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Review

A recipe for chaos: Extrachromosomal DNA and the hallmarks of cancer

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SUMMARY

Some aggressive cancers exhibit a level of rapid genome change and therapy resistance that is difficult to explain. Research over the past decade has shown that extrachromosomal DNA (ecDNA) can be the cause. When oncogenic genetic elements untether from chromosomes and no longer follow Mendelian inheritance, genomic chaos and accelerated evolution ensue, generating unique ecDNA biology and non-traditional therapeutic vulnerabilities distinct from traditional mutation-targeting approaches. Here, we put forward a holistic view where ecDNA is integrated into the broader Hallmarks of Cancer framework to better understand the problem and chart a path forward.

INTRODUCTION

The publication of the Hallmarks of Cancer in *Cell* 26 years ago was a watershed moment. By providing an organizing principle and shared language used by laymen and experts alike, the hallmarks concept has shaped cancer research from fundamental mechanisms to diagnostics and drug discovery. Since that time, the hallmarks have continued to evolve to parse cancer into a series of clear, understandable, and measurable features.[1–4] Recent technological advances and the ability to deploy them to study patients have also yielded some surprises, from rethinking the deterministic role of mutations in cancer to recognizing the differential impacts of mutations, structural variation, and chromosomal alterations to observing the critical interplay between genes, environment, and the immune system in cancer formation and progression. It may be time to again revisit the hallmarks of cancer in light of these new developments, including the increasingly recognized role for biological processes whose role in cancer was not previously appreciated. Extrachromosomal DNA (ecDNA) is one of them.

The past decade has revealed a surprisingly important role for ecDNA and its non-Mendelian inheritance in cancer. The application of modern molecular techniques to an observation made over 60 years ago has opened a new window and provided

a potentially unifying explanation for long-standing questions in cancer biology, such as why some cancers are so heterogeneous, how they achieve such high levels of oncogene amplification but can change DNA copy number so quickly to resist treatment, and why patients whose tumors have these ecDNAs have significantly shorter overall survival.[5] Beyond existing as a form of gene amplification, ecDNA is also an epigenetic problem that changes how genes are organized, regulated, and expressed. It also appears to exert influence on the tumor microenvironment (TME), playing a role in suppressing innate and adaptive immunity. Thus, the integration of ecDNA into the hallmarks of cancer framework is now warranted. This review will highlight the impact of ecDNA on the hallmarks of cancer, especially genome instability, a crucial enabling feature, in a way that affects many of the hallmarks of cancer. We will discuss what has been learned about ecDNA biology to date, its role in human cancers, and its enabling relationship to the hallmarks of cancer.

EcDNA

EcDNAs are megabase-sized DNA circles that can encode a combination of oncogenes, immune escape genes, and DNA regulatory elements,[6,7] driving high copy-number amplifications that are almost exclusively observed in cancerous but not in normal human genomes. Being unbound by chromosomes, ecDNAs lack centromeres and telomeres, enabling their rapid and uneven segregation into daughter cells through chromosomal hitchhiking at every successive round of mitotic cell divisions, stirring a level of remarkably heterogeneous genetic landscape.[8–10] Aside from driving heterogeneity at the copy-number level, distinct ecDNA species that encode different genetic elements can exist within a single cancer cell, further exacerbating genetic diversity at the genetic content level.[11] Unlike circular mitochondrial DNA (mtDNA) that lacks histone proteins and nucleosome structures, ecDNAs are packaged into highly accessible chromatin with a preponderance of active histone marks.[7] Despite the normal arrangement of the DNA into nucleosomes and an intact domain structure, it became clear that ecDNAs lack the ability to undergo higher-order compaction to exclude the transcriptional machinery. Thus, ecDNAs are powerful units that rewire genetic circuits to maximize oncogene expression, well-proven by the fact that ecDNA-encoded oncogenes are among the top 1% of genes expressed in the cancer genome even when normalized for gene copy number.[7]

Early cytogenetic observations of ecDNAs

Prior to the molecular era, the most common way to interrogate cancer cells was to look at them under a microscope and examine the morphology of their chromosomes during cell division. In 1965, Arthur Spriggs and colleagues did just that and reported "the presence of very small double chromatin bodies" in fresh tumor sample metaphase preparations,[12] which he called double minutes because they were often, although not always, doublets. It turns out that only about 30% of these are doublets,[6] hence the switch to the descriptive nomenclature of ecDNA. At the time, the significance of such ecDNA particles were not clear. Of note, human genomes are laced with small circular DNA elements, including rDNAs, microDNAs,[13] and extrachromosomal circular DNAs (eccDNAs).[14] They are rarely amplified, and most of them do not contain genes. Although eccDNAs may play a role in cancer, they will not be the focus of this perspective.

Between 1979 and 1981, Robert Schimke and colleagues, including Fred Alt, Rodney Kellems, and Daniel Haber, identified an "unstable" form of the amplified dihydrofolate reductase gene on double-minute chromosomes in mouse cancer cells and mouse fibroblasts that was associated with methotrexate resistance.[15–17] Following up on the idea that this could be relevant to human cancer, Garrett Brodeur and colleagues detected double-minute chromosomes in human neuroblastoma samples, and Kari Alitalo, Manfred Schwab, Chien-Chi Lin, Harold Varmus, and Michael Bishop identified c-MYC amplification on double minutes in a human colon cancer cell line,[18] while Alt and colleagues detected *MYCN* amplification on double minutes in a neuroepithelioma cell line.[19] Continued progress was made through the studies of Geoffrey Wahl, Elena Guilioto, Michelle Debatisse, Bernard Malfoy, George Stark et al.,[20,21] and Wahl, along with Dan Van Hoff and others, continued to find evidence to suggest importance in human cancer.[22] However, despite key work from a small number of researchers, including Noriaki Shimizu, Songbin Fu, and Clelia Tiziana Storlazzi, the relative importance of ecDNA remained unclear.[23–27]

As technologies advanced, the field moved from observing cells in metaphase to gene microarrays and then mapping of the entire human genome along with next-generation sequencing, and cancer biologists, unsurprisingly, shifted to reading and interpreting DNA sequences. In this process, the localization of cancer gene alterations became inferential, as short sequencing reads were mapped back to a normal reference genome. EcDNA largely disappeared from the literature, being considered a rare (1.4% of cancers) event of unclear significance.[28]

Chromosomal assumptions—A blind spot for ecDNA

Chromosomes are the terrain on which genes are written, providing the physical unit for genetic inheritance in all three kingdoms of life. Consequently, chromosomal inheritance underlies the most basic assumptions about how genes are passed during cell division. From Boveri's chromosomal theory of cancer[29] to the Philadelphia chromosome,[30–32] chromosomes play an outsized role in our basic understanding of cancer, serving as a reference point for interpreting DNA sequencing studies to decipher the mechanisms of tumor formation, progression, and treatment resistance. Advanced sequencing technologies rely on the implicit assumption that genes are on chromosomes, thereby following the rules of Mendelian inheritance. Advanced DNA sequencing technologies enabled the generation of remarkably detailed cancer genome maps of many different tumor types. To construct these maps, DNA sequence reads are mapped back to the reference human genome coming from normal cells, potentially mistakenly assigning ecDNA-based amplifications to the chromosomes from which the genes arose. Similarly, while techniques to study the clonal evolution of tumors using phylogenetic approaches remain effective for resolving the subclonal architecture based on chromosomal single-nucleotide variants (SNVs) and structural variants (SVs), they

implicitly assume chromosomal inheritance for all genomic elements. This assumption can result in misleading conclusions when inferring the genomic location and rapid evolutionary dynamics of genes amplified on ecDNA, which are key drivers of tumor fitness.

Revisiting the problem with new tools in hand

In 2014, a set of paradoxical observations about glioblastoma, a highly lethal brain cancer, brought the problem of ecDNA into stark new focus. Glioblastomas frequently harbor amplified *EGFRvIII*, a gain-of-function (GOF) deletion mutant of the epidermal growth factor receptor (*EGFR*) oncogene. First, the extent of intratumoral heterogeneity of EGFRvIII protein expression from cell to cell was too great to be easily explained by classical human genetics. It was postulated that this heterogeneity could contribute to treatment resistance, but it was unclear how. Second, when tumor cells that expressed either very high or very low levels of EGFRvIII protein were sorted and put into culture or into a mouse brain, they gave rise to tumor populations that recapitulated the extent of intratumoral heterogeneity found in the original tumor, which again was difficult to understand. Third, when *EGFRvIII*-amplified glioblastoma cells were treated with an EGFR tyrosine kinase inhibitor in a dish, in a mouse, or in patients, they quickly became resistant to treatment by dramatically lowering EGFRvIII levels, which rapidly returned once the drug was removed.[33] Looking at the chromosomes, rather than reading the sequences, unraveled the mystery. *EGFRvIII* was not encoded on chromosomes but rather on ecDNA elements. Treatment with an EGFR tyrosine kinase inhibitor resulted in relatively rapid resistance, concomitant with a large decline in *EGFRvIII* oncogene DNA and protein levels that returned to baseline upon discontinuation of drug treatment.[33] These results indicated that dynamic regulation of oncogene levels on ecDNA could be a major contributor to intratumoral genetic heterogeneity and targeted therapy resistance. Subsequent studies demonstrated that increased copy number of oncogenes amplified on ecDNA can also contribute to targeted therapy resistance, as has been demonstrated by *BRAF* or *NRAS* amplification on ecDNA in tumor cells from melanoma patients and patient-derived xenografts (PDXs) that developed resistance to mitogen-activated protein kinase (MAPK) inhibitors.[34,35]

At this point, the critical gaps in the collective understanding of oncogene amplification became apparent. How often are driver oncogenes amplified on ecDNA in cancer, and does the location of amplification matter? These important questions have now been addressed by leveraging powerful new DNA sequencing, computational, epigenetic, and imaging analysis tools.

EcDNA in human cancers

Through analysis of whole-genome sequencing, structural modeling, and cytogenetic analysis of 17 different cancer types on metaphase spreads, the prevalence of ecDNAs in human cancers was clarified. The most prevalently amplified driver oncogenes in cancer, including *EGFR*, *MYC*, *CCND1*, and *MYCN*, among others, are frequently found on ecDNA, and such ecDNA was not detected in the normal cells studied.[6] Importantly, ecDNA particles lacked centromeres. Mathematical modeling suggested that ecDNA would drive intratumoral genetic heterogeneity, which was empirically verified,[6,8–10] raising the possibility that it could play an important role in accelerated tumor evolution.

The development of AmpliconArchitect[36] and other computational tools made it possible to identify ecDNA from whole-genome sequencing data. With their application to public datasets, the prevalence of ecDNA across human cancers is now being recognized (Figure 1). In a study of sequencing data from nearly 15,000 adult patients of 39 different tumor types, ecDNA was detected in 17.1% of samples, with particularly high frequencies in glioblastoma, sarcomas, breast, lung, upper gastrointestinal (GI) tract, and genitourinary cancers, among others, and was significantly associated with advanced metastatic disease and shorter overall survival,[5] observations that were also identified in other datasets.[37,38] EcDNA is not confined to adult tumors, being similarly found in 18% of medulloblastoma in children, where it is also linked with significantly shorter survival.[39]

EcDNA in some pre-cancerous lesions

Recent work demonstrates that ecDNA can also be found in high-grade dysplasia before the onset of frank cancer[40] or in dysplastic regions adjacent to cancer.[42] In patients with Barrett's esophagus, in two clinical cohorts, ecDNA was detected in high-grade dysplasia, and its presence was tightly linked to the development of esophageal adenocarcinoma.[40] Similarly, the presence of ecDNA in regions of high-grade dysplasia adjacent to urothelial cancers suggests that it is involved in malignant transformation.[42] Houlahan et al. demonstrated that in high-risk estrogen receptor (ER)-positive and human epidermal growth factor receptor 2 (HER2)-positive breast cancers, cyclic ecDNA amplifications were already present in pre-invasive ductal carcinoma *in situ* (DCIS) lesions.[43] The study further revealed a unique mechanistic link where ER signaling induces the formation of R-loops, which subsequently serve as substrates for APOBEC3B-mediated editing and double-stranded breaks. These early structural scars suggest that ecDNA may be established well before invasive progression, acting as a primary engine for replication stress and immune evasion from the disease's inception. In light of these data and the role in transformation demonstrated in mouse genetic models, there is a growing recognition that ecDNA might be more broadly involved in cancer formation, although current knowledge is limited. There is a need to investigate its presence across other pre-invasive cancer types to determine its viability as a screening marker.

EcDNA AND THE HALLMARKS OF CANCER

EcDNA and the unstable genome

The cell has long been considered the unit for studying genome instability and variation. However, for cancers with ecDNA, the unit driving variation can be the ecDNA particle and its subsequent inheritance, as well as the epigenomic, regulatory, and functional consequences. New techniques, including single-molecule methods and live-cell imaging to track ecDNAs over time, have begun to provide unprecedented insight into how ecDNA directly and indirectly impacts several established

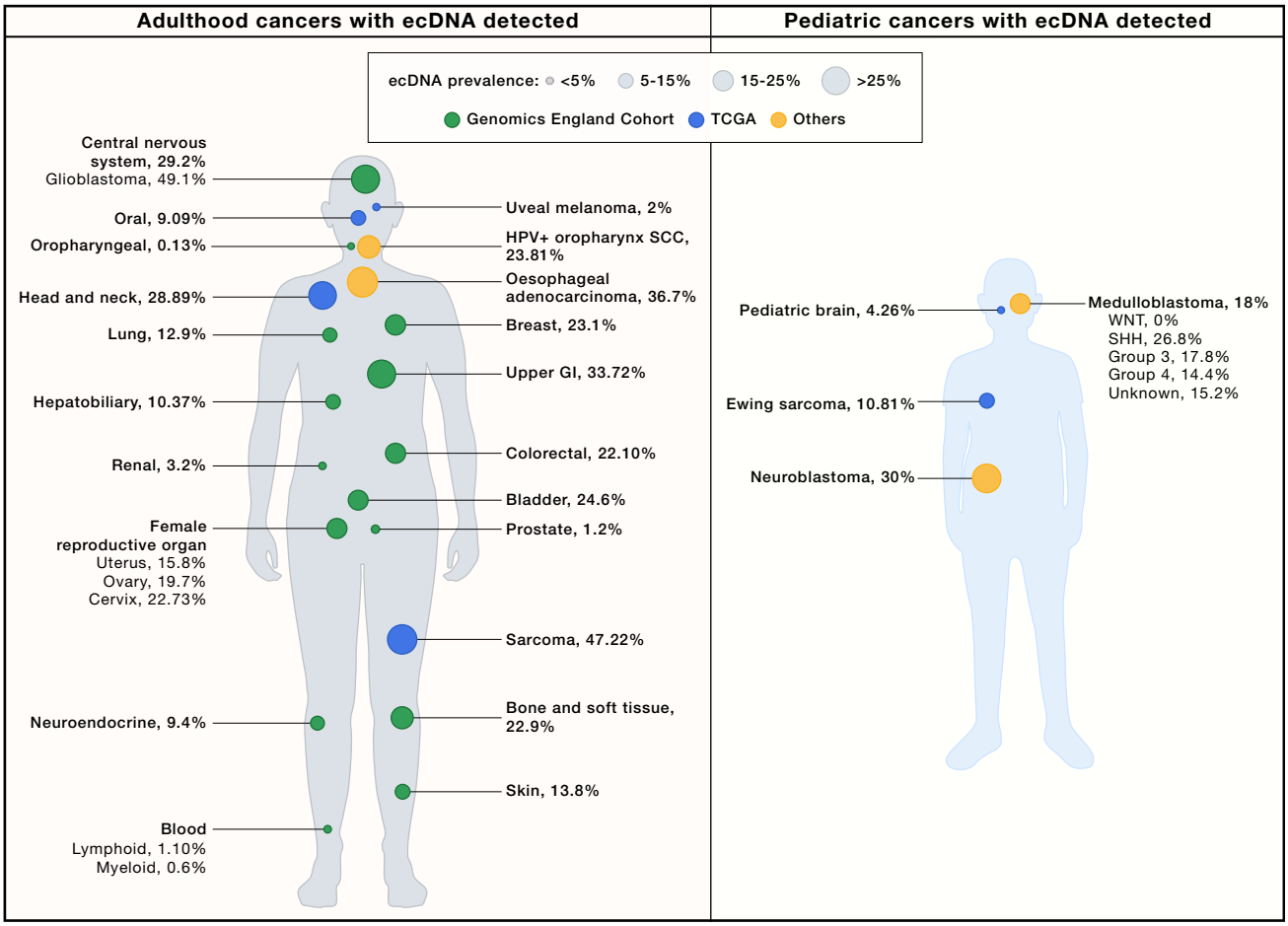

Figure 1. Body map of ecDNA's prevalence in adulthood and pediatric cancers

Body map displaying ecDNA prevalence in adulthood cancers (left) and pediatric cancers (right) reported from the Genomics England cohort,[5] TCGA cohort,[37] and others.[39–41]

hallmarks of cancers, highlighting ecDNA's contribution to fostering genome instability and mutation, sustaining proliferative signaling, evading growth suppressors, and avoiding immune destruction. This section on the life cycle of ecDNA (Figure 2) will explore the fundamental contributions of ecDNAs to the hallmarks of cancer.

EcDNA formation

The concept of genome instability encompasses a variety of different flavors of genomic alterations of DNA in cancer cells, including changes in sequence, copy number, structure, or topology. Remarkably, ecDNA is a potent contributor to each of these components, fundamentally contributing to genome instability and mutation. Genome instability and mutation are not only foundational hallmarks of cancer, but they are also enabling features that lead to the acquisition of other cancer hallmarks.[2] When Schimke and colleagues suggested that ecDNA was an unstable form of gene amplification,[15] they were presciently foreshadowing the pivotal role for ecDNA in promoting genome instability.

The extent of genome instability in ecDNA-containing tumors is vast, because it can be both a cause and a consequence of genome instability. Compelling correlative data suggest that features known to contribute to genome instability may also contribute to the initial formation of ecDNA. In their 2011 update, Hanahan and Weinberg pointed to the importance of a surveillance system that monitors genome integrity, including through the "guardian of the genome" *TP53*[54] and via the DNA maintenance machinery through "caretakers of the genome" with roles in (1) detecting DNA damage, (2) repairing DNA damage, and (3) suppressing mutagenic molecules.[55] Both the "guardians" and "caretakers" are impaired in tumors that have ecDNA. In addition, beyond the guardian's role, *TP53* is a canonical tumor suppressor that negatively regulates cell proliferation.[2] Thus, it serves as a key mechanism to evade growth suppressors. Corroborating ecDNA's contribution to this cancer hallmark, *TP53* loss is significantly associated with ecDNA, particularly in endometrial, renal, and luminal ER-positive breast cancer,[5,45] and also in patients with Barrett's esophagus who develop esophageal adenocarcinoma.[40] In some cancer types, *TP53* loss was not associated with ecDNA, such as in glioblastoma

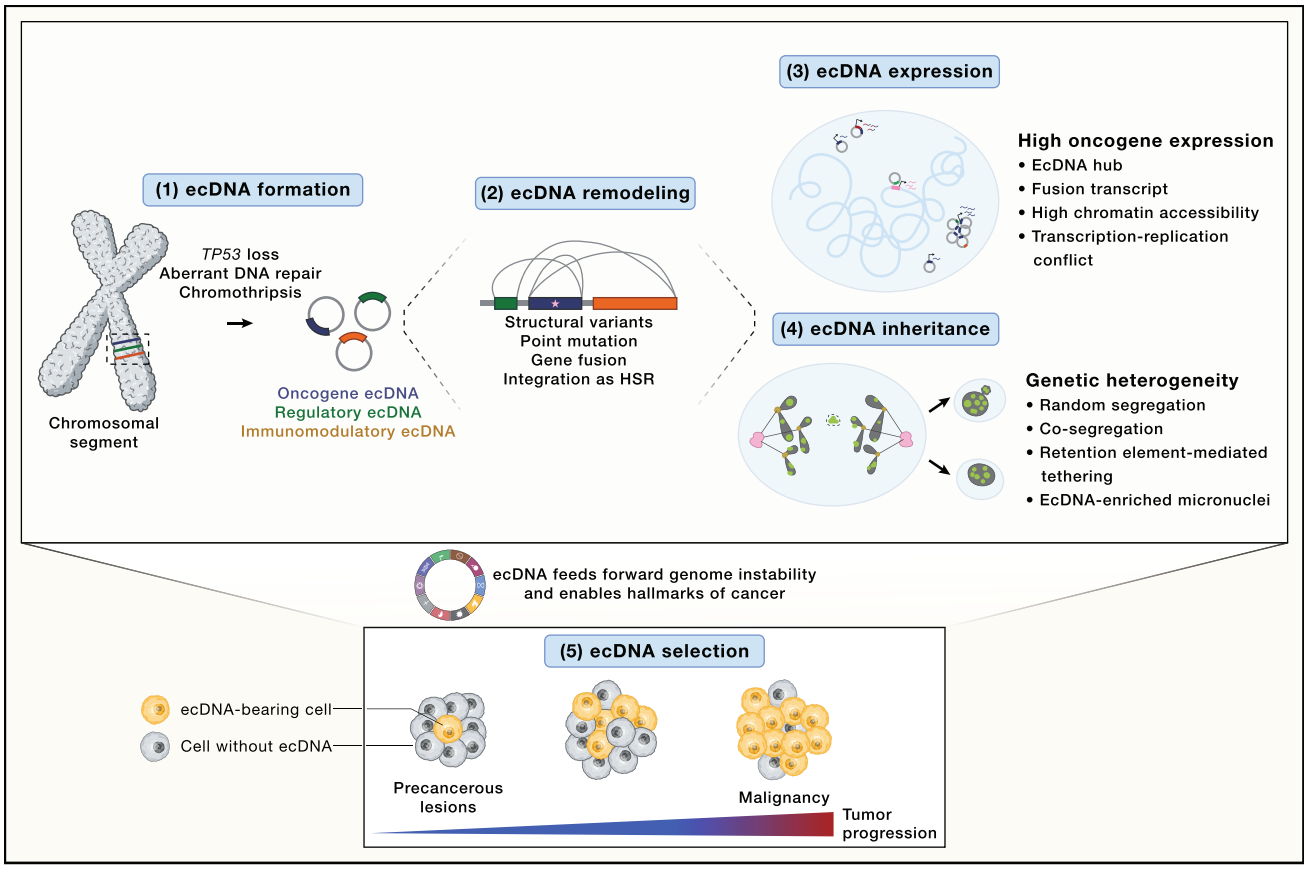

Figure 2. A genome-instability-centric view on ecDNA

EcDNA is both a cause and a consequence of genome instability.

(1) The life cycle of ecDNA begins with permissive genetic backgrounds and aberrant DNA repair events,[5,34,40,44–46] which generate diverse ecDNA species harboring oncogenes, regulatory elements, and immunomodulatory genes.[5,6,47]

(2) Dynamic genetic remodeling occurs on ecDNA, driving further structural rearrangements, resulting in SVs, gene fusions, and mutations.[5,33,48,49]

(3) ecDNA enhances oncogenic expression and rewires gene-gene interactions through unique mechanisms such as hub formation and *trans* gene interactions.[7,49–51]

(4) Its non-Mendelian inheritance,[9] driven by mechanisms like co-segregation principles,[11] retention element-mediated tethering,[52] and ecDNA-enriched micronuclei,[53] allows cancer cells to rapidly and unevenly inherit ecDNAs within a single cell division.

(5) Collectively, these forces promote genome instability and cancer hallmarks, creating a dynamic environment that accelerates tumor heterogeneity, evolution, progression, and drug resistance at an unprecedented pace.

and sarcomas, in which other tumor suppressor losses, such as *CDKN2A* and *NF1*, were detected.[5]

In addition to obvious tumor suppressor losses, the regulation of total genome copy number is often aberrant, with whole-genome doubling being strongly associated with ecDNA formation,[5] underscoring the underlying genome instability of ecDNA-containing cells. Interestingly, ecDNAs are less likely to be observed in tumors with underlying mismatch repair deficiency and DNA polymerase δ 1 or DNA polymerase ε deficiency (POLD1/POLEd).[5] The hypermutated genomic landscape and copy-number alteration status seemingly exist in mutual exclusivity in certain cancers, such as colorectal cancers, where some hypermutated colorectal cancers are relatively chromosomally stable with far fewer somatic copy-number alterations.[56,57] Such mutual exclusivity suggests hypermutation and copy-number alteration could be alternative mechanisms to confer proliferative advantage, which warrants future mechanistic studies to dissect whether they follow distinct evolutionary trajectories that result in functional redundancy.

EcDNA formation arising from paired double-strand breaks usually occurs in the context of a tumor suppressor loss, including *TP53*,[5,40] resulting in selection for ecDNAs bearing a GOF element, such as an oncogene, as illustrated in one type of *DHFR*/methotrexate model.[46] The causative role for paired double-strand breaks in ecDNA formation has been further experimentally established, as demonstrated using CRISPR-C[58] and, more recently, with Cre-Lox to generate ecDNA-driven cancer in autochthonous mouse models.[59] EcDNA has also been shown to arise from chromothripsis, in which shattered pieces of DNA resulting from lagging chromosomes that transit into micronuclei are subject to nuclease attack, after which DNA fragments can ligate into circular ecDNA structures.[46] Genetically engineering the Y chromosome and forcing it to mis-segregate in studies conducted by Peter Ly and Don

Cleveland's labs confirmed that chromothripsis can give rise to ecDNA. Through these ecDNA biogenesis models, specific DNA damage and repair proteins associated with ecDNA formation have been implicated, including those involved in non-homologous end joining,[34,37,46] the Fanconi anemia pathway,[44] and nucleases, including the recently described role for N4PB2 that appears to be important for ecDNA formation from lagging chromosomes in micronuclei.[60] We anticipate that the mechanism of ecDNA formation may vary by tumor tissue type.[5]

EcDNA remodeling

Time-inferred mutational signature analyses strongly suggest that ecDNAs continue to be remodeled after they are formed. This ongoing remodeling further enhances genome instability and mutation on ecDNA itself, which, through selection, can enhance tumor cell fitness. For instance, smoking and apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)-mediated cytosine deamination are frequently found in ecDNA-containing tumors, and signatures of homologous recombination deficiency and APOBEC activity become prominent once ecDNAs have formed.[5] In agreement with these findings, by mapping clustered mutations across 2,583 genomes, Bergstrom et al. found prominent clustered APOBEC3-mediated mutagenesis (kataegis) as a common feature on existing ecDNA particles,[48] resulting in strand-coordinated mutational bursts. In urothelial cancer, APOBEC3-induced mutations act as early clonal drivers, while platinum-based chemotherapy triggers late mutational bursts.[61] Crucially, they found that ecDNA-forming SVs, such as those amplifying *CCND1*, not only persist through systemic therapy but also increase in complexity and copy number. An additional layer of regulatory plasticity has been observed in a study involving paired high-grade serous ovarian cancer (HGSOC) samples, whereby ecDNAs in metastatic sites undergo active DNA demethylation, driving the expression of transposable elements such as long interspersed nuclear element-1 (LINE-1) and pathways associated with aggressive progression.[62]

EcDNAs are also a potent source for structural variation to enhance genome instability. Chromosomal rearrangements and translocations are a well-recognized source of gene fusions such as *BCR-ABL*, activating the biochemical activity of a proto-oncogene (i.e., the ABL tyrosine kinase activity). Until recently, the link between ecDNA and gene fusions was not known. Recent data demonstrate that genomic rearrangements resulting in amplified fusion transcripts are prominent on ecDNA. Integrated analysis of whole-genome and transcriptome sequencing data from cancer samples and cell lines of many different tumor types demonstrated that ecDNAs have the highest rate of oncogene fusion events of any copy-number alterations, establishing ecDNAs as the main source of gene fusions in solid tumors.[49]

EcDNAs can also generate genome instability by integrating into chromosomes as homogenously staining regions (HSRs)[63] that create new *cis*-regulatory interactions. The frequency of ecDNA chromosomal integration and its preferred sites, as well as its causes, are currently not well understood. Importantly, ecDNAs are not the only source for HSR formation. Other forms of amplification, including breakage-fusion-bridges, can also give rise to HSRs.[64]

Amplification of transposable elements on ecDNA generates yet another form of genome instability. 3D mapping of the architecture of *MYC*-amplified ecDNA in colorectal cancer cells demonstrated that transposable elements can be frequently amplified and reactivated on ecDNA and that they have functional enhancer activity that drives transcription.[65] Taken together, it is clear that there are multiple ways that contribute to the ongoing remodeling of ecDNAs in cancer. The rapid selection for ecDNAs that enhance tumor cell fitness is consistent with the finding that ecDNAs are more likely to be retained than chromosomal amplifications in longitudinal samples.[38]

EcDNA expression

As described above, oncogenes borne on ecDNAs, such as *EGFR*, *MYC*, *FGFR2*, *KRAS*, and *CDK4*, are among the top 1% of genes expressed in the cancer genome. The genetic elements encoded on ecDNA often regulate key signaling nodes that drive cancer cells toward ceaseless proliferation, fundamentally contributing to sustained proliferative signaling. EcDNAs are known to increase the transcriptional output of their oncogene cargo by mechanisms distinct from chromosomal DNA. First, ecDNAs have highly accessible chromatin, and such an open chromatin structure is accompanied by the lack of higher-order chromatin compaction. Enhanced ultra-long-range internal regulatory interactions on ecDNA are observed, as the circular topology relocates distal enhancers close to oncogenes in *cis* (also referred to as enhancer hijacking).[7] Second, multiple copies of ecDNAs often come into physical proximity to share regulatory elements.[50] Such cooperative intermolecular interactions of enhancers and gene promoters, which are encoded on ecDNA, facilitate the formation of micron-sized collections of 10 to 100 or more copies of ecDNA molecules in the cell nucleus, which are termed ecDNA hubs.[50] Compared with singleton ecDNAs, ecDNAs in hubs are more transcriptionally active, as revealed by fluorescence *in situ* hybridization (FISH)-based imaging. EcDNA hubs provide cancer cells with a massive transcriptional advantage because ecDNAs that originate from different chromosomes can congregate together and share gene regulatory elements in the same ecDNA hubs, thereby allowing transcriptional rewiring that is otherwise not possible for genes located on different chromosomes.[50] Importantly, targeting proteins that maintain ecDNA hub structure, such as BRD4 through JQ1 inhibition, greatly attenuates their transcriptional advantage. Third, since gene fusions are frequently found on ecDNAs,[49] fusion transcripts arising from these aberrant gene rearrangements may endow cancer cells with a growth advantage by sustaining proliferative signaling. A salient example is the frequent gene fusions linking the long non-coding RNA *PVT1* on the 5′ end to diverse oncogenes at the 3′ end on ecDNAs (e.g., *PVT1-MYC* and *PVT1-CASC11*). *PVT1* 5′ fusion was found to increase the messenger RNA stability of any linked short-lived transcript, revealing a new GOF mechanism to activate MYC and other oncogenes.[49]

Altogether, this form of altered *cis*-regulation, as well as the unique forms of *trans*-regulation described above, has profound effects on ecDNA-mediated gene transcription and cancer genome expression. This heightened transcription, in turn, creates increased opportunity for DNA damage, likely at least in

part through pervasive collisions between replication-transcription machineries on ecDNA that promote replication stress and damage to the ecDNAs themselves,[51] further exacerbating genome instability and mutation.

EcDNA inheritance

One of the most important aspects of ecDNA is what they lack—centromeres. In chromosomal inheritance, spindle fibers attach to centromeres through kinetochores to separate sister chromatids during cell division,[66] ensuring equal segregation of genetic information from mother to daughter cells. Without centromeres to equally distribute genetic information to daughter cells, ecDNAs are randomly segregated, resulting in intratumoral genetic heterogeneity[9] and fostering rapid genome evolution, as tumor cells that contain an ecDNA with elements that enhance fitness are rapidly selected, including resistance to treatment.[9] One can speculate that the random segregation of ecDNA in cancer cells bears some similarities to how genetic information can be asymmetrically inherited in bacteria through circular chromosomes and plasmids. Daughter cells do not always inherit the same DNA as the mother cell during cell division. Consequently, cell populations rapidly evolve, including to drive antibiotic resistance. The analogy is imperfect, in that horizontal transfer of ecDNA has yet to be established, but the concept of rapid evolution driven by unequal inheritance of circular DNA elements provides an illuminating analogy.

As cancer cells with ecDNA divide, the net effect of the numerical and structural evolution on ecDNA is dynamically and unevenly reset simply by how ecDNAs are inherited through non-Mendelian principles,[9] stimulating ongoing genome instability in terms of sequence, copy number, and structure. Of note, individual ecDNAs can contain single or multiple oncogenes, and multiple ecDNA species encoding combinations of oncogenes, immunomodulatory genes, and/or regulatory elements can be found within a single cancer cell.[5,11] Yet paradoxically, if ecDNAs gain a transcriptional advantage by congregating together in ecDNA hubs, random segregation would often break up advantageous combinations of ecDNA species in daughter cells, based on the expectation of chromosomal behavior of independent assortment, known as "Mendel's third law" of genetics. Instead, Hung et al. discovered that ecDNA hubs can be coordinately inherited by daughter cells after cell division, a process termed ecDNA co-segregation, thereby ensuring the continuity of fitness advantages across somatic cell generations.[11] During mitosis, chromosomes are dramatically condensed and cease transcription. By contrast, ecDNAs retain their open chromatin architecture, and the continuation of transcription initiation is required to sustain enhancer-promoter contacts between ecDNA species in ecDNA hubs that allow for their coordinated inheritance. *En masse*, the overall distribution of ecDNA particles remains random, but interacting ecDNA particles co-segregate by staying together during mitosis and being parceled randomly to daughter cells as a unit. This process allows winning combinations of genetic and epigenetic states to be transmitted across cell generations, thereby stabilizing oncogene cooperation that sustains proliferative signaling, fueling rapid tumor evolution under therapeutic pressure.[11] Such unique behavior of ecDNA inheritance may represent a vulnerability for targeted therapeutic strategies.

The absence of centromeres implies that ecDNAs are at risk of being left behind in the cytoplasm with every cell division. This challenge is analogous to the problem faced by DNA viruses, which have evolved specific DNA-binding proteins and viral DNA elements to tether viral episomes onto human chromosomes, a process termed mitotic hitchhiking. Sankar et al. discovered that human ecDNAs possess a family of DNA elements, termed retention elements, that promote the tethering of episomes to mitotic chromosomes.[52] Adding retention elements to heterologous episomes promotes their retention, and inhibiting the activity of retention elements in ecDNAs led to increased untethering and increased ecDNA loss. Notably, retention elements have a natural function: they are gene promoters that uniquely retain transcription factor binding during mitosis, which are termed mitotic bookmarks. When chromosomes condense during mitosis, chromosomal transcription is shut off, but the presence of DNA-binding factors on a subset of genes promotes the re-activation of these genes in the G1 phase of the next cell cycle, thereby propagating gene expression memory. Retention elements in ecDNAs contact enhancer elements on chromosomes that are also mitotically bookmarked. In effect, ecDNA retention is a co-option of the enhancer-promoter contacts involved in mitotic bookmarking.[52] Mitotic retention is critical for ecDNA immortality and thus represents a distinguishing feature of ecDNA biology that may be amenable to therapeutic targeting.

In addition, micronuclei are frequently found in ecDNA-containing cells, consistent with the notion that the presence of micronuclei is a feature of genome instability. Importantly, ecDNA amplicons are more frequently found inside micronuclei structures than chromosomal amplicons.[53] Such ecDNA-enriched micronuclei are often asymmetrically inherited into one of the daughter cells during mitosis, further driving the uneven inheritance of ecDNAs. Altogether, these interactions further enable ecDNAs to break Mendel's law of independent assortment, adding to the surprising tricks that ecDNA uses to generate genetic variation. Non-Mendelian inheritance is a central feature of ecDNA biology, explaining the surprising biology of some of the most aggressive forms of cancer—intratumoral genetic heterogeneity, massive oncogene copy number, rapid genome evolution, and treatment resistance.

EcDNA selection

The vastly heterogeneous genetic landscape facilitated by ecDNAs serves as a key principle, allowing for selection of cells with the best fitness corresponding to its TME. Analysis of ecDNA in human tumors over time[40] reveals that ecDNAs are constantly evolved to further stir genome instability and mutation. Once an oncogene is present in multiple copies of ecDNAs, each copy is subject to mutation and selection for GOF variants. This process does not only give cancer cells more shots on goal to obtain an advantageous mutation but also allows them to keep the elements that have the most GOF mutations, which are usually oncogenes, thus sustaining proliferative signaling. Well-known GOF mutations frequently found in cancer cells, such as EGFRvIII or KRAS(G12C), lock their downstream signaling proteins into a constitutively ON state. While these GOF mutations are also known to be present in ecDNA-

containing cells, it was unclear whether the GOF mutations are located on ecDNAs, chromosomes, or both. Recent development of methods to biochemically separate ecDNAs from chromosomal DNAs, followed by sequencing, confirmed the GOF mutation arises from ecDNA instead of the chromosomal copy.[47] Thus, the elevated copy number and the mutagenic nature of ecDNAs make ecDNA a powerful platform for rapid propagation of GOF mutants that confer cancer cell fitness.[47,67]

The dynamic nature of ecDNAs endows cancer cells with the ability to rapidly respond to stress or resist treatment. EcDNAs allow for rapid, almost real-time, dosage control, shedding the amplified oncogene when under drug pressure and re-amplifying when the drug is removed to regain proliferative signaling.[33] The reversible, dynamic copy-number change driven by ecDNA stands in stark contrast to stable chromosomal amplifications, suggesting that ecDNA could contribute to treatment resistance[11,33] and shorter survival in patients, as has been recently demonstrated.[5] Initially, many researchers suspected that ecDNA was a resistance mechanism that arises once tumors are established, along the lines of how *DHFR* amplification occurs in response to methotrexate treatment or *NRAS* and *BRAF* amplification on ecDNA becomes apparent in melanomas that develop resistance to MAPK inhibitors[34,35] or, for example, in prostate cancers that become resistant to anti-androgen therapy, in which *AR* or *MYC* amplification on ecDNA can be detected.[68] Through PD model systems such as organoids (PDOs) and PDXs, the role ecDNA plays in driving plasticity and treatment resistance has been demonstrated. In pancreatic ductal adenocarcinoma (PDAC), ecDNA is a primary driver of phenotypic plasticity through MYC heterogeneity.[69] By utilizing PDOs, the study demonstrated that *MYC*-bearing ecDNAs allow cancer cells to rapidly adapt to environmental stressors, such as the withdrawal of essential stromal niche factors like WNT. Similarly, in small-cell lung cancer (SCLC), PDX models have linked ecDNAs carrying *MYC*-family genes to acquired resistance against platinum-etoposide and olaparib-temozolomide. Notably, while non-*MYC* ecDNAs appear across all disease stages, ecDNAs harboring *MYC/L/N* amplifications are found almost exclusively in relapsed patients, suggesting they are a specific mechanism for surviving intensive DNA-damaging therapies.[70]

Taken together, these ecDNA-unique features create a powerful recipe for making tumors increasingly diverse through productive forms of genome instability and mutations, allowing ecDNA-bearing tumors to sustain proliferative signaling while evading growth suppressors' control (Figure 2).

The emerging nexus of ecDNA and immune evasion

EcDNA can be a powerful architect of the immune-evasive TME, functioning to avoid immune destruction. Initial pan-cancer genomic and transcriptomic analyses provided the first glimpse into this relationship. By integrating whole-genome sequencing with multi-platform immune infiltration data obtained from over 1,600 TCGA samples, we learned that ecDNAs can amplify immune regulatory genes that are associated with CD8 depletion and show reduced levels of cytotoxic $CD8^+$ T cells and attenuated cytolytic activity in ecDNA-containing tumors compared with their ecDNA-negative counterparts.[71] In addition, these tumors often exhibit a systematic downregulation of the major histocompatibility complex (MHC) class I and II antigen-presenting machinery, suggesting a fundamental defect in immune recognition.[71] A subsequent study employing more stringent ecDNA classification and robust gene feature-selection algorithms has reinforced these findings.[45] These analyses identified a core gene set predictive of ecDNA-associated immune evasion, characterized by the global suppression of lymphocyte activation and T cell-mediated signaling, suggesting a link between ecDNA and immune escape.[45]

While bulk sequencing provided a macroscopic correlation, it often obscured the intricate cellular heterogeneity of the TME. Advancements in single-cell RNA sequencing (scRNA-seq) and spatial profiling technologies have enabled higher-resolution investigations of ecDNA-driven immune evasion. In urothelial carcinoma, single-cell analysis has shown that ecDNA-positive tumors are specifically enriched for immunosuppressive regulatory T cells. Furthermore, these tumors exhibit frequent loss of heterozygosity at histocompatibility leukocyte antigen (HLA) class I loci and downregulated expression of beta-2-microglobulin (B2M), significantly impairing their antigen-presenting capacity.[42] Similar spatial and single-cell patterns have been observed in SCLC, where imaging mass cytometry revealed the physical exclusion of T cells and natural killer (NK) cells from ecDNA-rich tumor regions.[72] Collectively, these studies raise an important hypothesis that ecDNA may act as a TME remodeler.

The mechanisms by which ecDNA facilitates immune evasion likely stem from its high copy number, hyper-accessible chromatin, and unique genetic cargo. As carriers of oncogenes, such as *MYC*, *KRAS*, and *ERBB2*, as well as immunomodulatory genes, ecDNAs leverage their highly accessible chromatin and high copy number to reach elevated expression levels.[5,7] These oncogenes are well-known regulators of the immune response.[73] For example, MYC has been shown to regulate immune evasion, being capable of inducing PD-L1 expression, suppressing type I interferon signaling, and altering the cancer cell secretome to favor immunosuppression.[74]

As discussed above, ecDNAs may carry immunomodulatory genes. This was first highlighted in human papillomavirus (HPV)-associated head and neck cancers, where the *CD274* (PD-L1) gene was found co-amplified on human-viral hybrid ecDNAs.[41] More recent large-scale efforts, such as the Genomics England study, indicate that approximately 34% of ecDNA-containing tumors harbor immunomodulatory genes.[5] Intriguingly, 41.5% of these immunomodulatory ecDNAs lack canonical oncogenes. Tumors harboring immunomodulatory ecDNAs exhibit reduced T cell fraction compared with those containing oncogene-only ecDNAs.[5] This suggests a profound selective pressure to maintain ecDNA species that prioritize extrinsic immune evasion as well as cell-intrinsic proliferative signaling.

While a growing body of evidence supports a correlation between ecDNA and an immune-evasive phenotype, a fundamental question remains unanswered: why do ecDNA-driven cancers display more profound immunosuppression than those harboring other forms of genetic amplification? Other genomic alterations, such as enhancer hijacking due to chromosome translocation, chromosomal amplifications, or even

epigenetic derepression, can similarly drive massive oncogene expression and immunomodulatory gene activity. What is the ecDNA-specific factor that endows this unique immune-evasive capacity?

Addressing this question faces several difficulties. First, traditional clinical data derived from bulk-cell sequencing lacks the resolution to accurately deconvolute the cellular complexity of the TME. Second, while emerging single-cell and spatial profiling technologies offer high-resolution insights, establishing definitive causality in human tissues remains a critical challenge due to a persistent lack of isogenic controls. This makes it difficult to disentangle the specific biological effects of ecDNA from other forms of chromosomal amplification. Finally, although syngeneic mouse models carrying ecDNA-positive tumors are becoming available,[59] we have yet to generate the isogenic controls required for a direct comparison between ecDNA and chromosomal amplification.

A unique opportunity to bridge this causality gap has emerged from genetically engineered mouse models, such as the classic KPfC (*Kras*$^{LSL\text{-}G12D/+}$; *Trp53*$^{fl/fl}$; *Pdx1*$^{Cre/+}$) PDAC model, in which the mutant *Kras* oncogene may spontaneously amplify on ecDNAs or HSRs within the same tumor.[75] After isolating single-cell clones, a recent preprint study[75] demonstrates that although these isogenic cancer cell clones proliferate similarly *in vitro*, ecDNA-containing tumors are significantly more aggressive and establish an immune-evasive TME more rapidly *in vivo*. This divergent behavior is attributed to the random segregation of ecDNAs, which generates a subset of Kras super-expressor cells characterized by very high Kras copy numbers. These super-expressors reprogram the TME by secreting amphiregulin, an EGF-like growth factor that drives the expansion of myofibroblastic cancer-associated fibroblasts and subsequently blocks T cell infiltration.[75] By characterizing ecDNA-driven super-expressors as the primary architects of the TME, these findings offer a framework for studying the causal relationship between ecDNA and immune evasion.

The benefits of ecDNA-driven super-expression come at a metabolic and cellular cost, including elevated replication stress,[75] which is linked to genome instability.[76] To survive this oncogene overdose-induced stress,[77] ecDNA-driven cancers must systematically suppress the cell-intrinsic pathways that would otherwise detect such genomic instability. A key emerging observation is that the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) cytosolic DNA-sensing pathway is frequently silenced in these cancers to prevent the recognition of ecDNA as a pathological danger signal. Unlike stably inherited chromosomes, ecDNAs may occasionally be expelled into the cytosol, likely due to defective segregation during mitosis, a phenomenon recently described in a preprint study.[78] Consequently, another preprint study demonstrates that when cGAS expression is ectopically restored, it selectively recognizes these cytosolic ecDNAs, triggering a robust innate immune response to the ecDNA-positive cancer cells.[79] This selective vulnerability reveals a unique therapeutic opportunity. Leveraging mRNA lipid nanoparticle technology to deliver cGAS can effectively turn the unique genetic signature of ecDNA into a precision-targeted destruction signal, inhibiting ecDNA-containing tumors by utilizing their own genomic instability against them.[79] Interestingly, it was found that activation of the cGAS-STING pathway impedes the formation of ecDNA.[79] Although the molecular mechanism remains to be elucidated, this finding suggests that the cGAS-STING pathway may serve as a barrier to ecDNA-driven tumorigenesis.

These recent studies have begun to reveal the mechanism of ecDNA-mediated immune evasion; however, significant questions remain. Importantly, it remains to be determined whether the expression heterogeneity inherent to different ecDNA-encoded genetic elements universally drives the immune-evasive phenotype observed across various malignancies. Furthermore, it is not yet clear how cancer cells harboring traditional oncogenic ecDNAs might collaborate with those carrying specialized immunomodulatory and inflammatory genes to orchestrate TME remodeling. Beyond the impact of ecDNA-driven cancer on its surroundings, a provocative and unresolved question is whether a pre-existing immunosuppressive microenvironment facilitates the *de novo* formation and clonal expansion of ecDNAs. Addressing these complexities is a non-trivial task, as it will require high-fidelity modeling and precise isogenic controls, using both syngeneic mouse models and PDOs, to definitively disentangle causality from correlation.

EcDNA and other cancer hallmarks

Although the links between ecDNA and some of the other hallmarks of cancer have yet to be fully established, emerging data suggest that ecDNA is associated with most, if not all, of the hallmarks and enabling features (Figure 3). EcDNA represents a radical departure from traditional models of tumor promotion and mutation, driving a hyper-responsiveness to the microenvironment and facilitating rapid adaptation to external stimuli.

EcDNA-containing cells have shown traits indicative of deregulated cellular energetics. For example, glioblastoma cells with *EGFRvIII* amplification on ecDNA are relatively less sensitive to glucose withdrawal-mediated cell death than are their isogenic counterparts in which the amplicon has integrated into chromosomal HSRs, likely due to the ability of ecDNA glioblastoma cells to rapidly modulate oncogene copy number and proliferation rate.[9] Transcriptomic analysis on the TCGA cohort provided much insight on additional deregulated cellular processes of ecDNA-containing cells,[45] revealing their reduced pro-apoptotic gene expression (resisting cell death), enhanced pro-angiogenic gene expression (inducing angiogenesis), elevated proinflammatory gene expression (tumor-promoting inflammation), and upregulated epithelial-mesenchymal transition (EMT) gene expression (activating invasion and metastasis). Although the mechanisms remain to be elucidated, ecDNA is more frequently found in metastatic disease and in tumors that are pre-treated compared with newly diagnosed cancers.[5,38] The observation of ecDNA correlating with late-stage and treated disease has led to work focused on the mutational events that underlie this observation. Moreover, the recent discovery of ecDNA-borne *TERT*-encoding telomerase implies that ecDNA can contribute to the indefinite replication of the host cell,[80] supporting their role in enabling replicative immortality. Ongoing and future research is now poised to further dissect the molecular

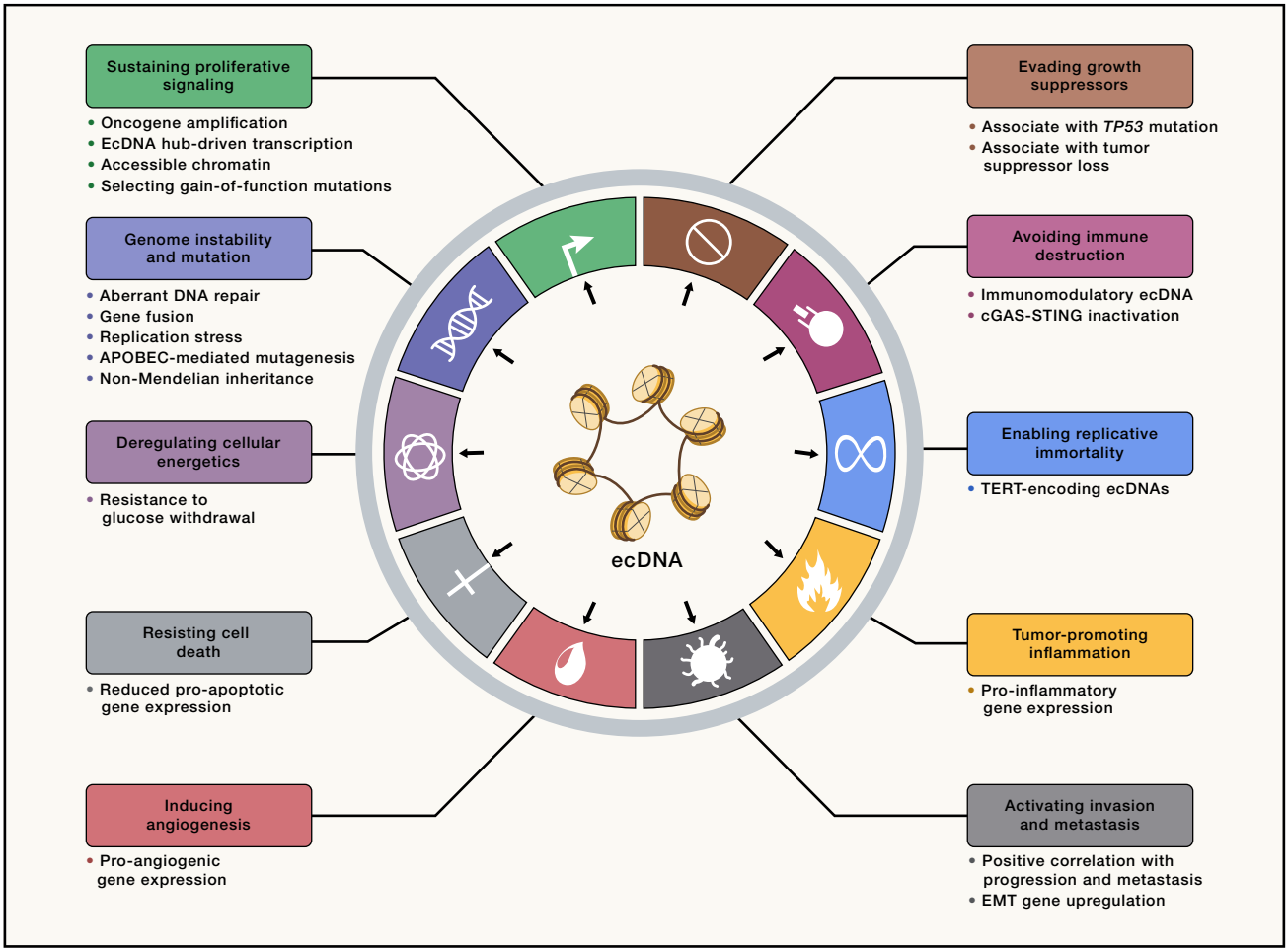

Figure 3. EcDNA can fuel hallmarks of cancer

The illustration depicts hallmarks and enabling characteristics proposed by Hanahan and Weinberg[2] and the contribution of ecDNA to each corresponding feature, which includes (1) genome instability and mutation,[9,34,44,45,48,49,51] (2) sustaining proliferative signaling,[5–7,49,50] (3) evading growth suppressors,[5,40] (4) avoiding immune destruction,[5,45,79] (5) deregulating cellular energetics,[9] (6) resisting cell death,[45] (7) inducing angiogenesis,[45] (8) activating invasion and metastasis,[38,45] (8) tumor-promoting inflammation,[45] and (9) enabling replicative immortality,[80] reported to date that occurs in some cancers (see also Figure 1).

mechanistic underpinnings on how ecDNA precisely drives these cancer hallmarks.

EcDNA as future cancer diagnostics

Driven by the observation that ecDNA is not a static genomic feature but a highly dynamic and resilient engine of somatic evolution, the clinical impact of ecDNA is warranted. Its increased frequency in late-stage and metastatic disease may be a direct reflection of selective advantages, which allow tumors to both persist through the bottleneck of intensive therapy and colonize distant microenvironments. Developing tools to detect ecDNA in blood will be critical for gaining more insight into when ecDNA arises and how it contributes to cancer progression.

Early research[81] suggested that the fragmentation patterns might also contain clues to epigenetic activity in the cell of origin and play a role in the detection of diseased cells, including tumor cells. These ideas have led to the development of methods for identifying circulating tumor DNA (ctDNA) detection using cfDNA sampled from blood. The methods rely on differences in "fragmentomic features," including fragmentation length patterns, location relative to nucleosomes, methylation, and GC content to enrich and/or detect ctDNA.[82,83] Other methods have utilized mutational patterns, including SNPs, copy-number variation, and aneuploidy signatures.[84,85] CtDNA analysis is increasingly utilized for early detection and minimal residual disease monitoring[86] in a variety of solid tumors, including gliomas, breast cancers, metastatic colorectal, metastatic castration-resistant prostate cancer,[85] SCLC,[87] non-SCLC,[88–90] and other cancer subtypes. Together, these developments raise the potential of ecDNA detection using cfDNA from patients.[91] In a recent study, cfChiP-seq (cell-free chromatin immunoprecipitation followed by sequencing) detected highly amplified oncogenes *MYC* and *MYCL* in SCLC patients, consistent with ecDNA observed in matched tumor tissue using whole genome sequencing and AmpliconSuite.[87] EcDNA are typically high in copy number, contain many SVs not found in normal genome, and have highly

accessible chromatin, likely leading to unique fragmentation. Furthermore, plasma cfDNA sequencing using tumor-informed or hybrid-capture approaches can identify focal high-copy amplifications and structural features consistent with ecDNA.[91] Together, these methodologies establish a framework for integrating ecDNA analyses across tissue and blood, with significant implications for prognosis, relapse and metastases prediction, and longitudinal disease monitoring.

The deeper lesson—EcDNA is a hallmark of some cancers

The Hallmarks of Cancer concept, from its initial presentation to its updates, has provided a shared lexicon for describing distinct, measurable features and enabling characteristics of cancer. How we think and communicate about cancer has changed in response, including how we diagnose tumors, what we measure, and even the processes that we aim to target. Therein lies the power of the hallmarks concept and why it so deeply resonates. In this review, we have described research developments and discoveries that have prompted a re-evaluation of some of our core ideas about the molecular basis of cancer and, through doing so, helped to explain some of the most puzzling aspects of its biology. The non-Mendelian inheritance achieved by ecDNA is a powerful concept with profound implications—accelerated evolution—that helps demystify apparently discordant features of cancer. Isn't it odd that a tumor can have a high oncogene copy number while still maintaining high levels of cell-to-cell variation in the expression of that same oncogene? How is it possible that you treat a tumor with a tyrosine kinase inhibitor and the oncogene copy number rapidly drops or rises, and when you remove it, it rapidly comes back to baseline again? Why do people whose cancers have ecDNA do so much worse than most other cancer patients? By framing new discoveries that have addressed these questions within the context of the Hallmarks of Cancer, we begin to see the importance of ecDNA.

Powerful new technologies, from single-cell to single-molecule approaches applied to tissue and blood, will surely change our ability to detect ecDNA, including early in the course of disease development or treatment resistance. As biology unravels, new targets will be uncovered that will likely require the most modern chemistry approaches, from activity-based protein profiling to molecular glues and degraders, for translation into effective treatments for some of the sickest of all cancer patients whose tumor types often fall into the untreatable category.[92] At present, a deeper lesson is clear. Cancers that have ecDNA-generated amplification, close to 20% of all people with cancer and likely more with advanced disease, are different, and the biochemical mechanisms that support ecDNA, which are distinct from other types of genome instability, are at the heart of its genomic chaos. Dare we say, for that subset of tumors, is ecDNA a new hallmark of cancer?

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DECLARATION OF INTERESTS

S.W. is a member of the scientific advisory board of Dimension Genomics. A.G.H. is a founder of Econic Biosciences. Z.J.C. is a scientific advisor of Brii Biosciences and a collaborator with ImmuneSensor Therapeutics. V.B. is a co-founder and serves on the scientific advisory boards of Boundless Bio, Inc. and Abterra, Inc. and holds equity in both companies. M.J.-H. has consulted for Astex Pharmaceuticals, Pfizer, and Achilles Therapeutics; is a member of the Achilles Therapeutics Scientific Advisory Board and Steering Committee; and has received speaker honoraria from Pfizer, Astex Pharmaceuticals, Oslo Cancer Cluster, Bristol Myers Squibb, Genentech, and GenesisCare. M.J.-H. is listed as a co-inventor on a European patent application relating to methods to detect lung cancer (PCT/US2017/028013). This patent has been licensed to commercial entities and, under terms of employment, M.J.-H. is due a share of any revenue generated from such license(s) and is also listed as a co-inventor on the GB priority patent application (GB2400424.4) with the title Treatment and Prevention of Lung Cancer. C.S. acknowledges grants from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Pfizer, Roche-Ventana, Invitae (previously Archer Dx Inc.—collaboration in minimal residual disease sequencing technologies), Ono Pharmaceutical, and Personalis. He is the Chief Investigator for the AZ MeRmaiD 1 and 2 clinical trials and is the Steering Committee Chair. He is also the Co-Chief Investigator of the NHS Galleri trial funded by GRAIL and a paid member of GRAIL's Scientific Advisory Board. He receives consultant fees from Achilles Therapeutics (also an SAB member), Bicycle Therapeutics (also an SAB member), Genentech, Medicxi, China Innovation Centre of Roche (CICoR), formerly Roche Innovation Centre—Shanghai, Metabomed (until July 2022), Relay Therapeutics (SAB member), Saga Diagnostics (SAB member), and the Sarah Cannon Research Institute. C.S. has received honoraria from Amgen, AstraZeneca, Bristol Myers Squibb, GlaxoSmithKline, Illumina, MSD, Novartis, Pfizer, and Roche-Ventana. C.S. has previously held stock options in Apogen Biotechnologies and GRAIL; currently has stock options in Epic Bioscience, Bicycle Therapeutics, and Relay Therapeutics; and has stock options and is a co-founder of Achilles Therapeutics. C.S. declares patent applications for methods to treat lung cancer (PCT/US2017/028013), targeting neoantigens (PCT/EP2016/059401), identifying patient response to immune checkpoint blockade (PCT/EP2016/071471), methods for lung cancer detection (US20190106751A1), identifying patients who respond to cancer treatment (PCT/GB2018/051912), determining HLA loss of heterozygosity (PCT/

GB2018/052004), predicting survival rates of patients with cancer (PCT/GB2020/050221), and methods and systems for tumor monitoring (PCT/EP2022/077987). C.S. is an inventor on a European patent application (PCT/GB2017/053289) relating to assay technology to detect tumor recurrence. This patent has been licensed to a commercial entity, and under their terms of employment, C.S. is due a revenue share of any revenue generated from such license(s). P.S.M., H.Y.C., and B.F.C. are co-founders and advisors of Boundless Bio. P.S.M. is a co-founder of S1 Oncology. H.Y.C. is an employee and stockholder of Amgen as of 16 December 2024. H.Y.C. is a co-founder of Accent Therapeutics, Cartography Biosciences, and Orbital Therapeutics and was an advisor of 10× Genomics, Arsenal Bio, Chroma Medicine, Exai Bio, and Vida Ventures until 15 December 2024.

REFERENCES

1. Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70. https://doi.org/10.1016/s0092-8674(00)81683-9.
2. Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646–674. https://doi.org/10.1016/j.cell.2011.02.013.
3. Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. Cancer Discov. *12*, 31–46. https://doi.org/10.1158/2159-8290.CD-21-1059.
4. Hanahan, D. (2026). Hallmarks of cancer-Then and now, and beyond. Cell. https://doi.org/10.1016/j.cell.2025.12.049.
5. Bailey, C., Pich, O., Thol, K., Watkins, T.B.K., Luebeck, J., Rowan, A., Stavrou, G., Weiser, N.E., Dameracharla, B., Bentham, R., et al. (2024). Origins and impact of extrachromosomal DNA. Nature *635*, 193–200. https://doi.org/10.1038/s41586-024-08107-3.
6. Turner, K.M., Deshpande, V., Beyter, D., Koga, T., Rusert, J., Lee, C., Li, B., Arden, K., Ren, B., Nathanson, D.A., et al. (2017). Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature *543*, 122–125. https://doi.org/10.1038/nature21356.
7. Wu, S., Turner, K.M., Nguyen, N., Raviram, R., Erb, M., Santini, J., Luebeck, J., Rajkumar, U., Diao, Y., Li, B., et al. (2019). Circular ecDNA promotes accessible chromatin and high oncogene expression. Nature *575*, 699–703. https://doi.org/10.1038/s41586-019-1763-5.
8. Lundberg, G., Rosengren, A.H., Håkanson, U., Stewénius, H., Jin, Y., Stewénius, Y., Påhlman, S., and Gisselsson, D. (2008). Binomial mitotic segregation of MYCN-carrying double minutes in neuroblastoma illustrates the role of randomness in oncogene amplification. PLOS One *3*, e3099. https://doi.org/10.1371/journal.pone.0003099.
9. Lange, J.T., Rose, J.C., Chen, C.Y., Pichugin, Y., Xie, L., Tang, J., Hung, K.L., Yost, K.E., Shi, Q., Erb, M.L., et al. (2022). The evolutionary dynamics of extrachromosomal DNA in human cancers. Nat. Genet. *54*, 1527–1533. https://doi.org/10.1038/s41588-022-01177-x.
10. Yi, E., Gujar, A.D., Guthrie, M., Kim, H., Zhao, D., Johnson, K.C., Amin, S.B., Costa, M.L., Yu, Q., Das, S., et al. (2022). Live-Cell Imaging Shows Uneven Segregation of Extrachromosomal DNA Elements and Transcriptionally Active Extrachromosomal DNA Hubs in Cancer. Cancer Discov. *12*, 468–483. https://doi.org/10.1158/2159-8290.CD-21-1376.
11. Hung, K.L., Jones, M.G., Wong, I.T.-L., Curtis, E.J., Lange, J.T., He, B.J., Luebeck, J., Schmargon, R., Scanu, E., Brückner, L., et al. (2024). Coordinated inheritance of extrachromosomal DNAs in cancer cells. Nature *635*, 201–209. https://doi.org/10.1038/s41586-024-07861-8.
12. Cox, D., Yuncken, C., and Spriggs, A.I. (1965). MINUTE CHROMATIN BODIES IN MALIGNANT TUMOURS OF CHILDHOOD. Lancet *1*, 55–58. https://doi.org/10.1016/s0140-6736(65)90131-5.
13. Shibata, Y., Kumar, P., Layer, R., Willcox, S., Gagan, J.R., Griffith, J.D., and Dutta, A. (2012). Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues. Science *336*, 82–86. https://doi.org/10.1126/science.1213307.
14. Noer, J.B., Hørsdal, O.K., Xiang, X., Luo, Y., and Regenberg, B. (2022). Extrachromosomal circular DNA in cancer: history, current knowledge, and methods. Trends Genet. *38*, 766–781. https://doi.org/10.1016/j.tig.2022.02.007.
15. Schimke, R.T., Kaufman, R.J., Alt, F.W., and Kellems, R.F. (1978). Gene amplification and drug resistance in cultured murine cells. Science *202*, 1051–1055. https://doi.org/10.1126/science.715457.
16. Kaufman, R.J., Brown, P.C., and Schimke, R.T. (1979). Amplified dihydrofolate reductase genes in unstably methotrexate-resistant cells are associated with double minute chromosomes. Proc. Natl. Acad. Sci. USA *76*, 5669–5673. https://doi.org/10.1073/pnas.76.11.5669.
17. Haber, D.A., and Schimke, R.T. (1981). Unstable amplification of an altered dihydrofolate reductase gene associated with double-minute chromosomes. Cell *26*, 355–362. https://doi.org/10.1016/0092-8674(81)90204-x.
18. Alitalo, K., Schwab, M., Lin, C.C., Varmus, H.E., and Bishop, J.M. (1983). Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-myc) in malignant neuroendocrine cells from a human colon carcinoma. Proc. Natl. Acad. Sci. USA *80*, 1707–1711. https://doi.org/10.1073/pnas.80.6.1707.
19. Kohl, N.E., Kanda, N., Schreck, R.R., Bruns, G., Latt, S.A., Gilbert, F., and Alt, F.W. (1983). Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell *35*, 359–367. https://doi.org/10.1016/0092-8674(83)90169-1.
20. Vogt, N., Lefèvre, S.-H., Apiou, F., Dutrillaux, A.-M., Cör, A., Leuraud, P., Poupon, M.-F., Dutrillaux, B., Debatisse, M., and Malfoy, B. (2004). Molecular structure of double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in gliomas. Proc. Natl. Acad. Sci. USA *101*, 11368–11373. https://doi.org/10.1073/pnas.0402979101.
21. Stark, G.R., Debatisse, M., Giulotto, E., and Wahl, G.M. (1989). Recent progress in understanding mechanisms of mammalian DNA amplification. Cell *57*, 901–908. https://doi.org/10.1016/0092-8674(89)90328-0.
22. Von Hoff, D.D., McGill, J.R., Forseth, B.J., Davidson, K.K., Bradley, T.P., Van Devanter, D.R., and Wahl, G.M. (1992). Elimination of extrachromosomally amplified MYC genes from human tumor cells reduces their tumorigenicity. Proc. Natl. Acad. Sci. USA *89*, 8165–8169. https://doi.org/10.1073/pnas.89.17.8165.
23. Utani, K., Kawamoto, J.K., and Shimizu, N. (2007). Micronuclei bearing acentric extrachromosomal chromatin are transcriptionally competent and may perturb the cancer cell phenotype. Mol. Cancer Res. *5*, 695–704. https://doi.org/10.1158/1541-7786.MCR-07-0031.
24. Shimizu, N., Hanada, N., Utani, K., and Sekiguchi, N. (2007). Interconversion of intra- and extra-chromosomal sites of gene amplification by modulation of gene expression and DNA methylation. J. Cell. Biochem. *102*, 515–529. https://doi.org/10.1002/jcb.21313.
25. Shimizu, N., Misaka, N., and Utani, K. (2007). Nonselective DNA damage induced by a replication inhibitor results in the selective elimination of extrachromosomal double minutes from human cancer cells. Genes Chromosomes Cancer *46*, 865–874. https://doi.org/10.1002/gcc.20473.
26. Storlazzi, C.T., Lonoce, A., Guastadisegni, M.C., Trombetta, D., D'Addabbo, P., Daniele, G., L'Abbate, A., Macchia, G., Surace, C., Kok, K., et al. (2010). Gene amplification as double minutes or homogeneously staining regions in solid tumors: origin and structure. Genome Res. *20*, 1198–1206. https://doi.org/10.1101/gr.106252.110.
27. Jin, Y., Liu, Z., Cao, W., Ma, X., Fan, Y., Yu, Y., Bai, J., Chen, F., Rosales, J., Lee, K.-Y., et al. (2012). Novel functional MAR elements of double minute chromosomes in human ovarian cells capable of enhancing gene expression. PLoS One *7*, e30419. https://doi.org/10.1371/journal.pone.0030419.
28. Mitelman, F., J.B., and M.F., eds. (2025) Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.
29. Hansford, S., and Huntsman, D.G. (2014). Boveri at 100: Theodor Boveri and genetic predisposition to cancer. J. Pathol. *234*, 142–145. https://doi.org/10.1002/path.4414.
30. Johansson, B., Fioretos, T., and Mitelman, F. (2002). Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol. *107*, 76–94. https://doi.org/10.1159/000046636.

31. Rowley, J.D. (2013). Genetics. A story of swapped ends. Science *340*, 1412–1413. https://doi.org/10.1126/science.1241318.

32. Nowell, P.C. (2007). Discovery of the Philadelphia chromosome: a personal perspective. J. Clin. Investig. *117*, 2033–2035. https://doi.org/10.1172/JCI31771.

33. Nathanson, D.A., Gini, B., Mottahedeh, J., Visnyei, K., Koga, T., Gomez, G., Eskin, A., Hwang, K., Wang, J., Masui, K., et al. (2014). Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science *343*, 72–76. https://doi.org/10.1126/science.1241328.

34. Dharanipragada, P., Zhang, X., Liu, S., Lomeli, S.H., Hong, A., Wang, Y., Yang, Z., Lo, K.Z., Vega-Crespo, A., Ribas, A., et al. (2023). Blocking Genomic Instability Prevents Acquired Resistance to MAPK Inhibitor Therapy in Melanoma. Cancer Discov. *13*, 880–909. https://doi.org/10.1158/2159-8290.CD-22-0787.

35. Xue, Y., Martelotto, L., Baslan, T., Vides, A., Solomon, M., Mai, T.T., Chaudhary, N., Riely, G.J., Li, B.T., Scott, K., et al. (2017). An approach to suppress the evolution of resistance in BRAFV600E-mutant cancer. Nat. Med. *23*, 929–937. https://doi.org/10.1038/nm.4369.

36. Deshpande, V., Luebeck, J., Nguyen, N.D., Bakhtiari, M., Turner, K.M., Schwab, R., Carter, H., Mischel, P.S., and Bafna, V. (2019). Exploring the landscape of focal amplifications in cancer using AmpliconArchitect. Nat. Commun. *10*, 392. https://doi.org/10.1038/s41467-018-08200-y.

37. Kim, H., Nguyen, N.-P., Turner, K., Wu, S., Gujar, A.D., Luebeck, J., Liu, J., Deshpande, V., Rajkumar, U., Namburi, S., et al. (2020). Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. Nat. Genet. *52*, 891–897. https://doi.org/10.1038/s41588-020-0678-2.

38. Kim, H., Kim, S., Wade, T., Yeo, E., Lipsa, A., Golebiewska, A., Johnson, K.C., An, S., Ko, J., Nam, Y., et al. (2024). Mapping extrachromosomal DNA amplifications during cancer progression. Nat. Genet. *56*, 2447–2454. https://doi.org/10.1038/s41588-024-01949-7.

39. Chapman, O.S., Luebeck, J., Sridhar, S., Wong, I.T.-L., Dixit, D., Wang, S., Prasad, G., Rajkumar, U., Pagadala, M.S., Larson, J.D., et al. (2023). Circular extrachromosomal DNA promotes tumor heterogeneity in high-risk medulloblastoma. Nat. Genet. *55*, 2189–2199. https://doi.org/10.1038/s41588-023-01551-3.

40. Luebeck, J., Ng, A.W.T., Galipeau, P.C., Li, X., Sanchez, C.A., Katz-Summercorn, A.C., Kim, H., Jammula, S., He, Y., Lippman, S.M., et al. (2023). Extrachromosomal DNA in the cancerous transformation of Barrett's oesophagus. Nature *616*, 798–805. https://doi.org/10.1038/s41586-023-05937-5.

41. Pang, J., Nguyen, N., Luebeck, J., Ball, L., Finegersh, A., Ren, S., Nakagawa, T., Flagg, M., Sadat, S., Mischel, P.S., et al. (2021). Extrachromosomal DNA in HPV-Mediated Oropharyngeal Cancer Drives Diverse Oncogene Transcription. Clin. Cancer Res. *27*, 6772–6786. https://doi.org/10.1158/1078-0432.CCR-21-2484.

42. Lv, W., Zeng, Y., Li, C., Liang, Y., Tao, H., Zhu, Y., Sui, X., Li, Y., Jiang, S., Gao, Q., et al. (2025). Spatial-Temporal Diversity of Extrachromosomal DNA Shapes Urothelial Carcinoma Evolution and Tumor-Immune Microenvironment. Cancer Discov. https://doi.org/10.1158/2159-8290.CD-24-1532.

43. Houlahan, K.E., Mangiante, L., Sotomayor-Vivas, C., Adimoelja, A., Park, S., Khan, A., Pribus, S.J., Ma, Z., Caswell-Jin, J.L., and Curtis, C. (2025). Complex rearrangements fuel ER+ and HER2+ breast tumours. Nature *638*, 510–518. https://doi.org/10.1038/s41586-024-08377-x.

44. Engel, J.L., Zhang, X., Wu, M., Wang, Y., Espejo Valle-Inclán, J., Hu, Q., Woldehawariat, K.S., Sanders, M.A., Smogorzewska, A., Chen, J., et al. (2024). The Fanconi anemia pathway induces chromothripsis and ecDNA-driven cancer drug resistance. Cell *187*, 6055–6070. https://doi.org/10.1016/j.cell.2024.08.001.

45. Lin, M.S., Jo, S.-Y., Luebeck, J., Chang, H.Y., Wu, S., Mischel, P.S., and Bafna, V. (2024). Transcriptional immune suppression and up-regulation of double-stranded DNA damage and repair repertoires in ecDNA-containing tumors. eLife *12*, RP88895. https://doi.org/10.7554/eLife.88895.

46. Shoshani, O., Brunner, S.F., Yaeger, R., Ly, P., Nechemia-Arbely, Y., Kim, D.H., Fang, R., Castillon, G.A., Yu, M., Li, J.S.Z., et al. (2021). Chromothripsis drives the evolution of gene amplification in cancer. Nature *591*, 137–141. https://doi.org/10.1038/s41586-020-03064-z.

47. Hung, K.L., Luebeck, J., Dehkordi, S.R., Colón, C.I., Li, R., Wong, I.T.-L., Coruh, C., Dharanipragada, P., Lomeli, S.H., Weiser, N.E., et al. (2022). Targeted profiling of human extrachromosomal DNA by CRISPR-CATCH. Nat. Genet. *54*, 1746–1754. https://doi.org/10.1038/s41588-022-01190-0.

48. Bergstrom, E.N., Luebeck, J., Petljak, M., Khandekar, A., Barnes, M., Zhang, T., Steele, C.D., Pillay, N., Landi, M.T., Bafna, V., et al. (2022). Mapping clustered mutations in cancer reveals APOBEC3 mutagenesis of ecDNA. Nature *602*, 510–517. https://doi.org/10.1038/s41586-022-04398-6.

49. Yi, H., Zhang, S., Swinderman, J., Wang, Y., Kanakaveti, V., Hung, K.L., Tsz-Lo Wong, I., Srinivasan, S., Curtis, E.J., Bhargava-Shah, A., et al. (2026). EcDNA-borne structural variants drive oncogenic fusion transcript amplification. Cell *189*, 906–921. S0092-8674(25)01422-9. https://doi.org/10.1016/j.cell.2025.12.009.

50. Hung, K.L., Yost, K.E., Xie, L., Shi, Q., Helmsauer, K., Luebeck, J., Schöpflin, R., Lange, J.T., Chamorro González, R., Weiser, N.E., et al. (2021). ecDNA hubs drive cooperative intermolecular oncogene expression. Nature *600*, 731–736. https://doi.org/10.1038/s41586-021-04116-8.

51. Tang, J., Weiser, N.E., Wang, G., Chowdhry, S., Curtis, E.J., Zhao, Y., Wong, I.T.-L., Marinov, G.K., Li, R., Hanoian, P., et al. (2024). Enhancing transcription-replication conflict targets ecDNA-positive cancers. Nature *635*, 210–218. https://doi.org/10.1038/s41586-024-07802-5.

52. Sankar, V., Hung, K.L., Gnanasekar, A., Wong, I.T.-L., Shi, Q., Kraft, K., Jones, M.G., He, B.J., Yan, X., Belk, J.A., et al. (2026). Genetic elements promote retention of extrachromosomal DNA in cancer cells. Nature *649*, 152–160. https://doi.org/10.1038/s41586-025-09764-8.

53. Brückner, L., Xu, R., Tang, J., Gnanasekar, A., Herrmann, A., Wong, I.T.-L., Zhang, S., Tu, F., Pilon, M., Kukalev, A., et al. (2025). Extrachromosomal DNA micronucleation constrains tumour fitness and improves patient survival. Preprint at bioRxiv. https://doi.org/10.1101/2025.04.15.648906.

54. Lane, D.P. (1992). Cancer. p53, guardian of the genome. Nature *358*, 15–16. https://doi.org/10.1038/358015a0.

55. Kinzler, K.W., and Vogelstein, B. (1997). Cancer-susceptibility genes. Gatekeepers and caretakers. Nature *386*, 761–763. https://doi.org/10.1038/386761a0.

56. Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1997). Genetic instability in colorectal cancers. Nature *386*, 623–627. https://doi.org/10.1038/386623a0.

57. Cancer; Genome; Atlas Network (2012). Comprehensive molecular characterization of human colon and rectal cancer. Nature *487*, 330–337. https://doi.org/10.1038/nature11252.

58. Rose, J.C., Belk, J.A., Wong, I.T.-L., Luebeck, J., Horn, H.T., Daniel, B., Jones, M.G., Yost, K.E., Hung, K.L., Kolahi, K.S., et al. (2025). Disparate Pathways for Extrachromosomal DNA Biogenesis and Genomic DNA Repair. Cancer Discov. *15*, 69–82. https://doi.org/10.1158/2159-8290.CD-23-1117.

59. Pradella, D., Zhang, M., Gao, R., Yao, M.A., Gluchowska, K.M., Cendon-Florez, Y., Mishra, T., La Rocca, G., Weigl, M., Jiao, Z., et al. (2025). Engineered extrachromosomal oncogene amplifications promote tumorigenesis. Nature *637*, 955–964. https://doi.org/10.1038/s41586-024-08318-8.

60. Krupina, K., Goginashvili, A., Baughn, M.W., Moore, S., Steele, C.D., Nguyen, A.T., Zhang, D.L., Koeppel, J., Trivedi, P., Malhotra, A., et al. (2025). Chromothripsis and ecDNA initiated by N4BP2 nuclease

fragmentation of cytoplasm-exposed chromosomes. Science *390*, 1156–1163. https://doi.org/10.1126/science.ado0977.

61. Nguyen, D.D., Hooper, W.F., Liu, W., Chu, T.R., Geiger, H., Shelton, J.M., Shah, M., Goldstein, Z.R., Winterkorn, L., Helland, A., et al. (2024). The interplay of mutagenesis and ecDNA shapes urothelial cancer evolution. Nature *635*, 219–228. https://doi.org/10.1038/s41586-024-07955-3.
62. Sun, R., Li, Z., Liu, Y., Hou, Y., Zhao, B., Chen, C., Fang, J., Guo, C., Zhou, Y., Qu, K., et al. (2025). Landscape of extrachromosomal DNA characteristics in high-grade serous ovarian cancer via long-read sequencing. Cell Rep. *44*, 116343. https://doi.org/10.1016/j.celrep.2025.116343.
63. Song, K., Minami, J.K., Huang, A., Dehkordi, S.R., Lomeli, S.H., Luebeck, J., Goodman, M.H., Moriceau, G., Krijgsman, O., Dharanipragada, P., et al. (2022). Plasticity of Extrachromosomal and Intrachromosomal BRAF Amplifications in Overcoming Targeted Therapy Dosage Challenges. Cancer Discov. *12*, 1046–1069. https://doi.org/10.1158/2159-8290.CD-20-0936.
64. Raeisi Dehkordi, S., Wong, I.T.-L., Ni, J., Luebeck, J., Zhu, K., Prasad, G., Krockenberger, L., Xu, G., Chowdhury, B., Rajkumar, U., et al. (2025). Breakage fusion bridge cycles drive high oncogene number with moderate intratumoural heterogeneity. Nat. Commun. *16*, 1497. https://doi.org/10.1038/s41467-025-56670-8.
65. Kraft, K., Murphy, S.E., Jones, M.G., Shi, Q., Bhargava-Shah, A., Luong, C., Hung, K.L., He, B.J., Li, R., Park, S.K., et al. (2025). Enhancer activation from transposable elements in extrachromosomal DNA. Nat. Cell Biol. *27*, 1914–1924. https://doi.org/10.1038/s41556-025-01788-6.
66. Cleveland, D.W., Mao, Y., and Sullivan, K.F. (2003). Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell *112*, 407–421. https://doi.org/10.1016/s0092-8674(03)00115-6.
67. Noorani, I., Haughey, M., Luebeck, J., Rowan, A., Grönroos, E., Terenzi, F., Wong, I.T.-L., Pradella, D., Lisi, M., Kittel, J., et al. (2025). Extrachromosomal DNA-Driven Oncogene Spatial Heterogeneity and Evolution in Glioblastoma. Cancer Discov. *15*, 2078–2095. https://doi.org/10.1158/2159-8290.CD-24-1555.
68. Watson, P.A., Arora, V.K., and Sawyers, C.L. (2015). Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer. Nat. Rev. Cancer *15*, 701–711. https://doi.org/10.1038/nrc4016.
69. Fiorini, E., Malinova, A., Schreyer, D., Pasini, D., Bevere, M., Alessio, G., Rosa, D., D'Agosto, S., Azzolin, L., Milite, S., et al. (2025). MYC ecDNA promotes intratumour heterogeneity and plasticity in PDAC. Nature *640*, 811–820. https://doi.org/10.1038/s41586-025-08721-9.
70. Pal Choudhuri, S., Girard, L., Lim, J.Y.S., Wise, J.F., Freitas, B., Yang, D., Wong, E., Hamilton, S., Chien, V.D., Kim, Y.J., et al. (2024). Acquired Cross-Resistance in Small Cell Lung Cancer due to Extrachromosomal DNA Amplification of MYC Paralogs. Cancer Discov. *14*, 804–827. https://doi.org/10.1158/2159-8290.CD-23-0656.
71. Wu, T., Wu, C., Zhao, X., Wang, G., Ning, W., Tao, Z., Chen, F., and Liu, X.-S. (2022). Extrachromosomal DNA formation enables tumor immune escape potentially through regulating antigen presentation gene expression. Sci. Rep. *12*, 3590. https://doi.org/10.1038/s41598-022-07530-8.
72. Zhang, J., Jin, Y., Lin, H., Deng, J., Ju, Y., Hu, X., She, J., Liang, Z., Dai, K., Qiu, M., et al. (2025). Amplification of Extrachromosomal MYC Paralogs Shapes Immunosuppressive Tumor Microenvironment in Small Cell Lung Cancer. Clin. Cancer Res. *31*, 4529–4542. https://doi.org/10.1158/1078-0432.CCR-24-3399.
73. van Weverwijk, A., and de Visser, K.E. (2023). Mechanisms driving the immunoregulatory function of cancer cells. Nat. Rev. Cancer *23*, 193–215. https://doi.org/10.1038/s41568-022-00544-4.
74. Dhanasekaran, R., Deutzmann, A., Mahauad-Fernandez, W.D., Hansen, A.S., Gouw, A.M., and Felsher, D.W. (2022). The MYC oncogene - the grand orchestrator of cancer growth and immune evasion. Nat. Rev. Clin. Oncol. *19*, 23–36. https://doi.org/10.1038/s41571-021-00549-2.
75. Qiao, K., Yang, Q.-L., Li, T., Chen, X., Yazgan, Z., Kim, Y.J., Gilbreath, C., Stanley Lim, J.Y., Xie, Y., Sun, X., et al. (2025). ecDNA-driven oncogene super-expressors shape immunoevasive tumor microenvironment. Preprint at bioRxiv. https://doi.org/10.1101/2025.11.15.688565.
76. Saxena, S., and Zou, L. (2022). Hallmarks of DNA replication stress. Mol. Cell *82*, 2298–2314. https://doi.org/10.1016/j.molcel.2022.05.004.
77. Shaw, K., Bernards, R., Stegmaier, K., Varmus, H., and Sellers, W.R. (2025). Prospects for understanding and exploiting the consequences of hyperactivation lethality. Trends Cancer *11*, 619–628. https://doi.org/10.1016/j.trecan.2025.04.009.
78. Xie, Y., Lim, J.Y.S., Liu, W., Gilbreath, C., Sun, X., Qiao, K., Kim, Y.J., and Wu, S. (2026). Cis and Trans Regulatory Mechanisms of ecDNA Segregation. Preprint at bioRxiv. https://doi.org/10.1101/2024.12.31.630921.
79. Li, T., Yang, Q.-L., Qiao, K., Zhang, A., Sun, C., Huang, H., Mischel, P.S., Wu, S., and Chen, Z.J. (2026). Innate immune sensing via the cGAS-STING pathway restricts extrachromosomal DNA–driven tumorigenesis. Preprint at bioRxiv. https://doi.org/10.64898/2025.12.31.697191.
80. Lee, J.J.-K., Salehi, S., Myers, M.A., Williams, M.J., Yao, M.A., Al-Rawi, D.H., Lee, J., Sun, E.G., Thol, K., Choi, S., et al. (2026). Evolution of oncogene amplification across 86,000 cancer cell genomes. Preprint at bioRxiv. https://doi.org/10.64898/2026.02.12.705658.
81. Snyder, M.W., Kircher, M., Hill, A.J., Daza, R.M., and Shendure, J. (2016). Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin. Cell *164*, 57–68. https://doi.org/10.1016/j.cell.2015.11.050.
82. An, Y., Zhao, X., Zhang, Z., Xia, Z., Yang, M., Ma, L., Zhao, Y., Xu, G., Du, S., Wu, X., et al. (2023). DNA methylation analysis explores the molecular basis of plasma cell-free DNA fragmentation. Nat. Commun. *14*, 287. https://doi.org/10.1038/s41467-023-35959-6.
83. Vavoulis, D.V., Cutts, A., Thota, N., Brown, J., Sugar, R., Rueda, A., Ardalan, A., Howard, K., Matos Santo, F., Sannasiddappa, T., et al. (2025). Multimodal cell-free DNA whole-genome TAPS is sensitive and reveals specific cancer signals. Nat. Commun. *16*, 430. https://doi.org/10.1038/s41467-024-55428-y.
84. Widman, A.J., Shah, M., Frydendahl, A., Halmos, D., Khamnei, C.C., Øgaard, N., Rajagopalan, S., Arora, A., Deshpande, A., Hooper, W.F., et al. (2024). Ultrasensitive plasma-based monitoring of tumor burden using machine-learning-guided signal enrichment. Nat. Med. *30*, 1655–1666. https://doi.org/10.1038/s41591-024-03040-4.
85. Knutson, T.P., Luo, B., Kobilka, A., Lyman, J., Guo, S., Munro, S.A., Li, Y., Heer, R., Gaughan, L., Morris, M.J., et al. (2024). AR alterations inform circulating tumor DNA detection in metastatic castration resistant prostate cancer patients. Nat. Commun. *15*, 10648. https://doi.org/10.1038/s41467-024-54847-1.
86. Pantel, K., and Alix-Panabières, C. (2025). Minimal residual disease as a target for liquid biopsy in patients with solid tumours. Nat. Rev. Clin. Oncol. *22*, 65–77. https://doi.org/10.1038/s41571-024-00967-y.
87. Pongor, L.S., Schultz, C.W., Rinaldi, L., Wangsa, D., Redon, C.E., Takahashi, N., Fialkoff, G., Desai, P., Zhang, Y., Burkett, S., et al. (2023). Extrachromosomal DNA Amplification Contributes to Small Cell Lung Cancer Heterogeneity and Is Associated with Worse Outcomes. Cancer Discov. *13*, 928–949. https://doi.org/10.1158/2159-8290.CD-22-0796.
88. Black, J.R.M., Bartha, G., Abbott, C.W., Boyle, S.M., Karasaki, T., Li, B., Chen, R., Harris, J., Veeriah, S., Colopi, M., et al. (2025). Ultrasensitive ctDNA detection for preoperative disease stratification in early-stage lung adenocarcinoma. Nat. Med. *31*, 70–76. https://doi.org/10.1038/s41591-024-03216-y.
89. Black, J.R.M., Karasaki, T., Abbott, C.W., Li, B., Veeriah, S., Al Bakir, M., Liu, W.K., Huebner, A., Martínez-Ruiz, C., Pawlik, P., et al. (2025). Longitudinal ultrasensitive ctDNA monitoring for high-resolution lung cancer risk prediction. Cell *188*, 7083–7098. https://doi.org/10.1016/j.cell.2025.10.020.

90. Normanno, N., Morabito, A., Rachiglio, A.M., Sforza, V., Landi, L., Bria, E., Delmonte, A., Cappuzzo, F., and De Luca, A. (2025). Circulating tumour DNA in early stage and locally advanced NSCLC: ready for clinical implementation? Nat. Rev. Clin. Oncol. *22*, 215–231. https://doi.org/10.1038/s41571-024-00985-w.

91. Behrouzi, R., Clipson, A., Simpson, K.L., Blackhall, F., Rothwell, D.G., Dive, C., and Mouliere, F. (2025). Cell-free and extrachromosomal DNA profiling of small cell lung cancer. Trends Mol. Med. *31*, 64–78. https://doi.org/10.1016/j.molmed.2024.08.004.

92. Wong, I.T.-L., Yi, H., Melillo, B., Cravatt, B.F., Chang, H.Y., and Mischel, P.S. (2026). Targeting extrachromosomal DNA in human cancers. Nat. Rev. Drug Discov. https://doi.org/10.1038/s41573-025-01369-0.