Science in Focus: Genomic instability and its implications for clinical cancer care

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What is genomic instability and why is it so common in cancer?

Genomic instability describes a state in which there is an increased tendency to acquire hereditable genetic alterations that may influence phenotype. It characteristically occurs as a consequence of deficient genome maintenance processes, such as DNA repair or cell-cycle checkpoints [1]. The genetic alterations that arise are often typified, both in terms of the mechanism of their formation and their functional consequences, by their size. At the smallest scale, the bases at individual nucleotides may be substituted. At the largest scale, entire chromosomes may be gained and lost. Cancers cells often harbour several types of genomic alteration that span the wide range of these limits [2] (Table 1).

Table 1 – Genetic alterations come in different shapes and sizes

Genetic Alteration	Description	Example(s) of causative genomic instability
Single Nucleotide Variant (SNV)	The substitution of the base at an individual nucleotide.	POLE proofreading mutation [3]
Insertion/Deletion (Indel)	The gain or loss, respectively, of one or a few nucleotides.	BRCA1/BRCA2 deficiency [4]
Copy Number Variant (CNV)	The gain or loss of copies of a segment of DNA, such as a gene.	Aurora A amplification [5] Transposon activity [6]
Translocation	The rearrangement of non-homologous chromosomes.	BRCA1/BRCA2 deficiency [4] AID/APOBEC activity [7]
Aneuploidy	The gain or loss of entire chromosomes	p53 deficiency [8]

The 2011 update detailing the Hallmarks of Cancer identified genomic instability as an enabling characteristic of cancer [9]. In principle, the acquisition of genomic instability facilitates carcinogenesis by enabling a cell and its descendants to alter their genomes. As each cell in this lineage acquires new genetic alterations, a group of cells that are genetically heterogeneous is formed. This sets the stage for the selection of cells that have acquired a growth advantage. Mutations emerge that enable cells to break free of homeostatic limits such as those involving proliferation, invasion, evading cell death *et cetera*, leading to classical cancerous behaviour.

Defining genomic instability as an enabling characteristic predicates that it must also occur early during tumourigenesis. In keeping with this notion, it is notable that disruption of p53, a protein notorious for its capacity to restrict several types of genomic instability, is the

most common founder event in cancer [8, 10]. It is also of note that a large proportion of cancer predisposition syndromes are attributable to defects in genes involved genome maintenance, and that carcinogenesis occurs precociously in affected individuals (reviewed in [11]). These findings are consistent with next-generation sequencing (NGS) data which consistently identify patterns of genomic instability at early stages of cancer development [12, 13].

These NGS approaches allow us to distinguish between ongoing genomic instability, and the 'scars' of past episodes of genomic instability that may be detected by sequencing. Thus, technically, genomic instability defines the rate of mutation whereas the readout from NGS generally provides a static snapshot of its impact. However, comparative sequencing of serially acquired or spatially distinct samples can provide a better reflection of mutational rates. In sum, these studies have confirmed that practically all cancers carry several mutations in their genome, although the burden of mutations varies across tumour types. In most solid tumours, on average 33 to 66 genes are significantly mutated, whilst in lung cancer and melanoma this number is nearer 200 [14].

The causes and consequences of genomic instability are diverse

Although the rationale for genomic instability can be explained relatively simply, its causes and consequences are far more nuanced. As described earlier – mutational burden, and by inference genomic instability, varies across different tumour types. In addition, beyond number of mutations, genomic instability can also differ based on the type of mutations present. Indeed, various mutational signatures have been deconvoluted from mixed patterns of SNVs in cancer genomes. In 2013, a seminal paper identified 21 distinct mutational signatures on the basis of 4,938,362 mutations from 7,042 cancers [15]. Certain signatures were associated with known mechanisms of mutagenesis. For instance, Signature 7 was frequently seen in malignant melanoma and was attributed to DNA damage from ultraviolet light. A smoking-related signature was also reported in lung cancer.

Further signatures were connected to with DNA repair defects. Within the cell, damaged DNA is repaired through multiple processes and the choice of repair pathway is characteristically associated with the type of DNA lesion. For instance, mismatch repair (MMR) acts on base-base mismatches and insertion/deletion mispairs, whereas homologous recombination (HR) repairs breaks in double-strand DNA. Defects in MMR (e.g. through MLH1 inactivation) and HR (e.g. through BRCA1 and BRCA2 inactivation) are classically associated with colorectal cancers or breast and ovarian cancers, respectively. However the reasons behind these tissue specificities have proven far harder to decipher. They may partly be explained by the fact that genes are expressed differentially across tissue types and that each site has a unique microenvironment and exposure to exogenous agents. For instance BRCA1 has been shown to mediate early differentiation of breast tissue and has also been implicated in the repair of double-stranded DNA breaks from estrogen and estrogen metabolites [4, 16, 17].

Together, these findings only reveal a limited view of the true sequence of events but reiterate that multiple mechanisms are likely to be involved. In fact, it has also become apparent that genomic instability does not continue at a steady and unrelenting rate

throughout tumour evolution [13]. This is important because such complexity amplifies manifold the challenge of translating our scientific understanding into improvements to clinical care. Indeed, as yet there are no reliable clinical biomarkers for genomic instability and this is in large-part due to its temporal dynamics (reviewed in [18]). This is because, as already discussed, current techniques used to measure features associated with genomic instability, e.g. aneuploidy, provide static snapshots which do not robustly correlate with the true nature of instability present.

How does the science of genomic instability translate into better patient outcomes?

Cancer prevention and early diagnosis

Identifying individuals who are predisposed to cancer due to inherited changes in genome maintenance processes is of significant clinical value. This is because these patients can then be targeted for cancer prevention strategies and/or screened in order to diagnose cancers at an early and potentially curable stage. In this cohort of patients one could hypothesise that relatively simple interventions such as smoking cessation could have profound effects on cancer risk reduction. If we were better able to understand the mechanisms behind cancer predisposition in these men and women more specific interventions could also be developed.

These principles are already being applied in the management of individuals carrying germline mutations in the *BRCA1* or *BRCA2* gene. Cancer predisposition in these cases is associated with increased genomic instability (reviewed in [4]). These individuals may benefit from cancer detection and prevention strategies. Women with *BRCA1* and *BRCA2* mutations are currently offered the choice between more intensive screening or bilateral mastectomy and oophorectomy. Recent clinical evidence also supports the use of tamoxifen in these cases as a cancer prevention strategy. A pooled observational cohort study of almost 1600 BRCA mutation carriers showed that tamoxifen use following a diagnosis of breast cancer reduced the risk of cancer by around a half in the contralateral breast [19].

Cancer treatment

One can argue that the genomic instability of cancer has long been a target of cancer therapy, based on the relative sensitivity of cancer cells to cytotoxic drugs and radiotherapy when compared to normal tissue. This notion is exemplified by the effectiveness of PARP inhibitors in BRCA deficient cells. This, alongside other advances in using DNA repair inhibitors for cancer treatment is reviewed elsewhere [20, 21]. Our review focuses on the implications of genomic instability as a whole on anticancer therapy.

How may we appraise the impact that genomic instability has upon cancer therapy? A study aimed at addressing this question used sequencing data from a broad range of tumour types and associated CNVs with clinical outcome. The results showed that tumours with either the lowest or highest rate of CNVs carried the most favourable outcomes [22]. This suggests that either too little or too much genomic instability can be detrimental to a cancer's survival. There are a number of possible explanations for these intriguing findings. Cancers require a minimum level of instability so that they are more easily able to evolve resistance

mechanisms to the therapeutic agent. Whilst in those cancers with high levels of instability (>75% CNVs), genome maintenance is impaired to the extent that the chances of producing viable daughter cells is significantly lowered. Moreover, these cells may be exquisitely sensitive to DNA damaging agents due to massively impaired DNA repair, and may also be more susceptible to destruction by the immune system (discussed later). These results suggest that we can stratify patients based on their profiles of genomic instability. However, as already discussed, an accurate biomarker of genomic instability remains to be discovered.

In 2017, the TracerX study published preliminary data from 100 lung cancer patients who had multiple spatially distinct surgical samples sent for sequencing [23]. The investigators were able to derive a measure of chromosomal instability by measuring "mirrored subclonal allelic imbalance". In simple terms this relies on comparing evolving genomic changes within the tumour specimens on the basis of subclonal changes in maternal and paternal alleles. The data confirmed that increased levels of chromosomal instability correlated with intratumoral heterogeneity as well as increased risk of recurrence or death. These results suggest that genomic instability can therefore inform prognosis, and patients at higher risk may benefit from more regular follow-up. Measuring circulating tumour DNA in these individuals may be particularly advantageous as it may allow diagnosis of relapse or residual disease prior to clinically evident metastases [24]. The extent to which these patients would benefit from more intensive adjuvant therapy is open to debate. However, based on the study described above one could argue that a proportion of these patients will be relatively sensitive to anticancer therapy.

Beyond informing clinicians as to which patients require treatment and their likely prognosis, our understanding of genomic instability may also guide the choice of therapeutic agent. Poly (ADP-ribose) polymerase 1 (PARP1) inhibitors have an established role in 1-5% of women with breast cancer who have inherited mutations in BRCA1 or 2. However a recent study identified 6 distinct mutational signatures predictive of BRCA deficiency which potentially increases the proportion of patients who may benefit from PARP1 inhibition to 22% [25]. These findings remain to be validated in clinical cohorts but form the basis for tangible and exciting translational clinical trials.

A greater understanding of the interaction of genomic instability and the immune response has also led to important clinical observations. Cancer cells deficient in mismatch repair acquire a large number of somatic mutations. This can lead to an increase in "non-self" immunogenic antigens which renders these cells more sensitive to immune-mediated therapies. Accordingly, in a phase 2 study of patients with MMR deficient colorectal (and non-colorectal) tumours had significantly better clinical outcomes after treatment with an anti–programmed death 1 (PD-1) immune checkpoint inhibitor, pembrolizumab [26]. There may, however, be a more complex relationship between mutational load and response to immunotherapy. For example, a recent study highlighted that mutations that occur early in evolution, and are therefore present in a greater proportion of subclones, improve immune recognition, as opposed to mutations that occur late and increase tumour diversity [27]. Moreover, aneuploidy has been associated with markers of immune evasion and poor clinical responses to immunotherapy [28].

A number of other treatment strategies leveraging the effects of genomic instability are also actively being pursued. For instance, aneuploid cells have been shown to be more sensitive to agents that increase metabolic stress [29]. Therapies that may reduce tolerance of genomic instability, such as reactivation of wildtype p53 function, have also been investigated but as yet remain clinically unproven (reviewed in [30]).

But what are the costs of targeting genomic instability in terms of toxicity? The most important consideration in this regard relates to the risk of secondary malignancy. One might hypothesise that interfering with genomic stability in normal cells might greatly increase second cancer risk particularly if DNA repair drugs are used in combination with DNA damaging agents. Most of our current data is in patients receiving the PARP inhibitor olaparib. There have been initial concerns that the drug might lead to myelodysplastic syndrome and therapy-related acute myeloid leukaemia (MDS/t-AML). However, the majority of these patients had been heavily pre-treated with platinum agents and were therefore already at significant risk of a second cancer. A proportion of olaparib-based studies have follow-up data beyond 5 years and pooled data from all studies suggest a cumulative incidence for MDS/t-AML of 0.5% [31]. Presently no causative association with the drug has been established. The data on newer anti-DDR drugs has yet to mature and importantly many cases of second cancers associated with cytotoxic drugs and radiotherapy did not emerge until decades after treatment. This is therefore an area that requires close monitoring.

To conclude, genomic instability is a key enabler of tumour evolution. In recent years it has become clear that its presence contributes to the complexity and heterogeneity of human cancer. Moreover, early strategies to leverage genomic instability in order to improve clinical outcomes have proved successful. It is likely that as our understanding of the phenomenon increases, our opportunities to exploit it will also increase.

References

[1] Lee JK, Choi YL, Kwon M, Park PJ. Mechanisms and Consequences of Cancer Genome Instability: Lessons from Genome Sequencing Studies. Annual review of pathology. 2016;11:283-312.
[2] Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. Nature genetics. 2013;45:1127-33.
[3] Roberts SA, Gordenin DA. Hypermutation in human cancer genomes: footprints and mechanisms. Nature reviews Cancer. 2014;14:786-800.
[4] Venkitaraman AR. Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. Science. 2014;343:1470-5.
[5] Anand S, Penrhyn-Lowe S, Venkitaraman AR. AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. Cancer cell. 2003;3:51-62.
[6] Belancio VP. LINE-1 activity as molecular basis for genomic instability associated with light exposure at night. Mobile genetic elements. 2015;5:1-5.

[7] Casellas R, Basu U, Yewdell WT, Chaudhuri J, Robbiani DF, Di Noia JM. Mutations, kataegis and translocations in B cells: understanding AID promiscuous activity. Nature reviews Immunology. 2016;16:164-76.
[8] Kastenhuber ER, Lowe SW. Putting p53 in Context. Cell. 2017;170:1062-78.
[9] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-74.
[10] Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502:333-9.
[11] Rahman N. Realizing the promise of cancer predisposition genes. Nature. 2014;505:302-8.
[12] Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature. 2010;467:1109-13.
[13] de Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, Yates L, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science. 2014;346:251-6.
[14] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr., Kinzler KW. Cancer genome landscapes. Science. 2013;339:1546-58.
[15] Alexandrov LB, Nik-Zainal S, Wedge DC, Campbell PJ, Stratton MR. Deciphering signatures of mutational processes operative in human cancer. Cell reports. 2013;3:246-59.
[16] Liu S, Ginestier C, Charafe-Jauffret E, Foco H, Kleer CG, Merajver SD, et al. BRCA1 regulates human mammary stem/progenitor cell fate. Proceedings of the National Academy of Sciences of the United States of America. 2008;105:1680-5.
[17] Savage KI, Matchett KB, Barros EM, Cooper KM, Irwin GW, Gorski JJ, et al. BRCA1 deficiency exacerbates estrogen-induced DNA damage and genomic instability. Cancer research. 2014;74:2773-84.
[18] Sansregret L, Vanhaesebroeck B, Swanton C. Determinants and clinical implications of chromosomal instability in cancer. Nature reviews Clinical oncology. 2018;15:139-50.
[19] Phillips KA, Milne RL, Rookus MA, Daly MB, Antoniou AC, Peock S, et al. Tamoxifen and risk of contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31:3091-9.
[20] Chalmers AJ. Science in Focus: Combining Radiotherapy with Inhibitors of the DNA Damage Response. Clinical oncology. 2016;28:279-82.
[21] O'Connor MJ. Targeting the DNA Damage Response in Cancer. Molecular cell. 2015;60:547-60.
[22] Andor N, Graham TA, Jansen M, Xia LC, Aktipis CA, Petritsch C, et al. Pan-cancer analysis of the extent and consequences of intratumor heterogeneity. Nature medicine. 2016;22:105-13.
[23] Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. The New England journal of medicine. 2017.
[24] O'Leary B, Turner NC. Science in Focus: Circulating Tumour DNA as a Liquid Biopsy. Clinical oncology. 2016;28:735-8.
[25] Davies H, Glodzik D, Morganella S, Yates LR, Staaf J, Zou X, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nature medicine. 2017;23:517-25.

[26] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England journal of medicine. 2015;372:2509-20.
[27] McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351:1463-9.
[28] Davoli T, Uno H, Wooten EC, Elledge SJ. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science. 2017;355.
[29] Tang YC, Williams BR, Siegel JJ, Amon A. Identification of aneuploidy-selective antiproliferation compounds. Cell. 2011;144:499-512.
[30] Bykov VJN, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. Nature reviews Cancer. 2018;18:89-102.
[31] Matulonis UA, Penson RT, Domchek SM, Kaufman B, Shapira-Frommer R, Audeh MW, et al. Olaparib monotherapy in patients with advanced relapsed ovarian cancer and a germline BRCA1/2 mutation: a multistudy analysis of response rates and safety. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2016;27:1013-9.

Infographic

Figure 1: Genomic instability and its implications for clinical cancer care