from each gene in a cell are magnified during replication. The authors exclude this noise using a previously reported technique[10] to add a unique molecular 'barcode' to each individual transcript before amplification. This enables the RaceID algorithm to determine whether high levels of gene expression are real or an artefact of amplification. Grün and colleagues demonstrated the efficiency of this strategy using a pool-and-split experiment. They pooled transcripts from 93 cells, split the RNA into 93 equal samples, which created 93 'average' single cells, then amplified and sequenced each sample separately; and no false positive rare cell types were detected.

RaceID identified the gene *Reg4* as being highly expressed specifically in enteroendocrine cells. Grün *et al.* isolated and sequenced a population of 161 *Reg4*-expressing cells. Using RaceID, they identified new enteroendocrine subtypes and validated them *in vivo* at the level of both RNA and protein. This confirmed that RaceID can be used for the identification of rare cell types.

There has been much debate about whether the intestinal stem-cell population is heterogeneous. Can RaceID find subtypes in this population, which is marked by expression of the gene *Lgr5*? Grün and colleagues sequenced transcriptomes from 288 *Lgr5*-expressing cells. RaceID identified these cells as largely homogeneous — the stem-cell population — mixed with a population of rare *Lgr5*-expressing secretory cells. However, as the authors note, it remains possible that the stem-cell population is heterogeneous, but that differences are below a level detectable even by RaceID.

The major limiting factor for RaceID is the accuracy of single-cell sequencing. It is still not possible to measure low-level gene expression accurately in a single cell, and the technical noise for detection of such genes will be too high to identify outliers. The genes for the transcription factors that determine a given cell type are generally not expressed as highly as those encoding hormones, for instance. This might prevent RaceID from discerning potentially functionally important rare cell types in which the differentially expressed genes are likely to mainly encode transcription factors, and may explain the fact that Grün *et al.* were unable to detect stem cells in the initial organoid analysis, because the cells express *Lgr5* at low levels.

The potential for falsely 'identifying' new rare cell types should also be considered. Care must be taken to avoid nucleic-acid cross-contamination or incomplete cell dissociation. It will be necessary to validate putative cell types at the RNA and even protein level.

In terms of sensitivity, accuracy and comprehensiveness, current single-cell sequencing techniques and bioinformatics tools are far from perfect. This is particularly true when it comes to discovering rare cell types. But through the unremitting efforts of Grün *et al.* and others, in the near future we may be able to chart a complete cell-lineage map of the human body. ■

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CANCER

A moving target

An *in silico*, three-dimensional model of tumour evolution suggests that cell motility is a key factor in the initial growth of a tumour mass. The model also reveals the dynamics of mutation spread. SEE LETTER P.261

NATALIA L. KOMAROVA

Evolutionary thinking is becoming an indispensable tool to understand cancer, and even to propose directions in the search for treatment strategies. In this issue, Waclaw *et al.*[1] (page 261) use mathematical modelling based on evolutionary principles to provide an explanation for the observed architecture of tumours, and to argue that cell migration might be the key to tumour shape, spread and drug resistance. This study opens up the possibility of treatments that target genes related to cell motility and adhesion, rather than the conventional targets of genes governing cell division, death and differentiation.

Cancer is an unwanted evolutionary process whereby cells, driven by random mutations, escape the orchestrated behaviour of a functioning tissue and enter a phase of abnormal growth and, later, metastasis (tumour spread). We still lack understanding of many aspects of this complex process, and researchers in different fields are collaborating to solve this ultimate riddle. Evolutionary biologists approach the study of tumours in a similar manner to the study of viruses, bacteria or animals: they seek the mutations that give rise to the ever-changing variety of tumour cells, and they look at the forces of natural selection that allow certain mutants to proliferate, replace their competitors and give rise to new waves of evolutionary change.

Waclaw *et al.* combined methods from evolutionary biology and ecology with current knowledge of the molecular biology of cancer to design a versatile mathematical model

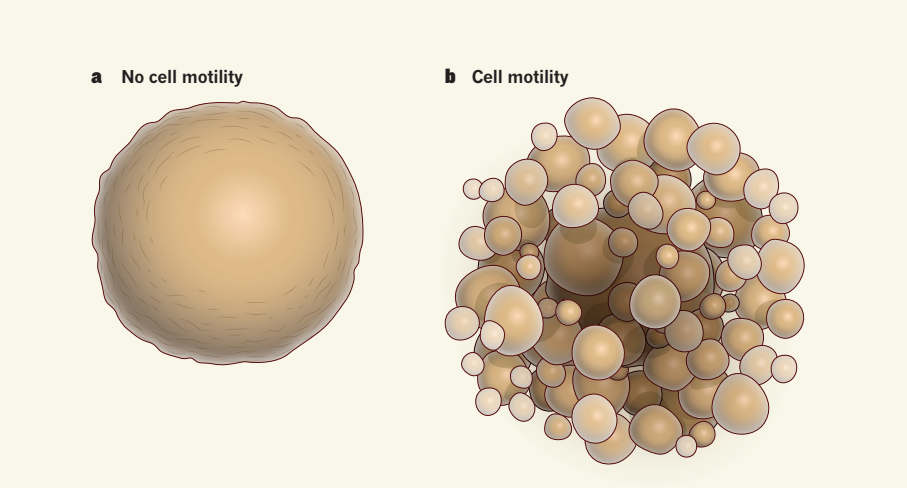

Figure 1 | Motility helps to explain tumour dynamics. Waclaw *et al.*[1] present an *in silico* model of 3D tumour architecture over the course of tumour evolution. **a**, When cells in the model are unable to move, a slow-growing tumour of largely spherical shape is predicted. **b**, By contrast, motile cells lead to a faster-growing tumour with a conglomerate structure more similar to that seen in clinical examples.

in which tumour-cell populations undergo evolutionary change, guided by realistic parameters. The authors used this model to study the growth laws of 3D *in silico* tumours, focusing on the tumours' geometry and cellular composition. If the tumour cells are relatively immobile, then, as they proliferate and form a malignant mass, they crowd each other out and thus slow their own replication. Soon the tumour can grow only at its surface, which results in a relatively slow expansion (the mass grows as a cubic power of time).

But this slow growth cannot account for the relatively fast tumour expansion observed in many clinical studies. The resolution of this apparent paradox lies in cellular motility. By giving each cell in the model the ability to migrate, the researchers observed a much faster, exponential, growth, which also yielded a different, more realistic, tumour shape (Fig. 1). This result is consistent with the earlier proposition[2] that migratory potential is a component of a cell's evolutionary fitness, in much the same way as is its replicative potential. However, it was previously thought that cell migration was mostly involved in the invasion of tissues by tumours or in metastasis. The direct, pivotal role of cell motility in tumour growth was under-appreciated and can now be considered a valid treatment target.

Another focus of the authors' study was tumour composition. In particular, they asked how quickly a particular mutation can propagate in a mass of cancer cells, thus changing the tumour's properties. Evolutionary processes and their outcomes are largely shaped by the environment in which they take place. For example, evolution in a well-mixed, homogeneous medium takes place at a different pace from evolution in an environment in which interactions are restricted by geometric constraints. And in the latter case, dimensionality is key. For example, it has been shown that inactivation of a tumour-suppressor gene (a two-hit evolutionary process in which the cells must first become less fit before becoming more fit) happens faster in 1D (a row of cells)[3,4] than in 2D (a layer), and this is in turn faster than in a fully mixed system with no spatial constraints[4–7]. By contrast, in two-step processes in which the intermediate mutant confers a slight selective advantage, the relationship is the opposite, and a non-spatial, fully mixed environment promotes the fastest pace of evolution[5]. These phenomena seem less surprising if one notes how reminiscent they are of other fundamental laws of nature in which space dimensionality changes how things work, such as the different fundamental solutions of Poisson's equations in 1D and 2D.

Waclaw *et al.* then set out to understand why, given the high overall degree of tumour heterogeneity, some mutations are so prevalent among the cells of a given tumour. In the context of tumour progression, two broad classes of mutation have been identified[8]. Cells with driver mutations are characterized by having a growth advantage over other cells, and such mutations are thought to be responsible for cancer initiation and progression. Passenger mutations are genomic changes that do not really alter the cells' growth properties, and do not have a causal role in cancer origin or progression. Waclaw and colleagues show that, in the presence of even a small amount of selective advantage (that is, a driver mutation), the affected cells sweep rapidly through a 3D cell population. This explains the observed composition of large tumours, in which almost every cell contains the same driver mutations, and heterogeneity resulting from passenger mutations accumulates later, during tumour progression.

This idea is crucial in the context of cancer therapy. A mutation that confers resistance to a drug is usually a passenger mutation before treatment; such mutations are 'hiding' inside any tumour and are generated simply by chance as a result of the constant background mutation rate. But resistant mutants immediately gain a selective advantage once treatment is applied. Waclaw and colleagues' paper illustrates how rapidly resistant cells can accumulate, leading to regrowth and treatment failure. This happens even faster in the presence of mutations that increase cellular motility.

How far are we from being able to use evolution to our advantage? Understanding evolution's intricate ways brings us a step closer to being able to reverse malignant processes, and to channel the dynamics in the direction we want. And can we use the genes responsible for cell motility or cell adhesion as targets in future cancer treatments? Waclaw and colleagues' theoretical study suggests that this is a possibility, and it is to be hoped that others will take up the challenge. ■

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CANCER

Mutant p53 and chromatin regulation

The finding that genes encoding enzymes that modify histone proteins are among the targets of certain mutant forms of the p53 protein sheds light on how these mutations cause cancer beyond p53 inactivation. SEE ARTICLE P.206

CAROL PRIVES & SCOTT W. LOWE

Mutations in the *TP53* tumour-suppressor gene are common in human tumours. Although these mutations invariably inactivate the normal activity of p53 (ref. 1), which is the transcription factor encoded by *TP53*, some mutations also endow p53 with 'gain-of-function' activities that promote cancer[2]. Whether diverse p53 mutants produce similar gain-of-function activities and how they do so remains a puzzle, but finding the answer might enable the design of strategies for treating many cancers. On page 206 of this issue, Zhu *et al.*[3] provide a possible explanation: they show that gain-of-function mutant p53 proteins induce the production of enzymes that modify the histone proteins around which DNA is packaged as chromatin, thus altering gene expression.

Experimentally altering the expression of gain-of-function mutant p53 affects the expression of myriad genes, enhancing the invasiveness and proliferation of tumour cells *in vitro*[4]. Moreover, mice harbouring key gain-of-function mutations in *TP53* develop tumours that differ from those lacking p53 (ref. 5). Lowering the level of gain-of-function p53 has antiproliferative effects *in vitro* and can reduce metastasis or trigger tumour regression *in vivo*[2,5,6]. A better understanding of these mutants is therefore desirable. Zhu *et al.* found that, in cultured human-cancer cell lines, gain-of-function mutant forms of p53 bind to different regions of DNA from the normal protein. In particular, the mutant proteins bind to the genes *MLL1* and *MLL2*. Gain-of-function p53 seems to be recruited to