better determining tumor heterogeneity.

There are some omissions in the description of the technique, which would make adoption of the technique easier for others. Specifically, the authors should state clearly the recommended thickness of the whole tumor slice, whether using a long-bladed knife or slicing machine. Clearly the slice thickness must fit in the tissue processing cassette - so probably less than 3 mm - do the authors think that 3 mm slices of a large mass will generally remain intact/not fall apart before being cut with the grid. The second, perhaps more important, point is the authors need to state clearly how much of a given tumor they believe should be sampled. In their 10 cm example, it seems like they would submit virtually the whole tumor but this is not clear. How much would they submit from a 20 cm tumor? Conversely, how big does a tumor need to be before this type of sampling technique is advised? Presumably smaller tumors (e.g. 5 cm) could be sampled in the conventional/routine fashion with larger pieces? Clarification of these points will enhance the value of the authors' proposal.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Report 07 July 2016

doi:10.5256/f1000research.9785.r14803

Thomas V. Colby
Department of Laboratory Medicine and Pathology, Mayo Clinic, Scottsdale, AZ, USA

A simple and practical proposal for tumor sampling tissue. One suggestion: give some guidance as to which among multiple tissue cubes would be put in the cassettes.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.