Tumor Heterogeneity: Will It Change What Pathologists Do?

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Keywords

Tumor heterogeneity · Molecular heterogeneity · Morphological heterogeneity · Differentiation · Clonal evolution · Sampling

Abstract

Although the notion that tumors are heterogeneous is well rooted in diagnostic pathology, the extent of this heterogeneity at the molecular level and its impact on (targeted) treatment choice will certainly influence the practice of pathology. Even though the consequences of tumor heterogeneity for cancer care are as yet incompletely understood, pathologists can contribute to solving major scientific and clinical problems related to tumor heterogeneity by rethinking guidelines for tumor sampling, to have a more comprehensive covering of intratumor heterogeneity in the available tumor tissue samples. They should develop guidelines and/or technology to adequately document sample characteristics such as percentage of tumor cells in a sample. They need to contribute to training of bioinformaticians in the field of cancer pathobiology and integrate such well-trained bioinformaticians in the workflow leading to a final pathology report. They also need to redefine post-graduate training programs in diagnostic pathology to address the need for in-depth training in molecular cancer

pathobiology. Pathologists might contribute more to making the public at large aware of the importance of data sharing. Finally, pathologists should support the creation of consortia in which clinical and molecular data are shared with the translational research community.

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Introduction

We are living the rediscovery of tumor heterogeneity. Pathologists and others who are used to look at the histology of tumors have long known that tumors are heterogeneous [1, 2]. To begin with, by nature a tumor is composed of neoplastic cells set in a tissue infrastructure which we call the tumor stroma, the visual reflection of what in terms of tumor biology is known as the micro-environment. This is provided by the host, is part of the tumor tissue and an important co-determinant of tumor behavior but not composed of truly neoplastic cells [3–5]. The neoplastic cells proper, in most tumors do not look the same throughout the tumor. Some areas may be well differentiated, as they look like the tissue of origin. Others may be undifferentiated, which has conceptually been associated with clonogenic propensity: undifferentiated cells will have maintained the capacity to proliferate.

There has been a tendency to call these “tumor stem cells,” a terminology which has generated much confusion but also stimulated a host of studies into tumor evolution [6]. Furthermore, in some tumors rather heterogeneous differentiation patterns occur, such as heterologous elements in a mixed Müllerian tumor of the uterus.

Things became more complicated when immunohistochemical dissection of the presence of proteins in tissue sections began. It became rapidly clear that for a variety of proteins, the staining pattern is not homogeneous. Some areas may be strongly positive, while others may be completely devoid of staining cells [7]. In other tumors, the immunoreactive cells are scattered throughout the tissue like stars in the sky. Rather often than not the mechanisms responsible for this type of intratumor heterogeneity are unknown. Intratumor heterogeneity in immunoreactivity patterns have complicated life for those interested in immunohistochemical marker studies: which methods to use to report heterogeneous staining for a marker, how to (semi)quantify and how to get to a reasoned cut-off value to dichotomize a population into negative and positive [8].

Things became even more complicated when molecular genetic analyses started to be “en vogue.” The implications of genome heterogeneity of tumors in daily (molecular) diagnostic practice will also be discussed herein.

Molecular Heterogeneity of Cancer

Much of the early reflection on the evolution of tumors and their genome abnormalities have been based on Nowells model, which emerged from his observations in leukemia. In the Nowell model of tumor progression [9], tumor cells continue to acquire new mutations and those which confer growth advantage are the initiators of a new clone of tumor cells that repopulates the tumor, ultimately overgrowing less favored tumor cell clones, a mechanism in population genetics known as selective (clonal) sweep. This model has been the prevailing paradigm for evolution and progression of cancer for a fairly long period of time [10]. Incidentally, as much as the term “clonal” has become part of the tumor-biological lexicon, a recently convened group of experts in the field had major difficulties in coming up with a consensus definition on the concept of a clone [11]. The Nowell model was interpreted during a lengthy period of time to lead to relatively homogeneous tumor cell populations within a given tumor, in terms of genome characteristics. This model was strongly promoted by Vogelstein’s studies on colorectal cancer, which led

to a linear stepwise progression model [12, 13]. In practical terms, this implied that a single sample would be representative for the entire tumor cell mass. It became clear, however, that this is a simplification of the dynamics of the cell populations comprised in any given tumor.

That this might not reflect the actual tumor development is a notion that emerged decades ago. Interested in the possibility that morphological heterogeneity in adenomas of the colon might be the result of differences in genetic make-up, we performed analysis of *KRAS* mutations in multiple samples from the same adenoma [14]. Remarkably, some samples carried a *KRAS* mutation while others from the same adenoma did not. Incidentally, this variability in *KRAS* mutation status was not associated with any morphological characteristic. We subsequently expanded these studies to colon carcinomas, and by taking up to 20 samples from the same tumor showed that in terms of molecular make-up several distinct subpopulations co-existed [15]. Simple attempts to reconstruct the findings in terms of clonal architecture of the tumors showed that heterogeneity was not random but clustered, which would be supportive of the existence of distinct sub-clones. However, these clusters seemed partly interwoven, suggesting that clones infiltrated each other. The difficulty we had in getting our papers published can be taken as evidence of the resistance the idea of intra-tumor molecular genetic heterogeneity met, just over 15 years ago. The findings led us to conclude that “taking into account a significant intra-tumor genetic heterogeneity of colorectal tumors, the use of (genetic) markers for prognosis management should be reconsidered” [15].

Gradually, classical Sanger sequencing studies of multiple tumor samples made it clear that tumors are more heterogeneous than earlier assumed, and with the advent of next generation sequencing (NGS) we now have to face the reality that at all levels all tumors are heterogeneous, with significant implications for targeted but also for more conventional forms of treatment [16].

Does Present Day Practice in Molecular Pathology Reflect Tumor Heterogeneity?

The short answer to this question is no [11]. The small endobronchial biopsy of a bronchial carcinoma might not be representative of a much larger lesion. The DNA in the sample might therefore not be representative of the whole tumor. Likewise, the single maybe 1 cm² but 5 µm thin tissue section sample of a colon cancer submitted for

determination of *KRAS* mutation status might not be representative of the whole tumor. To some extent, problems with representation can be resolved by morphological control. The problem of contamination of tumor DNA by a dominating stromal or inflammatory response or of necrotic tumor tissue can be mastered by microscopic control of the sample to be submitted for molecular analysis, at least when performed properly [18].

Does Molecular Heterogeneity Affect Patient Treatment?

The short answer to this question is: we do not know yet [11]. Information on the impact of different levels of tumor heterogeneity on clinical management of patients is very scanty. What we do know is that in general tumors are heterogeneous at the molecular level but the level of heterogeneity is strikingly different between different tumor types or even between different cases of the same tumor. The landmark paper by the Swanton group [18], reporting a high level of intratumor heterogeneity in renal cell carcinoma, caused a wave of concern in view of the possible complications of such striking heterogeneity in terms of treatment response. Heterogeneity has been documented in various tumor types at morphological, immunohistochemical and molecular level [19–22]. It rapidly became clear, however, that some tumors are much more homogeneous. Most colon cancers have a relatively stable genome [23], even though the emergence of *KRAS* mutated metastases in case of a *KRAS* wild-type primary has been reported [24]. Often, driver mutations in the primary tumor are also those found in (liver) metastases. A notable exception in colon cancer is the mismatch repair deficient/microsatellite instable case, which has a hypermutating genotype [25]. However, most of these mutations are (silent) passengers rather than drivers meriting to be targeted for therapeutical purposes. What we need now is much more detailed insight in the patterns of branching evolution/phylogeny of different tumor types. This will allow better understanding of which genome alterations are in the trunk of the (phylogenetic) tree, which are in the branches at which level and what drives the development of new branches [10, 11, 16, 26].

One might think that the large collections of molecular tumor data, in the context of the cancer genome atlas (TCGA) or the International Cancer Genome Consortium (ICGC), should provide some answers. The problem with these, however, is that as a rule single tumor samples have been analyzed, that decentral sampling is

rarely standardized and that follow-up data are relatively scanty. The combination of deep NGS and sophisticated bioinformatics does allow some insight into clonal heterogeneity. What we would really need is multiple samples on a sufficient number of primary tumors, long-term follow-up of patients with multiple (liquid or tissue) biopsies, tumor samples from metastatic sites and all of this with detailed documentation of clinical evolution and treatment response. All samples would have to be deep sequenced to follow molecular evolution of different tumor sites. In this way, clonal (trunk) mutations and events leading to branching subclones might be identified. Some clinical trials are underway, including this type of (elaborate) approach [27].

What Are the Consequences of Molecular Tumor Heterogeneity in Daily Diagnostic Pathology Practice?

In daily practice, pathologists (and not only those working in tertiary reference centers) are responsible for three distinct steps in the work-flow that leads to a final pathology report. First, there is the grossing of a specimen, which includes sampling of the lesion. This is ideally done based on grossing guidelines, such as those presently developed for global use by the International Collaboration for Cancer Reporting [28]. In the heterogeneity context, it is important to realize that most guidelines were developed before pathologists became aware of the potential clinical impact of molecular heterogeneity. The implication of classical sampling is “undersampling” of molecular heterogeneity. If the rule of thumb is to take one sample per centimeter max. dimension of a neoplastic lesion, this leaves open where the sample is taken and what the sample size is. Technicians dislike (for good reasons) large chunks of tissue for histological work-up and so 1.5×1.5 cm is a tissue sample of reasonable dimension. Where is this to be taken? There are no specific rules for this; the running definition of an appropriate sample is “representative” but what it needs to be representative for remains open. López et al. [29, 30] have come up with a simple but promising sampling approach. Rather than using a single 1.5×1.5 cm sample, this might be divided into nine 0.25-cm^2 samples, not from a single site but preferably randomly distributed over the tumor. Including these in a single block would increase the sampling rate without creating extra (technical and histopathological) efforts as the total sample dimension would not increase. There is no evidence that this is more efficient in detecting targetable driver mutations,

but studies in this group are underway and time will tell if increased sample number will generate the data we need to be able to handle tissue heterogeneity effectively in a therapy decision context.

Second step is the (histological) definition of the sample(s) on which molecular analysis is to be conducted. Even though when using NGS, with its much higher sensitivity in detecting mutations in a molecularly heterogeneous DNA sample, it is important for the pathologists responsible for the final report to know the percentage of tumor cells in a sample to come up with an appropriate interpretation of the findings. Very simple but rather important lessons are to be learned here. In a recent study, Lhermitte et al. [17] reported a painful lack of reproducibility in the estimated percentage of tumor in samples selected for molecular analysis. The single most effective approach to improve reproducibility was to clearly define what was to be assessed. Was this tumor cell area percentage relative to stroma in the lesion? Was this tumor tissue (which might include stroma) relative to pre-existing non-tumor tissue? Was this a percentage of tumor cell nuclei relative to non-tumor cell nuclei? It is not difficult to see that between the three, quite different results may be obtained. Fortunately, technology comes to the rescue: software algorithms are now available that allow machine-based definition of tumor versus non-tumor with much better reproducibility [31].

The third step is the interpretation of the results and incorporating them into a final pathology report. Major problem here is the extraction of clinically relevant findings from huge amounts of data. This problem might be manageable for a pathologist trained in the trade of molecular data interpretation, when it comes to approaches targeting a limited set of known cancer-associated genome aberrations. For exome sequencing, RNA sequencing and most obviously for whole genome sequencing, expert bioinformatics support is essential. This requires pathologists to be sufficiently comfortable with the matter so as to communicate effectively with bioinformaticians, and conversely bioinformaticians with some operational knowledge of cancer genomics and its application in a clinical context [32]. Pathologists need to be involved not only in training bioinformaticians in the pathobiology of cancer, but also in redefining the content for postgraduate training programs in diagnostic pathology. The (virtual) microscope will remain a core tool in pathology but a good level of understanding of molecular (tumor) pathology, more than the toolbox of the molecular biologist, is crucial for a pathologist in the 21st century.

Can Data Obtained in Diagnostic Practice Be Used to Improve Understanding of Molecular Mechanisms Involved in Cancer Progression and Treatment Response?

The wealth of data becoming available from diagnostic molecular analyses of human tumors first and foremost serves individual patients. It would be wasteful, however, not to use these data to increase our understanding of the molecular basis of tumor heterogeneity and its impact on clinical decision making, including finding ways to overcome the problem of resistance development. The obvious way to go is towards data sharing [11]. Ideally, cancer care centers should form consortia with translational research units, conceivably with participation of industry partners and supervised by a legal and ethics framework to guarantee patient safety and privacy protection. Molecular data are only useful when accompanied by clinical and pathological data, and within such consortia an optimal data set needs to be developed to complement molecular data. This implies a good level of coordination to ensure that the decentrally generated data are in a format that can be effectively shared. Such a program needs to be preceded by the development of new informed consent practices which will allow data sharing at this level. Pathologists will need to participate actively in such programs. Another important task for pathologists is to initiate or at least actively participate in campaigns to inform the public at large and more specifically the patient population. Productive interactions with patient advocacy organizations would be called for.

Conclusions

The cancer research and care providing community is gradually coming to grips with the realities of tumor heterogeneity. The consequences of tumor heterogeneity for cancer care are as yet incompletely understood. Pathologists can contribute to solving major scientific and clinical problems related to tumor heterogeneity by:

1. Rethinking guidelines for tumor sampling to have more comprehensive covering of intratumor heterogeneity in the available tumor tissue samples.
2. Developing guidelines and/or technology to adequately document sample characteristics such as percentage of tumor cells in a sample.
3. Contributing to the training of bioinformaticians in the field of cancer pathobiology.

4. Integrating well-trained bioinformaticians in the workflow leading to a final pathology report.
5. Redefining postgraduate training programs to address the need for in-depth training in molecular cancer pathobiology.
6. Contributing to making the public at large aware of the importance of data sharing.
7. Contributing to the creation of consortia in which clinical and molecular data are shared with the translational research community.

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