OPINION

A river model to map convergent cancer evolution and guide therapy in RCC

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Abstract | Intratumoural heterogeneity in clear cell renal cell carcinoma (ccRCC) complicates identification and validation of biomarkers and thwarts attempts to improve precision medicine. Efforts to depict intratumoural heterogeneity and to pinpoint strategies for disease control resulted in the creation of the trunk–branch model of mutational cancer evolution, which emphasizes targeting trunk mutations. However, most patients with ccRCC receiving current therapeutics that target these mutations, such as inhibitors of vascular endothelial growth factors, eventually develop resistance. A novel paradigm might improve depiction of cancer evolution and advise therapeutic selection: the river model is based on findings from multiregion sequencing in samples from exceptional responders to mTOR inhibitors. The accumulating data on genotypic and phenotypic convergence in renal cell carcinoma and other malignancies can be used to examine how a mutable river model might best describe clinically significant phenotype-convergent events that could guide effective cancer control. This model originates from studying exceptional responders and its generalizability awaits validation.

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Introduction

The success of imatinib, a tyrosine kinase inhibitor, for the treatment of chronic myelogenous leukaemia (CML) at the end of the past millennium spurred the development of targeted therapies for cancer.[1] Since then, marked progress has been seen in the treatment of multiple malignancies. In patients with gastrointestinal stromal tumours (also called GISTs), head and neck squamous cell carcinomas, non-small-cell lung cancer (NSCLC), melanoma, and HER2-positive breast cancer, the use of targeted agents against c-Kit and PDGFR-α,[2] EGFR,[3,4] EGFR[5] and anaplastic lymphoma kinase (ALK),[6] B-raf,[7,8] and HER2,[9,10] respectively, has resulted in profound improvements in overall and progression-free survival.

For clear cell renal cell carcinoma (ccRCC), two classes of targeted agents have been developed in the past decade whose clinical use has improved overall survival of patients with metastatic disease from approximately 1 year (when receiving cytokine therapy)[11] to nearly 3 years.[12,13] The two classes target distinct pathways involved in ccRCC tumorigenesis: the vascular endothelial growth factor (VEGF) pathway and the mTOR pathway.[14] Sunitinib, the second FDA-approved targeted agent for ccRCC, is now a first-line VEGF inhibitor for the treatment of metastatic disease.[15,16] The mTOR complex 1 (mTORC1) inhibitors temsirolimus and everolimus are category 1 recommendations[17] as first-line therapies for patients with ccRCC who have a poor prognosis and for those who fail first-line VEGF tyrosine kinase inhibitor (TKI) therapy, respectively.[11,18]

Despite therapeutic advances in the treatment of metastatic ccRCC, resistance inevitably develops, leading to progression and further spread of the disease. Two prevailing theories to explain therapeutic resistance exist: acquisition of new genetic and/or epigenetic aberrations and therapy-related selection of pre-existing resistant clones (termed intrinsic resistance).[19,20] The second hypothesis, proliferation of therapy-resistant clonal populations, is becoming increasingly accepted as a major cause of both primary and acquired resistance in cancer, especially in light of the extensive intratumoural heterogeneity that is characteristic of ccRCC and other malignancies.[21–23]

The existence of extensive intratumoural heterogeneity in patients with ccRCC poses an exceptional challenge to achieving effective treatment of this disease. Authors of a series of studies investigating intratumoural heterogeneity have suggested a few strategies to control disease progression, such as targeting ubiquitous trunk mutations.[24] In this Perspectives article, we propose a novel river model depicting cancer evolution based on our findings from multiregion sequencing performed on samples from exceptional responders to mTORC1 inhibitors. By capitalizing on the accumulating evidence of genotypic and phenotypic convergence in renal cell carcinoma (RCC) and other malignancies, we examine how a river model might best describe phenotypically convergent events that could guide effective cancer control.

Intratumoural heterogeneity

Pathologists have long recognized morphological variation between different regions of an excised renal tumour and, as a result, examination of multiple tumour sections but reporting only the highest grade detected has become common practice. In 2012, Gerlinger and colleagues[25] conducted the first multiregion sequencing analysis of primary renal masses and, through exome sequencing and copy number analysis of four large renal tumours, demonstrated that marked genomic heterogeneity was associated with histopathological variation. They found heterogeneity to be even more extensive than previously thought.[26] The investigators mapped two-thirds of the identified genetic aberrations to the subclonal branches; the remaining one-third were present ubiquitously throughout each tumour.[25] Similar distributions were found for copy number alterations affecting the short and long chromosomal arms in ccRCC.[27]

A subsequent analysis by Gerlinger *et al.*[28] again applied ultra-deep targeted exome sequencing to 79 samples derived from 10 patients with metastatic ccRCC,

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including the previously analysed four patients.[25] Intratumoural heterogeneity was detected in all patients and, in the majority of tumours, heterogeneity (that is, the number of detected mutations) increased with the number of biopsy samples analysed with no evidence of saturation. The investigators found that *VHL* mutations and chromosome 3p loss were the only ubiquitous events; other driver mutations, including aberrations in *PBRM1, SETD2* and *BAP1*, remained largely subclonal.[28] Of note, this enhanced multiregion analysis also found that single-biopsy approaches underestimated the prevalence of driver mutations when the researchers compared the cumulative prevalence of driver mutations per patient with the prevalence of driver mutations reported by the The Cancer Genome Atlas.[29] For example, *TP53* mutations, which were found in only 6% of single biopsies, comprised 40% of mutations when all tumour regions from a patient were considered cumulatively.[28] Similarly, mutations in the PI3K–Akt–mTOR pathway were found in 28% of individual biopsies but in 60% of patients included in this study.

From these multiregion studies, the tree model (or trunk–branch model) emerged to illustrate tumour growth.[24,30] In this model, ubiquitous mutations form the trunk, representing initiating and early driver mutations. For renal cancer, *VHL* mutation and heterozygous loss of chromosome 3p are well established to be initiating drivers, as nearly all ccRCC patients demonstrate these genomic alterations.[31,32] Early driver mutations that are present in the trunk include, for example, those affecting chromatin modification, namely *SETD2, BAP1, PBRM1* and *KDM5C*.[25,28,33,34] Heterogeneous mutations are assigned to the branches of the tree model, where their spatial and temporal variations and relationships are captured by further subdivisions. This model demonstrates the evolutionary progression occurring during tumour growth and highlights the complexity of clonal populations.

The presence of intratumoural heterogeneity emphasizes the limitations of single biopsies in providing adequate and appropriate information for clinical decision making, as many mutations that are present in spatially separate regions are missed.[35] Sequencing coverage is often insufficient to reliably detect subclones that are rare and have allele frequencies below a certain threshold in one specific biopsy sample, but are common in other parts of the tumour.[35,36] Only through massively deep sequencing can single biopsies or single-region analyses provide valuable information regarding subclonal populations. In four patients with ccRCC, Gerstung *et al.*[23] identified subclonal variants with frequencies as low as 1:10,000 alleles when sequencing tumour and matched normal samples with a median coverage of >100,000× for mutations in *VHL, PTEN, TP53,* and *CDKN1B*. Like multiregion investigations, detection of subclones in a single specimen using massively deep sequencing analyses quantified clonal evolution and genetic diversity, identified loss of heterozygosity and clearly demonstrated intratumoural heterogeneity.[23]

Intratumoural heterogeneity might also indicate disease prognosis.[37] In lung adenocarcinomas, investigators found that patients who relapsed had higher global intratumoural heterogeneity—specifically, larger subclonal fractions in their primary tumours—compared with patients who did not relapse.[38] Intratumoural heterogeneity might also be responsible for drug resistance, as undetected subclones that are resistant to a given therapy could proliferate under specific selection pressures, leading to eventual relapse of disease.[39] Ongoing research in both NSCLC and CML corroborate the role of intratumoural heterogeneity in drug resistance. Two studies independently showed that the EGFR mutation Thr790Met, also known as the gatekeeper mutation T790M, which is found in >50% of patients who develop secondary resistance to gefitinib, might also contribute to primary resistance.[40,41] In treatment-naive patients with NSCLC, the investigators found subpopulations of cells containing the T790M resistance mutation and these patients had shorter progression-free survival (PFS) with TKI therapy directed against EGFR compared with patients who did not have cells with this mutation.[40,41] Similarly, in imatinib-resistant CML, two studies found minority subpopulations of drug-resistant cancer cells in treatment-naive patients.[42,43] In aggregate, intratumoural heterogeneity affects tumour biopsy strategy, determination of actionable targets, treatment planning and drug resistance.

Challenges of heterogeneity

The mounting evidence of intratumoural heterogeneity in ccRCC highlights two challenges that both clinical oncologists and researchers face. Firstly, if intratumoural heterogeneity is pervasive, obtaining a comprehensive genomic profile of any one tumour might be impossible without sampling multiple tumour regions; however, the number of regions that must be sampled to obtain an adequate characterization of a patient's tumour is unclear. Secondly, given the extensive intratumoural heterogeneity, appropriately fulfilling the prerequisites of precision medicine—prescribing targeted therapeutics, predicting response and thwarting resistance—seems unattainable. In the past year, data that address the first challenge are beginning to emerge. After sampling 3–5 spatially separate regions of a primary renal mass and conducting targeted exome sequencing for a specific subset of ccRCC driver mutations, Sankin and colleagues[44] recommended that at least three regions should be sampled to be able to detect aberrations in *VHL, PBRM1, SETD2, BAP1* and/or *KDM5C* with 90% certainty. Although this work provides a foundation for future multiregion biopsy analyses, the selected driver mutations are among the most commonly found aberrations in ccRCC (*VHL* 52.3%, *PBRM1* 32.9%, *SETD2* 11.5%, *BAP1* 10.1% and *KDM5C* 6.7%).[29] Further investigations are critical for appropriate detection of less prevalent but therapeutically targetable driver mutations (for example, those involving *MTOR* and/or *TSC1*).

Various strategies have been proposed to achieve appropriate therapy in the context of intratumoural heterogeneity. For example, based on the tree model, one reasonable method is targeting trunk mutations.[24] In ccRCC, this approach translates into targeting the dysregulated von Hippel–Lindau disease tumour suppressor (VHL)–hypoxia-inducible factor (HIF) axis and its ultimate phenotypic outcome: VEGF-mediated angiogenesis.[45,46] VEGF inhibitors, encompassing the TKIs and the monoclonal antibody bevacizumab, have contributed to prolonged partial or complete responses in some patients.[47] Unfortunately, the beneficial effects of VEGF inhibitors are not durable and resistance inevitably emerges. In these situations, intratumoural heterogeneity and underlying genetic and epigenetic flexibility are probably responsible for continued tumorigenesis through development of drug-resistant clones and/or the annexation of alternate pathways that circumvent drug inhibition.[36,39,48]

In addition, the effectiveness of the trunk-targeting strategy can also be impaired

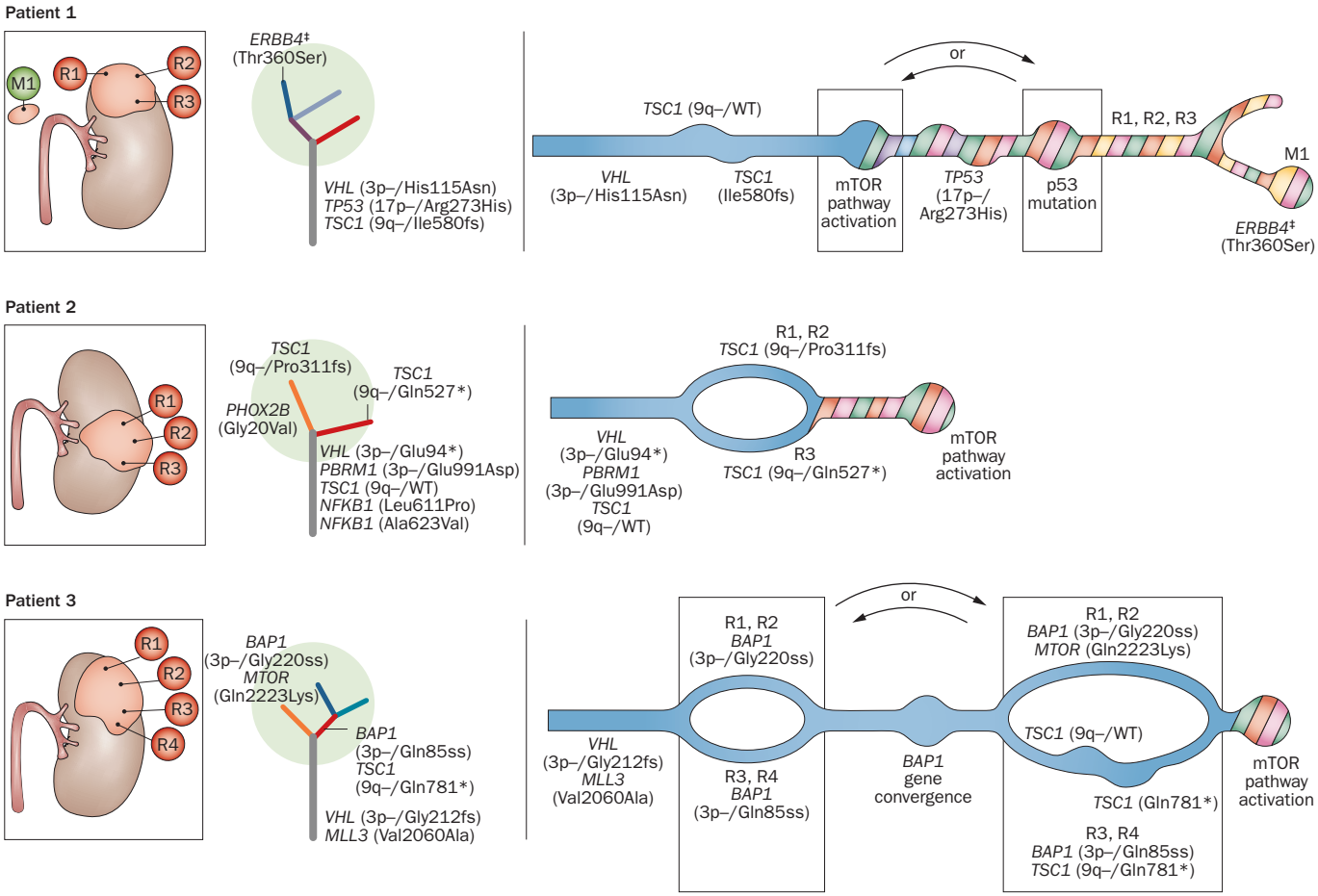

Figure 1 | Mutations demonstrating convergent evolution. Multiregion sequencing of biopsy samples from patients with metastatic renal cell carcinoma revealed specific mutations in three exceptional responders to mTORC1 inhibitors.[50] The tree models depict mutations found using the MSK–IMPACT assay and categorize them into trunk and branch based on their prevalence. This model does not differentiate between drivers and passengers; hence, mutations without a phenotypic effect are included. The passenger mutations had low allele frequencies and/or were only seen in single branches, for example the *PHOX2B* mutation in patient 2. When depicted in the river model, analyses demonstrate early convergent mutation of subclones resulting in mTOR activation, which explains the beneficial effect of treatment with mTORC1 inhibitors temsirolimus and everolimus. In patients 1 and 3, the chronology of mutation acquisition could not be definitively characterized. ‡*ERBB4* mutation, J. J. Hsieh, unpublished work. Abbreviations: fs, frame shift; M, metastasis sampled; mTORC1, mTOR complex 1; R, tumour region sampled; ss, splice site; WT, wild type.

when new driver mutations arise in the trunk or branches, owing to changing environmental conditions and selection pressures.[30] Such a development was demonstrated in patients with NSCLCs positive for *ALK* rearrangements.[49] Treatment with ALK TKIs conferred short-term disease control but, invariably, relapse occurred within 1 year owing to drug resistance.[49] Through analysis of biopsy samples obtained from 18 patients who developed secondary resistance to the ALK TKI crizotinib, the investigators determined that drug resistance arose from novel heterogeneous mutations scattered throughout the tumour branches.[49] Interestingly, the pretreatment driver trunk mutations themselves did not change during therapy, suggesting that the resistant mutations conferred an additive phenotypic effect.

Given these seemingly insurmountable obstacles to achieving effective therapy posed by intratumoural heterogeneity, the finding that some patients experience exceptional responses to the same targeted therapeutic agents to which others rapidly develop resistance deserved particular investigation. Our study on a cohort of five patients with metastatic ccRCC who exhibited prolonged response to the mTORC1 inhibitors temsirolimus or everolimus elucidates some of the factors that contribute to this uncommon phenomenon.[50]

Convergent evolution in ccRCC

In metastatic ccRCC, the median PFS of patients receiving mTORC1 inhibitors is <6 months;[11] however, some patients experience markedly longer survival periods. For example, the outlier cohort of five patients with metastatic ccRCC that we selected for further analysis had a median PFS of 28 months.[50] To determine the mechanisms underlying the durable responses of these patients, we first conducted whole exome sequencing followed by custom targeted exome sequencing of 231 cancer genes, encompassing tumour suppressors, oncogenes and therapeutic targets.[51] The MSK–IMPACT (Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets) assay was performed on samples from multiple regions of these patients' primary tumours and, when available, from metastases. In three patients, multiregion analysis revealed marked intratumoural heterogeneity; however, at the same time, results revealed phenotypic convergence upon mTOR pathway activation across regions (Figure 1).[50]

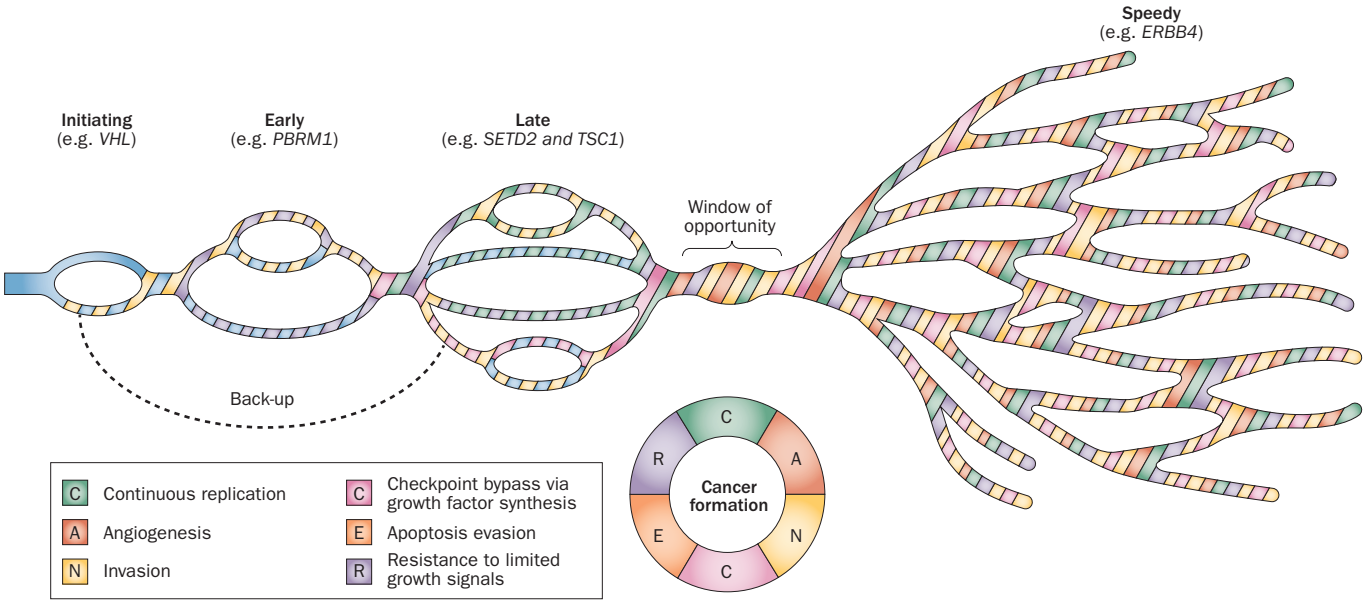

Figure 2 | The braided river model of convergent cancer evolution. The model illustrates parallel and convergent events occurring throughout tumorigenesis. Starting from initiating mutations, it depicts the stepwise accumulation of different driver mutations, the distinct time point when all cancer hallmarks have been acquired and the tipping point beyond which targeted therapies have limited effectiveness, owing to the presence of aggressive subclonal 'speedy' drivers conferring treatment resistance. Application of this paradigm enables delineation of phenotypically important mutations and pathways. With accumulating data, it might determine the most appropriate therapy, taking into account convergent events present in a patient's tumour. Expanding knowledge in cancer genomics might define a therapeutic window of opportunity, which describes a period in which the primary tumour and/or metastases predominantly rely on one targetable pathway. Targeted treatment becomes much more difficult when the rate of mutagenesis increases and the prevalence of mutations conferring resistance (speedy drivers) grows.

Patient 1 had three regions sequenced within their primary tumour and one region within a metastatic site. All regions had mutations in *VHL, TP53* and *TSC1*. The metastatic site demonstrated an additional mutation in *ERBB4* (J. J. Hsieh, unpublished work). In patient 2, three regions sampled from the primary tumour had mutations in *VHL* and *PBRM1*; two clonal populations with distinct inactivating mutations in *TSC1* were also found. Finally, in patient 3, four regions within the primary tumour contained two dominant clones with distinct mutations in *BAP1*, *TSC1* and *MTOR*. Further functional investigation demonstrated that the mutations in *TSC1* and *MTOR* resulted in mTOR pathway activation in all three patients.[50] The kinase mTOR is a coordinator of cell growth and metabolism and its activation results in reduced sensitivity to nutrient-deprived conditions.[52] The convergence upon mTOR activation evident in these exceptional responders implies a critical role for this pathway in ccRCC tumorigenesis,[53] which is also supported by the general therapeutic benefit conferred by mTOR inhibitors in patients with ccRCC.

Although the trunk–branch model can accurately depict intratumoural heterogeneity within a mass, it lacks the capability to describe the functional relevance of the innumerable heterogeneous mutations involved and to illustrate what might be clinically significant convergence events on both the genetic level and the pathway level. Hence, based on the findings of convergent evolution from Voss and colleagues,[50] cancer growth might be better visualized as a braided river, with the capacity to diverge and converge, rather than an ever-branching tree (Figure 2). The source of the river is analogous to the trunk mutations and contains the ubiquitous driver events, generally the initiating and early drivers. For example, considering patient 3, the beginning of the river includes mutation of *VHL* and heterozygous loss of chromosome 3p (Figure 1). The heterogeneous mutations previously ascribed to the branches of the tree model become tributaries along the river, retaining both the capability to become driver mutations as well as to converge with other spatially or temporally distinct mutations that affect the genes along critical oncogenic or tumour suppressor pathways of a given cancer type. In patient 3, two distinct and spatially separate mutations in *TSC1* and *MTOR* both affect the PI3K–Akt–mTOR pathway and activate mTOR kinase through different mechanisms—hence, with a view on the river model, they join together in affecting this pathway. Such analysis of the mutational data from the exceptional responder cohort shows that the braided river model enables a novel visualization of subclonal events and has the potential to summarize heterogeneous genetic and epigenetic alterations into clinically relevant information.

A window of opportunity

Often, the acquisition of metastatic potential is thought of as the ultimate cancer hallmark and once this phenotype exists the possibility of cure—or even just control of disease—becomes remote. Despite this principle, cytoreductive nephrectomy is one recommendation for patients with metastatic ccRCC who are good candidates for surgery.[18] As metastatic cells derive from subclones within the primary mass, resection of that mass ostensibly removes a source of development of new treatment-resistant subclones, of emergence of the mutator phenotype (if not already present) and of continued metastatic seeding and dispersal.[36,48,54] Despite resection, compelling evidence of continued genetic

divergence within metastatic sites exists, which has been shown in several malignancies including ccRCC, pancreatic cancer,[55] breast cancer[56] and many others. These findings explain the difficulty in treating metastatic disease. Not only do we not yet understand the genetic basis of metastasis, but further mutations within metastases ensure an increased likelihood of emergence of driver mutations (so-called back-up drivers) that circumvent dependency on the therapeutically targeted pathways and confer resistance.[57]

However, as demonstrated by the analysis of exceptional responders, some patients can derive benefit from treatment despite metastatic dissemination of disease.[50] In one of the patients, in whom we analysed a metastatic site (patient 1), we even found an instance of a distinct *ERBB4* mutation in one metastasis (J. J. Hsieh, unpublished work), suggesting continued genetic divergence after acquisition of metastatic potential (Figure 1).[50] Protein overexpression from *ERBB4* has been shown to activate the PI3K–Akt–mTOR pathway independently from mTOR-related mechanisms in patients with lung, breast or colon cancer.[58–60] In addition, in breast cancer, *ERBB4* mutations can mediate resistance to ERBB2 inhibitors.[58]

The observation of durable therapy responses in the exceptional responders might indicate that, in select patients, a window of opportunity after acquisition of metastatic potential and dissemination exists during which the immigrant cancer cells continue to rely on drivers acquired in the primary mass.[36,61] Hence, during the window of opportunity, even in the presence of additional potential driver mutations, such as *ERBB4* in metastases, the metastases still heavily depend on the drivers that led to premetastatic tumorigenesis. For example, in patient 1, the metastases were reliant on driver mutations involving *VHL* and *TSC1*.[50] Hypothetically, therapy was given during a window in which, despite mutations in *TP53* and *ERBB4*, activation of the mTOR pathway dominated the tumour phenotype and, thus, imparted sensitivity to temsirolimus. Although further analysis of metastases at a later time point was not conducted, one could speculate that metastatic subclones containing the *ERBB4* mutation proliferated, turning this mutation into a back-up driver that could impede or reduce the effects of temsirolimus and result in the eventual disease progression. Alternatively, the *ERBB4* mutation might also represent the first detectable 'speedy' driver among many new mutations present in the metastatic lesions, as the rate of mutagenesis and prevalence of treatment-resistant mutations race out of control. As more driver mutations occur and proliferate, the ability to target specific pathways becomes increasingly challenged and patient prognosis becomes increasingly dire.

Convergence in other cancers

Voss *et al.*[50] are the first investigators to describe therapeutically beneficial pathway-convergent events in ccRCC. However, a series of multiregion genomic analyses in RCC have also reported the presence of gene and pathway convergence in key RCC driver genes (*SETD2, KDM5C, PTEN, BAP1, PBRM1* and *PIK3CA*) and components of the SWI/SNF chromatin remodelling complex (*PBRM1, ARID1A* and *SMARCA4*).[25,28,62]

In 2015, Juric *et al.*[63] identified pathway convergence in response to therapeutic selection pressure in one patient with metastatic breast cancer. PCR-based genetic sequencing identified a mutation in *PIK3CA*. The patient was subsequently enrolled in a phase I clinical trial of a novel PI3Kα inhibitor (BYL719), and treatment with this inhibitor resulted in a partial response lasting 9.5 months.[64] Disease progression occurred in the lungs, prompting discontinuation of BYL719, and the patient rapidly succumbed to the disease. Tissue samples from 14 metastatic sites were collected during rapid autopsy and underwent whole genome sequencing followed by IMPACT sequencing to identify mechanisms of resistance to BYL719. Investigators found single copy loss of *PTEN* in all metastatic sites.[63] Strikingly, in 10 of the 14 metastatic sites, they detected six distinct alterations in the remaining copy of *PTEN* leading to immunohistochemistry-confirmed loss of PTEN protein, including four different exon-level deletions, a splice site mutation at Lys342 and a frameshift indel at Pro339. Thus, multiregion sequencing identified convergent evolution upon PTEN loss as a mechanism of resistance to the PI3Kα inhibitor BYL719.

In malignancies other than ccRCC and breast cancer, the relationship between genetic convergence and clinical outcome has yet to be investigated. However, convergence itself has been reported in several cancers, including chronic lymphocytic leukaemia, multiple myeloma and colorectal cancer. In a longitudinal analysis of clonal evolution in patients with chronic lymphocytic leukaemia, investigators found evidence of convergent evolution in two of the 12 study participants.[65] Mutations affecting the same gene were present in different subclones. In one patient (designated CLL11), sequencing identified two major subclones. One subclone was characterized by a nonsense mutation in *NOTCH1* and a frameshift indel in *DDX3X*. The second subclone contained an independent frameshift in *DDX3X*, as well as two distinct mutations in *NOTCH1*. A second patient (designated CLL33) had two subclones and almost complete subclonal replacement occurred during therapy until the time of relapse.[65] At baseline, the major clone contained mutations in *DDX3X* and *SF3B1*. The much smaller subclone, comprising only 0.4% of the leukaemic cells, contained independent mutations in *DDX3X* and *SF3B1* in addition to further mutations in *NOTCH1* and *TP53*. Furthermore, both subclones demonstrated different breakpoints on del(11q22). At time of relapse, the smaller subclone dominated the malignancy, comprising >99% of cancer cells.

In six patients with multiple myeloma, single-cell genotyping and copy number analysis revealed two patients who had distinct mutations in the Ras–MAPK pathway.[66] In these patients, independent acquisition of mutations in *KRAS* and *NRAS* were found in spatially separate clones with a convergent phenotype, resulting in constitutive Ras–MAPK pathway activation. The authors did not discuss whether this finding affected therapeutic decision making for these patients.

Finally, in metastatic colorectal cancer, resistance mechanisms to EGFR-targeted antibody therapy demonstrated marked heterogeneity.[67] Although the most common mutation involved *KRAS*, research has implicated numerous genes to be involved in both primary and secondary resistance, for example *NRAS, ERBB2, BRAF, MET, PIK3CA* and *PTEN*.[67] Regardless of the gene or mutation involved, the convergent phenotypic result was constitutive activation of mitogen-activated protein kinase kinases (MEK) and mitogen-activated protein kinases (ERK), which identifies another area of research and a pharmacological target for therapy in patients with tumours resistant to EGFR-targeted agents.[67]

Conclusions

The convergence phenomenon documented in an increasing number of malignancies lends further credibility to the evolutionary basis of cancer development: external selection pressures dictate emergence of the most adaptive phenotypes and, in turn, these phenotypes configure the genetic mutations that accumulate within proliferating cancer cells.[48,68] From this evolutionary perspective, the pervasiveness of intratumoural heterogeneity—and, similarly, the lack of clinically significant biomarkers—is not surprising.[69] Nevertheless, through continued multiregion analyses and further longitudinal sequencing investigations in exceptional therapy responders, the pathways on which tumorigenesis is dependent might yet be elucidated.

Exceptional responders present a unique opportunity to understand tumour biology, and determining the basis of their distinct therapeutic response is developing into a new branch of cancer research. Such analyses, however, have their limitations, despite the useful biomarker data they provide. Often, exceptional responders represent a very small subset of patients and, as such, conclusions from analyses of their tumours might not be generalizable to the larger population of patients with cancer.

Furthermore, although pathway convergence dictated therapy responses in patients with ccRCC or breast cancer, establishing the clinical significance of convergent events requires validation in a large cohort of patients and in other malignancies. Thus, the braided river model—based on observation of pathway-convergent events—is limited by the cohort of patients studied. Moreover, the model cannot, at this time, provide temporal information regarding sequential mutations. Still, the paradigm offers many advantages in comparison with the trunk–branch model. As many studies are beginning to show, convergence of mutations on the gene and pathway level are not uncommon events. The current trunk–branch model, although useful in describing the abundance of subclonal populations, cannot represent these convergent events, which occur between, and sometimes within, subclones. These convergent events, as the exceptional responders demonstrate, might have critical roles in clinical decision making and response to targeted therapy. Through analysing convergent events and recognizing the functional interconnections between tributaries of the river, the ability to bridge the gap from phenotype to genotype and vice versa can be achieved.

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Author contributions

Both authors researched data for the article, substantially contributed to discussion of the content, wrote and reviewed and/or edited the article before submission.