Discovering intratumor heterogeneity: the next frontier for pathologists

J.I. LÓPEZ[1], G. DE PETRIS[2]

[1] Department of Pathology, Cruces University Hospital, Biomarkers in Cancer Unit, Biocruces Research Institute, University of the Basque Country (UPV/EHU), Barakaldo, Spain; [2] Department of Pathology and Laboratory Medicine, Penrose St Francis Hospital, Colorado Springs, CO, USA

Key words

Summary

Discovering intratumor heterogeneity is a crucial issue in modern oncologic medicine. Highly sophisticated technology such as high-throughput DNA sequencing has demonstrated the real dimension of the problem. The overwhelming majority of malignant tumors show high levels of intratumor heterogeneity when thoroughly studied. Intratumor heterogeneity develops both in temporal and spatial domains and its distribution is not deterministic making each case truly unique and unrepeatable. Pathologists are main actors in intratumor heterogeneity detection since they are the medical specialists who sample the tumors. Recent evidences have shown that currently applied sampling protocols are insufficient for a reliable intratumor heterogeneity detection. Pathologists must adapt classic sampling to the new times thus continuing being key pieces in the multidisciplinary approach to neoplasia that modern medicine demands.

Introduction

Intratumor heterogeneity (ITH), namely, the fact that a given neoplasm is qualitatively different along its different regions, is an intrinsic characteristic of human malignancies with high impact in modern medicine [1][2]. ITH is responsible for most of the current therapeutic failures and constitutes the main obstacle to improve survival in patients with cancer [3][4]. Unveiling ITH is not an easy task in clinical practice. It is a complex and multifactorial problem involving basic researchers and clinical specialists [5].

Pathologists are main actors on the clinical side of the care of cancer patients, since they are responsible for the initiation of the solution to the problem. Pathologists handle surgical specimens and decide which specific parts of the tumor are going to be analyzed, and this is a crucial decision for the patient. Any non-detected molecular alteration in an insufficient or incorrect tumor sampling will be lost forever when the remaining of the surgical specimen is eventually discarded.

This mini-review intends to call the attention of pathologists to the imperative need of identifying ITH providing scientific evidence helping pathologists to abandon old habits and move forward to design more informative sampling.

Scientific approach

Pathologists look at the tumors by the naked eye and under the microscope. Maybe because of that (and usually under a high clinical pressure), pathologists have a biased perception of what is actually a neoplasm. Diagnostic routine tends to lead the pathologist to consider tumors as mere static two-dimensional groups of cells with distinctive morphologies and architectural arrangements. There is a permanently updated body of knowledge translating morphologic data to pathological diagnoses which, in turn, will conditionate the implementation of specific treatments. However, a neoplasm is something more complex.

A neoplasm is a community of billions of cells dynamically interacting with each other. The collective behav-

Correspondence

José I. López, Department of Pathology, Cruces University Hospital, plaza de Cruces s/n, 48903 Barakaldo, Bizkaia, Spain - Tel. +34.600.6084 - Fax: +34.600.6132 - E-mail: jilpath@gmail.com

ior of these modified cells has deep roots into theoretical physics and follows nonlinear oscillatory far from the equilibrium self-regulated models condensed in power laws [6][7]. Following these models, a neoplasia, a colony of bacteria, and a community of bees, for instance, display similar behavioral patterns. These patterns are condensed in the concept of *swarming* [8]. Swarming refers to a collective unison behavior of large collectivities of insects, fishes, birds (and cells) without any centralized coordination. Swarming behavior is not exclusive of biological phenomena; events as diverse as earthquakes, robotics, traffic in big cities and stock exchange fluctuations follow similar behaviors [7]. There are mathematical models demonstrating that the underlying rules for tumor metastatic settlements and the establishment of new ant colonies are the same. In fact, the laws of physics that govern neoplasia are the same that regulate not only all biology, but also the entire universe. Actually, physicists call *dark matter* the set of ions and proteins contained in the cell attributing them central roles in the development of many of their activities [9].

The possibilities for developing mutations in such a huge community of cells are practically infinite but the rules that govern this phenomenon are totally unknown so far. The classical approach establishes that the evolution of malignant cells in a neoplasm follows a Darwinian pattern [10]. However, non-Darwinian models of evolution have been detected in highly heterogeneous neoplasms [11], thus indicating that the *decisions* adopted by malignant cells to accomplish ITH might be much more complex than believed [12], since they are not genetically determined [13].

The development of ITH is unclear and the collected data are contradictory so far. ITH is a stochastic spatial and temporal event [3][14]. Spatial ITH leads to tumor regionalization resulting in different tumor areas with different mutational statuses. This leads to the development of branching patterns specific of each tumor [14]. Temporal ITH, however, is made of not time-dependent events that seems to develop at very early stages of carcinogenesis [15]. These findings contradict the widespread belief that a tumor is more heterogeneous the greater and more evolved it is and places the focus of interest in small tumors, many of them incidentally discovered in daily practice.

Clinical approach

No doubt, ITH is the next frontier of modern oncology. An enormous number of scientific papers have been trying to decipher it in the recent years, but the problem is far from being fully elucidated. When examining current approaches, it seems that modern medicine has not noticed the importance of the discovery of ITH for the patient. For instance, ITH is right now the main obstacle for the development of targeted therapies in many types of cancer. The success of these therapies depends on the non-responder component of the tumor, that is some cases has not been identified, sampled, or characterized enough. This situation leads to oncologists to demand urgent solutions [5]. To overcome these limitations, various authors have recently developed algorithms to assess ITH when there is very little material to analyze [16-19].

The problem is generalized and goes beyond pathologists. On one hand, it is a technological problem. Massive sequencing devices are still very expensive and data mining requires specialization in bioinformatics, two aspects that are far from being adopted by many public hospitals. On the other hand, the problem is the representativeness of the samples. Are endoscopic, transthoracic or transrectal biopsies representative enough so as to give something else than a morphological diagnosis? [20][21], Can a targeted therapy be initiated with the molecular information obtained from small tissue fragments representing less than 1% of the tumor? [22].

To overcome this hurdle, and to make easier obtaining a sample by non-invasive methods, the liquid biopsy is being postulated as a safe alternative. Liquid biopsy can detect free DNA and circulating neoplastic cells in peripheral blood and its usefulness is right now being tested in colorectal [23] and breast [24] cancers. However, its real applicability is still to be demonstrated.

Recent literature shows many examples of confusing results obtained in molecular analyses of the same tumor types and, even more important, deep disagreements in the appropriateness of providing expensive therapies. In this specific scenario, pathologists should first wonder how many of these apparent inconsistencies might be simply due to incomplete/inadequate tumor samplings.

Even after the problem of the sample representativeness is considered (and understood as not yet solved), the clinician must then face another issue: Individual intrinsic tumor resistance to treatments. The presence of cell clones resistant to targeted therapies can be either *de novo* or therapy induced [25-29]. Another strategy in development is the so called adaptive therapy, in which the objective is to maintain stable the mass of cells sensitive to therapy via variations in the treatment [30]. The goal here is to achieve, if not cure, at least the non-progression of the tumor.

Pathological approach

Most targeted therapies are introduced once the pathologist report is available. When a neoplasm cannot be studied *in toto* due to its large size, pathologists make a selection of the samples to be analyzed based on accepted and recommended protocols. However, these protocols were designed in a dogmatic fashion when ITH was not an issue and therefore need urgent updating.

Pathologists around the world face the same questions when facing a large tumor: where to sample and when to stop sampling? Deficiencies in molecular ITH detection, together with the lack of an appropriate therapeutic response, are weakening the position of pathologists in the diagnostic process. The adoption of appropriate guide-

lines to identify ITH in pathology laboratories seems mandatory and urgent.

The main efforts of pathologists are directed towards microscopic and molecular diagnoses while tumor sampling is perceived as a task of somewhat lesser importance. The question seems simple, but it is still not answered: What is a correct tumor sampling?

Fukuoka et al. [31] have developed a new method called spiral array that improves ITH detection in formalin-fixed paraffin-embedded samples of lung [32] and prostate [33] cancers. Spiral array allows tissue optimization in biopsies with scarce material in their retrospective analysis. However, spiral array does not resolve tumor sampling in a prospective manner.

Multi-site tumor sampling (MSTS) [34-36] has demonstrated to be a feasible and efficient alternative to current sampling protocols without extra costs. Briefly, MSTS is based in the divide-and-conquer algorithm [37] and consists on increasing six to eightfold the number of samples obtained from a tumor placing them in the same number of cassettes that would be used by routine protocols through diminishing their size [34]. In this manner, a 10 cm in diameter tumor would make use of the same 10 cassettes (1 cassette per centimeter of tumor) with six to eight small fragments on each cassette. Common sense, an *in silico* analysis [34], and a preliminary clinical validation [36], demonstrate that 60-80 small samples (in 10 cassettes) obtained from many tumor areas across the tumor detect much more ITH than 10 large samples (in 10 cassettes). Should molecular analysis confirm this data MSTS could be an affordable solution balancing sustainability and efficiency.

Conclusions

Heterogeneity is inherent to life, and tumors are no exception. Pathologists have the main responsibility for the detection of ITH since they are the specialists handling surgical specimens. Current sampling protocols are inefficient at detecting ITH and must be updated. The success of many expensive targeted therapies depends on the ITH identification. For these reason, discovering intratumor heterogeneity is the next frontier for pathologists.

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