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REVIEW

Somatic variation in normal tissues: friend or foe of cancer early detection?

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Background: Seemingly normal tissues progressively become populated by mutant clones over time. Most of these clones bear mutations in well-known cancer genes but only rarely do they transform into cancer. This poses questions on what triggers cancer initiation and what implications somatic variation has for cancer early detection.

Design: We analyzed recent mutational screens of healthy and cancer-free diseased tissues to compare somatic drivers and the causes of somatic variation across tissues. We then reviewed the mechanisms of clonal expansion and their relationships with age and diseases other than cancer. We finally discussed the relevance of somatic variation for cancer initiation and how it can help or hinder cancer detection and prevention.

Results: The extent of somatic variation is highly variable across tissues and depends on intrinsic features, such as tissue architecture and turnover, as well as the exposure to endogenous and exogenous insults. Most somatic mutations driving clonal expansion are tissue-specific and inactivate tumor suppressor genes involved in chromatin modification and cell growth signaling. Some of these genes are more frequently mutated in normal tissues than cancer, indicating a context-dependent cancer-promoting or -protective role. Mutant clones can persist over a long time or disappear rapidly, suggesting that their fitness depends on the dynamic equilibrium with the environment. The disruption of this equilibrium is likely responsible for their transformation into malignant clones and knowing what triggers this process is key for cancer prevention and early detection. Somatic variation should be considered in liquid biopsy, where it may contribute cancer-independent mutations, and in the identification of cancer drivers, since not all mutated genes favoring clonal expansion also drive tumorigenesis.

Conclusion: Somatic variation and the factors governing homeostasis of normal tissues should be taken into account when devising strategies for cancer prevention and early detection.

Key words: somatic evolution, driver gene, clone selection, healthy tissues, cancer initiation, cancer early detection

INTRODUCTION

Cancer has long been referred to as a disease of the genome because of the pivotal role played by genetic alterations in driving its initiation and progression.[1] Only recently, however, cancer mutational screens have revealed the extent of cancer genomic modifications that often accumulate over several years.[2,3] These studies have greatly expanded our knowledge on the genetic basis of cancer. The analysis of thousands of cancer exomes and genomes has led to the identification of >3000 putative driver genes.[4] Almost 600 of these genes have experimental confirmation of their cancer role, while the rest are predictions of statistical approaches that measure the evolutionary forces acting on mutant genes or the effect and properties of their alterations.[5,6] With only a few notable exceptions, the vast majority of known or predicted cancer drivers promote cancer only in specific tissues.[4] Moreover, the majority of cancer genomes bear mutations in more than one driver, supporting early theoretical work on the need of multiple hits to initiate tumorigenesis.[7]

In addition to identifying the driver events, cancer mutational screens have been used to infer the mutational processes active in cancer cells and formulate models of cancer evolution. Phylogenetic trees based on alteration clonality[8] enable reconstruction of the evolutionary paths of individual cancer samples from the seeding cell to the time of sequencing. These can then be used to interpret and predict future evolutionary trajectories, including response to therapy.[9] Knowing the genome sequence of fully fledged tumors, however, does not inform on events predating cancer transformation. In fact, it tells very little about the early phases of tumor formation, namely the events and

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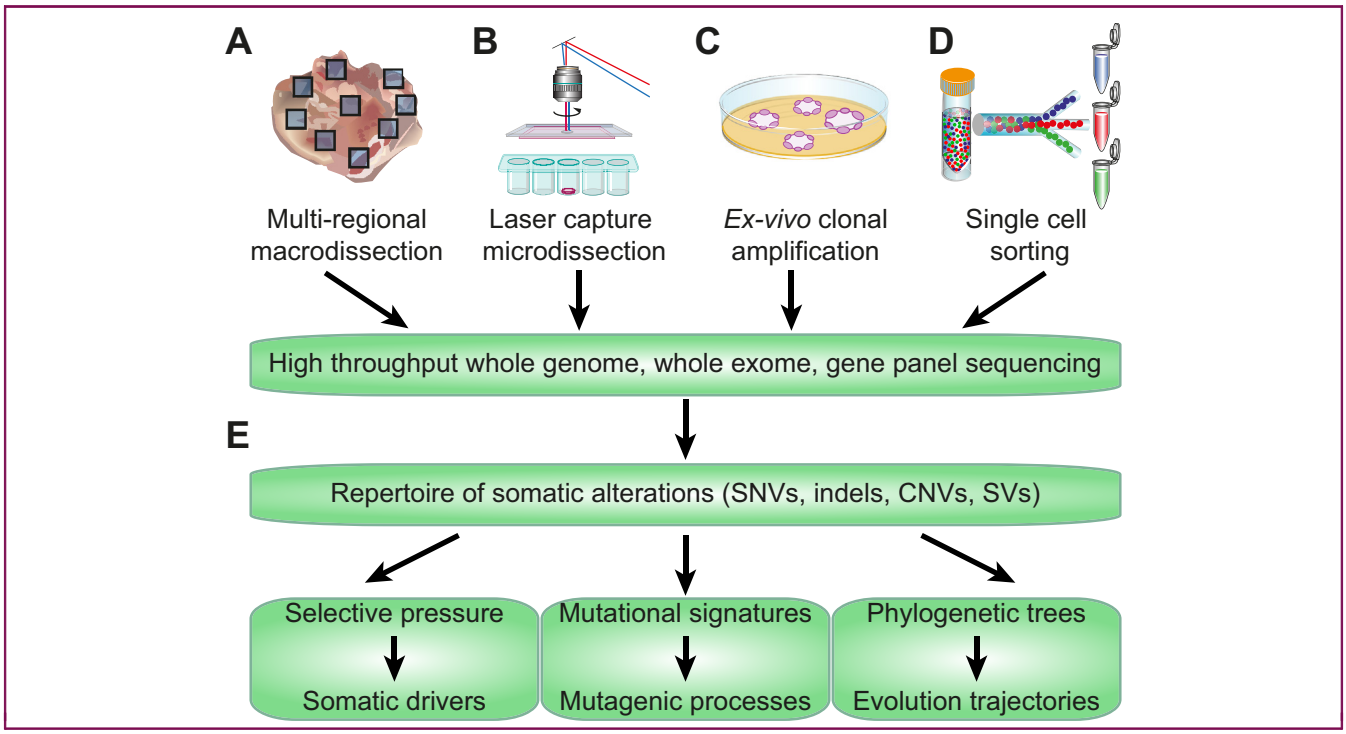

Figure 1. Approaches to detect and analyze somatic mutations in normal tissues. DNA extracted from (A) macro- or (B) micro-dissected tissues, with or without subsequent targeted bulk resequencing, (C) *ex vivo* clonal expansion of isolated cells, and (D) single-cell sorting is sequenced using next-generation sequencing approaches that allow high-throughput detection of somatic mutations that can then be used to identify the drivers of clonal expansion, the mutational processes causing them, and to trace tissue evolution (E).
CNVs, copy number variants; SNVs, single-nucleotide variants; SVs, structural variants.

conditions that promote transformation of normal cells into cancer cells.

One of the main challenges in detecting pre-cancer mutations is that, before the clonal expansion associated with cancer, they hit only a small fraction of cells. These mutations are therefore diluted within the tissue and their frequency is usually below the detection power of conventional sequencing methods. Until the advent of high-throughput sequencing technology, only a few somatic alterations occurring in apparently normal tissues were documented. Among these were the inactivation of cytochrome c oxidase and *TP53* in colon and skin detected through immunostaining or conventional Sanger sequencing.[10-13] However, the extent of somatic variation occurring in the human genome has started to be fully appreciated only recently.[14,15] High-throughput sequencing coupled with bioinformatic analysis has finally enabled quantification of low-frequency alterations occurring in phenotypically normal tissues.

In this review, we summarize the results of mutational screens in non-cancer tissues, focusing on what they have revealed about the origin of somatic mutations and their impact on tissue homeostasis and disease. We then discuss the relevance of somatic variation for cancer initiation and how it can help or hinder strategies to improve cancer detection and prevention.

THE MUTATIONAL LANDSCAPE OF HISTOLOGICALLY NORMAL TISSUES

Recent advances in DNA sequencing technologies and computational approaches for data analysis have enabled detection of somatic mutations occurring in only a few cells within adult tissues. DNA extracted from macro-dissected tissue slides (Figure 1A), microscopically identifiable clonal structures (Figure 1B), clones expanded *ex vivo* (Figure 1C), or single-cell populations (Figure 1D) can be sequenced at high depth to identify rare alterations. The resulting repertoire of somatic mutations can then be used to quantify the selective pressure driving clonal expansion, identify the underlying mutagenic processes, and rebuild tissue somatic evolution in time and space (Figure 1E). Although enabling detection of rare mutations, all these approaches have limitations (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.09.156), which should be considered when interpreting the results.

During life, the homeostasis of most tissues is preserved through the asymmetric divisions of adult stem cells, which enable the maintenance of a stem cell pool while sustaining tissue renewal through the progressive differentiation of progenitor cells (Figure 2A). The acquisition of somatic alterations in the genome of stem or progenitor cells may result in their increased fitness that fuels the clonal expansion of their progenies, which eventually populate part of the tissue (Figure 2B).

Somatic variation has shown recurrent features across the tissues sequenced so far. For example, the mutational load as well as the number and size of mutant clones increase with age, in the presence of inflammatory conditions and upon exposure to mutagens (Figure 2C). Moreover, somatic clones only rarely acquire copy number alterations, structural rearrangements, or chromosomal abnormalities.

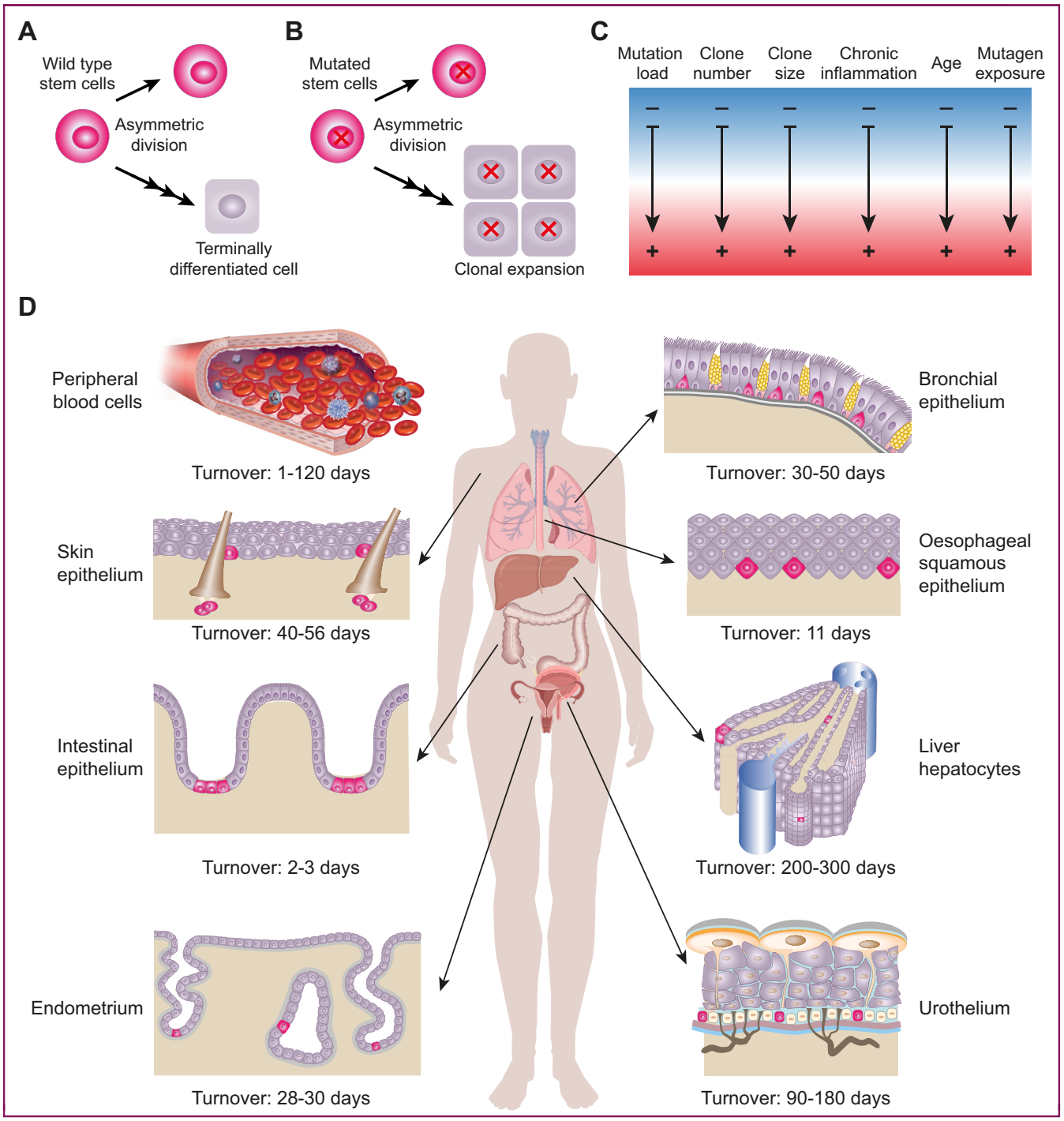

Figure 2. Somatic evolution of normal tissues. (A) Tissue homeostasis is maintained through asymmetric division of wild-type stem cells. (B) Somatic mutations conferring fitness advantages result in clonal expansion of the mutant progenies. (C) Recurrent features of somatic evolution across normal tissues. (D) Schematic representation of the structure and turnover of histologically normal human adult tissues. Turnover data were taken from Bowden,[106] Pan et al.,[107] Cousins et al.,[108] Khandelwal et al.,[109] and Milo et al.[110]

Despite these commonalities, the number and size of clones vary substantially across tissues suggesting that their proliferative potential does not depend uniquely on the intrinsic advantages contributed by mutations. The architecture of the tissue and the frequency of its turnover (Figure 2D) also likely play major roles in determining the fate of the mutant clone. Hematopoietic stem cells produce thousands of mature blood cells every day and mutant clones can in principle expand freely in the bloodstream. Accordingly, age-dependent clonal hematopoiesis, i.e. the expansion of mutant hematopoietic cells sharing a common origin, is highly diffuse in the general population.[16-18]

Unlike the blood, solid tissues pose spatial barriers to clone expansion. For example, the intestinal epithelium is organized into well-defined clonal structures known as crypts that undergo continual renewal during life. Despite the high tissue turnover, clonal expansion beyond the single crypt (a phenomenon known as 'crypt fission') rarely occurs

in healthy gut.[19-23] Normal liver also usually hosts relatively few mutant clones,[24-26] possibly due to the low turnover and the lobular structure of the tissue. The mutational landscape of both gut and liver changes drastically in the presence of inflammatory disorders such as inflammatory bowel disease or cirrhosis, which positively correlate with the number of mutant clones.[24,26]

An increased number of clones is also observed in endometriotic endometrium,[27-29] confirming that chronic inflammation remodels adult tissues through continuous cycles of destruction and repair that favor clone outgrowth. Unlike normal colon and liver, mutant clones almost completely replace non-inflamed endometrium by menopause.[29-31] This is likely facilitated by the 'rhizome' structure of the endometrial epithelium, in which vertical glands acquire additional mutations during every menstrual cycle.[32]

The epithelia of skin and esophagus also progressively become a patchwork of mutant clones during life.[33-38] In both tissues, the stem/progenitor cell compartments are localized above the basement membrane of the epithelium (Figure 2D), which poses a weaker barrier to the propagation of mutant clones than intestinal crypts or hepatic lobes. As expected due to the higher exposure to external mutagens, skin accumulates around 10-fold more mutations than esophagus.[36] Interestingly, recent observations suggest that the mutagenic effect of some exogenous insults, and the consequent expansion of mutant clones, may be reversible. For example, despite the mutation burden being generally higher in tobacco smokers or ex-smokers than in never smokers, high variability has been observed across and within individuals. In particular, some clones show comparably low mutational burden in current, former, and never smokers,[39] indicating that their stem cells are less susceptible to (or are shielded from) smoking mutagens. Lowly mutant clones are fourfold more frequent in ex-smokers than in current smokers and can repopulate the bronchial epithelium once the exposure to smoking ends. Although further studies are needed to explain reasons and mechanisms of this decrease, these results may suggest that the fitness advantage of somatic mutations is context-dependent and varies with circumstances.

Extensive inter- and intra-individual variation in the mutational spectrum has also been observed in the urothelium of bladder and ureter, which, despite the relatively low turnover, become substantially populated by mutant clones over time.[40,41]

GENES AND MUTATIONAL PROCESSES DRIVING SOMATIC CLONAL EXPANSION

Genes acquiring somatic mutations that increase cell fitness and drive clonal expansion (somatic drivers) are identified using approaches similar to those used for cancer drivers, preferentially detecting frequently mutated genes.[4] So far, these approaches have identified 147 somatic drivers across nine tissues (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.09.156). Almost 90% of these genes are well-known (canonical) or predicted (candidate) cancer drivers and tumor suppressors outnumber oncogenes (Figure 3A). This is in line with the prevalence of somatic point mutations and small indels that are more likely to inactivate tumor suppressors. It should be noted however that the use of driver detection methods developed for cancer genomics may result in detection bias and overestimation of the overlap between cancer and somatic drivers.

Functionally, somatic drivers that are also cancer drivers are typically signaling genes mediating cell growth or chromatin modifiers (Figure 3B). Given their role in cell differentiation,[42] it is tempting to speculate that mutations in chromatin modifiers promote cell dedifferentiation and self-renewal that, in turn, favor the clonal expansion of mutant cells. The few somatic drivers that are not cancer drivers do not show any significant functional enrichment, indicating no convergence toward the disruption of any particular biological process.

Unlike cancer, where the higher the size of the analyzed cohort the more drivers become detectable,[4] the number of somatic drivers does not increase with sample or donor size (Figure 3C). For example, clonal expansion in blood is driven by a similar number of genes as in intestine or diseased endometrium, despite 20-fold more blood samples having been sequenced. This suggests that the early phases of somatic clonal expansion tend to be promoted by the same genes driving cancer, but the extent of inter-individual heterogeneity of the somatic driver repertoire may be more limited. This also confirms that clonal expansion depends on the features of the tissue as well as its exposure to mutagens, in addition to the intrinsic advantages of the mutant cells. Comparisons across tissues should however take into account the experimental and analytical approaches used to detect somatic mutations, since each of them has biases and limitations (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.09.156). Moreover, it is likely that a sizable fraction of somatic drivers remain still unknown.[43]

Even considering these technical caveats, the somatic driver landscape shows high tissue specificity. For example, only 13 genes drive clonal expansion in three or more tissues (Figure 3D). An extreme case is again blood that shares only *TP53* with other tissues, indicating that clonal hematopoiesis is promoted by a small and tissue-specific set of somatic drivers. There are clear differences even across solid tissues. For example, multiple mutational screens of skin and endometrium have reported alterations in the same drivers (*NOTCH1*, *FAT1*, and *PIK3CA*, *KRAS*, respectively, Figure 3D), due to parallel or convergent evolution. In the former case, clones carry distinct, inactivating mutations (*NOTCH1* or *FAT1*), while in the latter they converge toward the same activating mutation (*KRAS* or *PIK3CA*). This does not occur in other tissues, where different screens identified different drivers.

Intriguingly, a few well-known cancer drivers, notably *KRAS* in endometrium, the *NOTCH* genes in skin and esophagus, and the *ERBB* genes in colon, are more frequently altered in normal tissues than in the

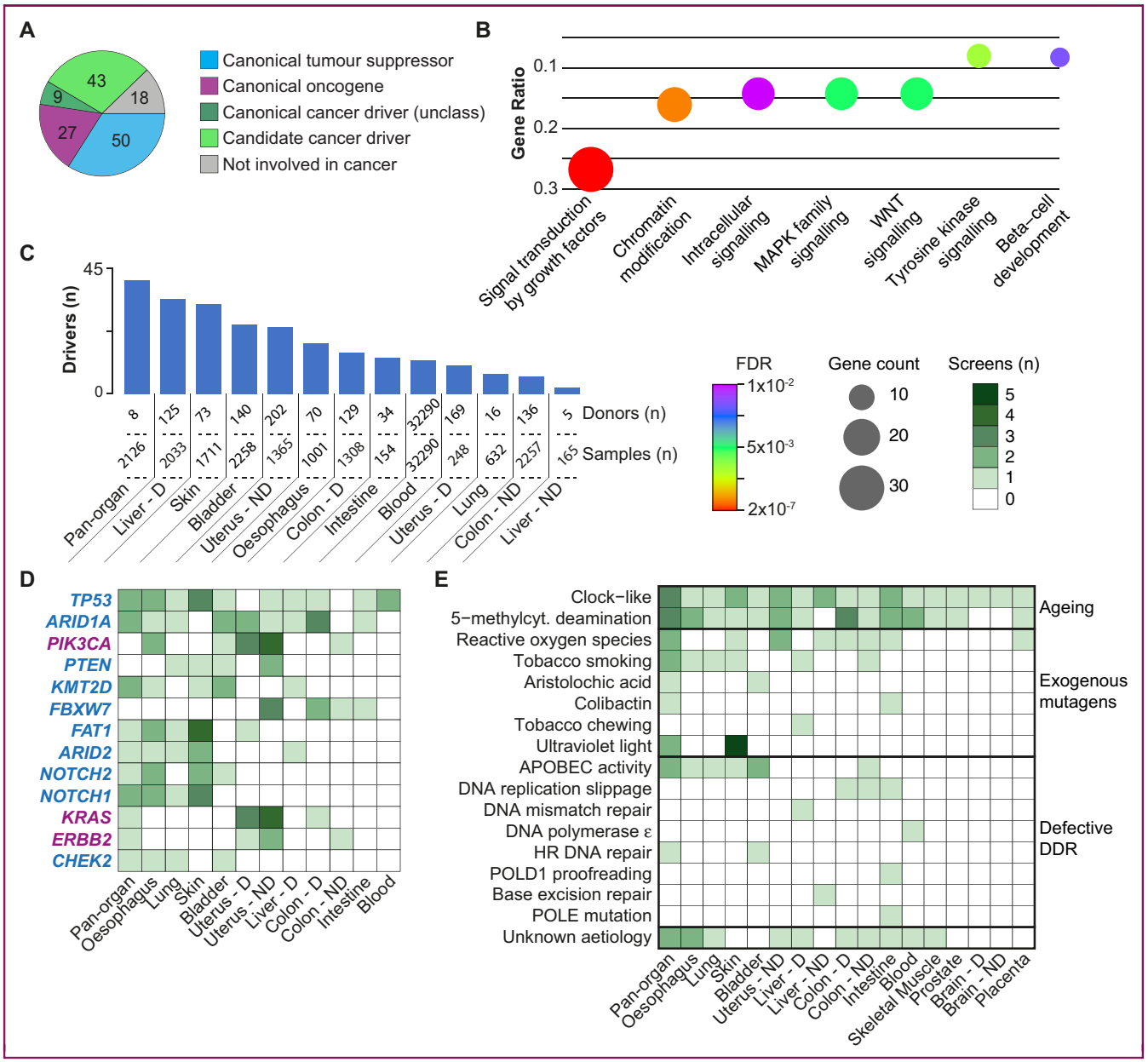

Figure 3. Somatic driver repertoire. (A) Breakdown of somatic drivers that are also cancer drivers or that have not been associated with cancer. Canonical and candidate cancer drivers were derived from the NCG database (http://www.network-cancer-genes.org/).[4] (B) Gene set enrichment analysis of somatic drivers in level 2 Reactome pathways v.72[111] as compared to the other cancer drivers from the NCG database. Enrichment was calculated using one-sided Fisher's exact test corrected with Benjamini–Hochberg for multiple testing. Gene ratio represents the proportion of somatic drivers over the total. Circle size indicates the gene count per pathway. (C) Number of unique somatic drivers per tissue. (D) Somatic drivers recurring in three or more tissues. (E) Etiologies of somatic mutations as derived from the signatures reported in Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.09.156. The etiologies were assigned using COSMIC v.2 and v3.2[112] and grouped based on similarities. Screens on diseased uterus did not describe any signature.

5-Methylcyt., 5-methylcytosine; APOBEC, Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like cytidine deaminase; D, diseased (non-cancer) tissue; DDR, DNA damage response; FDR, false discovery rate; HR, homologous recombination; ND, non-diseased tissue; POL, polymerase.

corresponding cancers (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.09.156). This suggests that some cancer drivers may have either a cancer-promoting or a cancer-protective role depending on the context and time of their alteration.

Notably, placenta represents an exception in terms of clonal expansion occurring in healthy tissues. Instead of being driven by mutations that increase the cell fitness, placenta mosaicism results from the developmental expansion of trophoblast progenitors carrying early embryogenic mutations.[44] Placenta also shows higher mutation rate and frequent copy number alterations compared to other tissues (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.09.156). This could be due to distinct prenatal and postnatal mutational process or the lack of genome-protecting mechanisms in placental trophoblasts.

The patterns of mutations occurring in the genome of mutant clones, known as mutational signatures, are indicative of the processes responsible for somatic mutagenesis. Signatures related to endogenous mutational processes are prevalent in all tissues sequenced so far (Figure 3E). Some of these mutations are likely acquired in the first few cell

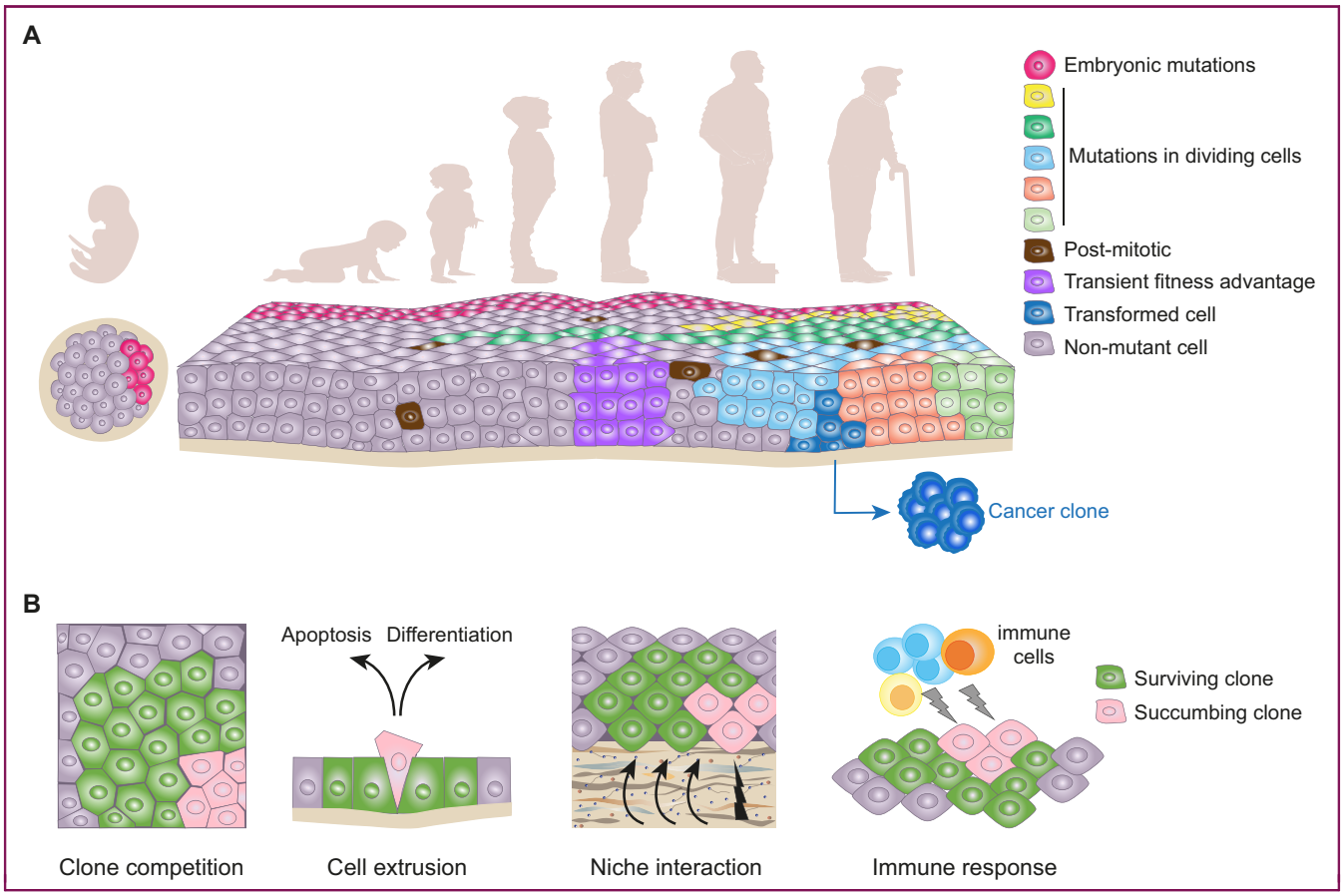

Figure 4. Origin, evolution, and fates of somatic clones. (A) Somatic mutations accumulate throughout life resulting in somatic mosaicism. Some mutations are acquired during embryo development and fixed by neutral drift. Other mutations arise in post-mitotic or actively dividing cells due to the exposure to endogenous or exogenous insults. Mutations that confer fitness advantages initiate clonal expansion of actively dividing cells. In some cases, these advantages are transient and the clone disappears when the insult is removed. Some mutant cells may acquire transforming potential and start the tumorigenic process. (B) Clone selection and expansion are driven by several intrinsic and extrinsic factors such as competition between mutant cells with different fitness, cell extrusion leading to apoptosis or differentiation, active responses of the stromal niche, and selective pressure of the immune system.

divisions of embryonic development, when the mutation rate per generation is very high,[45,46] and continue to accumulate throughout life. The pervasiveness of endogenous signatures indicates that the main source of mutational variation in somatic tissues is related to aging. Signatures induced by reactive oxygen species, and tobacco smoking are also relatively frequent. Other external mutagens, such as UV light, aristolochic acid, or colibactin, are instead specific to skin, urothelium, and intestine, respectively. This is consistent with their cancer-promoting role in these organs confirming that, at least in these cases, normal clonal expansion and cancer initiation have the same mutagenic origins.

Together with the mutational signatures found in cancer, normal tissues show several novel signatures that have never been described before (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.09.156). These may be hidden by the prevalence of stronger mutational processes that take over during cancer evolution or may indicate a different origin of somatic mutations that do not eventually evolve into cancer. None of these novel mutational signatures have a known etiology, which prevents from discriminating between these two scenarios.

ORIGINS AND CONSEQUENCES OF SOMATIC MUTATIONS IN AGING AND DISEASE

Somatic mutations are acquired from early development throughout adult life, with clones growing in number and size over time (Figure 4A).

Interestingly, mutation rate is higher during fetal development than in postnatal cells,[47-50] especially in the first three embryonic divisions.[45,48,51] This is likely due to the absence of transcription-associated DNA repair[52,53] and a higher tolerance toward DNA damage due to the lack of apoptosis[53-55] during very early development. Fixation of embryonic mutations often occurs by neutral drift rather than selection and mutant cells can eventually populate large portions of one or more tissues, as in the case of the same mutations found in brain and spleen.[49]

Somatic mutations that promote clonal expansion during embryonic development or adult life hit dividing cells that most likely are stem or progenitor cells. However, mutations may occur also in post-mitotic tissues and affect slowly or non-dividing cells, such as visceral smooth muscle and neurons.[56,57] For example, the post-mitotic expansion of CAG repeats in neurons is known to cause Huntington's disease.[58] Recent technical innovations, including single-cell[59] and

single-molecule[57] DNA sequencing, have shown that post-mitotic neurons accumulate mutations at a similar rate than mitotically active cells. This surprising result indicates that, together with errors generated during cell divisions, mutations can continuously arise from non-mitotic insults. Although the signatures of post-mitotic mutations do not point toward any specific etiology, their linear accumulation over time suggests that they are the result of a dynamic equilibrium between DNA damage and repair throughout life.[57]

Do somatic mutations result always in disease conditions? While a clear link exists between mutation accumulation and cancer, as extensively discussed below, still relatively little is known about their role in other diseases. Embryonic mutations that disrupt Mendelian genes may result in similar but less severe syndromes than germline mutations. Examples include overgrowth syndromes where somatic mutations confer growth advantages to mutant cells located in specific areas of the body[60,61] and almost 10% of mutations causing autism spectrum disorder.[62] Moreover, tissue phylogeny and lineage tracing have shown that mutations arising during fetal development can modify known cancer drivers leading to the expansion of cancer-precursor clones that eventually initiate childhood tumors, including Wilms' tumors,[63] pediatric liver cancers,[64] and malignant rhabdoid tumors.[65]

Clonal hematopoiesis is a known risk factor in cardiovascular disease due to a combination of increased inflammation and mutation-specific effects,[66] while somatic mutations in immune cells may favor the onset of immune disorders.[67] Despite these examples, however, the widespread diffusion of somatic mutations in the normal population and the phenotypically normal appearance of mutated tissues suggest that most mutations, even when favoring clonal expansion, are not pathogenic.

In addition to disease, the accumulation of mutations has long been associated with aging. Mutations are thought to favor the progressive decline of cell functions,[68-70] although the molecular basis of this remains largely elusive. It has been proposed that somatic mutations could reduce the efficiency of gene regulatory networks and increase cell-to-cell transcriptional heterogeneity.[70,71] However, the high somatic mutation burden observed in carriers of germline *POLE/POLD1,*[23] *MUTYH*,[72] or mismatch repair gene[73,74] defects does not lead to any appreciable sign of accelerated aging, suggesting that a more complex relationship likely exists between mutation and aging. It is likely that multiple, and mostly independent, forms of molecular and tissue damage synergistically contribute to aging-related functional decline. In this multifactorial context, somatic mutations may favor cell type imbalances in tissue composition due to the prevalence of cell proliferation over differentiation.[75] Intriguingly, a putative beneficial role of somatic variation in sustaining the renewal capacity of exhausted stem cells over time has also been proposed.[76] This multitude of interpretations indicate that the functional role of somatic variation is still mostly unknown and warrants further studies.

SOMATIC MUTATIONS AND CANCER TRANSFORMATION

The pervasiveness of mutant clones in phenotypically normal tissues poses the questions of how these clones form, grow, and survive and under what circumstances they transform into cancer.

In the early phases of clone formation, competition between mutant cells with different fitness is a key factor for their survival.[77,78] Lineage tracing of mutagen-driven clone formation in the mouse esophagus has shown that *NOTCH1*-mutant clones have higher fitness and outcompete *NOTCH1* wild-type clones, causing their extrusion from the basal epithelium.[77] *NOTCH1*-mutant clones become progressively selected for in the normal esophagus and this could explain why *NOTCH1* mutations are more frequent than in esophageal cancer.[33,34] As alluded to earlier, an intriguing speculation is that mutations in cancer drivers may have different roles and consequences depending on the context and time of alteration. In the case of *NOTCH1*, early mutations may create a decoy fitness peak that reduces the chances of malignant transformation.[79,80] This variable role is further supported by the effect of conditional heterozygous deletion of somatic drivers in liver, including the two tumor suppressors *ARID1A* and *KMT2D*. Their deletion promotes liver regeneration and reduces damage susceptibility in the presence of injury.[25] Therefore, as likely suggested by the reduction in the number of mutant clones in the lung of ex-smokers,[39] the selective advantage of *NOTCH1*, *ARID1A*, and *KMT2D* may be transient and context-dependent.

It is likely that additional mechanisms also contribute to clone selection (Figure 4B). Mutant cells can be extruded from the epithelium through the activation of cytoskeletal proteins in neighboring cells, leading to apoptosis[81] or differentiation.[82] Moreover, cell-extrinsic factors, whose contribution has been investigated only marginally, are also likely to support or hinder clonal expansion. Active responses of the stromal niche surrounding the mutant cells, including a mesenchymal activation or a change in the composition of the extracellular matrix, may influence the expansion of certain clones and favor the clearance of others. For instance, increased mechanical stiffening of the extracellular matrix is thought to attenuate the extrusion of mutant cells from the epithelium.[83]

Finally, the role of the immune system during clonal expansion remains largely unknown. The immune system acts as an additional bottleneck during cancer evolution by exerting a selective pressure on cancer cells and shaping their immunogenicity.[84] Since it is now clear that mutant clones in normal tissues only rarely evolve into tumors, it is tempting to speculate that immunosurveillance starts well before cancer transformation. It may be that only non-immunogenic clones survive, while the others are eliminated by a concerted innate and adaptive immune response. Once established, somatic clones may reach a dynamic equilibrium with the immune system that keeps their size at bay or may evolve immune evasion mechanisms to survive and continue to grow. It should be noted, however, that

mutations in immune evasion genes are not under selection in normal skin.[85] Moreover, measuring negative selection in somatic genomes is challenging because it requires extensive datasets and careful correction for confounding factors that may lead to over- or underestimations.[86] As a consequence, it is currently unclear whether these forces can[87] or cannot[40] shape the normal genome.

When and how do mutant cells transform into cancer cells? The most striking differences between somatic and cancer clones are the number of mutated drivers and the extent of chromosomal instability. Usually, somatic clones have at most two drivers (although a higher number has been reported in a small proportion of endometrial glands[31]) and usually lack copy number alterations. In contrast, multiple drivers are needed for tumorigenesis.[7] Moreover, while chromosomal instability is a hallmark of pre-cancer to cancer transition,[88] somatic drivers show no functional enrichment in pathways related to genome stability (Figure 2B). Therefore, a prerequisite for transformation may be the acquisition of multiple hits that may favor the onset of chromosomal instability.

The order by which driver alterations are acquired is likely to be another required factor to promote transformation. Individuals with clonal hematopoiesis have a higher risk of developing acute myeloid leukemia if they bear *TP53* mutations compared to mutations in other genes.[89] Similarly, progressive mutations in *APC*, *KRAS*, and *TP53* are paradigmatic of the adenoma to carcinoma transition in colon but are not observed in the normal colonic epithelium. It should be noted, however, that *APC* mutations are very rare in normal colonic crypts. Finally, the overall genotype of the mutant cell as well as the phenotype of the surrounding niche, including the interplay with the immune system, may decide the fate of the clone toward transformation.

IMPLICATIONS OF SOMATIC VARIATION FOR CANCER PREVENTION AND EARLY DETECTION

The accumulation of cancer driver mutations long before the appearance of cancer represents both opportunities and challenges for cancer prevention and early detection.

A better understanding of the endogenous and exogenous factors that trigger transformation of mutated but still normal cells into cancer cells has the potential to open avenues to improve or develop prevention strategies. For example, it could improve the sensitivity and specificity of cancer risk prediction algorithms, thus allowing clinicians to restrict cancer surveillance only to individuals at highest risk.[90] A deeper knowledge on the determinants of transformation could also point toward preventive therapies aimed at actively interrupting or at least delaying the carcinogenic process. Long-term use of aspirin has been associated with reduced risk of gastrointestinal cancers.[91] Although the molecular mechanism is not fully understood, the anti-inflammatory action of aspirin is probably a major component of its cancer-prevention effect. Similarly, the inhibition of the proinflammatory cytokine interleukin 1 has been proposed as a potential cancer-preventive strategy.[92]

The pervasiveness of somatic clones also poses some challenges for cancer detection and monitoring. A prime example is liquid biopsy, a non-invasive approach increasingly used for tumor early detection and for monitoring response to therapy.[93] Liquid biopsy is based on the identification of circulating DNA fragments bearing driver mutations, which are usually thought to derive from dead cancer cells or extracellular vesicles. Circulating DNA from mutated but normal cells can act as a confounding factor particularly in older patients who are likely to bear a high number of mutant clones and experience age-induced cell death. For example, *TP53* mutations were detected in the circulating DNA of 49% lung cancer patients but also in 11% non-cancer controls.[94] Circulating fragments of mutated DNA in healthy individuals can derive from clones originally resident in solid tissues or, more often, from mutant blood cells. Clonal hematopoiesis is a known source of noise in liquid biopsy,[95,96] but it can be efficiently accounted for through the parallel sequencing of matched leukocyte DNA.[97] Circulating DNA of mutant cells from solid tissues is more difficult to distinguish. In this case, focusing on cancer methylation patterns in addition to mutations, as in the case of the GRAIL test,[98] could improve the test specificity, although the occurrence of methylation changes during somatic evolution cannot be excluded. Overall, however, the performance of liquid biopsy in detecting early stage cancer is poorer than for advanced disease.[96]

Another challenge concerns the identification of cancer driver genes and how they can be reliably distinguished from somatic drivers that increase cell fitness in normal cells but may not necessarily contribute to tumorigenesis. This may be the case of the frequent *NOTCH1* mutation occurring in normal esophagus as well as esophageal cancer. Despite positive selection being reported for *NOTCH1* mutations in esophageal cancer,[99] its early mutations in normal esophagus could impair tumor growth.[100] Therefore, identifying cancer drivers based uniquely on their recurrence across cancer samples may lead to false positives. High mutation frequency may in fact result from the recurrent mutation of somatic drivers in healthy individuals, some of whom will develop cancer independently from (or even despite) that driver. There are both experimental and analytical strategies that could mitigate this noise. For example, deep sequencing of normal tissues surrounding the tumor will help assess if the same somatic drivers are altered in the tumor-precursor cells. Analytically, cohort-level approaches based on recurrence or positive selection should be complemented with driver detection methods that predict drivers in individual samples, for example, identifying their network deregulations[101-103] or applying machine learning to identify mutant genes that resemble cancer drivers.[104,105]

FUTURE PERSPECTIVES

The ability to precisely quantify the extent of genomic variation occurring in seemingly normal tissues is radically changing our understanding of somatic evolution. The idea of a stable genome inherited from germline cells and

maintained strictly unaltered throughout adult life does not hold true. Rather, the genome of somatic cells undergoes continuous modifications, some of which confer fitness advantages that can initiate clonal expansion. This results in dynamic tissue remodeling that starts during embryo development, where it is mostly driven by neutral drift, and continues as we age, where the fittest clones undergo positive selection.

The long-term fate of somatic clones depends on the interplay between the intrinsic features of the host tissue and the extrinsic features of the surrounding ecosystem, which are likely to change over time. Ending the exposure to damage and stress may reduce the fitness of previously selected clones, causing their shrinkage and clearance. Alternatively, clones may persist for a long time in equilibrium with the surrounding ecosystem. The disruption of this equilibrium may result in gaining transforming capacity.

Currently, very little is known on what regulates the homeostatic equilibrium within tissues, and this limits our understanding of the initial phases of cancer initiation and the efficacy of early clinical intervention. Further studies are needed to define the functional activity of driver genes in different contexts, including the role of epigenetic alterations and mutations in non-coding regions during somatic evolution. Moreover, a detailed knowledge of the functional composition of the niche surrounding mutant clones will reveal key extrinsic factors supporting their survival. Finally, new model systems are needed to follow the fate of mutant clones exposed to changing conditions. Addressing these fundamental questions will advance novel cancer detection and prevention programs.

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DISCLOSURE

The authors have declared no conflicts of interest.

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