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Single cell analysis of cancer genomes

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Genomic studies have provided key insights into how cancers develop, evolve, metastasize and respond to treatment. Cancers result from an interplay between mutation, selection and clonal expansions. In solid tumours, this Darwinian competition between subclones is also influenced by topological factors. Recent advances have made it possible to study cancers at the single cell level. These methods represent important tools to dissect cancer evolution and provide the potential to considerably change both cancer research and clinical practice. Here we discuss state-of-the-art methods for the isolation of a single cell, whole-genome and whole-transcriptome amplification of the cell's nucleic acids, as well as microarray and massively parallel sequencing analysis of such amplification products. We discuss the strengths and the limitations of the techniques, and explore single-cell methodologies for future cancer research, as well as diagnosis and treatment of the disease.

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Introduction

Cancer is a disease caused by changes to the DNA, whereby the cancer genome is shaped by the interplay of processes of DNA damage and repair, cellular selection and clonal expansions [1,2]. Tumour evolution is classically thought of as a series of clonal expansions that are each triggered by new driver mutations conferring a selective advantage [3,4], hence 'new' cells undergo Darwinian evolution, very much like how species develop [5,6]. Over the past decades, we have learnt much about how cancers develop from studying their genomes, most notably through the introduction of massively parallel sequencing. Comparison of cancer samples from different sites or different time points is increasingly painting a picture of cancers undergoing branching evolution, resulting in competition between different subclones [7–13]. In solid tumours, this picture is further complicated by a topological component [8,14], with potentially different selection forces operating at different locations of the tumour.

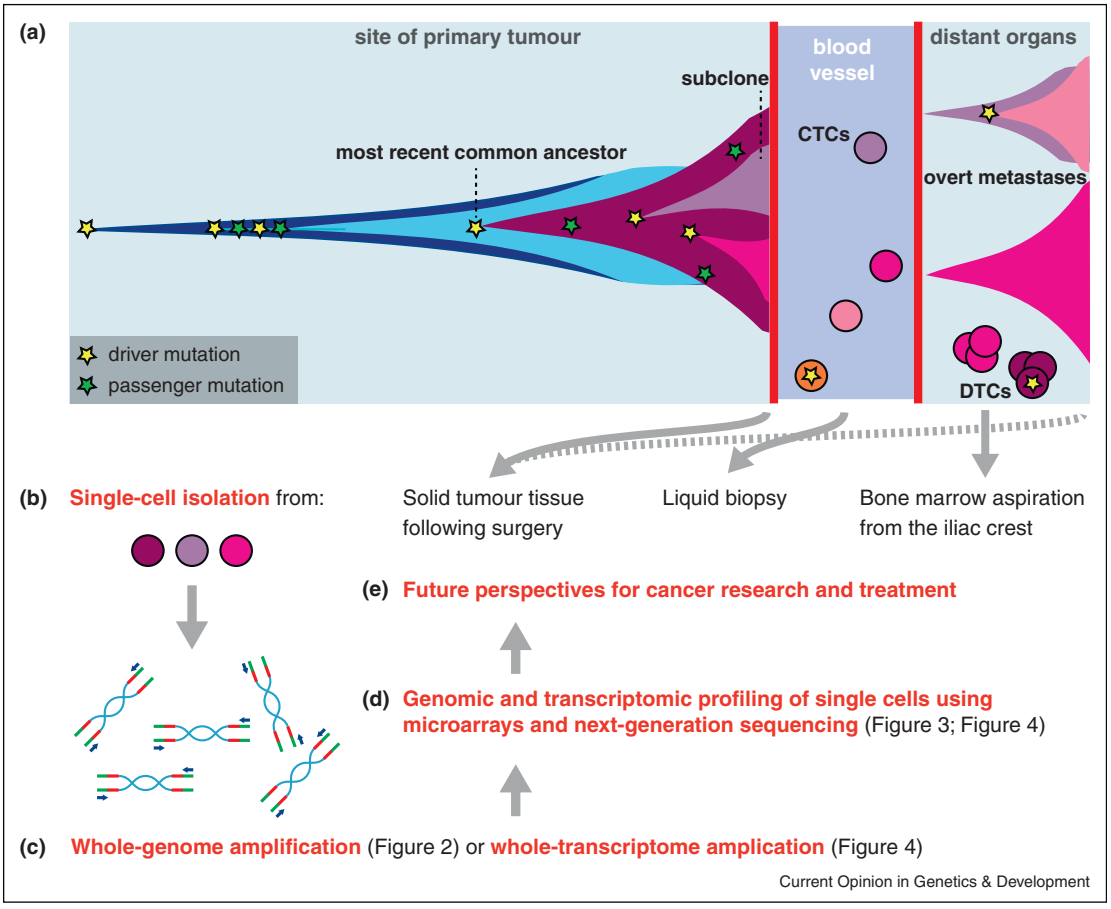
Most current large-scale cancer genome sequencing studies use DNA extracted from millions of cells. The resulting genome sequences therefore contain intermixed sequences from different tumour clones, as well as from admixed normal cells. Computational methods can determine which mutations are clonal (present in all tumour cells) and which are subclonal [15]. In addition, by analyzing point mutation and copy number data further with bioinformatics algorithms, phylogenetic trees of different tumour subclones can be inferred [12]. Although these methods provide important information on the genomes of distinct cell populations within the tumour, the number of tumour cell populations they can disentangle is limited, and inferring rare subclonal populations remains difficult.

Recent advances have made it possible to profile the genomes of single cells. The isolation of single cancer cells, followed by amplification of the DNA and array profiling or next-generation sequencing (Figure 1), opens avenues to study tumour subclonal architecture and tumour evolution in unprecedented depth. Here, we provide an overview of current methods to profile genomes of single cells. We discuss their strengths and limitations and the perspectives they offer for cancer research and therapy monitoring.

Isolation of individual cancer cells

To isolate single cells from solid tumours, two main approaches have been developed. The first method exploits the precision of modern flow cytometry to sort nuclei from single cells [16,17••]. Tissue-cubes of ~1 mm^3, cut off a (frozen) solid tumour, are teased apart in cell lysis buffer, containing DAPI, a fluorescent DNA-intercalator, and the resulting single nuclei are flow-sorted based on DNA content. This technique provides the advantage of allowing identification and isolation of tumour subpopulations on the basis of ploidy [16,17••]. Although the cytoplasm is lost, extensions to analyses of

Figure 1



Single-cell analysis of the cancer genome. **(a)** Cancers arise due to the acquisition of driver mutations resulting in successive clonal expansions of nascent tumour cells. Driver mutations that occur after the emergence of a most recent common ancestor will give rise to tumour subclones. Solid tumours also shed cells in a patient's blood stream (circulating tumour cells or CTCs) and cells disseminating to distant organs (disseminated tumour cells or DTCs), which may cause overt metastases. **(b)** To study tumour evolution and intra-tumour genetic heterogeneity, individual tumour cells can be isolated using a variety of techniques. Furthermore, CTCs can be isolated from peripheral blood, and DTCs from the bone marrow, a frequent homing-niche of DTCs. **(c)** Following isolation, single cells are lysed and their DNA or RNA is amplified using whole-genome amplification (WGA) or whole-transcriptome amplification (WTA) techniques, respectively. **(d)** The WGA and WTA products can be profiled using microarray or massively parallel sequencing platforms, **(e)** providing important perspectives for future cancer research and cancer treatment.

the transcriptome *per se* are possible [18]. However, this approach also entails limitations. In particular, micronuclei may be lost. Micronuclei are not merely by-products from genomic instability but are likely prone to DNA-replication stress and further DNA-mutational processes [19] and therefore may be important players in tumour evolution.

A second method disperses the tissue from fresh solid tumour biopsies in a single-cell suspension, using enzymatic treatments, including, for example, collagenases [20•]. Intact individual cells can subsequently be isolated using (mouth-controlled) pipetting, modern cell-sorting or microfluidics systems with or without applying immunocytochemistry. Microfluidics devices provide the advantage that in addition to capturing individual cells, they also provide nanoliter reaction chambers to further process the nucleic acids of multiple individual cells in parallel under highly standardized conditions at significantly reduced reagent costs. Both of the above technologies have the disadvantage that precise topological information about the cell is lost, which can be overcome by using laser capture microdissection from fixed tissues or cryosections. However, capturing a full cell or nucleus can be problematic [21].

Solid tumours also shed cells in a patient's blood stream (circulating tumour cells or CTCs) and cells disseminating to distant organs (disseminated tumour cells or DTCs) (Figure 1). DTCs can remain dormant over a prolonged period of time following resection of the primary tumour, before giving rise to overt metastases [22]. Investigating CTCs and DTCs is important not only for understanding tumour evolution and progression, but also as liquid