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Review

Aging and Reproductive Cancers: An Integrative View on Cell-Free DNA and Transposable Elements

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Abstract

Aging is one of the strongest risk factors for cancer, and its impact is particularly evident in malignancies of the reproductive system. Ovarian, endometrial, cervical, vulvar, prostate, and penile cancers are mainly diagnosed in older adults and often show different clinical and biological features compared with the same tumors in younger patients. Aging is associated with hormonal changes, immune decline, epigenetic alterations, and accumulation of DNA damage, all of which contribute to cancer development and progression. At the same time, many older patients have frailty and multiple comorbidities, which can limit the use of screening programs and invasive diagnostic procedures. This often leads to delayed diagnosis and worse outcomes. Cell-free DNA (cfDNA) is a minimally invasive biomarker that can be obtained from blood samples and provides molecular information on both tumor and host tissues. Circulating DNA reflects tumor-specific alterations but is also influenced by aging-related changes in DNA release, fragmentation, and methylation. For this reason, aging must be considered when cfDNA-based biomarkers are applied in clinical practice. In this review, we describe how aging influences the biology of reproductive system cancers and how these processes are mirrored in cfDNA profiles. We focus on the clinical use of cfDNA for cancer detection and monitoring in older and fragile patients. Special attention is given to repetitive elements in cfDNA, which are strongly affected by aging and tumor-related epigenetic changes and can be detected with high sensitivity even when the tumor fraction is low. We propose an integrative mechanistic framework in which age-related epigenetic and genomic changes influence both tumor biology and cfDNA composition, with transposable elements acting as a central link between aging and cancer.

Keywords: aging; reproductive cancers; cell-free DNA; liquid biopsy; cancer biomarkers; early detection; frailty; elderly patients; transposable elements

1. Introduction

Cancers of the reproductive system represent an increasing global challenge, particularly as populations age. Across malignancies, cancer risk rises steadily with advancing age, reflecting the gradual accumulation of biological alterations that accompany aging. The average age at cancer diagnosis is now approximately 65.7–66.7 years for men and 64.7 years for women, highlighting the disproportionate impact on older individuals [1,2,3]. This relationship is evident in reproductive system cancers. Ovarian cancer incidence increases sharply after age 45, with the main affected age group ranging between 55 and 75 years [4,5]. Endometrial cancer peaks in postmenopausal women (60–64 years), and mortality rises further in older ages [6]. Cervical cancer, although mainly driven by persistent Human Papillomavirus (HPV) infection, also shows an age-related pattern, as older women are more likely to experience viral persistence and progression [6]. In men, prostate cancer displays one of the clearest age-related trajectories, with most diagnoses occurring after 65 years and influenced by androgen changes, chronic inflammation, and the accumulation of genomic and epigenomic alterations [7,8]. Although some urogenital cancers may appear earlier in life, prostate cancer remains the predominant malignancy of the male reproductive system in older age [9]. Beyond epidemiology, aging profoundly reshapes the biological context in which cancers arise. DNA damage progressively accumulates, DNA repair efficiency declines, and epigenetic regulation drifts toward global hypomethylation and dysregulated gene expression [10,11]. Immune function deteriorates (immunosenescence), reducing the clearance of emerging tumor cells and persistent infections, while aging tissues develop chronic low-grade inflammation (inflammaging), promoting genomic instability and tumor initiation [12]. These age-related processes shape not only cancer risk but also tumor behavior, treatment response, and outcomes in older patients. These hallmarks of aging provide a conceptual framework for this review and are not

reiterated in detail in subsequent sections, where they are discussed in relation to specific mechanisms relevant to reproductive cancers and cfDNA biology.

Early detection is essential for improving survival, yet older adults often face difficulties in accessing or tolerating standard diagnostic procedures. Frailty, multimorbidity, and reduced physiological reserve frequently limit the feasibility of imaging, biopsy, and surgery, emphasizing the need for minimally invasive diagnostic tools [13,14]. Compliance with screening may decline with age, and some tools lack sufficient sensitivity for early disease detection. In this context, cell-free DNA (cfDNA) has emerged as a promising approach. cfDNA consists of short DNA fragments released into the bloodstream from dying or stressed cells during normal cellular turnover and pathological processes, including cancer [15,16]. Cell-free DNA includes fragments derived from both unique and repetitive regions of the genome, reflecting the global chromatin landscape of the cells from which it originates. As it can be obtained through a simple blood draw, cfDNA provides a minimally invasive readout of disease biology and captures signals from multiple tumor sites simultaneously [17]. Advances in cfDNA analysis, including fragmentation patterns and methylation profiling, allow sensitive detection of tumor-derived material. cfDNA integrity indices correlate with tumor burden [18,19,20], whereas tissue-specific methylation signatures enable detection even when tumor-derived cfDNA is scarce [21,22]. In reproductive system cancers, cfDNA biomarkers increasingly distinguish malignant from benign conditions and support earlier diagnosis and longitudinal monitoring. Given the clinical heterogeneity and frequent late-stage presentation of these tumors, cfDNA may be particularly useful in older adults. cfDNA can be viewed as a circulating molecular “diary”, capturing tissue stress, tumor evolution, and the broader influence of aging. While several reviews have examined liquid biopsy in cancer, the role of aging and frailty has received far less attention. Here, aging is treated as a fundamental clinical factor shaping cancer risk, tumor biology, and biomarker performance.

Population aging is one of the most significant demographic trends of recent decades, driven by increased life expectancy and declining fertility rates. Individuals aged ≥60 years now represent a rapidly expanding global demographic, projected to surpass 2.1 billion by 2050 [23]. The oldest-old (≥80 years) show the fastest growth and the highest vulnerability to frailty and chronic disease. This demographic shift is mirrored by disease burden: aging is the strongest risk factor for major chronic conditions, including cancer, cardiovascular disease, metabolic disorders, and neurodegeneration [24]. In oncology, approximately two-thirds of new cancer diagnoses and over 70% of cancer deaths occur in individuals ≥60 years [23], with rising case numbers driven primarily by population aging. Biologically, aging reflects cumulative time-dependent processes that undermine tissue integrity. Somatic mutations accumulate due to replication errors and oxidative damage, whereas DNA repair efficiency declines [10]. Dysregulated apoptosis and senescence permit the persistence of damaged cells [11]. Epigenetic remodeling contributes further instability: aging is associated with global hypomethylation, focal hypermethylation of tumor suppressors, and loss of chromatin organization [10,11]. Immune function declines (immunosenescence), reducing tumor surveillance and impairing viral clearance, while chronic low-grade inflammation (inflammaging) drives oxidative stress and pro-tumorigenic signaling [12]. These changes increase cancer risk and affect tumor progression and treatment outcomes.

Clinically, multimorbidity, polypharmacy, and frailty complicate diagnosis and treatment in older cancer patients [25]. Standard diagnostics, including biopsy and extensive imaging, may be poorly tolerated or infeasible. Tumor evolution and intratumor heterogeneity further complicate management, and a single biopsy often fails to capture dynamic tumor changes, increasing the need for minimally invasive and repeatable biomarkers such as cfDNA [15,17]. In this review, we move beyond a descriptive overview and propose an integrative conceptual framework linking aging biology, transposable element deregulation, and cfDNA-based biomarkers. We hypothesize that aging-associated epigenetic drift, including heterochromatin loss and transposable element derepression, directly influences cfDNA release, composition, and fragmentation patterns. These processes generate circulating DNA signals that reflect both tumor-specific alterations and age-related background noise. Within this framework, transposable element–derived cfDNA emerges as a critical interface between aging and cancer, representing both a diagnostic opportunity and an analytical challenge, particularly in older and frail patients. This review specifically addresses the lack of integration between aging-associated epigenetic drift and the interpretation of cfDNA biomarkers, a gap that remains insufficiently explored in the current literature. In this context, we highlight transposable element–derived cfDNA as a potential amplified biomarker signal in low tumor fraction settings, particularly relevant for aging populations.

2. Age-Associated Cancers of the Reproductive System

To improve conceptual clarity, the manuscript is organized around key biological mechanisms linking aging, cancer, and cfDNA, rather than treating these topics separately. The following section first provides an overview of age-associated reproductive system cancers and then discusses female and male tumors separately to highlight both shared and sex-specific features. Cancers of the reproductive system represent a major and growing component of the global cancer burden in aging populations. As life expectancy increases worldwide, more women and men de-

velop malignancies of the ovary, uterus, cervix, prostate, and other reproductive organs at older ages, a trend driven not only by demographic expansion but also by the biological processes of aging, which progressively alter tissue homeostasis, immune function, and genome stability [10,26]. These tumors, despite their distinct histology and etiology, share several age-linked features: their incidence rises sharply in later decades of life, they often present at advanced stages, and they display considerable molecular and epigenetic heterogeneity due to accumulated DNA damage, epigenetic drift, and immune-mediated selection [10]. As introduced above, aging is characterized by a set of interconnected biological hallmarks that progressively alter tissue homeostasis and increase cancer susceptibility [10,26]. These changes not only increase the likelihood of malignant transformation but also shape tumor behavior and therapeutic response. In women, most gynecologic cancers exhibit a clear age-dependent pattern. Ovarian cancer, although relatively uncommon, is the most lethal gynecologic malignancy and is predominantly diagnosed after menopause, with incidence rising markedly after age 45 and peaking between the sixth and seventh decades of life [4,5]. Endometrial cancer shows an even stronger association with aging, reflecting prolonged unopposed estrogen exposure, metabolic dysfunction, chronic inflammation, and cumulative genomic and epigenomic alterations within the endometrium [6]. Cervical cancer, driven primarily by persistent high-risk HPV infection, also displays age-dependent dynamics: younger women often clear HPV, whereas immune decline and reduced DNA repair capacity in older age favor viral persistence and progression [12]. Less common malignancies such as vulvar and vaginal cancers occur mainly in older women, reflecting long-term epithelial damage, immune dysfunction, and cumulative exposure to oncogenic stimuli [27,28].

A similar age-linked pattern is observed in men. Prostate cancer represents one of the most age-dependent malignancies, with incidence increasing exponentially with advancing age and the highest burden in men over 65 years [7,8]. Age-related alterations in androgen signaling, chronic prostatic inflammation, accumulation of somatic mutations, and widespread epigenetic dysregulation contribute to the stepwise emergence of malignant clones [29,30,31]. Penile cancer remains rare, yet its incidence increases in older age groups, likely due to immune decline, chronic inflammation, and delayed diagnosis [32]. Across sexes, several interconnected biological processes explain why reproductive tissues become more vulnerable with age. Progressive genomic instability increases the probability of oncogenic mutations [10], while epigenetic drift, global hypomethylation coupled with focal hypermethylation of tumor suppressor genes, further destabilizes gene regulation. Immunosenescence impairs tumor immune surveillance and reduces clearance of oncogenic infections, whereas chronic low-grade inflammation (inflammaging) promotes a tumor-supportive microenvironment [12]. Hormonal transitions, such as menopause in women and age-related androgen decline in men, further weaken tissue homeostasis and increase cancer susceptibility.

Clinically, the burden of reproductive system cancers in older adults is strongly influenced by multimorbidity, polypharmacy, and varying degrees of frailty, which complicate diagnostic pathways and therapeutic decision-making [14,33]. Invasive procedures, including biopsy, extensive imaging, or surgical staging, may be poorly tolerated or pose significant risks in very old or frail patients, often leading to delayed diagnosis and suboptimal treatment. In parallel, these cancers evolve dynamically under treatment pressure, generating resistant subclones that are not always captured by a single tumor biopsy. These challenges highlight the need for minimally invasive and repeatable diagnostic strategies, fueling growing interest in liquid biopsy approaches based on circulating biomarkers, particularly cfDNA [15,17].

2.1 Reproductive System Cancers in Women

Cancers of the female reproductive system are strongly influenced by aging and represent a major clinical challenge in older women. In addition to their clinical features, these tumors provide a useful model to understand how aging-related processes such as chronic inflammation, hormonal imbalance, and immune decline influence cfDNA release, fragmentation patterns, and the contribution of transposable element–derived sequences. Their incidence increases clearly after menopause, when hormonal changes, immune decline, and cumulative genomic and epigenomic damage progressively alter tissue homeostasis and cancer susceptibility [10].

Ovarian cancer is the most lethal gynecologic malignancy [34,35] and illustrates well the consequences of age-associated cancer biology. The high mortality rate of ovarian cancer is mainly due to late diagnosis and the occurrence of therapy resistance, significantly worsening patient outcomes [36,37]. Although it represents only a small proportion of all female cancers, most cases are diagnosed after menopause, with the main affected age group ranging between 55 and 75 years of age [4,5]. Early symptoms are often vague and nonspecific, including abdominal bloating, early satiety, pelvic discomfort, or subtle urinary and bowel changes, which frequently lead to delayed diagnosis [38]. As a result, approximately 70% of patients present with advanced-stage disease, when survival remains poor [39]. Aging also contributes to tumor heterogeneity and treatment resistance, complicating clinical management in older patients.

Endometrial cancer shows one of the strongest correlations with aging among gynecologic tumors. Most cases occur in postmenopausal women and are closely linked to prolonged estrogen exposure unopposed by progesterone,

metabolic alterations, and chronic inflammation of the endometrium [6]. These age-related changes promote genomic instability and epigenetic dysregulation in the endometrial epithelium, thereby increasing both cancer risk and the likelihood of aggressive disease in older women [40].

Aging profoundly affects the immune system: as immune competence declines and senescent immune cells accumulate, the capacity for immune surveillance weakens, increasing susceptibility to cancer initiation and progression [12,41]. At the same time, age-associated chronic low-grade inflammation sustains a tumor-promoting microenvironment that further impairs antitumor responses and contributes to disease progression [42]. As a result, a significant proportion of cervical cancer burden and mortality occurs in middle-aged and older women, especially in populations with reduced screening participation [43]. Vulvar and vaginal cancers are less common but are predominantly diagnosed in older women, with incidence increasing markedly after the age of 65. These malignancies are often associated with long-term epithelial damage, chronic inflammatory conditions, and impaired immune surveillance, which together contribute to malignant transformation and delayed clinical detection [44,45]. Overall, female reproductive system cancers clearly demonstrate how aging affects cancer risk, tumor biology, and clinical presentation. Their strong age dependence, frequent late diagnosis, and biological heterogeneity make them particularly suitable models for studying age-adapted diagnostic strategies, including minimally invasive approaches such as cfDNA analysis. From a biomarker point of view, these age-related changes can directly affect cfDNA profiles. Chronic inflammation and increased cell turnover can lead to higher release of cfDNA into the blood, while immune dysfunction may reduce its clearance, causing an accumulation of circulating DNA fragments. At the same time, epigenetic changes, especially global hypomethylation of repetitive elements, may increase the amount of TE-derived sequences in cfDNA. These mechanisms suggest that, in older patients, cfDNA contains not only tumor-related signals but also a background influenced by aging. The main clinical characteristics of these cancers are summarized together with male tumors in Table 1 [4,5,6,7,8,27,28,32].

2.2 Reproductive System Cancers in Men

Cancers of the male reproductive system show a strong association with aging, and their incidence increases clearly in older age groups [46]. These tumors also provide a relevant context to explore how age-associated biological changes, including inflammation, alterations in androgen signaling, and epigenetic drift, shape cfDNA dynamics and the molecular characteristics of circulating DNA, including repeat-derived signals. Among these tumors, prostate cancer represents the most frequent and the most age-dependent malignancy, while penile cancer remains rare but occurs mainly in elderly men. Prostate cancer is one of the clearest examples of a malignancy that develops in close relation with aging. Its incidence rises steadily with age, and most diagnoses occur in men older than 65 years, with the highest disease burden observed in the seventh and eighth decades of life [7,8]. Although many prostate tumors follow an indolent course, a relevant proportion of cases progress to aggressive disease, particularly in older men. Age-related alterations in androgen signaling, chronic inflammation of the prostate, accumulation of somatic mutations, and widespread epigenetic dysregulation are thought to contribute to the gradual emergence of malignant clones over time [29]. These processes also increase tumor heterogeneity, complicating risk stratification and treatment decisions [47]. Large genomic profiling studies have shown that prostate tumors diagnosed in older men more frequently harbor complex copy number alterations, DNA repair defects, and epigenetic abnormalities associated with aggressive clinical behavior and resistance to therapy [48]. In parallel, experimental models have demonstrated that age-related changes in the prostate microenvironment, including increased inflammatory signaling and altered stromal–epithelial interactions, can actively promote the selection of more aggressive tumor clones over time [49]. Clinically, these biological features are associated with a higher risk of progression and metastasis in a subset of elderly patients, despite similar histological grading at diagnosis [50]. From a clinical perspective, prostate cancer in older men often presents significant management challenges. PSA-based screening has contributed to earlier detection, but its interpretation may become more complex in the presence of advanced age, comorbidities, and biological heterogeneity. In this context, clinical decision-making is frequently individualized, particularly in frail patients, for whom invasive diagnostic procedures may be less feasible [33,51]. Penile cancer is uncommon, but its incidence increases with age, and most cases are diagnosed in men older than 60 years [52]. Age-related immune decline, chronic inflammatory conditions, and delayed access to healthcare contribute to this pattern, often leading to diagnosis at advanced stages [53]. Molecular studies have shown that HPV-independent penile cancers, which are more frequent in older men, are driven by chronic inflammation and display more aggressive biological features [54,55]. Clinically, population-based analyses indicate that older patients are more likely to be diagnosed at advanced stages and have poorer outcomes compared with younger men [56]. Overall, male reproductive system cancers illustrate how aging influences cancer risk, tumor biology, and clinical management. Their strong age dependence and diagnostic complexity further highlight the need for minimally invasive and repeatable diagnostic approaches that can better capture tumor dynamics in older and fragile patients. Similarly, in male reproductive cancers, age-related changes such as inflammation, alterations in androgen pathways, and epigenetic remodeling can af-

Table 1. Main clinical characteristics of age-associated reproductive system cancers discussed in the main text.

Cancer	Predominant age range	Key age-related drivers	Typical clinical issue
Ovarian	55–75 [4,5]	Epigenetic drift, menopause	Late diagnosis
Endometrial	>60 [6]	Estrogen imbalance, metabolic stress, chronic inflammation	Comorbidities and treatment risk
Cervical	>50 [6]	HPV persistence, immunosenescence	Screening drop-off
Vulvar	>65 [27,28]	Chronic epithelial damage, chronic inflammation, impaired immune surveillance	Delayed detection
Prostate	>65 [7,8]	Androgen decline, chronic inflammation, somatic mutations, epigenetic dysregulation	Risk stratification difficulty
Penile	>60 [32]	Immune decline, chronic inflammation	Late presentation

"Predominant age range" refers to the main affected age group that accounts for the majority of clinical diagnoses based on current epidemiological data.

fect both tumor biology and cfDNA features. Increased genomic instability and activation of transposable elements may lead to the release of fragmented DNA rich in repetitive sequences, while changes in chromatin structure with age can influence cfDNA fragmentation patterns. These observations suggest that cfDNA signals in older patients should be interpreted within the general biological context of aging. Table 1 summarizes the main clinical characteristics of the reproductive system cancers described in the main text.

Taken together, reproductive system cancers in both women and men share several common aging-related biological mechanisms, despite their tissue-specific differences. These include immunosenescence, chronic low-grade inflammation, epigenetic drift, and progressive genomic instability, all of which contribute to tumor initiation, progression, and heterogeneity. Importantly, these same processes also influence the release, composition, and molecular features of cfDNA, suggesting that age-related systemic changes may represent a common biological background across different tumor types. This shared framework supports the development of broadly applicable cfDNA-based biomarker strategies tailored to aging populations.

2.3 Biological Mechanisms Linking Aging to These Tumors

Building on the general hallmarks of aging introduced above, this section focuses on the specific biological mechanisms most relevant to reproductive tissues and their reflection in cfDNA. Hormonal alterations also play a key mechanistic role in shaping cancer risk and progression at the cellular level in aging reproductive tissues. In reproductive organs, these mechanisms act on tissues that undergo lifelong hormonal modulation and repeated structural remodeling, making them particularly vulnerable to age-related molecular instability. Although these processes contribute to cancer risk across tissues, their impact is particularly pronounced in reproductive organs, where hormonal signaling and tissue remodeling play central roles. One of the central mechanisms linking aging to cancer is the progressive accumulation of genomic instability. With advancing age, DNA damage accumulates because of replication errors, oxidative stress, and environmental exposures, while the efficiency of DNA repair pathways gradually declines. This imbalance increases the likelihood that oncogenic mutations will persist and expand, thereby promoting clonal evolution in reproductive tissues [10]. In parallel, age-related defects in DNA repair and chromosomal maintenance contribute to structural genomic alterations, including copy number changes, which are commonly observed in ovarian, endometrial, and prostate cancers [57]. These alterations often accumulate over decades, reflecting the long latency of reproductive system cancers. Aging is also characterized by profound epigenetic remodeling. Over time, global DNA hypomethylation and focal hypermethylation of tumor suppressor genes progressively alter gene expression programs, a phenomenon often referred to as epigenetic drift [57]. These epigenetic changes are particularly relevant in reproductive system cancers, where they affect hormonal response pathways, cell differentiation, and genome stability [58]. Importantly, age-associated hypomethylation disproportionately affects repetitive elements, contributing to genomic instability and aberrant transcriptional activity, features that are frequently observed in both aged tissues and tumors [59]. Because repetitive elements constitute a large fraction of the genome, their deregulation leaves strong molecular signals that can be detected in circulating DNA. Changes in immune function represent another key link between aging and cancer. Immunosenescence leads to reduced immune surveillance, impaired clearance of premalignant cells, and diminished control of oncogenic viral infections, such as HPV in cervical and penile cancers [12]. At the same time, aging tissues develop a chronic, low-grade inflammatory state known as inflammaging, which promotes oxidative stress, DNA damage, and a tumor-supportive microenvironment. Together, immunosenescence and inflammaging, a concept originally introduced by Franceschi and colleagues, create conditions that not only facilitate cancer initiation but also support tumor progression and immune es-

cape [60]. Hormonal alterations further contribute to age-dependent cancer risk in reproductive organs. In women, menopause is associated with profound changes in estrogen and progesterone signaling, which influence endometrial and ovarian tissue homeostasis [61]. In men, age-related androgen decline and altered androgen receptor signaling affect prostate epithelial biology and may contribute to tumor initiation and progression [62]. These hormonal shifts interact with genomic and epigenomic changes, thereby amplifying vulnerability to malignant transformation in aging tissues [63].

Finally, aging affects the tissue microenvironment, including stromal cells, extracellular matrix composition, and vascular function. Experimental and translational studies have shown that aging of the tissue microenvironment, including increased inflammatory signaling and altered stromal–epithelial interactions, can actively promote tumor growth and the selection of more aggressive clones over time [64]. This concept is particularly relevant for reproductive system cancers, which develop over long periods and are strongly influenced by cumulative microenvironmental changes [65]. Together, these mechanisms explain why reproductive system cancers are strongly linked to aging and exhibit more complex biology in older patients. Although not all molecular alterations are detectable in circulation, a growing body of evidence indicates that many of them can be identified through cfDNA analysis, especially in age-associated cancers. These mechanisms are not only central to tumor biology but are also reflected in circulating cfDNA, where age-related genomic instability, epigenetic remodeling, and TE deregulation may shape both background signals and tumor-derived signatures.

3. cfDNA in Aging and Cancer

Although cfDNA biology is often described from a mechanistic perspective, in aging populations these features have direct clinical relevance, as they can be readily detected in blood and may emerge as early tumor-related signals.

3.1 Origin and Biology of cfDNA

cfDNA refers to extracellular DNA fragments that circulate outside cells [66]. Under physiological conditions, genomic DNA is almost entirely confined to the nucleus, with a smaller fraction in mitochondria [67,68]. Most cfDNA arises as a by-product of cell death, mainly apoptosis and necrosis, although a minor portion derives from active secretion. Through these processes, limited amounts of DNA are released into the bloodstream and interstitial spaces, collectively constituting cfDNA [66,69]. In healthy individuals, cfDNA concentrations are kept low by a tightly regulated balance between DNA release and clearance [70,71]. When this balance is disrupted due to increased release, impaired clearance, or both, cfDNA accumulates and may contribute to the development or progression of pathological conditions [72,73]. Several cfDNA categories have been described based on structural features, including extrachromosomal circular DNA (eccDNA), single-stranded cfDNA (scfDNA), and double-stranded cfDNA (dcfDNA) [74,75]. Depending on their structural state, cfDNA fragments can circulate as freely soluble molecules, be enclosed within extracellular vesicles, or form part of DNA–macromolecule complexes [17,76,77,78]. Another widely used classification is based on biological origin and distinguishes nuclear, mitochondrial, and microbial cfDNA species [79,80,81]. In clinical settings, disease-associated cfDNA subtypes include tumor-derived circulating tumor DNA (ctDNA), fetal cfDNA in maternal plasma (cffDNA), and donor-derived cfDNA (dd-cfDNA) released from transplanted organs [82,83,84,85].

Circulating cfDNA shows substantial variability in structure, conformation, and fragment length, reflecting chromatin organization, histone association, and the mechanisms of release and degradation. A large fraction of plasma cfDNA is present as cell-free nucleosomes, as demonstrated by ChIP-seq approaches that sequence DNA associated with specific histone marks [86,87]. The characteristic modal peak at ~166 bp, together with a recurring 10 bp periodicity, is consistent with nuclease-mediated cleavage between and within nucleosomes during apoptosis and subsequent DNA processing [88,89].

Recent progress in long-read sequencing, including PacBio SMRT and Oxford Nanopore Technologies (ONT), has revealed the presence of cfDNA fragments longer than 500 bp, often appearing in regularly spaced peaks that suggest polynucleosomal structures [90,91]. Their generation has been linked to DNA fragmentation factor subunit beta (DFFB) during apoptosis, as supported by the overrepresentation of A- and G-ended fragments that match DFFB cleavage motifs [90,92,93]. These long fragments are enriched in euchromatic regions and correlate positively with transcriptional activity [71] indicating that nuclear architecture and fragmentation dynamics influence cfDNA release patterns. Beyond nuclear-derived cfDNA, mitochondrial DNA (mtDNA) is also detectable in plasma. mtDNA is generally shorter than nuclear cfDNA [94,95] and lacks nucleosomal organization due to its circular, histone-free structure. Both circular and linear forms are present, with relative abundance depending on tissue origin; for example, liver- and fetal-derived mtDNA are mainly linear, whereas bone marrow–derived mtDNA is predominantly circular [95,96]. Extrachromosomal circular DNA (eccDNA) represents another cfDNA species, identified in healthy individuals, pregnant subjects, and cancer patients [97,98,99]. EccDNA often shows two prominent size peaks around 202 bp and 338 bp, probably corresponding to one or two nucleosomes plus linker DNA [97]. Its formation has been attributed to homologous recombination, replication- and transcription-associated processes, and DNA repair pathways [100].

Single-stranded cfDNA species have also been described. Using magnetic-bead purification and ssDNA library preparation, ultrashort cfDNA fragments of ~50 nt were identified. These fragments are enriched near transcription start sites, promoters, CpG islands, and 5′ UTRs [101,102,103]. Their stability may depend on sequence features such as G-quadruplex formation [101] or protection by transcription factor binding to single-stranded DNA [102], although their precise origin and persistence remain incompletely understood. More recently, improved enrichment protocols have enabled isolation of short double-stranded cfDNA fragments (20–60 bp), which are strongly enriched at transcription factor and chromatin-associated protein binding sites, suggesting that protein–DNA interactions can protect these fragments from plasma degradation [104].

3.2 Age-Related Changes in cfDNA Profiles

Aging is a complex process characterized by gradual and irreversible physiological decline involving multiple organs and systems [105]. This deterioration of physical and cognitive function reflects cumulative alterations in molecular, cellular, and physiological pathways [10,11] and is often associated with increased frailty and heightened susceptibility to environmental and endogenous stressors [106].

During aging, increased release of senescent cells, metabolites, and cellular debris from different tissues triggers immune activation and persistent inflammation [107]. As cfDNA originates from multiple tissues undergoing damage or turnover, it becomes a sensitive readout of overall health status. Because cfDNA release and clearance are highly dynamic, it has emerged as an attractive biomarker for various conditions, including cancer, prenatal disorders, autoimmune diseases, and aging itself [88]. Over the past decades, several studies have examined the relationship between cfDNA and chronological age, supporting its potential role as an aging biomarker [108]. Meddeb et al. [109] reported significantly higher cfDNA levels in healthy older individuals compared with younger controls. Similar findings were observed in studies comparing healthy nonagenarians and younger adults, with cfDNA concentrations positively associated with age and frailty [110]. Ørntoft et al. [111] documented increased cfDNA copy numbers in older participants. Umetani et al. [112] evaluated both cfDNA quantity and integrity, proposing cfDNA integrity as a candidate biomarker, and later work showed that reduced integrity correlated with higher in-hospital mortality in elderly patients with COVID-19 [112,113].

Senescence and cell death are major drivers of increased inflammation in older adults, leading to higher cfDNA release. A meta-analysis showed that elevated total and unmethylated cfDNA levels are associated with systemic inflammation and frailty in nonagenarians [114]. cfDNA also participates in innate immune activation, particularly via TLR9 signaling. In this context, cfDNA can act as a damage-associated molecular pattern (DAMP), interacting with receptors such as RAGE and cooperating with mediators like HMGB1 to enhance inflammatory signaling pathways, including the induction of chemokines such as MCP-1/CCL2 that recruit immune cells [115,116]. Excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), two key mediators of oxidative stress–induced diseases [35,117,118,119,120], induces further DNA damage, thereby enhancing cfDNA release [121]. Higher cfDNA levels have been reported in several age-related diseases, including stroke [122,123], sepsis [124], diabetes [125,126], insulin resistance [127], and chronic kidney disease [128]. Elevated cfDNA has also been associated with in-hospital mortality in middle-aged intensive care patients [129]. In cardiovascular disease, particularly acute coronary syndromes, cfDNA levels rise and correlate with lesion burden and myocardial injury and can help stratify patients at higher risk [130,131,132,133,134].

Aging is also associated with extensive epigenetic remodeling, including global genomic hypomethylation and focal hypermethylation, which contribute to genomic instability and altered cfDNA release. These changes are modulated by lifestyle and environmental factors (smoking, alcohol, physical activity, diet, sex), contributing to interindividual variability in cfDNA profiles [135,136]. Heyn et al. [137] reported global cfDNA hypomethylation with age, while Erichsen et al. [138] observed decreased LINE/Alu methylation in plasma from older individuals. cfDNA nucleosome landscapes also change with age, showing redistribution of heterochromatin and region-specific gains and losses [139]. Li et al. [140] proposed a cfDNA methylation clock capturing age acceleration and health status, showing that older and unhealthy centenarians display higher cfDNA levels and global hypomethylation.

In summary, cfDNA accumulation and qualitative changes (fragment size, structure, fragment ends, sequence features, and genetic and epigenetic marks) represent an inherent feature of aging and provide a rich source of diagnostic information. Given its minimally invasive nature, cfDNA analysis can complement or sometimes surpass traditional diagnostics, enabling early detection, disease classification, prognosis, treatment monitoring, and longitudinal follow-up. These age-related changes in cfDNA quantity and quality are not only biological markers of aging, but also define the background noise against which tumor-derived signals must be detected in older patients.

3.3 cfDNA as a Biomarker in Reproductive System Cancers

Cancer remains a leading cause of death worldwide, making early detection a critical goal [9]. Several screening tests, such as colonoscopy, PSA testing, cervical cytology, and mammography, have improved outcomes but still have limitations [141,142,143,144]. cfDNA, released into the

bloodstream during cell death and other processes, reflects somatic mutations, methylation changes, and fragmentation signatures, thereby providing a minimally invasive tool for early cancer detection [145]. In healthy individuals, cfDNA levels are usually <10 ng/mL, whereas cancer patients show higher and more variable levels, partly due to ctDNA, which often exhibits shorter fragment sizes (~143 bp) [88,146]. cfDNA and ctDNA can be quantified and profiled using ddPCR and NGS, enabling detection of tumor-specific mutations, copy number changes, and methylation patterns [145,147]. ctDNA-based liquid biopsy is now recognized as a minimally invasive approach for early detection, monitoring of tumor heterogeneity, and clonal evolution [148]. Elevated ctDNA correlates with advanced stage, poor prognosis, and disease progression, while declining levels are associated with treatment response and remission [149,150,151,152,153].

In ovarian cancer, ctDNA levels mirror tumor stage, burden, CA125 levels, and imaging findings [154]. ctDNA sequencing has revealed BRCA1/2 reversion mutations in platinum-resistant or refractory disease, clarifying mechanisms of resistance to platinum-based therapies and PARP inhibitors [155,156,157]. Higher ctDNA levels are observed in ovarian cancer compared with benign tumors and healthy controls, and combining ctDNA with CA125 and HE-4 improves diagnostic sensitivity to approximately 90% [158,159]. Several clinical trials are evaluating ctDNA for early detection, minimal residual disease, and treatment monitoring in epithelial ovarian cancer (e.g., NCT06249308, NCT05693987, NCT03691012, NCT06071286).

In endometrial cancer, ctDNA levels correlate with disease stage, progression, and treatment response, sometimes with accuracy >90% [160,161]. Increased ctDNA is often associated with high-risk or advanced tumors [162,163,164,165,166]. Shintani et al. [167] used ddPCR to identify PIK3CA and KRAS mutations in ctDNA, demonstrating the feasibility of mutation-based ctDNA biomarkers in endometrial cancer. Several ongoing trials (e.g., NCT06083779, NCT05049538, NCT05504161) are assessing cfDNA/ctDNA for early detection and prognostic stratification.

Prostate cancer, a highly age-associated malignancy, also exhibits robust ctDNA signals. Higher circulating cfDNA concentrations and altered integrity have been documented in metastatic compared with localized disease [168,169,170,171]. ctDNA can distinguish malignant prostate cancer from benign prostatic hyperplasia [172] and provides valuable information during follow-up [173,174].

Taken together, these data support ctDNA as a versatile, non-invasive biomarker for screening, prognostication, and longitudinal monitoring in reproductive system cancers. Its ability to capture dynamic genomic and epigenomic changes in real time makes cfDNA a key tool for precision oncology, especially in aging and frail populations.

4. Repetitive Element Deregulation in Reproductive Cancers and cfDNA Signatures

From a diagnostic perspective, repetitive elements represent an ideal target for cfDNA-based assays because their abundance and deregulation generate high-amplitude signals even when tumor-derived DNA is scarce. TEs are mobile genomic sequences that exploit host cellular machinery to replicate within eukaryotic genomes. They constitute a substantial fraction of the genome in these organisms [175]. The concept of TEs was first introduced by Barbara McClintock in the 1940s, when she hypothesized that these sequences might function as regulatory components influencing development, differentiation, and even contributing to genome restructuring. At that time, her ideas were met with skepticism, mainly due to the limited technologies available. Only several decades later her work fully recognized, culminating in the Nobel Prize in 1983 for demonstrating that transposition can lead to genomic rearrangements. Nevertheless, many of the broader regulatory functions she had proposed were not conclusively demonstrated at that time [176]. With the development of advanced genomic methodologies, it has become possible to systematically explore the roles of TEs in human development and disease [177,178].

4.1 Classification of Transposable Elements Based on Transposition Mechanisms

Although TEs are highly diverse, they are typically classified according to their transposition mechanisms. DNA transposons, originally discovered by McClintock, represent around 3.4% of the human genome and transpose through a “cut-and-paste” mechanism. These elements are now considered inactive in mammals [179]. Retrotransposons, in contrast, mobilize via a “copy-and-paste” strategy, in which they are first transcribed into RNA and then reverse-transcribed into cDNA that reintegrates into the genome (Fig. 1). In humans, retrotransposons account for more than 90% of all TEs, whereas DNA transposons represent a minor fraction [180].

Retrotransposons are further sub-classified into long terminal repeat (LTR) and non-LTR elements. LTR retrotransposons, including human endogenous retroviruses (HERVs), are characterized by LTR sequences flanking them and require integrase proteins to insert their cDNA into host genomic DNA [181,182]. Non-LTR retrotransposons include LINE-1 (L1) and Alu elements, and they utilize target-primed reverse transcription for replication [181,182,183] (Fig. 1).

Alu elements are primate-specific short interspersed nuclear elements (SINEs) with approximately one million copies, whereas L1 sequences cover about 17% of the genome, despite being present in fewer copies [184]. A defining feature of L1 elements is that full-length copies contain two open reading frames: ORF1, which encodes an RNA-binding protein, and ORF2, which provides reverse

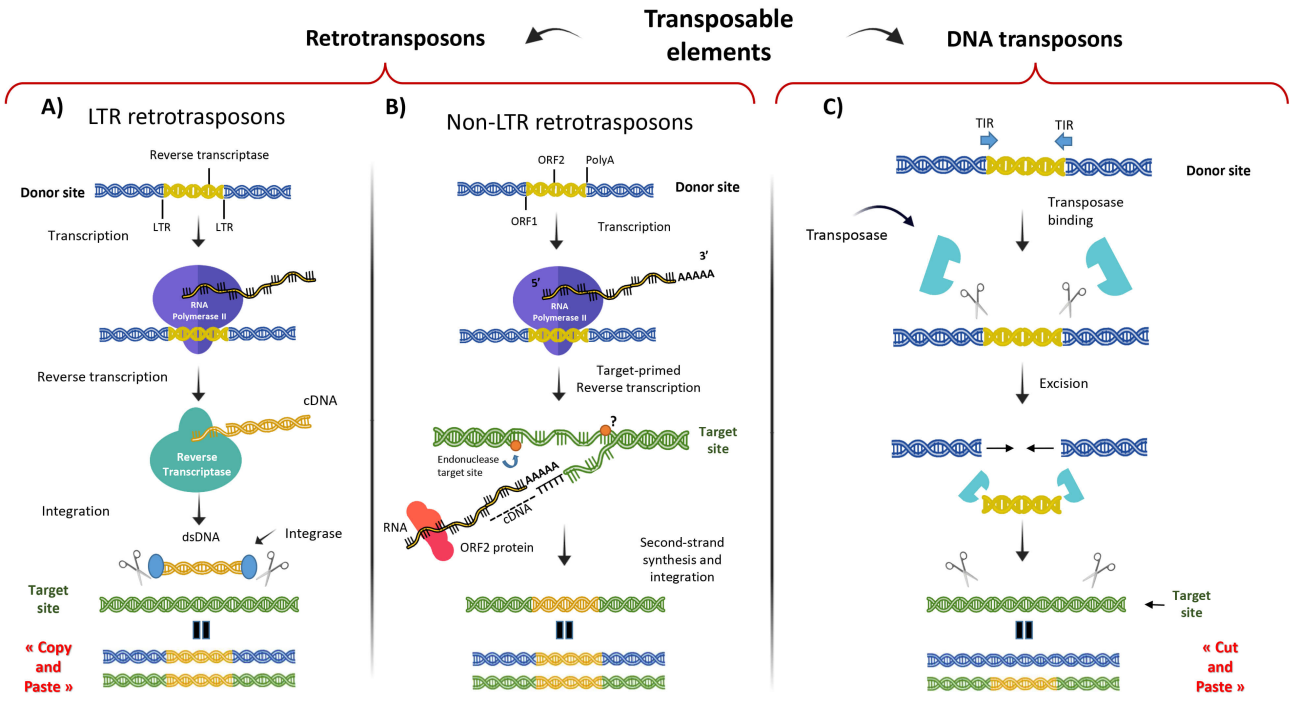

Fig. 1. Classification and transposition mechanisms of human transposable elements. Schematic overview of the major pathways underlying transposable element mobilization. Retrotransposons (A,B) transpose through replicative mechanisms that require reverse transcription of an RNA intermediate. (A) *LTR retrotransposons.* These elements contain two long terminal repeats (LTRs) and encode both reverse transcriptase and integrase, which are essential for retrotransposition. The 5′ LTR harbors a promoter recognized by host RNA polymerase II, which drives transcription of the TE mRNA. Reverse transcriptase then copies this mRNA into a full-length cDNA, and integrase (purple circles) inserts the resulting double-stranded DNA into a new genomic target site. (B) *Non-LTR retrotransposons*. These elements lack LTRs and encode two open reading frames, ORF1 and ORF2. Their mobilization occurs through target-primed reverse transcription (TPRT). As with LTR retrotransposons, transcription by RNA polymerase II produces a full-length mRNA with a 3′ poly(A) tail. This transcript is translated into ORF1p (which acts as an RNA-binding chaperone protecting the transcript from degradation) and ORF2p (with endonuclease and reverse transcriptase activities). The ORF2p endonuclease cleaves the genomic DNA at poly(T)-rich target sequences on both strands; the question mark indicates that the precise mechanism of second-strand cleavage remains unclear. The exposed poly(T) sequence anneals to the RNA poly(A) tail, allowing reverse transcription and cDNA synthesis while the RNA template is degraded. The cDNA free end is then ligated to the endonuclease-generated overhang, followed by second-strand synthesis and repair. (C) *DNA transposons*. These elements are typically flanked by terminal inverted repeats (TIRs; blue arrows) and encode a transposase that mediates a “cut-and-paste” mobilization mechanism (scissors). The transposase binds at or near the TIRs, excises the transposon from its original genomic locus, and inserts it into a new target site.

transcriptase activity, thereby enabling their activity in the human genome [185]. In contrast, Alu elements are non-autonomous and depend on L1-encoded machinery for mobilization. Other active elements in humans include SVA (SINE-R/VNTR/Alu) sequences and some HERV-K copies [186]. Dysregulation of these elements has been increasingly associated with cellular stress, immune activation, and several human diseases, notably age-related disorders and cancer [180,187,188].

TE Activity in the Germline: Rates and Disease Associations

In humans, TEs make up ~45% of the genome, and their expression is tightly regulated during development. They are particularly active in embryonic stem cells, early embryos, blastomeres, pluripotent stem cells, and the brain, where they may contribute to neuronal diversity, while remaining largely epigenetically repressed in differentiated tissues [189,190,191,192,193]. This repression is essential for maintaining genomic stability, given that TE mobilisation can introduce mutations and contribute to genetic variability [194,195].

A subset of TEs remains active, with expression controlled by a fluctuating balance between activation and repression [196]. Active elements include L1 and non-autonomous retrotransposons that depend on L1-encoded proteins [197,198,199]. Estimated germline retrotransposition rates are ~1 in 21 births for Alu [200] and ~1 in 95 births

for L1 [201]. Somatic retrotransposition, once considered rare, is now recognized in multiple organisms [202]. In humans, L1 activity is detected in early embryos, stem cells, and neural tissues [191,192,203,204,205], although mapping somatic insertions remains technically challenging and often requires single-cell approaches [206,207,208].

Somatic TE activity is also common in cancers, where tumors may accumulate hundreds of new L1 insertions [209,210,211,212]. These events arise from a small number of highly active "hot" L1 loci that vary across individuals, tumor types, and stages of clonal evolution [212,213]. Although some *de novo* insertions disrupt tumor suppressor genes or oncogenes [212], many appear to be incidental [187]. Importantly, host mechanisms that restrict TE activity weaken with aging, potentially increasing TE mobilization later in life [214].

4.2 Aging-Associated Deregulation of the Repeatome

A major driver of functional decline associated with aging is the accumulation of senescent cells, which promotes tissue dysfunction and reduces organismal survival and reproductive fitness. From an evolutionary perspective, aging reflects the age-dependent weakening of natural selection, first proposed by Haldane and later formalized by Medawar, wherein the force of selection decreases after reproductive age [215]. This forms the basis of the mutation accumulation hypothesis, whereby deleterious late-acting mutations persist because purifying selection becomes less efficient, and the antagonistic pleiotropy hypothesis, which posits that traits beneficial early in life may be selected despite detrimental late-life effects [216].

Among genomic factors associated with aging, TEs have gained attention due to their capacity to generate mutations and disrupt cellular processes [68,217,218]. The idea that TEs contribute to aging through cumulative mutagenesis, aligned with error- and damage-based aging theories, was first proposed by Shmookler Reis and Goldstein (1983) [219] and later supported by findings from Vijg et al. (1985) [220]. Building on this, Murray introduced the transposon aging model, suggesting that an exponential rise in TE copies may impair cell and organismal viability by affecting the expression of essential genes [221].

Experimental evidence across species supports this framework: in Drosophila and mice, elevated TE activity affects lifespan by increasing DNA damage and genomic instability [222,223]. More recently, aberrant TE activity has been implicated in age-related neurodegenerative, autoimmune, and neoplastic disorders, all of which negatively affect longevity [224]. Under physiological conditions, TE expression is tightly regulated by epigenetic mechanisms, including DNA methylation, repressive histone marks, and small RNA pathways [225]. However, aging progressively erodes these regulatory mechanisms, resulting in increased TE expression and mobilization. Consistent with this, age-associated TE upregulation, and in some cases active retrotransposition, has been documented across multiple organisms and somatic tissues [226,227,228,229].

Mechanisms of TE Reactivation in Aging

In young and healthy cells, TEs and satellite repeats are efficiently suppressed by a multilayered surveillance system acting at both transcriptional and post-transcriptional levels. These mechanisms include DNA methylation, repressive histone modifications, stable heterochromatin formation, small noncoding RNA pathways, and several restriction factors that collectively prevent aberrant TE transcription and mobilization [224,225]. During aging, this regulatory network progressively deteriorates, leading to a widespread derepression of repetitive genomic regions. One of the earliest and most prominent age-related changes is global DNA hypomethylation, which preferentially affects TE-rich regions and directly contributes to increased retrotransposon transcription and mobilization potential [230,231]. Concurrently, aging is associated with the loss of constitutive heterochromatin, as evidenced by reduced levels of H3K9me3 and decreased recruitment of HP1 at repetitive loci. This chromatin relaxation results in transcriptional activation of LINE elements and satellite repeats [232,233]. In parallel, aging is accompanied by a decline in the fidelity of histone modification maintenance, leading to epigenetic drift and a further reduction of repressive chromatin marks at repeat-rich genomic regions [234,235,236].

These epigenetic alterations are further exacerbated by the accumulation of oxidative DNA damage. Repetitive sequences are particularly vulnerable to such damage due to their sequence composition [237,238]. Aged and senescent cells often exhibit elevated TE transcription together with abortive or incomplete retrotransposition events, leading to the accumulation of cytoplasmic TE-derived nucleic acids, including double-stranded DNA, RNA–DNA hybrids, and double-stranded RNA [239,240]. These nucleic acid species are sensed by pattern recognition receptors such as cGAS–STING and RIG-I-like receptors, triggering type I interferon signaling and pro-inflammatory responses that contribute to chronic sterile inflammation, commonly referred to as inflammaging [240,241,242]. Collectively, these findings support a mechanistic model in which age-associated epigenetic erosion and DNA damage drive the derepression of repetitive elements, promoting genomic instability and sustained activation of innate immune pathways. This persistent inflammatory signaling reinforces cellular senescence and contributes to organismal aging [224].

4.3 Repeatome Alterations in Cancer and Reproductive Malignancies

Cancer cells exhibit greater disruption of the repeatome compared with normal and aged tissues, reflecting extensive epigenetic and chromatin changes linked to malignant transformation. A characteris-

tic feature of cancer genomes is widespread DNA hypomethylation, which predominantly affects repetitive elements, especially L1 retrotransposons, leading to their transcriptional activation and enhanced mobilization [194,243,244,245,246,247,248]. Loss of methylation-driven repression promotes chromatin loosening and breakdown of heterochromatin, particularly in centromeric and pericentromeric regions, causing expansion and irregular transcription of satellite repeats [243,249]. Elevated retrotransposon activity contributes to genomic instability through insertional mutagenesis, double-strand breaks, and structural rearrangements, thereby supporting tumor evolution [211,244].

TEs influence gene expression by inducing transcriptional activation or repression when they integrate into regulatory regions [250,251,252]. ERV insertions upstream of the AMY1 gene, for example, can activate cryptic promoters and drive tissue-specific expression [253]. Somatic retrotransposon insertions occur in approximately 50% of tumors [250], with L1 insertions representing the most common somatic structural variant in esophageal adenocarcinoma and the second most frequent in colorectal and head and neck cancers [254]. In a recent analysis of 1024 lung adenocarcinoma patients, the indel signature 2 (ID2) type, showed short latency and high LINE-1 activity associated with L1 promoter demethylation; L1 reactivation likely contributes to the ID2 mutational signature by regulating the ZNF695 transcription factor of the KZFP5 family [248]. Aberrant L1 mobilization may cause tumor suppressor deletions and oncogene amplifications [255]. Single nucleotide resolution studies indicate that somatic L1 retrotransposition occurs mainly in epithelial tumors, preferentially in hypomethylated regions, affecting cancer-relevant genes and reshaping gene expression [210]. TEs can additionally modulate transcription through epigenetic mechanisms [256], and escape from epigenetic repression, together with hypomethylation and abnormal chromatin states, promotes L1 mobilization in cancers such as pancreatic ductal adenocarcinoma and esophageal squamous cell carcinoma [257].

Findings from gynecological and urogenital cancers underscore the relevance of recurrent repeatome deregulation for tumor biology and biomarker discovery. In ovarian cancer, several recent studies have reported alterations in the expression levels of L1 and ERV-K elements, with differential expression patterns observed also between cisplatin-sensitive and cisplatin-resistant cellular models, suggesting a potential role for these transposable elements in ovarian tumor biology and chemotherapy response [252,258,259,260]. Genome-wide methylome studies in endometrioid adenocarcinoma and uterine papillary serous carcinoma reveal extensive differential methylation, with TEs constituting a substantial fraction of both hypermethylated and hypomethylated regions, highlighting the involvement of specific TE families in endometrial cancer development and enhancer remodeling [261,262] (Fig. 2).

Likewise, hypomethylation of LINE-1 and Alu is frequently observed in cervical cancer and promotes genomic instability [245]. RNA-seq analyses reveal extensive stage-dependent ERV dysregulation, including notable ERV3 protein changes [263]. In prostate cancer, HERV-K gag mRNA and protein are significantly more abundant in malignant than in benign tissue [264]. Collectively, these findings suggest that repeatome alterations represent a common feature of malignant transformation across diverse cancer types rather than tissue-specific phenomena [263,264]. Importantly, these widespread and recurrent repeatome alterations generate molecular patterns that are particularly well suited for detection in plasma, making repetitive elements attractive biomarkers for early and low-shedding cancers.

4.4 Repeat-Based Biomarkers for Early Cancer Detection

Repetitive genomic elements, including Alu, L1 retrotransposons, ERV, and satellite DNA, represent a substantial fraction of the human genome and constitute highly informative targets for ctDNA analysis due to their copy number, genomic dispersion, and sensitivity to cancer-associated epigenetic and structural deregulation [145,265]. At the analytical level, PCR- and ddPCR-based quantification of Alu and LINE-1 elements remains a widely used approach to estimate total cfDNA concentration and fragmentation, exploiting the differential amplification of short (≈90–115 bp) versus long (≈250–300 bp) amplicons to derive cfDNA integrity indices (cfDI) that reflect tumor-associated necrotic DNA release and correlate with tumor burden and clinical outcome [266,267,268]. Beyond quantitative metrics, epigenetic dysregulation of repetitive elements is a central hallmark of cancer, and hypomethylation of LINE-1, Alu, and ERV loci serves as a surrogate of global DNA hypomethylation and chromosomal instability; in plasma, reduced LINE-1 methylation has been repeatedly associated with cancer presence, advanced stage, and poor prognosis, while derepression of ERV loci reflects loss of epigenetic silencing and altered innate immune signaling [59,269,270]. With the advent of next-generation sequencing–based liquid biopsy approaches, repetitive elements are increasingly leveraged as bioinformatic anchors in shallow whole-genome sequencing and fragmentomics, enabling inference of genome-wide copy number alterations, tumor fraction, and nucleosome positioning from ctDNA fragmentation profiles, which show altered end motifs, and non-random breakpoint enrichment across repeat-rich regions [271,272]. In this context, ERV-derived sequences have gained renewed interest, as their aberrant transcription, hypomethylation, and copy number variation in cancer reflect large-scale epigenomic instability; recent studies suggest that ERV and satellite derived ctDNA fragments may contribute to cancer-specific signals detectable in plasma, although their repetitive nature poses challenges for accurate mapping and quantitative standardization [243,249]. Collectively, although repetitive element–

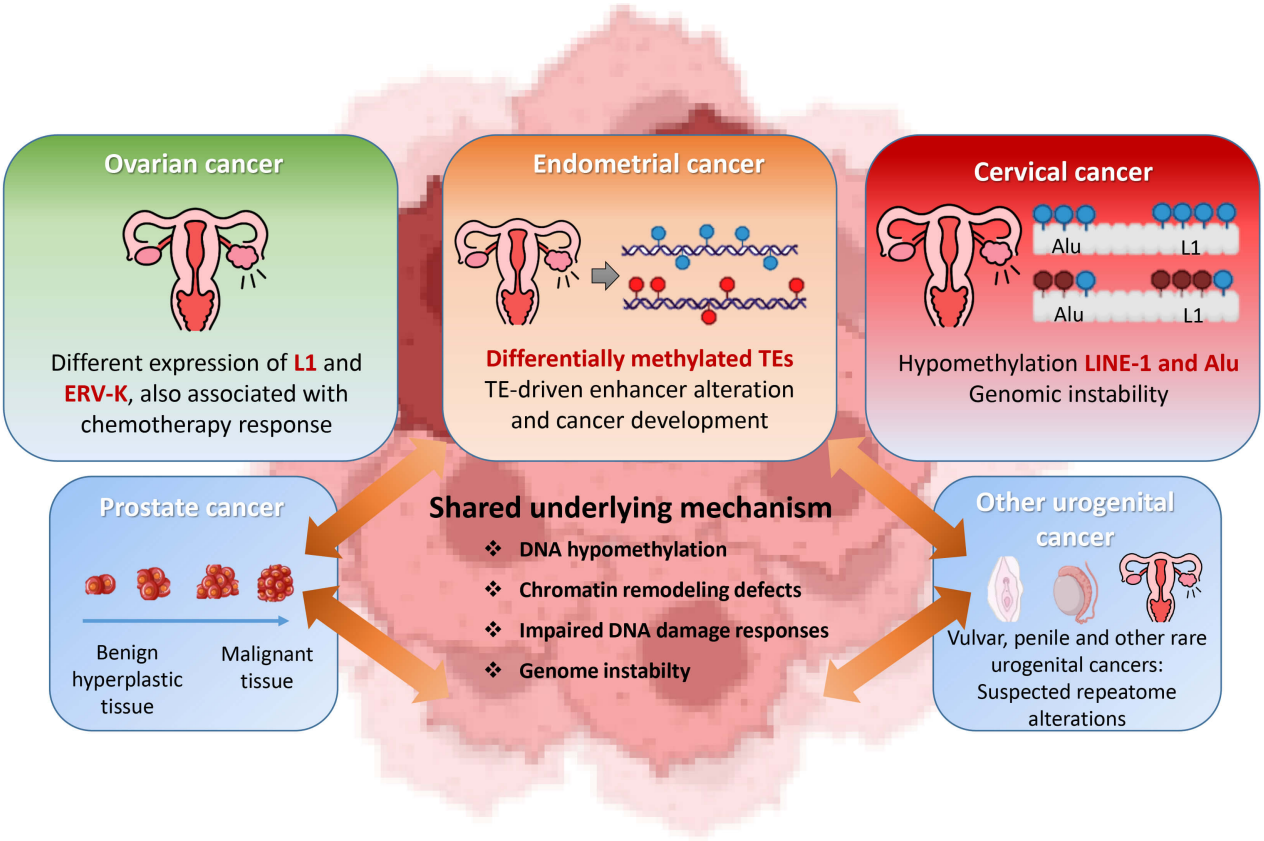

Fig. 2. Repeatome deregulation across reproductive cancers. Overview of major repeatome alterations in urogenital malignancies. Ovarian, endometrial, cervical, prostate, and rare urogenital cancers show recurrent dysregulation of LINE-1, Alu, and ERV elements through hypomethylation, abnormal chromatin remodeling, and altered TE expression. These changes contribute to genomic instability, enhancer rewiring, and tumor-specific transcriptional programs. Shared mechanisms, including DNA methylation loss, chromatin loosening, and impaired DNA-damage responses, indicate that TE deregulation is a common molecular feature across urogenital cancers.

based assays lack single-locus mutational specificity, they provide orthogonal and biologically grounded information on ctDNA abundance, fragmentation dynamics, epigenetic deregulation, retrotransposon reactivation, and chromosomal instability, supporting their integration with mutation- and locus-specific methylation profiling in comprehensive liquid biopsy frameworks [145,265]. Collectively, these findings demonstrate that repeat-based cfDNA biomarkers provide a high-amplitude, biologically grounded signal for early cancer detection and monitoring, complementing mutation-based liquid biopsy strategies and extending their sensitivity to early and low-shedding tumors [145,148]. DNA methylation profiling provides higher sensitivity and tissue specificity, enabling cancer detection even when ctDNA levels are low, but it requires complex analytical pipelines and may be influenced by age-associated epigenetic drift, which can introduce background noise and reduce specificity [137,269]. cfDNA fragmentomics represents a complementary approach, capturing genome-wide fragmentation patterns and nucleosome positioning. While this strategy can detect tumor-derived signals independent of specific mutations, it remains less standardized and may also be affected by age-related changes in chromatin organization and cell turnover [88,271,272]. In this context, repeat-based approaches offer a distinct advantage due to the high copy number and widespread distribution of transposable elements, which generate amplified signals even at low tumor fractions [265]. However, these signals are also highly sensitive to aging-related epigenetic changes, potentially reducing their specificity when used in isolation [59,137]. Taken together, these considerations suggest that no single cfDNA approach is sufficient on its own. Instead, integrative strategies combining mutation detection, methylation profiling, fragmentomics, and repeat-derived signals are likely to provide the most robust and clinically relevant biomarkers, particularly in elderly populations characterized by increased biological variability and lower tumor-derived cfDNA fractions.

4.5 Transposable Elements as Mediators of Aging and Cancer

To provide a more integrative view, we propose a mechanistic framework linking aging-associated heterochromatin loss, transposable element derepression, cy-

tosolic DNA accumulation, cfDNA release dynamics, and activation of innate immune pathways such as cGAS–STING and TLR9. TEs, such as Alu sequences, LINE-1 retrotransposons, and ERVs/HERVs, undergo dynamic epigenetic and transcriptional changes throughout the human lifespan and represent important mediators at the interface between aging and cancer. Aging is associated with a global epigenetic drift, including a progressive hypomethylation of repetitive elements, which contributes to increased retrotransposition activity, genomic instability, and alterations in gene regulatory programs [59,273]. At the same time, age-related reductions in DNA repair efficiency, combined with the accumulation of oxidative DNA damage, further increase the somatic mutational burden and help create a cellular environment that is more permissive to malignant transformation. In cancer, many of these aging-related epigenetic changes become even more pronounced. Hypomethylation of LINE-1, Alu, and ERV sequences frequently correlates with aneuploidy, copy number variation, and aberrant activation of innate immune pathways, suggesting that repetitive elements function not only as contributors to tumor evolution but also as potential biomarkers of oncogenic processes [249,270]. More recent studies have combined repetitive element metrics with epigenetic clock models, such as Horvath's DNAmAge or PhenoAge, to measure biological aging and cancer risk simultaneously. For instance, LINE-1 or Alu methylation levels can be integrated into multivariate epigenetic aging models, improving their predictive ability for both chronological age and susceptibility to cancer [274]. In parallel, analyses of plasma cfDNA show that altered methylation and fragmentation profiles of repetitive elements reflect both accelerated epigenetic aging and early tumor-associated genomic instability. These observations support their use as dual biomarkers for age-related cancer risk and early, potentially preclinical, detection [138,265]. Overall, current evidence suggests that repetitive elements act as molecular integrators of aging and oncogenesis, connecting epigenetic drift, genomic instability, and immune modulation. At the same time, these properties provide a conceptual basis for combining cfDNA-based repeat profiling with epigenetic clocks to identify individuals at higher risk for developing cancer (Fig. 3).

In the context of aging, transposable element–derived cfDNA represents a dual and context-dependent signal. On the one hand, age-associated derepression of transposable elements amplifies repeat-derived sequences in circulation, potentially increasing the sensitivity of cfDNA-based assays, particularly in cancers with low tumor shedding [226,230,265]. This amplification effect may be especially advantageous in older and frail patients, where conventional mutation-based approaches are often limited by low tumor fraction [145]. On the other hand, aging itself is associated with global epigenetic drift and widespread hypomethylation of repetitive elements, leading to increased baseline levels of repeat-derived cfDNA that are not necessarily tumor-specific [10,59,138]. This creates a critical analytical challenge, as age-related background signals may reduce specificity and complicate the interpretation of cfDNA-based biomarkers. To overcome this limitation, integrative analytical strategies are required. These include combining repeat-derived cfDNA signals with mutation-based ctDNA detection to improve specificity [145], integrating DNA methylation profiling to distinguish cancer-associated epigenetic alterations from physiological aging [269,270], and leveraging cfDNA/ctDNA fragmentomics to identify tumor-specific fragmentation patterns [88,271,272]. In addition, machine learning approaches incorporating age as a biological variable may further improve discrimination between tumor-derived and age-related cfDNA/ctDNA signals. Moreover, age-associated accumulation of cytosolic nucleic acids derived from transposable elements may activate innate immune pathways such as cGAS–STING and TLR9, further linking TE derepression, inflammation, and cfDNA dynamics [240,241]. Within this framework, transposable element–derived cfDNA should not be considered a standalone biomarker, but rather a high-amplitude component of a multimodal liquid biopsy strategy, particularly suited for aging populations.

5. Conclusions and Future Perspectives

Aging profoundly shapes the development and progression of reproductive system cancers through genomic instability, epigenetic drift, immune decline, chronic inflammation, and hormonal alterations. In many older and frail patients, standard diagnostic procedures are difficult to perform or poorly tolerated, highlighting the need for minimally invasive and repeatable diagnostic strategies. cfDNA helps address this gap by providing accessible molecular information on mutations, methylation, fragmentation, and genomic instability from blood samples. However, aging itself alters cfDNA levels, integrity, and epigenetic patterns, making age an essential parameter for interpreting circulating biomarkers. Transposable elements, including LINE-1, Alu, and ERVs, generate particularly strong signals in cfDNA. Their age-related hypomethylation, derepression, and enhanced mobilization are further amplified in cancer, creating molecular patterns with high diagnostic potential. Together, these observations support the use of repeat-based cfDNA and ctDNA profiling as a practical diagnostic strategy for aging populations, where early detection must rely on sensitive, non-invasive, and robust molecular signals. Looking ahead, integrating repeat-based metrics with mutational, methylation, and fragmentomic analyses may provide deeper insights into the underlying mechanisms of age-associated reproductive system cancers and enhance our understanding of their biological evolution.

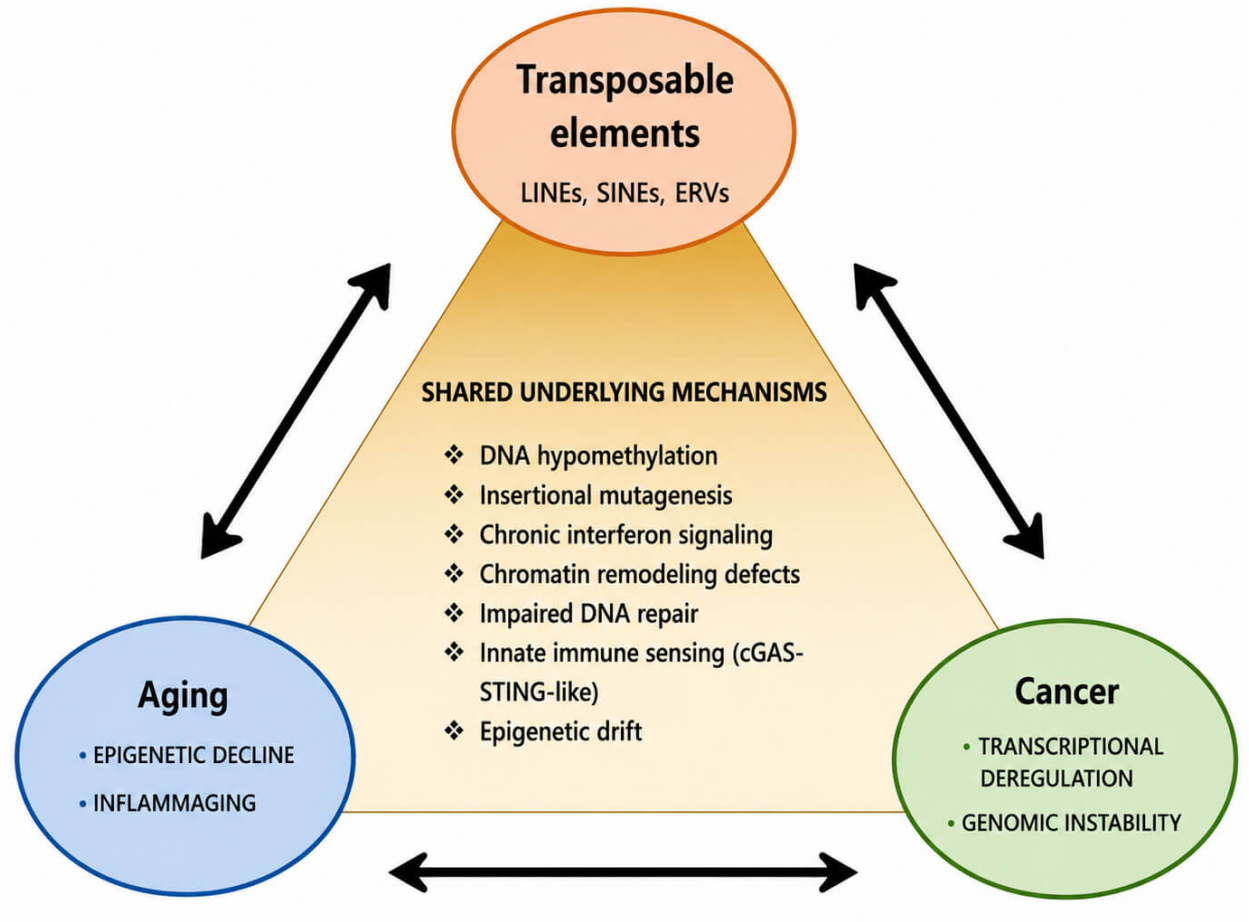

Fig. 3. Conceptual interplay between TEs, aging, and cancer. Schematic representation of the interplay between TEs, aging, and cancer. Shared underlying mechanisms, including DNA hypomethylation, insertional mutagenesis, chronic interferon signaling, chromatin remodeling defects, impaired DNA repair, innate immune sensing, and epigenetic drift, highlight their interconnected biological impact positioning TEs as key molecular bridges between aging and oncogenesis.

Clinical Translation and Challenges in Aging Populations

From a clinical perspective, the translation of cfDNA-based biomarkers into routine practice in aging populations presents several challenges. Older individuals often exhibit increased baseline cfDNA levels due to comorbidities, chronic inflammation, and enhanced tissue turnover, which may reduce the specificity of cancer detection. In addition, low tumor fractions, particularly in early-stage disease or in frail patients, may limit the sensitivity of mutation-based approaches. In this context, repeat-derived cfDNA signals may offer an advantage due to their high copy number and amplification potential, although their interpretation requires careful distinction between tumor-related and aging-associated background signals. Addressing these challenges will require integrative analytical strategies combining mutation detection, DNA methylation profiling, fragmentomics, and repeat-based features, together with the development of age-adjusted diagnostic thresholds. Future research should focus on the development of cfDNA-based models that explicitly incorporate aging as a biological variable, including the integration of transposable element–associated methylation changes with established epigenetic clocks to enable simultaneous assessment of biological aging and cancer risk. In addition, machine learning approaches incorporating age-related cfDNA features may improve the discrimination between tumor-specific signals and physiological aging-related background noise. Prospective clinical studies specifically designed for elderly and frail populations are urgently needed to validate these approaches and facilitate the development of more sensitive, specific, and minimally invasive liquid biopsy strategies for reproductive system cancers in aging populations. Future studies should specifically test whether integrating transposable element–associated methylation signals with mutation-based cfDNA assays improves early cancer detection in older individuals. In addition, systematic comparisons of combined cfDNA features, including mutations, methylation, fragmentomics, and repeat-derived signatures, are needed to identify the most informative biomarker combinations in aging populations.

Author Contributions

Conceptualization, SF, MCecati, FM and MCardelli; literature analysis and interpretation, LC, RC, FM, SV and SF; writing-original draft preparation, MCecati, SV, RC, LC; writing-review and editing, SV, SF, RC, FM and MCecati; supervision, FM and MCardelli; visualization, SV and MCecati; methodology, SV. All authors have read and agreed to the published version of the manuscript. All authors contributed to editorial changes to the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During manuscript preparation, OpenAI GPT-4.1 was used for language editing and grammar checking. The authors reviewed and edited all text and take full responsibility for the content of the published work.

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