Spotlight

Pathology and the evolutionary dynamics of clear cell renal cell carcinoma

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ABSTRACT

Cancer is an ecosystem whose intrinsic mechanisms do not show up under the microscope of pathologists. However, the information provided by pathologists is absolutely necessary for the correct implementation of personalized treatments. This short paper seeks to analyze this apparent paradox, i.e. static snapshots for making crucial decisions in essentially dynamic diseases, taking clear cell renal cell carcinoma as a paradigmatic example of tumor variability. We seek to call the attention of pathologists and other cancer-related medical specialists to extend knowledge of the evolutionary features of the disease to help obtain a better understanding of why cancer behaves as it does.

Cancer is a dynamic disease whose evolution depends on multiple intrinsic factors (genetic, epigenetic, and microenvironmental). Unfortunately, many of these factors have not been yet identified, so predicting the evolution of each individual case still remains a difficult task in the routine practice. Personalized therapies currently rely essentially on the histological and molecular data provided by pathologists. That information reflects the status of the tumor exactly at the time when the biopsy is taken from the patient, so by definition it is static in time and partial in extent. While the sequential temporal histological analysis of tumors is not possible in humans, such thorough analyses are perfectly suitable and depend on appropriate specimen handling. Multisite tumor sampling [1,2], for example, guarantees a complete molecular analysis to assure the correct identification of intratumor heterogeneity (ITH) at sustainable costs. ITH is a crucial point in oncological practice because it remains constant in most malignant tumors and is a major cause of therapeutic failure. However, the next generation sequencing tools and other sophisticated devices needed for this purpose are expensive and not yet universally affordable, so their generalized implementation remains pending.

Clear cell renal cell carcinoma (CCRCC) is a paradigmatic example of ITH. It ranks in the top-ten list of the most frequent malignant neoplasms in adults in Western countries. It remains a health problem of major concern due to its intrinsic biological aggressiveness and frequent resistance to therapy, with high 5-year mortality rates usually in the context of metastatic disease. These somewhat disappointing clinical results are showing the level of complexity of this tumor and advise to

incorporate to current approaches additional scientific tools like those provided by Mathematics (Quantitative Analysis, Game Theory, Bioinformatics), Biophysics, System Biology, Biotechnology, Ecology, and others, to cancer study. Such a multidisciplinary sum of efforts will allow a better understanding of oncogenesis and tumor evolution in the next years thus improving survival expectancies.

Main clinical, pathological, and genetic features of CCRCC have been recently updated [3]. This tumor displays a wide range of histological appearances frequently intermingled within the same tumor, which makes morphological standardization of diagnosis a difficult task. Pathological stage (pTNM), Fuhrman grade, sarcomatoid dedifferentiation, extrarenal extension, and tumor necrosis are classic histological parameters which correlate with survival. However, the broad spectrum of architectural arrangements and the wide vascular patterning are very difficult to systematize. Aside from the classical histopathological parameters, two different prognostic groups can be identified in CCRCC patients depending on the immunohistochemical expression of anti-BAP1 and anti-PBRM1 proteins. As a rule, BAP1 mutant tumors seem to behave more aggressively than PBRM1 mutant cases.

Despite the inherent limitations associated with the static study of an essentially dynamic process, histological snapshots of tumors still provide crucial data for understanding cancer behavior. For example, some authors have described that different microscopic findings are associated with specific mutational profiles in CCRCC [4]. Thus, tumors with an inflamed histological phenotype characterized by heavy intratumor lymphocytic infiltration seem to be associated with *BAP1* gene
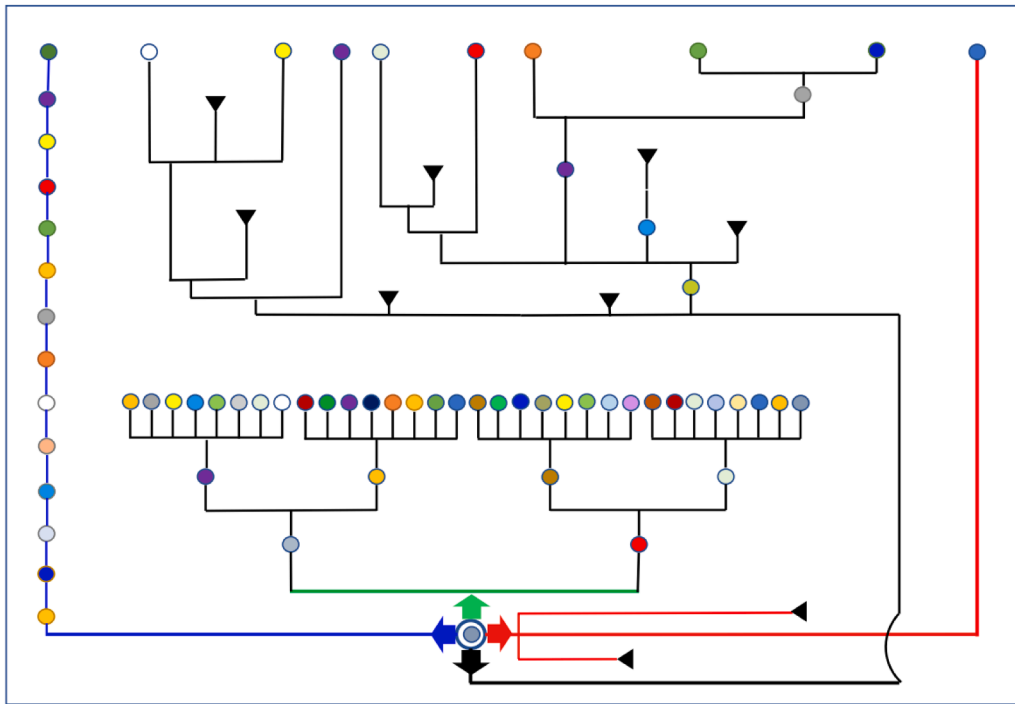
Fig. 1. Schematics showing tumor evolution models. The blue arrow represents linear evolution, where driver mutations conferring stronger selective advantages occur in a step-wise process (successively generated clones appear represented by circles with different colors). A linear evolution model gives rise to tumors without intratumor heterogeneity. The red arrow shows the punctuated evolution, where a clone with high fitness fixes early in the tumor evolution and dominates tumor expansion (the clone with highest fitness dominates tumor expansion, with extinct clones represented by black triangles). The punctuated model generates tumors with low intratumor heterogeneity. The black arrow depicts the branched-type evolution, where clonal and sub-clonal diversification develops following Darwinian patterns [new clones/subclones appear (circles) and disappear (triangles) during tumor evolution]. The branched pattern creates tumors with high levels of intratumor heterogeneity. The green arrow illustrates the neutral evolution model, where a huge number of passenger mutations with no clinical significance appear in a non-Darwinian manner (high number of clones coexist represented by circles with many colors). Typically, neutral evolution generates tumors with extremely high levels of intratumor heterogeneity.
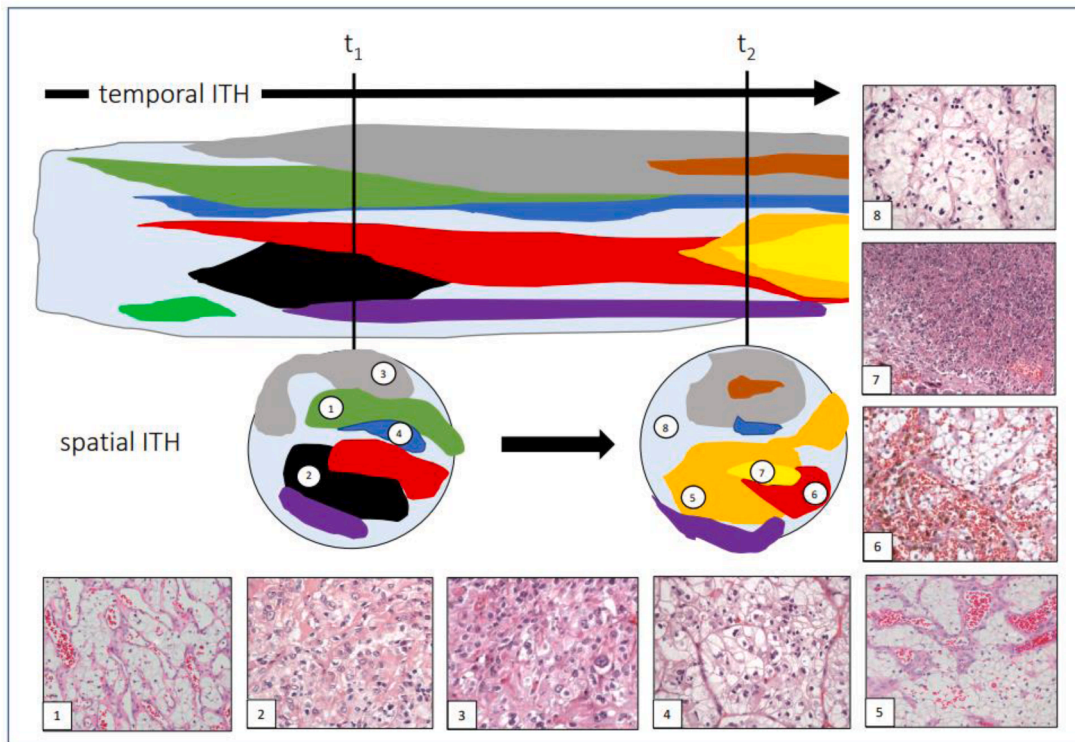

Fig. 2. Representation of the dynamic temporal and spatial intratumor heterogeneity distribution in the branching evolutionary model of clear cell renal cell carcinoma, where clones and sub-clones appear and disappear. Spatial clonal and sub-clonal diversification over time is depicted at two different static points of evolution, with significant differences shown between them. As reflected in the histological pictures of eight different areas sampled, intratumor heterogeneity is high and shows different vascular patterns (samples 1, 5, and 8), low (samples 1, 5, 6, and 8) and high (samples 2, 3, and 4) grade areas, hemorrhage (sample 6), and necrosis (sample 7).

mutations, a genetic disorder associated with the aggressive forms of CCRCC. On the other hand, an angiogenic histology characterized by prominent intratumor neovascularization is linked to *PBRM1* gene mutations, in which tumors behave less aggressively. The therapeutic implications of this simple histological distinction are clear: Inflamed tumors are expected to respond better to immune checkpoint blockage inhibition whereas angiogenic tumors respond better to anti-angiogenic drugs. Again, patient selection for administering these therapies is performed under the pathologist's microscope. Actually, the assessment of the anti-PD-1/PD-L1 expression in the tumor lymphocytic infiltration places the pathologist once again at the core of therapeutic decision making. This analysis, however, is far from being perfect because the use of different antibodies with different assessment methods, insufficient sampling, and high inter- and intra-observer variability introduce inconsistencies [5].

Historically, there have been many attempts to test the ability of pathologists to predict the clinical evolution of tumors based exclusively on histological and immunohistochemical findings. Some authors have taken into consideration the sum of cytological and architectural findings, along with microenvironmental features (tumor capsule invasion, characteristics of the inflammatory infiltrates, tumor necrosis, vascularization, and others) for such a purpose. However, the high histological variability detected in most CCRCC and the unavoidable subjectivity in the inter- and intra-observer microscopic assessment mean that such predictions, by themselves, are not very reliable. To make matters worse, genomic analyses have shown that a subset of small CCRCC bear early *BAP1* gene mutations and are wolves in sheep's clothing, thus reflecting major phenotypic/genotypic divergencies. Indeed, additional histological/molecular discrepancies have been recently reported, for instance that ability for tumor invasion is not necessarily associated with the most aggressive clone [6].

A step forward in cancer understanding is achieved by pathologists and oncologists when the dynamic perspective of the disease is taken into account as is the case with ecology. Considering neoplasms as communities of individuals (cells) interacting with each other has provided a new perspective on cancer evolution. As a result, the term eco-oncology has been coined [7]. Three Darwinian (linear, punctuated, and branched) models of tumor evolution and one non-Darwinian (neutral) model, have been described from this perspective (Fig. 1) [8]. Linear evolution gives rise to tumors without ITH and appears when driver mutations conferring stronger selective advantages occur in a step-wise process. By contrast, the punctuated model is characterized by a clone with high fitness fixing early in the tumor evolution which dominates tumor expansion, generating aggressive neoplasms with low ITH. In the branched-type evolution there is clonal and sub-clonal diversification, resulting in tumors with high levels of ITH. Finally, in the non-Darwinian (neutral) model, where a huge number of passenger mutations with no clinical significance appear, tumors display extremely high levels of ITH.

The branched model of tumor evolution is the best example of clinically significant high ITH occurring in CCRCC. Here, clones and sub-clones appear and disappear over time during tumor development (Fig. 2). This leads to high temporal and spatial variability of both histological appearance and genetics. As mentioned above, the static picture depends specifically on the exact time when the tumor is removed, so the practical conclusion is that the same tumor may show two totally different faces at two different stages of its evolution. No matter CCRCC can became very different in different phases of their respective evolution, the start point seems to have a common pathway, as reflected in a recent study by Mitchell et al. [9]. In this interesting study, the authors demonstrate that a chromothripsis event causing 3p loss and concurrent 5q gain in one allele occurs in a few hundred of renal cells as early as childhood or adolescence. These cells remain dormant for decades until the *VHL* gene of the other allele is damaged, this way starting tumor development. On the other hand, further knowledge in identifying the origin of renal cell carcinoma and its microenvironment is being recently

provided by single-cell genomics [10,11].

Although the process was initially considered as stochastic, a recent study on a series of 101 CCRCC based on a multiregional histological and genomic paired analysis has shown that there is some degree of predictability in their evolution [12]. This study demonstrates that branched-type tumors have mutations constrained to *VHL, PBRM1, SETD2, PI3K* genes, display high levels of genomic ITH, present low levels of chromosomal complexity, pursue an attenuated clinical course, and develop late, solitary metastases. This association between the level of ITH and tumor aggressiveness has been corroborated in a recent histological study of 28 exhaustively sampled CCRCC [13].

It is now clear that ecological principles play a key role in governing cancer evolution [14]. This has been demonstrated again in two recent studies which find significant genomic differences across different tumor regions [15,16]. For example, ITH seems to be higher in the tumor periphery whereas tumor center areas remain relatively homogeneous [15]. Local microenvironmental pressures induce the development of metastatic competences specifically in the tumor interior, where the hypoxia is greatest, thus making the struggle for cell survival fierce [16]. Here, the options are to escape or die. This finding correlates well with the central tumor necrosis detected by pathologists in routine practice, an otherwise well-known histological feature of tumor aggressiveness which characterizes Grade 4 CCRCC. Conversely, tumor edges are better vascularized and develop local invasive competencies. A similar conclusion is reached in another study in which the authors have shown that the invasive fraction of CCRCC does not correspond to the metastatic competent clones [6]. This particular tumor regionalization seems to be a constant event in many tumors. From a practical point of view, this distribution of competencies between tumor center and periphery should guide the needle of radiologists in obtaining tumor tissue at the preoperative stage and the scalpel of pathologists in sampling CCRCC in the grossing room [2]. This new evolutionary perspective, however, needs to be confirmed in larger series.

In conclusion, this overview seeks to concentrate evidence showing that tumors are complex ecosystems with many players in action, the deciphering of which is hindered by the limitations inherent in a static approach to intrinsically dynamic processes. Pathologists must keep this in mind when analyzing tumor specimens and adapt sampling protocols to assure at least an accurate spatial identification of ITH. Finally, analyzing neoplasms from an ecological viewpoint may help to understand why tumors evolve as they do and how to deal with them more efficiently.

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