Snapshot: Tumour Evolution

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Understanding how tumours grow and evolve over time is crucial to help shed light on the underlying reasons why treatments fail and tumours metastasize. This Snapshot provides a brief introduction into the main concepts of tumour evolution.

Modes of tumour evolution:

Tumours contain different subpopulations of cancer cells (subclones) which are governed by the principles of evolution by natural selection, whereby heritable variation influences cancer cell survival. The accumulation of somatic genomic alterations such as point mutations and somatic copy number alterations as well as microenvironmental changes can cause differences in the fitness of cancer cells, leading to fluctuations in the subclonal growth dynamics over time.

Selection is one of the key forces in evolution and describes the differential survival of cells due to differences in phenotype. **Positive selection** in cancer can be manifested as the accumulation of beneficial somatic mutations that result in a fitness advantage and lead to expansion of subclones. A clonal sweep takes place if a subclone outcompetes its neighbouring cells, thus ultimately leading to a reduction of diversity and a homogeneous cancer cell population. **Negative selection**, on the other hand, results in the elimination of cells which have accumulated fitness reducing mutations coupled with environmental pressures, for example the removal of potent neo-antigens. Negative selection is measured by an absence of deleterious mutations.

During **neutral evolution** individual subclones grow at similar rates, with the frequency of mutations reflecting the time of their occurrence rather than their fitness. Neutral evolution can be seen as evolution in the absence of positive selection. Following a clonal sweep, some tumours may evolve predominantly neutrally, or with negative selection, while others might evolve with a combination of neutral evolution and selection. Genetic drift describes the fluctuations in the frequency of subclones due to random birth and death events and is more pronounced when the population size is small.

Cancer evolution, by definition, is **branched**, due to the continuous accumulation of mutations leading to genotypic divergence. However sometimes only one cell lineage may dominate a population over time, resulting in the appearance of **linear evolution**. This phenomenon may be overestimated due to **sampling bias,** which describes the confounding effect of limited sampling applied to the tumour. If all subclones are sampled, the full evolutionary trajectory

can be reconstructed, even without presence of the ancestral clonal population, as each subclone bears the footprints of all its ancestral somatic alterations. If only one subclone of a heterogeneous tumour is sampled, all alterations appear clonal, even those that are in fact subclonal within the whole tumour (**clonal illusion**). If only a subset of subclones is sampled this can lead to an **illusion of linear evolution**.

The accumulation of adaptive alterations can occur gradually or in bursts, which is referred to as **punctuated evolution**. Punctuated evolution results in a rapid clonal sweep and is likely induced by large-scale alterations such as whole genome doubling events.

Due to selection, evolution may converge upon the same phenotype through different genetic mechanisms occurring on separate branches resulting in parallel subclonal expansion (**parallel evolution**). Additionally, **multiple hits** of the same gene within the same cell lineage (same branch of the tree) can occur throughout tumour evolution, e.g. in the case of multiple hits to a tumour suppressor gene.

Different genomic events, including point mutations, copy number alterations and structural variants, and non-genomic events, including disruption to DNA methylation, chromatin accessibility and transcription factors, can engender subclonal **diversity** thus providing the fuel for selection. In addition, the tumour microenvironment has a large impact on cancer evolution. Subclones presenting neo-antigens may be recognised by the immune system and eliminated, which equates to negative selection. Thus, adaptive variations leading to escape from the immune response such as loss-of-heterozygosity (LOH) of the Human Leucocyte Antigen (HLA) locus or up-regulation of immune checkpoint molecules, may result in beneficial fitness effects and positive selection of the corresponding subclones. The cooperation between subclones and the interaction of cancer and stromal cells can lead to environmental advantages for a subset of cells which can promote selection of that population, shaping the evolution of the tumour.

Seeding patterns:

Throughout tumour evolution, cancer cells can disperse from the primary tumour to distant organs in the body and form metastases. This dispersal can occur once or multiple times throughout tumour development. Furthermore, cells can disseminate **early** during tumour evolution when the cancer only consists of a small number of cells or **late**. However, due to limited sampling, timing divergence of metastases in absolute time is often not possible. Therefore, one approach is to time metastatic divergence relative to the last clonal sweep. If the cells disseminated before the last clonal sweep, it can be considered relatively early divergence. Conversely, if divergence occurred after the last clonal sweep, this can be considered relatively late. The metastasising subclone includes both unique mutations and all mutations from the most recent common ancestor of the primary tumour and metastasis. Thus, all truncal mutations are necessarily also present in the metastasis.

If a single subclonal population acquires metastatic potential and seeds all metastatic sites, this is referred to as **monoclonal seeding** from the primary. Only one branch of the tree is involved in metastatic seeding in this case and therefore this is considered monophyletic dissemination. Conversely, if multiple subclones of the primary tumour seed one or more metastatic sites, this is referred to as **polyclonal seeding** from the primary. If all subclonal populations with metastatic potential arise along one branch of the phylogenetic tree, this is

considered **monophyletic** dissemination while **polyphyletic** dissemination describes seeding from subclones from different branches of the tree.

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Declaration of Interests

N.M. has received consultancy fees and has stock options in Achilles Therapeutics. N.M.. holds European patents relating to targeting neoantigens (PCT/EP2016/ 059401), identifying patient response to immune checkpoint blockade (PCT/ EP2016/071471), determining HLA LOH (PCT/GB2018/052004), predicting survival rates of patients with cancer (PCT/GB2020/050221).

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