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SURVEY AND SUMMARY

Contributions of DNA interstrand cross-links to aging of cells and organisms

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ABSTRACT

Impaired DNA damage repair, especially deficient transcription-coupled nucleotide excision repair, leads to segmental progeroid syndromes in human patients as well as in rodent models. Furthermore, DNA double-strand break signalling has been pinpointed as a key inducer of cellular senescence. Several recent findings suggest that another DNA repair pathway, interstrand cross-link (ICL) repair, might also contribute to cell and organism aging. Therefore, we summarize and discuss here that (i) systemic administration of anti-cancer chemotherapeutics, in many cases DNA cross-linking drugs, induces premature progeroid frailty in long-term survivors; (ii) that ICL-inducing 8-methoxy-psoralen/UVA phototherapy leads to signs of premature skin aging as prominent long-term side effect and (iii) that mutated factors involved in ICL repair like ERCC1/XPF, the Fanconi anaemia proteins, WRN and SNEV lead to reduced replicative life span *in vitro* and segmental progeroid syndromes *in vivo*. However, since ICL-inducing drugs cause damage different from ICL and since all currently known ICL repair factors work in more than one pathway, further work will be needed to dissect the actual contribution of ICL damage to aging.

INTRODUCTION

Each human cell has to repair the large numbers of different DNA damages encountered each day: around 50 000 single-strand breaks (SSB), 10 double-strand breaks (DSB), 10 000 depurinations, 600 depyrimidations, 2000 oxidative lesions, 5000 alkylating lesions and 10 interstrand cross-linking events (1). Although rare, DNA interstrand cross-links (ICLs) are among the most deadly types of damage. The cross-linking of the two complementary DNA strands prevents replication as well as transcription and prevents the use of information encoded by the complementary strand for repair. Thus, ICL formation poses a major challenge for the cellular repair systems, also reflected by the fact that estimated 40 ICLs in repair deficient mammalian cells are sufficient to induce cell death (2). ICLs are considered to be mainly sensed during replication in S-phase, where they lead to collapse of replication forks and DSBs, while little is known on transcription-coupled sensing and repair of ICLs. Surprisingly, ICL repair seems also absent in mitochondrial DNA (3).

The mechanisms that lead to repair of ICLs are still not well understood in mammalian cells, but two major pathways have been identified. The minor pathway depends on ERCC1/XPF and translesion bypass by Rev3 and is error-prone (4). The major pathway depends again on ERCC1/XPF and error-free homologous recombination repair (5). Excellent recent reviews summarizing ICL repair are available for yeast (6,7) as well as for mammalian cells (8–11).

While other DNA damage repair pathways like transcription-coupled nucleotide excision repair (NER) have well-established links to aging of cells, tissues and organisms (12), it is not yet clear if and to what extent ICLs are involved in causing or contributing to progeroid functional decline. Therefore, we here summarize several findings suggesting that exogenous exposure to ICL inducing agents or endogenous ICL repair deficiencies are associated with signs of premature aging.

PREMATURE AGING AS SIDE EFFECT OF CHEMOTHERAPIES

ICL inducing agents used in tumour therapy

Most of our current knowledge on ICL repair derives from the use of ICL-inducing chemicals in biochemical or genetic analysis of cells and cell lines on the one hand and from their wide and successful use as anticancer chemotherapeutics (13) on the other hand. Common to all of these chemical compounds is their bifunctional character that allows them to react with both DNA

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strands. Although this is widely accepted as major cytotoxic effect, it should be noted that the individual ICL-inducing agents induce different specific steric DNA-adduct structures and that they generate other than ICL damage like DNA monoadducts, intrastrand cross-links, damage to lipids, RNA and proteins. Furthermore, different reactive intermediates can be formed by cellular metabolism. For a detailed review, see Ref. 7. The most important substance classes used in cancer therapies are briefly summarized in the following.

Platinum compounds, the most famous of which is cisplatinum diammine dichloride II (CDDP) was one of the first chemotherapeutics originally identified as inhibitor of bacterial cell division (14). Since then it has been used to treat a wide range of different tumours (15,16) and second-generation drugs are intensely worked on (17). The damage to the DNA mainly consists of intrastrand cross-links as well as around 5–8% ICL of total adducts (18,19), which are responsible for the main cytotoxic effects (20).

Bis(2-chloroethyl)methylamine (HN2) and other members of the nitrogen mustard family are as well widely used as anti-cancer drugs (21). Again the majority of damage consists of monoadducts to the DNA, however, the 1–5% ICLs are responsible for the high cytotoxicity (22). Oligonucleotides conjugated to nitrogen mustards can be used to introduce ICLs at specific sites in the genome (23).

One of the most used chemotherapeutics of the nitrosurea class is bis(2-chloroethyl)nitrosurea (BCNU, carmustine), which decomposes in aqueous phase to so far uncharacterized reactive bifunctional molecules (24). The number of ICLs formed by this drug is estimated to be around 8% of all adducts, and again this seems to be the main cytotoxic component (25).

Mitomycin C (MMC) is a quinine-containing antibiotic isolated from streptomycetes. Only its intermediates that are formed after several intracellular metabolic activation steps generate ICLs, which make up 5–14% of all adducts (26). The ICLs mainly affect dCpG sequences in the minor groove of DNA. A recent derivative, aziridinomitosene 4, has been shown to have very high ICL-forming activity without prior metabolization (27). Besides forming adducts, MMC also induces production of reactive oxygen species (ROS), which also contributes to its cytotoxicity (28).

Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBD) are a family of DNA interactive anti-tumour antibiotics derived from various *Streptomyces* species. One of the most promising derivatives thereof is SJG-136, which displays a 440-fold higher ICL formation activity than the nitrogen mustards (29,30). ICLs are targeted to the minor groove of the DNA even in a non-reductive environment (31).

Early onset of progeroid frailty after chemotherapy

Only now, after several decades of using ICL-inducing drugs in chemotherapy against cancer, sufficient patients with more than 10 years survival are available for studying long-term side effects. Several years after the initial treatment, patients suffer from a variety of problems that usually occur later in life like decline of cognitive functions, visual deterioration, musculoskeletal decline, osteoporosis, skin changes, chronic fatigue and sexual dysfunction (32) as well as cardiovascular complications (33). We therefore propose to refer to this side effect of chemotherapies as acquired premature progeroid syndrome (APPS) in analogy to the term premature progeroid syndromes for hereditary diseases that resemble accelerated aging (34). While it is clear that a large proportion of cancer patients received ICL-inducing chemotherapeutics, the data so far have not been apportioned according to the drugs used. Thus, it is not yet clear if and how the individual cross-linking agents differ in their long-term effects and if and how they differ from chemotherapeutics with other modes of action.

Similarly, it is not yet clear what causes APPS as a long-term side effect. One possibility is the exhaustion of proliferative potential of stem and progenitor cells as well as of normal differentiated cells by the cytotoxic drugs. In this scenario, DNA damage induces cellular senescence and/or apoptosis in damaged cells, forcing the surrounding undamaged cells to undergo repeated proliferation in order to maintain tissue homoeostasis. This idea is supported by several observations.

Increased apoptosis as well as senescence after chemotherapy has been reported in many studies (35), and senescent cells accumulate in different tissues and organs with age (36–38) and even in tumours (39). One trigger of senescence is critically short, uncapped telomeres (40) and indeed accelerated telomere shortening has been observed in chemotherapy-treated patients versus age-matched controls (41). Furthermore, deficiencies in DNA repair have been shown to impair haematopoietic stem cell function (42) or to even deplete the pool of haematopoietic stem cells with age (43). Therefore, APPS might be caused by a general decline of tissue regeneration and repair capacity in consequence to chemotherapy.

PSORALEN/UVA-INDUCED ICLs AND PREMATURE SKIN AGING

Psoralens belong to the furocoumarins, bifunctional agents that form ICLs as well as thymine monoadducts upon UVA activation and are among the most potent interstrand cross-linking agents. Upon selection of different wavelengths up to 40% of the monoadducts can be converted to ICLs. Psoralen cytotoxicity is clearly linked to ICL-forming activity, since exposure of cells to psoralens with UV wavelengths that do not induce ICLs or monofunctional psoralens not able to form ICLs are markedly less toxic (44).

For studying response to and repair of specific ICLs, targeted single ICLs can be introduced into the genome using either oligonucleotides forming triplex DNA at the complementary sites or peptide nucleic acids conjugated to dimeric bis-psoralen (45,46). Furthermore, a digoxigenin-4,5',8-trimethylpsoralen conjugate enables visualization of ICLs in cultured cells (47).

The clinical conditions for which 8-methoxy-psoralen/UVA treatment (PUVA) has been widely and successfully used over decades are skin diseases like psoriasis, vitiligo and mycosis fungoides. The therapeutic effect depends on

formation of ICLs the massive formation of which has been observed in treated tissues (48). One prominent side effect of repeated PUVA treatment is premature aging of the skin (49–51).

As a model to study the underlying mechanisms, human fibroblasts and keratinoycytes have been subjected to PUVA treatment. These studies suggest that premature skin aging might be due to induction of a cellular senescence programme triggered specifically by ICL formation (51–54) resembling a combined DNA damage and stress-induced phenotype at least at the transcriptional level (55).

PUVA-induced senescence is signalled by ATR (56), whose importance for ICL repair is emphasized by data from *Saccharomyces cerevisiae*. Yeast ATR's homologue Mec1 is activated by the heterotrimeric Rad17–Mec3–Ddc1 complex (57). Surprisingly, MEC3 has recently been identified to be allelic to Pso9, mutations in which render yeast cells sensitive to PUVA (58). Furthermore, the human Rad17–Mec3–Ddc1 homologue called Rad9/Rad1/Hus1 (911) complex localizes to telomeres and modulates telomere length and telomerase activity (59).

While in the short-term cell cycle arrest is telomere-independent, after 28 days after recovery from PUVA treatment, senescence is still maintained with DNA damage foci persisting mainly at telomeres as detected by co-staining of γ-H2AX with telomere-specific fluorescence *in situ* hybridization. In contrast, intrachromosomal DNA damage has largely been repaired during the recovery (56). It is not clear why the damage foci persist at the telomeres and what might be the nature of this damage. In this regard, it is of interest that telomeric t-loops are efficiently maintained after psoralen cross-linking (60), and that telomeric sequence contains the TA basepairing within the TTAGGG repeats that are prime targets of 8-methoxypsoralen (61). This suggests that the telomeres might be exquisitely susceptible to ICLs and that PUVA treatment might cause more ICL per kilobase DNA at the telomere than within genomic sequences, and/or that ICL repair is less efficient at the telomeres.

Besides senescence, apoptosis might be involved in the reduction of the proliferative capacity of skin cells, since *in vitro* and *in vivo* PUVA has been shown to induce apoptosis in epidermal cells via p53 and Fas ligand (62).

DOES ENDOGENOUS FORMATION OF ICLs INCREASE WITH AGE?

So far, ICL formation by exogenous sources is undoubted, but how do ICLs arise spontaneously within cells and tissues? One of the few currently known endogenously generated molecules causing ICLs is the bifunctional lipid peroxidation product malondialdehyde. Various studies have identified specific cross-link structures by malondialdehyde with DNA *in vitro* (63) as well as *in vivo* in a variety of human tissues (64–66).

ROS necessary for peroxidation of lipids to malondialdehyde arise from intrinsic cellular pathways, above all from cell respiration, but also during prostaglandin biosynthesis, and oxidative burst of immune cells. Extrinsic sources like UV light, or heavy metal ions contribute to ROS production as well (67).

Free radicals have been postulated to be a major cause of aging in the 'free radical theory of aging' (68) and there is little doubt that ROS contribute to deterioration of cell (69) and organ function, e.g. brain (70), kidney (71,72), liver (73) or heart (74). Increased formation of ROS (75), lipid peroxidation products and reactive aldehydic molecules (one of which would be malondialdehyde) has indeed been observed during aging (76–78). In addition, lipid peroxidation products have been suggested as one parameter in a possible set of clinical aging markers (79).

However, direct evidence for an increase of malondialdehyde and in consequence malondialdehyde-ICLs has not yet been provided, since the age-comparative studies so far were based on quantification of the bulk of reactive aldehydes only, e.g. using thiobarbituric acid reactive substances (TBARS) assay.

Might there also be a difference between fast induction of ICLs versus slow gradual increase as expected during aging due to gradual ROS increase (80,81)? Two studies suggest that slow accumulation of DNA damage indeed results in higher cytotoxicity than short-term high-dose exposure. In the first study, HCT 116 cells were treated for 24 h with low doses of the ICL-inducing agent SJG-136, leading to gradual formation of ICLs, and limited p21-induced cell cycle arrest. This resulted in significantly higher cytotoxicity than a 1 h treatment with high doses of SJG-136 that caused full DNA damage response, although dose and time of treatment were carefully chosen to yield similar final levels of ICLs within the cells (82). Similarly, in the second study, low doses versus high doses of the DNA-damaging agents, hydroxyurea and UV were compared in three cell lines partially deficient in different components of ATR-mediated signalling. Again, low doses were found to cause significantly more cell death accompanied with slow/insufficient activation of damage signalling and repair (83).

ICL REPAIR DEFICIENCY CONTRIBUTES TO SIGNS OF ACCELERATED AGING

Although ICL repair is still not fully understood in higher eukaryotic cells, several central players have been identified during the last years including, ERCC1/XPF, the Fanconi anaemia proteins, but also the RecQ helicases WRN and BLM. Patients and corresponding animal models with mutations in these factors display various grades of segmental progeroid syndromes. In addition, other factors contributing to ICL repair like SNM1/hPso2 or SNEV have been connected to cellular aging and telomere biology. However, it has to be kept in mind that all of the ICL factors described so far work in more than one DNA repair pathway or exert more than one function.

ERCC1/XPF

ERCC1/XPF is a structure-specific heterodimeric endonuclease essential in NER, but also during ICL repair.

Incisions near the ICL site that 'unhook' the cross-linked oligonucleotide specifically depend on ERCC1/XPF (84,85). Mutations in both of its subunits have been found to cause segmental progeroid syndromes in humans. Similarly, mouse models deficient in ERCC1 (86,87) as well as in XPF (88) show a congruent severe progeroid phenotype that is quite distinct in severity from most other mouse models deficient in NER only. ERCC$^{-/-}$ mice show ataxia, kyphosis, osteopenia, weight loss, skin atrophy, sarcopenia and hepatocellular polyploidization (89) and the fibroblasts are exquisitely sensitive to cross-linking agents but also to UV light (87).

Recently, the first patient deficient in ERCC1 has been identified, displaying a severe disease phenotype of cerebro-oculo-facio-skeletal syndrome that also in part resembles premature aging and resulted in early death (90). In contrast to the knockout mouse model, cells of this patient, showed only intermediate sensitivity to UV and MMC treatment, comparable to other NER-deficient cells (90).

This finding suggests that XPF/ERCC1 functions besides NER repair might confer the severity of the mutation. Indeed, XPF/ERCC1 is required for meiotic and mitotic homologous recombination in mouse and fly (91,92) and also implicated in telomere processing, responsible for removing the 3' overhang of uncapped telomeres (93). Surprisingly, the endonuclease function required for both ICL and NER is separated from the telomere processing function of XPF, since a point mutation that abrogates DNA repair does not interfere with 3' overhang removal in cell culture experiments (94). Furthermore, NER and ICL repair functions of XPF might be separable as well (95).

This is consistent with the clinical appearance of the currently known XPF mutations. Most of them result in mild forms of xeroderma pigmentosa (XP), a cancer-prone syndrome characterized by high UV sensitivity. In contrast, one patient with a dramatic progeroid phenotype has been identified bearing a novel mutation in XPF (R153P9) interfering with formation of ERCC1 heterodimers (96). Primary fibroblasts of this patient are much more sensitive to ICL-inducing MMC as compared to XPA-derived cells, while they are only similarly sensitive to UV irradiation (96). This finding would also support a specific role of deficient ICL repair distinct from NER deficiency in accelerating the aging process. Clearly, further work is required for dissecting the contributions of different mutations in XPF and ERCC1 in the observed progeroid features. It would for example be of high interest, to complement XPF-deficient mice with constructs harbouring the various mutants, to see if and to what extent ICL, NER, and dysfunctional telomere processing of XPF contribute to their progeroid phenotype.

A completely different and much unexpected link between ERRC1 deficiency and aging has been discovered recently. Suppression of IGF1 signalling is one of the very few conserved mechanisms that prolongs life span in a wide range of model organisms from *S. cerevisiae* (97), *Caenorhabditis elegans* (98), *Dorsophila melanogaster* (99), and mouse (100,101). Surprisingly, this suppression of IGF1 signalling was found in livers of ERCC1-deficient mice (96). Similar suppression of the IGF1/GH axis is seen after exposure of wild-type mice to chronic genotoxic stress using MMC (96). This would suggest that high levels of ICL damage provide a feedback signal to suppress growth at the organism level, probably in order to allocate more energy to cellular maintenance and repair in order to prolong the life span (96). Absence of IGF1 suppression in XPA or Cockayne syndrome B-deficient mice would argue against ERCC1's NER function as reason for developing progeroid phenotypes. It would be interesting to test if impaired IGF1-signalling backgrounds [e.g. in Ames or Snell dwarf mice (102)] would additionally reduce the life span and increase severity or accelerate the appearance of progeroid symptoms observed with ICL repair deficiency.

Further contributions to a premature aging phenotype might derive from increased apoptosis as observed in liver tissue (103), decreased replicative potential of ERCC1$^{-/-}$ embryonic fibroblasts (87) as well as depletion of hematopoietic stem cells, which again is not observed in XPA mutant mice (104).

An experimental setting that might allow for addressing ERCC1 deficiency in humans possibly arises from the finding that ERCC1 is transcriptionally repressed by fludarabine treatment (105,106), and increases ICLs synergistically with cisplatin or oxaliplatin (107,108). Fludarabine is a chemotherapeutic drug mainly used against haematological malignancies (109). It would be of interest to analyse if this drug also leads to APPS in long-term survivors.

FA pathway

FA is a disorder showing developmental and bone marrow defects, as well as cancer predisposition (110). This rare hereditary disease is caused by mutations in one of currently 13 proteins constituting 13 complementation groups [FANCA, B, C, E, F, G, L and M forming a core complex, D1, D2(BRCA2), H, I, J]. FAAP24 has recently been proven as an additional FA complex member, although it has not been found mutated in FA patients yet (111). Recent progress in understanding the functions of FA proteins and the 'FA pathway' has been reviewed in detail (9–11,112,113).

Although not being ranked among the segmental progeroid syndromes in the initial listing by George Martin (34), there still seems to be a segmental premature aging component in FA. This consists of progressive bone marrow failure, squamous cell carcinomas of the oral cavity and genital area much earlier in life than in normal individuals, impaired gametogenesis and premature reproductive aging. Additionally, >80% of FA patients are prematurely affected by endocrine abnormalities including hyperinsulinaemia, hypothyroidism and growth hormone deficiency, all of which are normally associated with advanced age (114). Decline of growth hormone is of note, since this leads to less IGF signalling similar to ERCC1/XPF deficiency, supporting the idea of a general switch from growth to repair upon (ICL?) damage.

Furthermore, cells of FA patients show signs of accelerated cellular senescence. PBMCs have accelerated individual annual telomere-shortening rates *in vivo* (115–117) while fibroblasts derived from FA patients show accelerated telomere shortening *in vitro* (118), consistent with a reduced replicative life span and earlier entry into cellular senescence (119,120). This accelerated telomere erosion, however, is not due to faster replicative shortening, but to increased telomere breakage (121). Together with an increase in apoptosis of haematopoietic stem cells (122,123), this might also contribute to the progressive bone marrow failure in patients (124) as well as in knockout mouse models (125–127).

To what extent are the FA proteins involved in ICL repair? While indeed hypersensitivity against ICLs by MMC and diepoxybutane is a common hallmark of all FA cells and used as standard diagnosis of FA, there is a broad spectrum of additional sensitivities against genotoxic damage including γ-irradiation, bleomycin, UV and methyl methane sulphate depending on the cell type of the same patient (128) as well as on the complementation group (129). For example, FANCG null Chinese hamster ovary (CHO) cells are similarly sensitive against monoalkylating agents as against ICL-inducing agents (130). Furthermore, monoubiquitination of FANCD2, a crucial step in activation of the 'FA protein pathway' is also induced by chemically blocking replication forks (131). These findings led to the proposal that the FA proteins—rather than being specifically necessary for ICL—might act more globally on stabilizing collapsed replication forks that do not exclusively arise due to ICL (11). Collapse of replication forks leads to formation of DSB, which have recently been suggested to be a prerequisite for HR-dependent repair of ICL (132). The FA proteins might prevent the DSBs from being repaired by non-homologous end joining by keeping the broken strands in close proximity. Thus, the FA pathway might largely counteract genomic instabilities by favouring base substitutions and small deletions over larger deletions and chromosomal rearrangements (10,11,133). Still, FA proteins are needed together with Msh2, ERCC1/XPF and Rev3 in HR-dependent repair of single psoralen-induced ICLs (132).

Further work is necessary to dissect if and to what extent reduced ICL repair, failed stabilization of replication forks or other DNA damage contribute to the progeroid symptoms in FA. To further complicate things, FA cells also show elevated ROS levels and increased sensitivity against ROS (123). Therefore, it cannot be excluded that ROS cause or additively contribute to premature aging in FA patients.

BLM and WRN helicases

Besides its function in base excision DNA repair (134), the RecQ helicase member WRN has also been implicated in ICL repair. Cells from Werner syndrome patients show sensitivity to ICL-inducing drugs (135,136) and WRN helicase activity has been shown necessary for repair of PUVA-induced ICLs (137).

WRN activity might be necessary at different points of ICL repair. It interacts with the SNEV-complex (see below) in early steps of repairing single psoralen ICLs *in vitro* (138), while in the later HR repair step it interacts with a complex containing Rad51, ATR, Rad54 and Rad54B (139) localizing to stalled replication forks (140). Another protein–protein interaction linking WRN to ICL repair derives from yeast, where its homologue sgs1 interacts with Pso5/rad16 (141), involved in ICL repair and global NER (142).

A second RecQ helicase family member, which also physically and functionally interacts with WRN (143), is BLM. Fibroblasts derived from Bloom's syndrome patients show sensitivity to MMC treatment (144) and to cisplatin (145). Both helicases have also been found to interact with members of the FA complex subunits and with HR factors (137,146–148). Furthermore, FA core complex assembly is necessary for BLM phosphorylation and localization to nuclear foci upon ICLs (144). The unwinding activity of BLM also enhances Mus81 endonuclease activity (149), which converts ICLs to DSBs (150). Genetic interaction between Mus81 and BLM homologues in *D. melanogaster* further supports their function in a common pathway (151).

Mutations in both helicases cause prominent segmental progeroid syndromes. WRN mutations are the cause of Werner syndrome (152). High genomic instability is observed in cells of Werner syndrome patients due to massive loss of telomeric sequences during replication (153), also leading to a reduced replicative life span *in vitro* (154).

Similarly, Bloom syndrome, is prominently ranked among the segmental progeroid disorders (152) and BLM, like WRN, is necessary for telomere functionality (155). Again, a clear attribution of accelerated aging to ICLs is not possible in the background of WRN and BLM mutations, since their functions are not limited to ICL repair.

hPSO2 (SNM1)

The nomenclature of the *Pso* genes is derived from yeast cells displaying sensitivity to 8-methoxy-psoralen/UVA treatment (142). Yeast Pso2 is involved in translesion synthesis repair of ICL during G1 (156). The five homologues in humans are SNM1, SNM1B/Apollo, and SNM1C/Artemis, ELAC2 and CPSF73, all of them containing a β-CASP/metallo-β-lactamase domain (157). Sensitivity to ICL has been established for SNM1 in knockout mice (158) and for SNM1B/Apollo in human cells by siRNA-mediated knockdown (159). SNM1 knockout mice-derived cells show MMC sensitivity (158) as well as increased tumour incidence and immune deficiency (160). However, only weak resemblance to aging is observed in these mice.

The second homologue, SNM1B/Apollo interacts with TRF2 and thus localizes to telomeres (161–163). Its knockdown in human fibroblasts leads to rapid loss of telomeric sequences, accelerated entry into replicative senescence and formation of γ-H2AX DNA damage

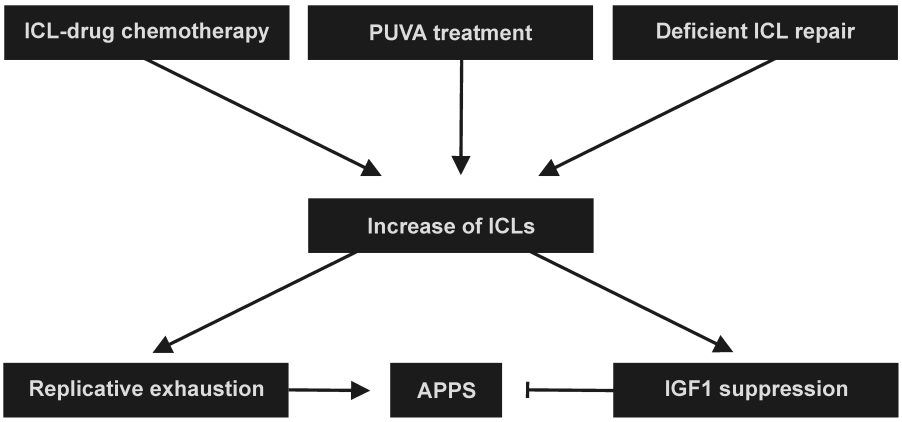

Figure 1. Overview of a proposed contribution of DNA interstrand cross-links (ICLs) to aging: increased formation of ICLs leads to acquired premature progeroid syndrome (APPS) by exhaustion of replicative potential of stem and progenitor as well as normal cells, while suppression of IGF1 signalling redirects energy from growth to repair and maintenance.

foci. If SNM1B/Apollo mutations also affect organismal aging has not been analysed yet.

SNEV (hPSO4)

The SNEV core complex consisting of CDC5L, SNEV (hPSO4, hNMP200, hPRP19), SPF27 (BCAS1) and PLRG1 together with WRN helicase is essential in early steps of ICL repair *in vitro* using single psoralen cross-linked plasmids as substrate for fractionated HeLa nuclear extracts (138). Furthermore SNEV binds dsDNA and might accumulate upon MMC, but also upon γ-irradiation and bleomycin treatment in cell cultures (164), while it clearly is ubiquitinated upon MMC and methyl-methan-sulphonate treatment (165).

SNEV's involvement in DNA repair is consistent with the role of its yeast orthologue Pso4 (Prp19) (166,167), where the temperature-sensitive mutant strain pso4-1 displays a pleiotropic phenotype that includes sensitivity to 8-methoxy-psoralen/UVA treatment (168). In yeast, Pso4 has been assigned to epistasis groups rad6 and rad52, emphasizing its pleiotropic nature (169,170).

How is SNEV connected to aging? It was originally isolated as mRNA that decreases during replicative senescence of endothelial cells (171), while upon over-expression it extends the replicative life span and reduces basal apoptotic levels (172). Targeted disruption of SNEV is early embryonic lethal, but haploinsufficiency causes mouse embryonic fibroblasts to enter early into replicative senescence *in vitro* (173). In addition, we recently found a decrease in the self-renewal capacity of haematopoietic stem cells derived from $SNEV^{+/-}$ mice as well as from senescence accelerated SAMP8 mice. Haematopoietic stem cells from both have significantly reduced SNEV levels as compared to wild-type or long-lived SAMR1 controls (174). This further supports a link between DNA repair, low replicative life span and the regenerative capacity of stem cells.

However, the multiplicity of SNEV's functions as an essential pre-mRNA splicing factor (167,175), as ubiquitin E3 ligase (176,177) and lipid droplet-binding protein (178), again makes it very difficult to dissect if its ICL repair function is connected to cellular aging. If SNEV haploinsufficient mice show premature progeroid symptoms and reduced life span like the SAMP8 mice is currently under investigation.

CONCLUSIONS

Three different types of conditions that induce increased levels of ICLs have been summarized here: chemotherapeutic treatment of cancer using ICL-inducing drugs, PUVA treatment of skin diseases and increase of endogenously formed ICLs by impaired ICL repair. All of these conditions lead to more or less pronounced progeroid features, clearly indicating that DNA damage is among the driving forces of aging and age-associated pathologies. Although it seems clear that ICLs contribute to aging-like loss of functions, their specific contribution remains unknown due to the facts that all ICL-inducing drugs cause additional damage other than ICL and all currently known proteins involved in ICL repair have other functions as well. Similarly, several other factors conferring hypersensitivity to ICL-inducing agents have not been linked to aging yet, e.g. the other Pso proteins like Pso1/Rev3 or the Rad51 paralogues XRCC2, XRCC3 and Rad51C.

How is ICL damage translated to aging of organisms? A major contributor might be the exhaustion of replicative potential of stem, progenitor and normal cells due to increased apoptosis and senescence upon damage, while suppression of the IGF1 signalling might be a counteractive measure aimed at funnelling energy to repair and maintenance of the damaged cells as summarized in our model (Figure 1).

While our model is consistent with the idea that aging is accelerated by stochastic damage but counteracted by genetically programmed repair (179), it is so far only based on induction of premature progeroid syndromes and shortening of life span. An important unanswered question therefore is if reduced ICL induction or improved ICL repair, e.g. by overexpression of ICL

repair factors would be able to prolong the life and health span of organisms.

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