Chapter

Chromosome Segregation Defects in Scleroderma

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Abstract

Fibrosis in systemic sclerosis (SSc or scleroderma) is characterized by an abundance of chromosome segregation defects and chromosome instability (CIN) that lead to overactivation of autoimmunity and inflammation. This chapter will emphasize the most recent findings on the involvement of centromere and telomere dysfunction in scleroderma. We will discuss how centromere and telomere dysfunction contribute to CIN, fibrosis, and cellular autoimmunity in scleroderma. We will also summarize how chromosome segregation defects in the form of aneuploidy and micronuclei formation activate the Cyclic GMP–AMP synthase (cGAS) Stimulator of interferon genes (STING) pathway of cellular immunity. Activation of this pathway induces production of inflammatory cytokines IFNβ and IL6. Finally, we will summarize the most recent therapies to block the cGAS-STING pathway and treat fibrosis.

Keywords: centromeres, chromosome instability, telomeres, fibrosis, cGAS-STING pathway, micronuclei, IFNβ, IL6

1. Introduction

1.1 Pathogenesis and diagnosis of scleroderma fibrosis

Scleroderma results from increased production and accumulation of collagen and other extracellular matrix (ECM) proteins in body tissues culminating in fibrosis, a thickening of the connective tissue [1]. Fibroblasts, the resident cells of connective tissue are key players of fibrosis in SSc [2]. After tissue damage and immune cell activation, fibroblasts in the ECM differentiate into secretory myofibroblasts in a tissue microenvironment rich in fibroblast growth factor (FGF), transforming growth factor beta (TGFβ), interferon beta (IFNβ) and interleukins 1 and 6 [1, 2]. Buildup of myofibroblasts is responsible for the disproportionate production and accumulation of collagen and ECM proteins in SSc fibrosis [1–4]. Excessive buildup of collagen in the skin, gastrointestinal tract, lungs, kidneys, heart, or other tissues together with microvascular damage and cell-mediated autoimmunity lead to organ dysfunction.

Scleroderma is subclassified into limited cutaneous (lcSSc) or diffuse cutaneous (dcSSc) based on the degree of hardening and thickening of the skin and organ involvement. LcSSc usually affects the skin of the face and lower limbs, while dcSSc

affects large areas of the skin and major organs. Although both types of SSc are very incapacitating, dcSSc patients have higher morbidity and mortality due to increased inflammation and fibrosis of internal organs, especially interstitial lung disease (ILD) and heart fibrosis [5–8]. The cause of SSc pathogenesis is yet to be uncovered, although a combination of factors, including immune system dysregulation [8], genetic and epigenetic factors such as mutations in HLA genes [9–11], and reactive oxidizing species (ROS) [12] play a major role. Exposure to environmental factors such as silica, bleomycin, aromatic and chlorinated solvents, ketones, and trichloro-ethylene have also been linked to the condition in some individuals [2–4, 8, 13–16].

The diagnosis of SSc relies on clinical evaluation and detection of antibodies that recognize the nucleus of the patient's own cells, the so-called antinuclear antibodies (ANAs) [17, 18]. ANAs recognize nuclear components and are detected in as many as 95% of SSc patients [19]. Interestingly, some of these antibodies localize to centromere regions, the middle portion of chromosomes. Anticentromere antibodies (ACAs) were first identified in the lcSSc CREST variant (CREST: calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) [20] and served as crucial reagents to study centromere biology. ACAs are found in up to 43% of lcSSc (mostly all cases of CREST), recognize many centromere proteins (CENPA, CENPB, CENPC, and so on, to CENPT), and are associated with a generally more favorable prognosis [20]. However, ACA-positivity is associated with the development of pulmonary arterial hypertension [18–20]. CENPB autoantibodies are present in almost all ACA positive lcSSc patients [21]. CENPB autoantibodies can also be found in Sjogren syndrome and neoplasia [21, 22]. CENPA autoantibodies appear to be more specific for SSc patients at risk of pulmonary vascular disease. Autoantibodies that recognize CENPC alone are associated with Sjogren syndrome and CENPF autoantibodies are associated with cancers [22].

ANAs specific for SSc recognize topoisomerase I, an enzyme that breaks single stranded DNA, relaxes supercoiled DNA, and facilitates chromosome condensation [18]. Antitopoisomerase-I antibodies (ATAs; anti-Scl-70) are mostly found in dcSSc patients (prevalence 20–40%), and correlate with poor prognosis, lung fibrosis, and disease progression [17, 18]. Anti-RNA polymerase III autoantibodies are detected in dcSSc patients (prevalence 10–25%), and serve as markers of rapid disease development and renal crisis [17, 18].

Recent studies have also identified ANAs that localize to telomeres, the protective cap of chromosomes, in 9% of SSc patients [23, 24]. These antibodies recognize the shelterin telomeric repeat binding factor 1 (TERF1) protein and are linked to severe lung disease [23]. Despite their clinical value for specific diagnosis and prognosis of SSc, the etiology of ACAs, ATAs, and anti-telomere antibodies in SSc patients is unknown.

1.2 Centromeres

Centromeres are structural units that modulate the proper division of chromosomes. Destabilization of centromere function results in chromosome instability (CIN), a hallmark of birth defects, cancers, and fibrosis [25–28]. Centromeres have two vital functions; (i) recruit centromere proteins to form the kinetochore, and (ii) keep sister chromatids together before chromosome segregation [29, 30]. Defects in either of these vital functions results in structural and numerical CIN, presented in the forms of lagging chromosomes, dicentric chromosomes, aneuploidy, and micronuclei (**Figure 1**) [31, 32].
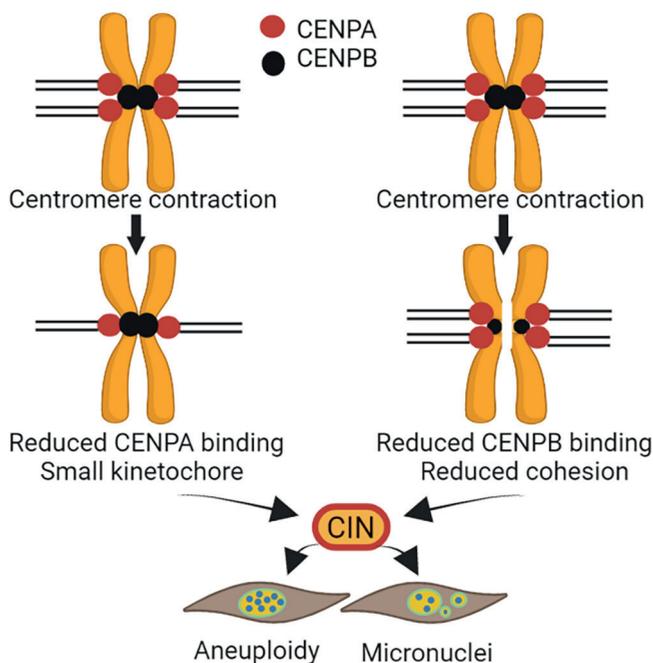
Figure 1.
Centromere function. The H3 CENPA deposits to centromere DNA to initiate the assembly of the kinetochore, which is necessary for microtubule attachment and chromosome segregation. CENPB binds to arrays in the centromere sequence and interacts with CENPA and other centromere proteins to facilitate centromere function. CENPB also binds pericentromere arrays to facilitate chromatid adhesion. Destabilizing these processes, such as centromere sequence deletion or contraction, could reduce CENPA or CENPB binding, leading to CIN. This figure was created with BioRender.com.

Centromere DNA sequences contain arrays of high order repeats (HORs), which can extend several megabases in length (**Figure 2**) [33]. The size of centromere arrays varies among individuals and disease processes. HORs are composed of 171 bp α-satellite units organized in a head-to-tail fashion [33]. During the evolution of hominids, centromeric α-repeats in each chromosome became homogeneous. Today, the α-repeats in each centromere array have 98–100% similarity, yet they have only ~75% similarity to the α-repeats of other centromeres arrays [34, 35]. The centromere core is composed of 1 or 2 arrays [36], while the periphery of the centromeres, the pericentromere, is more diverse, with shorter arrays or monomers of alpha and other repeat types, as well as transposon-like elements [36–38]. Centromeres have been difficult to study because of their repetitive nature, but the genomic assembly of human centromeres has recently been achieved [39–41]. Even so, the human population has a large variation in centromere size and content [41–43].

The function of the centromere is modulated epigenetically by CENPA, a H3-histone variant that forms nucleosomes with H2A, H2B, and H4 at active centromere arrays or elsewhere and defines the location of kinetochore formation (**Figure 2**) [29]. CENPB, a protein that binds to specific DNA sequences in centromeres (CENPB boxes), enhances this centromere chromatin interaction. CENPB interaction with H3K9me3 helps maintain sister chromatid cohesion [30, 44, 45]. Despite the prevalence of ACAs in SSc, the role that centromere sequences and proteins play in the pathogenesis of SSc has remained unexplored.
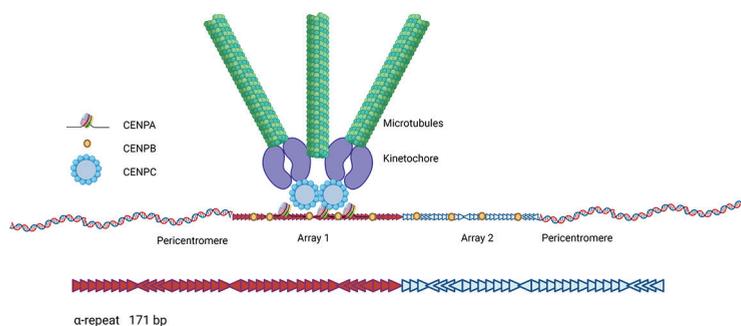
Figure 2.
Organization of human centromeres. Centromere sequences are composed of α-repeats, ~171 bp units, organized in a head-to-tail fashion (arrows). Centromeres sequences are composed of one or two arrays of HORs. Pericentromere sequence contains α- and other satellites. The H3 histone CENPA deposits to the largest array of the core to start the assembly of the kinetochore, which is necessary for microtubule attachment and chromosome replication. H3K9me3 remains abundant at the pericentromeres. CENPB binding to centromere sequences and its interaction with CENPA and CENPC stabilize the formation of the kinetochore. CENPB also binds pericentromere arrays to facilitate chromatid cohesion. This figure was created with BioRender.com.

1.3 Telomeres

Telomeres are specialized chromatin structures that protect chromosome ends. Telomere sequences are regions of TTAGGG repeats that shrink in dividing cells during cell replication [46–49]. The main functions of the telomeres are to (i) protect the terminal regions of the chromosomes from progressive degradation, and (ii) prevent chromosome fusion by ensuring that the DNA repair systems do not mistakenly recognize the very ends of the DNA as DSBs. Excessive telomere shortening or telomere uncapping can produce telomere fusions, which results in CIN, mostly in the forms of dicentric, lagging chromosomes, and micronuclei (**Figure 3**). Telomerase holoenzyme helps maintain telomere length by synthesizing telomeric DNA [46]. Shelterin
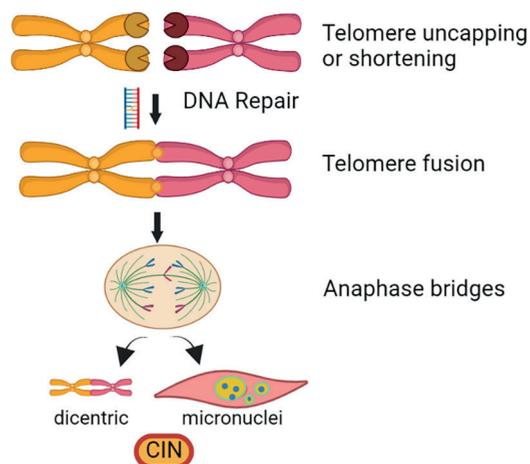

Figure 3.
Telomere function. The telomeric chromatin structure protects chromosome ends from recombination, repair, and fusion. Telomerase holoenzyme counteracts telomere DNA degradation during replication by adding DNA to telomeres, thus preserving telomeres for future cell division. Deletion or degradation of telomeres produces CIN. Recombination and subsequent repair of degraded telomeres can fuse chromosomes, leading to dicentric and/or micronuclei formation. This figure was created with BioRender.com.

complex proteins bind and protect telomeres from degradation and allow access of telomerase and helicase to telomere DNA [46–49]. Although telomerase activity is reduced in somatic cells, it is upregulated in cells that undergo rapid expansion, such as the cells involved in ILD and fibrosis [47, 48]. Thus, it is a distinct possibility that telomerase plays a crucial role in SSc fibrosis.

1.4 Chromosome instability and activation of cGAS-STING/type I interferon pathway

cGAS is an enzyme that senses cytosolic double-stranded DNA from pathogens or damaged self-DNA, such as micronuclei, as part of the innate immune response (**Figure 4**) [50–53]. Upon binding to cytosolic DNA, cGAS produces cGAMP, which interacts with STING to trigger downstream activation of the type I interferon pathway [52, 53]. STING recruits TBK1 to phosphorylate the transcription factor IRF3, which translocates to the nucleus and induce production of type I interferons including IFNβ [53]. Activation of STING also triggers RELA (p65), a component of the NFKB that induces IFNβ and pro-inflammatory cytokines, such as IL6 (**Figure 4**) [49]. cGAS-STING pathway activation is impeded by BANF, which competes with cGAS binding to intracellular DNA, and TREX1, which associates with cGAS and degrades the DNA [54–56]. Fibroblasts from cGAS KO mice do not produce IFNβ following DNA transfection [56]. Aberrant detection of cytosolic DNA has been implicated in cancers and autoimmune diseases [57]. It has also been suggested that cGAS-STING is overactivated in autoimmune diseases such as systemic lupus erythematosus (SLE) [58, 59], and interferons are known to be increased in SSc [60, 61]. Additionally, there are recent findings showing that the cGAS-STING pathway independently triggers fibrosis [62, 63]. As we show below, lesion fibroblasts from SSc patients show increased activation of the cGAS-STING/ type I Interferon pathway [64].

2. Chromosome abnormalities in scleroderma cells

Several investigators have noted chromosomal abnormalities in SSc [28, 64–70], including numerical and structural CIN. These findings included micronuclei formation, lagging chromosomes, aneuploidy and polyploidy, chromatid aberrations with gaps and breaks, acentric chromosome fragments, rearrangements, dicentric, and ring forms. These aberrations were specific to lymphocytes, lesion fibroblasts, and bone marrow cells. These cytogenetic abnormalities were more common in advanced forms of SSc, but not in CREST or Raynaud phenomena. Our studies showed increased aneuploidy in forearm lesion skin fibroblasts from dcSSc but not lcSSc patients, which is in agreement with these findings [64]. However, we did not see chromosome abnormalities in lymphocytes or macrophages from the same patients and, the reason for these inconsistencies remains to be clarified. Abnormal nuclear morphology and micronuclei formation were the most common CIN structural abnormalities found in involved skin fibroblasts from SSc patients, suggesting that lagging chromosomes and chromosome fragments failed to be incorporated into the nucleus and were enveloped separately with the nuclear membrane [64, 69, 70]. The clastogenic agent bleomycin further increased micronuclei formation in SSc patient fibroblasts from affected and unaffected skin, but not in skin fibroblasts from healthy controls [69]. The increased chromosomal breakage in dcSSc was not associated with
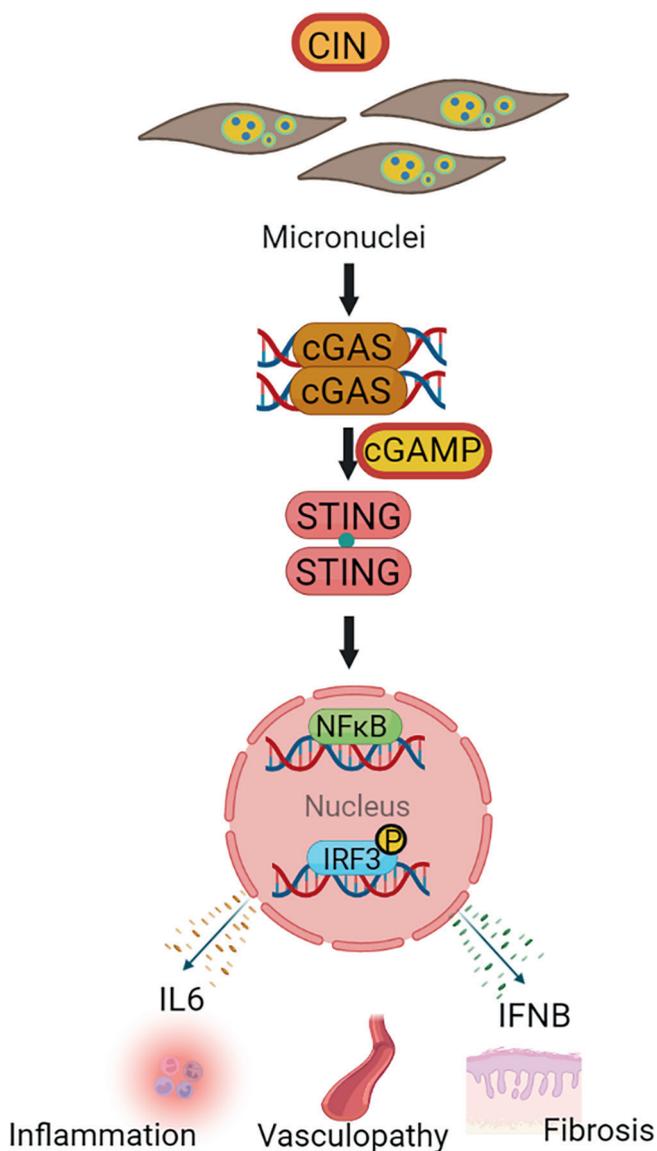
Figure 4.
Function of cGAS-STING pathway. cGAS senses cytosolic DNA from viruses as well as damaged DNA or micronuclei from CIN. Binding of cGAS to cytosolic DNA produces cGAMP, a molecule that interacts with STING to activate the type I interferon pathway. STING activates the expression of NF-κB and phosphorylates IRF3 to induce the production of IL6 and IFNβ, cytokines that mediate inflammation, vasculopathy, and fibrosis.

cancer development or chromosome breakage syndromes [67], although it appears to also be common in SSc family members [65].

3. Centromere defects in scleroderma

Abnormal centromere assembly, localization, and function may lead to errors in chromosome segregation and production of centromere antibodies. Recent studies

in our laboratory demonstrated that centromere DNA sequences are shorter at several chromosomes in SSc patients, particularly in lesion dermal fibroblasts [64]. Although changes in the length of centromeres among human populations have been reported [41–43], such dramatic centromere deletion in several chromosomes is pervasive in SSc. Centromere deletions appear to dominate the landscape of several chromosomes in dcSSc, yet centromere arrays in chromosomes 1 and 2 were consistently found deleted in SSc patients [64]. It is unclear why these centromere arrays were particularly deleted, and it is not known whether the deletions contribute to SSc pathology. Further studies indicated these centromere changes were not the result of culture conditions or the previous therapy received by the patients. It remains to be explored whether changes at centromere sequences directly affect the deposition and strength of the kinetochore during chromosome segregation. However, centromere deletions in SSc were found to correlate with abnormal CENPA expression and deposition, which lead to CIN. In fact, SSc fibroblasts show a lack CENPA deposition in micronuclei with CENPB boxes, suggesting that centromere DNA sequences influence centromere function and chromosome segregation as shown in **Figure 1**.

A second important observation was understanding of how centromere changes in SSc patients lead to the production of ACAs [64]. Our studies identified specific centromere defects in lcSSc patients that produce ACAs. Nuclear proteins CENPA and CENPB leaked and colocalized into the cytoplasm and cell membrane of lcSSc dermal fibroblasts. The integrity of the nuclear membrane appears to be one critical factor that contributes to the leaking of centromere proteins, although it is expected that other factors play a role in the specificity of centromere protein misplacement. It remains to be explored what factors, other than rupture of the nuclear membrane, lead to leaking of CENP proteins only in lcSSc patients that produce ACAs. CENPB in the cytoplasm of lcSSc lesion fibroblasts co-localizes with MHC class II molecules DRB1 and DRB5, which suggests a likely mechanism for ACA production in lcSSc [64]. It is possible that the size and complexity in structure of CENPB and perhaps other CENP proteins are more immunogenic. Whether lesion fibroblasts are the sole drivers of aberrant autoimmunity in lcSSc or whether other cell types also act as antigen-presenting cells is unknown.

4. Telomere defects in scleroderma

Studies aimed at understanding the role of telomeres in SSc have found that, similar to centromere deletions, telomere attrition is detected in SSc patients. These recent findings were evident in a subset of SSc patients and telomere shortening was also found to be more prominent in patients with ILD [71, 72]. Whether such telomere deletions also exist in involved skin fibroblasts remains to be studied. Other studies revealed that shorter telomeres in lymphocytes of SSc patients correlated with the production of autoantibodies targeting telomere-associated proteins (shelterin) in a subset of patients. Further, the production of telomere antibodies also correlated with ILD [23, 24]. The recent discovery of telomere autoantibodies in SSc patients may not have been possible before due to the spatiotemporal expression of these proteins and tissue specificity [73]. In contrast, ACAs were discovered in CREST patients even before we understood the structure of centromeres. It is possible that telomere attrition leads to an excess of shelterin proteins that leak to the cytoplasm and act as antigens in a subset of SSc patients. Taken together, these studies suggest

that telomere dysfunction affects chromosome segregation and may promote inflammation and fibrosis in SSc as indicated in **Figure 4**.

5. Chromosome instability and cGAS-STING activation in scleroderma and fibrosis

Production of micronuclei and release of DNA into the cytoplasm can activate the cGAS-STING pathway of inflammation and fibrosis [50–53]. Previous reports have shown that SSc fibroblasts produce increased amounts of IL6 and IFNβ, the final molecules activated in the cGAS-STING/type I interferon pathway, although they can be activated by other cellular and immune pathways [74, 75]. Although IL6 and IFNβ may be promising targets to treat SSc, the molecular mechanisms of how these proteins are activated was only recently discovered. The production of IL6 in serum of patients and dermal fibroblasts appears to be specific to SSc clinical phenotypes of dcSSc, such as ILD, pulmonary arterial hypertension, gastrointestinal involvement, and cardiac involvement [76, 77]. On the other hand, increased production of IFNβ and activation of IFN-regulated genes has been observed in the serum and involved skin tissue of patients with SSc [75]. Furthermore, the IFNβ score correlated with markers of disease severity [75]. We observed that increased amounts of micronuclei in SSc dermal fibroblasts strongly correlated with the expression of *cGAS* (r = 0.6095), *IFNB* (r = 0.9562), *IL6* (r = 0.5665), and other-related proinflammatory and profibrotic cytokines [64]. These observations suggest that IL6 and IFNβ are produced by sensing of cytosolic DNA released from micronuclei by cGAS to trigger the cGAS-STING pathway cascade [52]. Increased levels of cGAS colocalized to micronuclei in lesion fibroblasts. cGAS sensing of micronuclei produced increase amounts of 2′3' cGAMP, a second messenger directly produced by cGAS after sensing cytosolic DNA. We further detected the phosphorylation and translocation to the nucleus of downstream factor IRF3, the active Ser-396 phosphorylated form, and detected in the nuclei Phospho p65 (RELA). Finally, we identified the increased IFNβ secretion into the SSc fibroblasts' cell supernatants. Therefore, blocking the cGAS-STING pathway appears to be a promising therapeutic approach to treat SSc. Ex vivo and in vivo studies need to be developed in order to validate this approach and its potential to treat SSc fibrosis.

Although inhibitors of the cGAS-STING pathway have been only examined in autoimmunity in SLE, blocking the final cytokines of the pathway have been explored in SSc. Blockage of IL6 has shown to be promising for reversing the production of TGFβ, a cytokine involved in dermal fibrosis [78]. Further, the efficacy of IL6 inhibitors, particularly Tocilizumab in clinical trials, finishing phase 3, has been satisfactory in preserving lung function, although the primary skin fibrosis endpoint was not met [79, 80]. IFNβ inhibitors per se have been suggested but never studied in SSc [81]. We believe that by blocking the pathway early would prevent the release of both IL6 and IFNβ, attacking the fibrotic and inflammatory component of the disease with a synergistic effect.

We confirmed that cGAS activation was responsible for the IFNβ production in SSc skin cells, treating lesion fibroblasts with G150, a cGAS-specific inhibitor [63]. G150 treatment strongly reduced the cGAS-induced enzymatic (2′3' cGAMP) activity and the production of IFNβ, although the levels of IL6 were not tested. Future research needs to address if cGAS or STING inhibition blocks both cytokines, but also reduces fibrosis of the skin and other organs. Even though current cGAS-STING pathway inhibitors show promising efficacy, they present toxicity to the cells and may

harm the body in the long term [82, 83]. Recently, novel CGAS-STING inhibitors have been discovered showing different degrees of specificity for cell types and phenotypes [82, 83]. Therefore, the specificity of new molecules for treating SSc subtypes and phenotypes deserves to be addressed thoroughly for optimal efficacy and safety.

6. Conclusions

We find that telomere and centromere defects at the genetic and epigenetic levels are prevalent in SSc patients and may contribute to chromosome missegregation. Centromere and telomere sequence deletions appear to be important factors that contribute to chromosome segregation defects, and hence, lead to CIN. Such centromere and telomere deletions may have stemmed from ROS or perhaps from exposure to environmental factors that damage the DNA and/or interfere with DNA repair pathways. This review brings to light a new target pathway in SSc, the cGAS-STING/IFNβ pathway, which can contribute to fibrosis. Targeting the cGAS-STING/ IFNβ pathway with newly-developed molecules could prove to be a viable route to treat SSc fibrosis.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

cGAS	Cyclic GMP–AMP synthase
STING	Stimulator of interferon genes
SSc	Systemic Sclerosis
lcSSc	limited cutaneous systemic sclerosis
dcSSc	diffuse cutaneous systemic sclerosis
ROS	reactive oxidizing species
ECM	extracellular matrix
FGF	fibroblast growth factor
TGFβ	transforming growth factor β
IFNβ	interferon beta
IL1	interleukin 1
IL6	interleukin 6
ANAs	antinuclear antibodies
ACAs	anticentromere antibodies

CREST	calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia
ATAs	antitopoisomerase-I antibodies
DSBs	double-stranded breaks
TERF1	telomeric repeat binding factor 1
CENPB	centromere protein
CIN	chromosome instability
HORs	high order repeats
TBK1	TANK-binding kinase 1
RELA or p65	REL-associated protein
NFKβ	Nuclear factor kappa B
IRF3	Interferon regulatory factor 3
BANF	BAF Nuclear Assembly Factor 1
TREX1	Three Prime Repair Exonuclease 1
SLE	Systemic lupus erythematosus

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