Tumor heterogeneity: next-generation sequencing enhances the view from the pathologist’s microscope

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The term heterogeneity covers many aspects of the variability in tumor phenotypes, which are a characteristic of human malignancies. Morphologists of the late 19th century first described the multiple cell types composing tumors and began to recognize cancers of different types. Over the past half century the molecular underpinnings of the variability in human cancers has been gradually revealed but within the last 5 years there has been an explosion in our ability to determine and learn from cancer heterogeneity, through the use of next-generation sequencing and related methods. The complexity and variation in the structure of cancers can seem daunting, but important lessons in cancer biology and the approaches to therapy can be learned from studying how much of the complexity is subject to change and how much is a consequence of stochastic rather than deterministic processes. The evolution of clones, individual variation in response to therapy, distinct biological subtypes of cancer and tumor immune responses are all examples of the heterogeneous nature of human cancers. Self evidently, when heterogeneity is used to describe any aspect of a cancer, it is important to know which variational feature is being addressed.

Experimental approaches to heterogeneity

A review by Hiley et al. [1] sets the stage for the research studies of heterogeneity published in this special issue, by updating the reader regarding how the use of next-generation sequencing and clever experimental design have increased our understanding of genomic and regional heterogeneity in cancers. The special collection provides several research-based studies of tumor heterogeneity, encompassing the variation between individuals (the tumor subtype) in breast cancers (Ali et al. [2]) and, by contrast, the lessons that can be learned from longitudinal study of single patient (‘N of 1’) cases (Fisher et al. [3], Nadauld et al. [4]). These studies provide contrasts between the approaches needed to determine disease groupings in populations, where many hundreds or thousands of patients must be studied, with the approaches to pursuing the moving target of individual cancers. The latter can be effectively studied in smaller numbers with informative consequences when evolution is used to sift the features undergoing selection and fixation. An Opinion piece by Good et al. [5] provides a scientific and philosophical perspective on the N of 1 paradigm.

Methodological approaches to heterogeneity

Of equal importance to the experimental approaches used to study heterogeneity are the methods used to evaluate heterogeneity across the spectrum of variation that can be measured in cancer by different assay types. These encompass next-generation sequencing methods for analyzing tumor and normal cell composition, the analysis of clonal populations in cancers (Qiao et al. [6]), and methods addressing epigenetic plasticity (Zheng et al. [7]). Hence, several important tools and approaches are presented that facilitate answering critical questions about heterogeneity, and an opinion piece contributed by Russnes et al. [8] advocates for data integration to better interpret heterogeneity data.

Single cell approaches to heterogeneity

Resolving structure and function with single cell approaches is also becoming important, both in studies of clonality as well as for functional assessment of tumor cell populations. Learning how to reconcile whole tumor cell-based population approaches with the single cell data will be important. Nicholas Navin’s review [9] of single cell sequencing in cancer studies provides a survey of this rapidly developing area.
RNA-based studies of heterogeneity
Next-generation sequencing-based studies of RNA populations from cancer cells are revealing important aspects of transcriptional activity and its role in cancer. A review by Patrick Nana-Sinkam and Carlo Croce [10] sets the stage by discussing the role of microRNAs in gene regulation in cancers. White et al. [11] present important new descriptions of long non-coding RNAs (lncRNAs) in lung cancers, and Wyatt et al. [12] describe transcriptomes in the context of therapy response in high-risk prostate cancers. The method for detecting allele-specific expression contributed by Mayba et al. [13] will also yield important insights into which variants detected by DNA sequencing are actually being expressed in the transcriptome of cancer cells. The role of the epigenome in contributing to the patterning of transcriptomes and the possibility of modulating RNA expression is emphasized in three primary research articles exploring this aspect of tumor heterogeneity (Lund et al. [14], Fleischer et al. [15], and Charlton et al. [16]).

Clinical aspects of heterogeneity
As our underlying knowledge about cancer genomics and heterogeneity improves, the need to translate this information into informed cancer care for patients is an obvious next step. Berger and Varghese have contributed an Opinion piece [17] to describe the translation of cancer genomics in the clinic, and contributions from de Bono [18] and Bardelli [19] outline the use of circulating tumour cells (CTCs) and circulating free DNA (cfDNA), respectively, as approaches to monitoring tumor progression. These blood-based or 'liquid biopsy' approaches present an exciting new paradigm in contrast to conventional and less sensitive imaging-based approaches to monitor patients. Deininger also reviews [20] an important area to clinical therapeutics that is often identified by genomic information, providing an overview of therapy response and resistance to targeted therapies. The genomic variability between patients is highlighted in the research article of Ali et al. [2], delineating molecular subtypes in large cohorts of breast cancer patients.

Data and more data
Finally, in the genomic era, sharing of data and complete descriptions of analytic methods, to the level of providing code in addition to data, will prove crucial to continued success. Boutros et al. [21] provide a novel look at crowdsourcing algorithms for cancer analysis, and Bartha Knoppers and colleagues [22] present a critically important Opinion regarding the legal framework for genomic data sharing. Hence, the special collection provides a wide-ranging overview of cancer heterogeneity in its many manifestations, from fundamental methods-based approaches to study heterogeneity to the use of genomic information for response and progression monitoring. We think the breadth of cancer studies that have been impacted by next generation sequencing, including improved understanding and characterization of cancer heterogeneity, is setting the stage for major breakthroughs in our biological understanding of this vexing and complicated disease.

Competing interests
The authors declare that they have no competing interests.

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References
1. Hiley C, de Bruin EC, McGranahan N, Swanton C: Deciphering intratumor heterogeneity and temporal acquisition of driver events to refine precision medicine. Genome Biol 2014, 15:453.
2. Ali HR, Rueda OM, Chin SF, Curtis C, Dunning MJ, Aparicio SAJR, Caldas C: Genome-driven integrated classification of breast cancer validated in over 7,500 samples. Genome Biol 2014, 15:431.
3. Fisher R, Horswell S, Rowan A, Salm M, De Bruin E, Gulati S, McGranahan N, Stares M, Geilingler M, Varela I, Crockford A, Favero F, Quidvive I, Andre F, Navas C, Gronopoulos E, Nicol D, Hazi S, Hrouda D, O'Brien T, Matthews N, Phillimore B, Begum S, Rabinowitz A, Biggs J, Bates PA, McDonald NQ, Stamp G, Spencer-Dene B, Hsiek JJ, et al.: Development of synchronous VHL syndrome tumors reveals contingencies and constraints to tumor evolution. Genome Biol 2014, 15:433.
4. Nadauld LD, Garcia S, Natsoulis G, Bell JM, Aparicio SAJR, Mardis ER: SubcloneSeeker: a computational framework for reconstructing tumor clone structure for cancer variant interpretation and prioritization. Genome Biol 2014, 15:443.
5. Zheng X, Zhao Q, Wu H, Li W, Wang H, Meyer CA, Qin QA, Xu H, Zang C, Jiang P, Li F, Hou Y, He J, Wang J, Wang J, Zhang P, Zhang Y, Liu XS: MethyLPurify: tumor purity deconvolution and differential methylation detection from single tumor DNA methylomes. Genome Biol 2014, 15:419.
6. Russnes HG, Lanning PE, Barresen-Dale AL, Lindaes OR: The multitude of molecular analyses in cancer: the opening of Pandora's box. Genome Biol 2014, 15:447.
7. Aparicio and Mardis Genome Biology 2014, 15:463
8. http://genomebiology.com/2014/15/9/463
10. Nana-Sinkam SP, Croce CM: MicroRNA regulation of tumorigenesis, cancer progression and interpatient heterogeneity: towards clinical use. Genome Biol 2014, 15:445.

11. White NM, Cabanski CR, Fisher-Silva JM, Dang HK, Govindan R, Maher CA: Transcriptome sequencing reveals altered long intergenic non-coding RNAs in lung cancer. Genome Biol 2014, 15:429.

12. Wyatt AW, Mo F, Wang K, McConneyey B, Brahmbhatt S, Jong L, Mitchell DM, Johnston RL, Haegert A, Li E, Liew J, Yeung J, Shrestha R, Lapuk A, McPherson A, Shukin R, Bell RH, Anderson S, Bishop J, Hurtado-Coll A, Xiao H, Chinnaiyan AM, Mehra R, Lin D, Wang Y, Fazli L, Gleave ME, Volik SV, Collins CC: Heterogeneity in the inter-tumor transcriptome of high risk prostate cancer. Genome Biol 2014, 15:426.

13. Mayba O, Gilbert HN, Liu J, Haverty PM, Jhunjhunwala S, Jiang Z, Watanabe C, Zhang Z: MBASED: allele-specific expression detection in cancer tissues and cell lines. Genome Biol 2014, 15:405.

14. Lund K, Cole J, VanderKraats ND, McConneyey T, Pchelintsev NA, Clark W, Copland M, Edwards JR, Adams PD: DNMT inhibitors reverse a specific signature of aberrant promoter DNA methylation and associated gene silencing in AML. Genome Biol 2014, 15:406.

15. Fleischer T, Frigessi A, Johnson KC, Edvardsen H, Touleimat N, Klajic J, Riis MLH, Haakensen V, Wännberg F, Naume B, Helland Å, Barresen-Dale AL, Tost J, Christensen BC, Kristensen VN: Genome-wide DNA methylation profiles in progression to in situ and invasive carcinoma of the breast with impact on gene transcription and prognosis. Genome Biol 2014, 15:435.

16. Charlton J, Williams RD, Weeks M, Sebire NJ, Popov S, Vujanic G, Mifsud W, Alcaide-German M, Butcher LM, Beck S, Pritchard-Jones K: Methylome analysis identifies a Wilms tumor epigenetic biomarker detectable in blood. Genome Biol 2014, 15:434.

17. Varghese AM, Berger MF: Advancing clinical oncology through genome biology and technology. Genome Biol 2014, 15:427.

18. Mateo J, Gerlinger M, Rodrigues D, de Bono JS: The promise of circulating tumor cell analysis in cancer management. Genome Biol 2014, 15:448.

19. Siravegna G, Bardelli A: Genotyping cell-free tumor DNA in the blood to detect residual disease and drug resistance. Genome Biol 2014, 15:449.

20. Eiring AM, Deininger MW: Individualizing kinase-targeted cancer therapy: the paradigm of chronic myeloid leukemia. Genome Biol 2014, 15:461.

21. Boutros PC, Margolin AA, Stuart JM, Califano A, Stolovitzky G: Toward better benchmarking: challenge-based methods assessment in cancer genomics. Genome Biol 2014, 15:462.

22. Kosset P, Dove ES, Baggaley C, Westin EM, Cate FH, Kaye J, Harris JR, Knoppers BM: Building a data sharing model for global genomic research. Genome Biol 2014, 15:430.