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Interconnection between DNA damage, senescence, inflammation, and cancer

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1. ABSTRACT

In order to deal with endogenous and exogenous factors, including radiation or pathogens, cells evolved different strategies. This includes highly complex processes such as DNA damage response, senescence, cell death, and inflammatory reactions. Recent research indicates an interconnection between the mentioned cellular pathways whilst all of them seem to play a role in induction and progression, but also the prevention of cancerous diseases and therefore qualify for potential prevention and treatment strategies. On the basis of their pivotal functions in cancer biology in general, each of the cellular processes represents promising single therapeutic targets. Further, due to their strong interconnection, targeting all of them in a multimodal approach could be another promising strategy to treat cancer. We, therefore, review the mechanisms of DNA damage induction, detection and repair as well as the induction of cell death. Further, features of senescence and mechanism of inflammation induction and abrogation are outlined. A special focus is set on how senescence and inflammation are related to diseases and how targeting them, could contribute to improvement of cancer therapies.

2. INTRODUCTION

Distinct impacts of external or internal origin induce various cellular responses. One of those stressors is ionizing radiation and since the DNA is the most radiosensitive structure within a cell, radiation predominantly induces DNA damage and consecutive DNA damage responses. As a consequence, DNA repair, cellular senescence, cell death and in a worst-case scenario also malignant transformation of cells are the outcome. Additionally, endogenous stressors such as inflammation exhibited by the organism itself exist. An inflammatory reaction consists of an initiation and a resolution phase. It is triggered by immune cells upon exposure to dangerous or foreign material and represents in first line a protective event. An interconnection between DNA damage, inflammation and senescence has become more and more evident in inflammatory and malignant diseases and is in the focus of this review.

3. DNA DAMAGE: INDUCTION, DETECTION, REPAIR, AND CELL DEATH

Organisms are exposed to a variety of exogenous and endogenous harmful insults during their lifespan. The DNA in a single cell contains all information necessary for fundamental processes of the organism. Therefore, it is important for any organism to prevent DNA damage and where not possible to quickly and reliably repair the damage, as damage-related mutations on the long run can lead to cancer and other diseases (1).

DNA damage can be induced by physical (e.g. ionizing radiation) and chemical agents (e.g. cytotoxic

cancer drugs). In addition, also during normal cellular metabolism, reactive chemical by-products can harm the DNA. Further, genomic damage can occur due to errors in DNA replication due to misincorporation of bases by DNA polymerases (2). Ionizing radiation for example can induce single strand breaks (SSB), double strand breaks (DSB), oxidation of DNA bases or inter-/intrastrand crosslinks, respectively (3-5). The damage is induced either by direct interaction of a particle with DNA or in biological systems mostly secondary by chemical processes triggered by ionizing radiation (5). DNA damaging agents such as reactive oxygen species (ROS), which include O_2^-, H_2O_2 or $OH^\bullet$ or reactive nitrogen species (RNS) such as NO_3^- or $NO_2^\bullet$ are the key players. In addition, an induced DNA-damage itself can lead to the formation of ROS through the H2AX-Nox1/Rac1 pathway (6).

Different types of DNA damage involve different ways of sensing and repair mechanisms including a plethora of molecules also in dependence of the cell cycle phase. Double strand breaks (DSB) and single strand breaks (SSB) are detected by MRE11-RAD50-NBS1 (MRN) and replication protein A (RPA) complexes, respectively (7). The initial recognition step leads to the recruitment of DNA damage response (DDR) kinases ataxia telangiectasia mutated (ATM) by MRN or ATM and rad3-related (ATR) by RPA (8-12). Subsequently through the mediating function of proteins like p53-binding protein1 (53BP1; ATM) or topoisomerase-binding protein 1 (TopBP1, ATR) the effector kinases CHK1 and CHK2 are activated which further impact on transcription factors, cell cycle regulators, DNA repair or the apoptotic machinery such as p53, BRCA1 or MDM2 (13-18).

Although being very quick and reliable in general DDR can fail. In that case, a transmission of DNA damage during cell division should be prevented. For this purpose, cells evolved several different cell cycle checkpoints leading to slow-down of cell cycle progression or to cell cycle arrest in case of un- or misrepaired DNA damage. This gives cells time for repairing the damage before proceeding in cell cycle (for comprehensive review see (19-21).

In parallel or after established cell cycle arrest/slow-down DNA repair processes are initiated. A variety of DNA repair mechanisms are available, and their utilization depends on the type of DNA damage that is induced. DNA repair mechanisms includes base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and non-homologous end-joining (NHEJ) (reviewed in (22)). A DSB can result in chromosomal aberrations and genomic instability that can lead to malignant transformation and therefore represents one of the most dangerous types of damage. HR and NHEJ are the pathways by which DSB are repaired (23-26). In human cells the HR is restricted to the late S- and G2 phase of the cell cycle as this repair mechanism relies on the intact sister chromatid as a template and, therefore, is regarded as nearly error-free (reviewed in (27)). The more error-prone NHEJ is performed during all cell cycle phases, although showing differences in the importance according to the cell cycle phase and repairs DSB by direct ligation of the broken ends ((28) and reviewed in (29)).

Although highly functional and efficient repair mechanisms do exist, cells are often not able to repair all of the DNA damage. This is especially true for complex or prevalent quantity of damage. These events require the eradication of the damaged cells by cell death as the dissemination of possible mutations and related malignant transformation has to be prevented. Possible cell death forms include mitotic catastrophe, apoptosis, necrosis, and autophagy (reviewed in (30)).

4. SENESCENCE: INDUCTION AND CONSEQUENCES

Despite the different cell death options, cells evolved a bypass to deal with persistent DNA damage and potential cancer induction, the so-called (stress-induced) senescence. More than 50 years ago senescence was firstly described by Hayflick and colleagues, who showed that human fibroblasts do not proliferate until infinity in culture (31, 32). This phenomenon was termed replicative senescence due to the erosion of telomeres during replication. In addition to stress-induced and replicative senescence a third “type” has been identified as oncogene-induced senescence (OIS). In this case, oncogenes like RAS or BRAF can activate senescence inducing pathways culminating in cell cycle arrest, making OIS another cellular strategy of the cells to avoid malignant outgrowth (33-35). Also the loss of tumor suppressor genes like PTEN, RB1 or INPP4B can result in induction of senescence (36-38).

Senescence pathways are initially induced through altered expression of cell cycle inhibitors or activators including cyclin-dependent kinase inhibitors (CDKI) like p21 or p16 (39-41). It is interesting to note that p21 is supposed to be important for the initial cell cycle arrest and p16 for the manifestation of the latter (42). Senescent cells display several characteristics such as the absence of proteins driving proliferation, morphological changes (increase in volume, flattened morphology if adherent) or expression of senescence-associated ß-galactosidase (SA-ßgal) (31, 43). Additionally senescent cells harbor telomere-dysfunction foci (TIF) or DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS). These segments contain several DNA damage foci like 53BP1 that are not attributable to the initial DNA damage (44, 45). Although senescent cells do not proliferate, they remain in a metabolically active status and thereby cannot be defined as dead (46). Above all, the expression profile

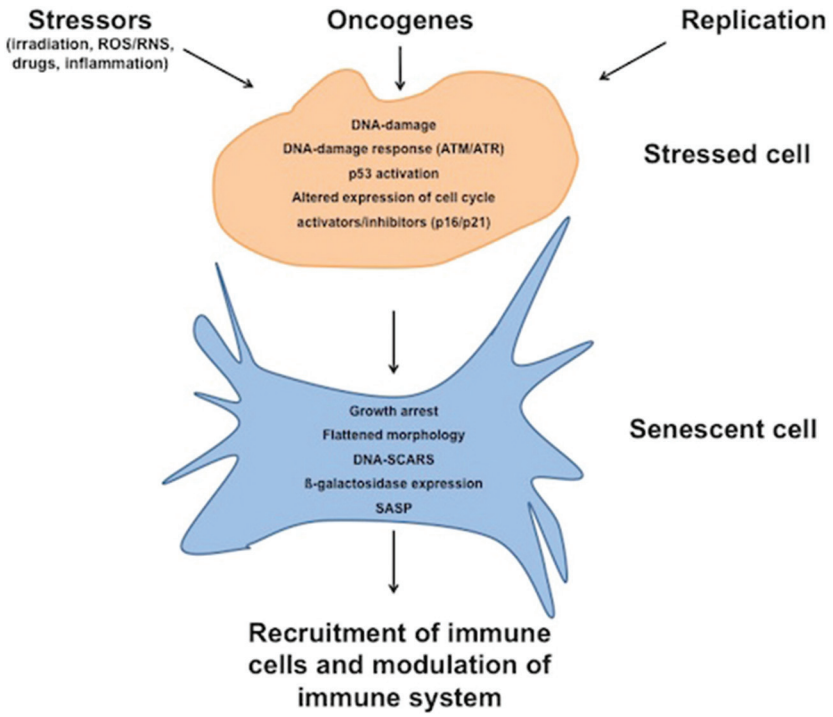

Figure 1. Inducers and features of senescence. Several influences such as stressors (radiation, reactive oxygen species (ROS), reactive nitrogen species (RNS), drugs or inflammation), oncogenes, and replication have the potential to stress a cell. The DNA damage and related DNA damage response via the kinases ataxia telangiectasia mutated (ATM) or ATM and rad3-related (ATR) can lead to p53 activation and subsequently to an altered expression of cell cycle activators or inhibitors (e.g. p16/p21). These cellular events have the potential to induce a senescent cell which can be characterized by a persistent growth/divisional arrest, a flattened morphology, DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), ß-galactosidase expression as well as the expression/secretion of the senescence-associated secretory phenotype (SASP). The latter can lead to the recruitment and modulation of the immune system.

and the secretion of distinct proteins change dramatically during the induction and establishment of senescence. This so-called "senescence-associated secretory phenotype" (SASP) is induced with different qualities and robustness merely upon DNA damage, oxidative stress or dysfunctional telomeres (41, 45, 47-52). The SASP includes pro-inflammatory cytokines (e.g. Interleukin (IL)-1α, IL-1ß, and IL-6), chemokines (e.g. IL-8), and growth factors (e.g. vascular endothelia growth factor (VEGF)) (53, 54). Figure 1 summarizes the inducers and features of senescence.

Key molecules in the induction of the SASP are nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) and C/EBP-ß, which are activated upon DDR-induced expression of the membrane-bound IL-1α and subsequent binding to its plasma-membrane-associated receptor IL-1R (53, 55-57). Furthermore, the mitogen-activated protein kinase (MAPK) p38 and the mammalian target of rapamycin kinase are involved in the induction of the SASP (55, 57, 58). Consecutively the NF-κB signaling cascade is activated, representing a hallmark pathway for the expression of pro-inflammatory cytokines/chemokines such as IL-1ß, IL-6, IL-8 and tumor necrosis factor alpha (TNFα) (reviewed in (59)). It is important to note that in case of senescence-associated cytokine/chemokine expression and related receptors induced by oncogenes, these released mediators support and maintain the senescence growth arrest (53, 54). Acosta *et al.* showed for example that in premature senescence the NF-κB and C/EBP-ß pathways induce the expression of chemokine receptors (CXCR), namely CXCR1 and CXCR2 in a p53-dependent manner (54). This upregulation leads to an increased DNA damage/DDR and further enforcement of the oncogene-induced senescence and the SASP including ligands for the chemokine receptors. The subsequent interaction of CXCRs with their cognate ligands could act as positive feedback loop to further manifest growth arrest (54).

Cytokines, chemokines, and growth factors are components of the SASP and can also act as key players in inflammatory reactions. Therefore, an interconnection between senescence and inflammation is apparent.

5. INFLAMMATION: INVOLVED CELLS, INDUCTION, AND RESOLUTION

Since the cytokines and chemokines secreted for the clearance of senescent cells attract a plethora

of immune cells, inflammatory response is one of the hallmarks of senescence (60). In general, inflammation is a protective process of the immune system against intruders from outside (host defense) and for repairing endogenous tissue damage (tissue homeostasis). Five classical signs normally accompany inflammation: heat, swelling, redness, pain, and loss of function. These macroscopic signs reflect the vasodilatation and extravasation of immune cells into the inflamed tissue triggering and sustaining the acute local inflammation. Such an acute inflammation consists of induction, regulation and resolution, followed by tissue repair. Nevertheless, if one of these highly regulated processes fails to terminate, a chronic inflammation might persist.

An inflammatory microenvironment is characterized by an overproduction of cytokines, chemokines and toxic reagents, such as ROS or RNS, released to eradicate microbial invaders. However, these highly potent effectors are not able to discriminate between host and foreign; thus collateral damage is unavoidable (61). Consequently, a persisting inflammation leads to prolonged tissue degeneration accompanied by loss of function, which might lead to a benign, inflammatory disease or tumorigenesis, or both (62-64).

Inducers of inflammation can be divided into exogenous factors, such as pathogens, allergens or toxic compounds, and endogenous ones, including damaged, stressed or otherwise malfunctioning cells and/or tissues (summarized in (65)). For a swift and effective detection of microbial infections, the immune system developed pattern recognition receptors (PRRs) as sensors for detecting a conserved set of pathogen-associated molecular patterns (PAMPs; e.g. lipoproteins, dsRNA, β-glucans) that are expressed exclusively by foreign microorganisms. Likewise, receptors have developed for sensing damage-associated molecular patterns (DAMPs; e.g. HMGB-1, ATP, RNA), which are abundant intracellular components released by damaged cells upon disintegration of the plasma membrane. Furthermore stressed cells might release intracellular content through an active and caspase-1-dependent process that likewise is sensed by residential immune cells (66). The PRRs encompass two classes of transmembrane proteins including Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), as well as two classes of cytosolic proteins, the so called retinoic acid inducible gene-I-like receptors (RLRs) and nucleotide-binding oligomerization domain like receptors (NLRs) (summary of PRRs and following signal cascades is given in (67)). Upon receptor-molecule interaction adaptor proteins (e.g. MyD88, Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)) are recruited and, depending on cell type and PRR, various signaling cascades are activated, involving different MAPK pathways (e.g. p38 MAPK pathway, ERK-pathway). All these signaling cascades can culminate in the activation and translocation of transcription factors (e.g. NF-κB, activator protein 1, interferon regulatory factors, C/EBPβ) into the nucleus to activate the transcription of pro-inflammatory genes (68). The latter are also regulated by chromatin remodeling and epigenetic modifications (69, 70). The NF-κB pathway in particular plays a central role in regulation of genes for inflammatory cytokines, adhesion molecules, angiogenic factors and cell survival (summarized in (71-73)). In the light of tumorigenesis, NF-κB is described to be deregulated in a relevant number of tumors and thus promoting tumor cell survival and proliferation (summarized in (74)).

An acute inflammation is a very complex, coordinated and regulated process with the involvement of many different molecules and cells. The initiation, regulation and resolution of an acute inflammatory response are mainly determined by the innate immune system, which also defines the extent of subsequent adaptive immune mechanisms (75). In the initial detection of DAMPs or PAMPs tissue residential macrophages, dendritic cells, mast cells, endothelial cells and epithelial cells play a crucial role. Upon recognition, macrophages produce and release, among others, inflammatory mediators such as cytokines (e.g. TNFα, IL-6, IL-1) and chemokines (e.g. IL-8, CCL2, CXCL8), which further recruit immune cells. In parallel, residential mast cells release amines causing vasodilatation and an increased vascular permeability. As a consequence, additional immune cells including neutrophils, monocytes, eosinophils and basophils are recruited by chemotaxis from the blood. Besides, plasma containing antibodies and other soluble mediators (e.g. complement) enter the area of inflammation. Cytotoxic molecules (e.g. defensins, ROS, RNS) released from neutrophils and macrophages in combination with phagocytosis lead to the eradication of the pathogens (76). In parallel, dendritic cells migrate into draining lymph nodes presenting processed antigens to T and B cells, and thus, induce a specific adaptive immune reaction (77). To avoid chronic inflammation, its trigger has to be eliminated. If that fails, the inflammation persists and might contribute to tumorigenesis.

The resolution of inflammation is an actively regulated process involving the reduction and termination of tissue infiltration of immune cells, counter-regulation of cytokines and chemokines, apoptosis of effete immune cells with subsequent clearance by macrophages, as well as the return of remaining immune cells into the blood or lymphatics (summarized in (78)). The resolution process is initiated by the release of pro-resolving mediators including lipids (e.g. lipoxins, resolvins, protectin, maresins), proteins (e.g. annexin A1, prostaglandins), peptides, gaseous mediators (e.g. carbon monoxide, hydrogen sulfide) and neuromodulators (summarized in (78)). The extravasation of immune cells is blocked by annexin A1 (79), which also accelerates neutrophils apoptosis (80) and clearance by macrophages (81-83). The macrophages transform from a pro-inflammatory M1

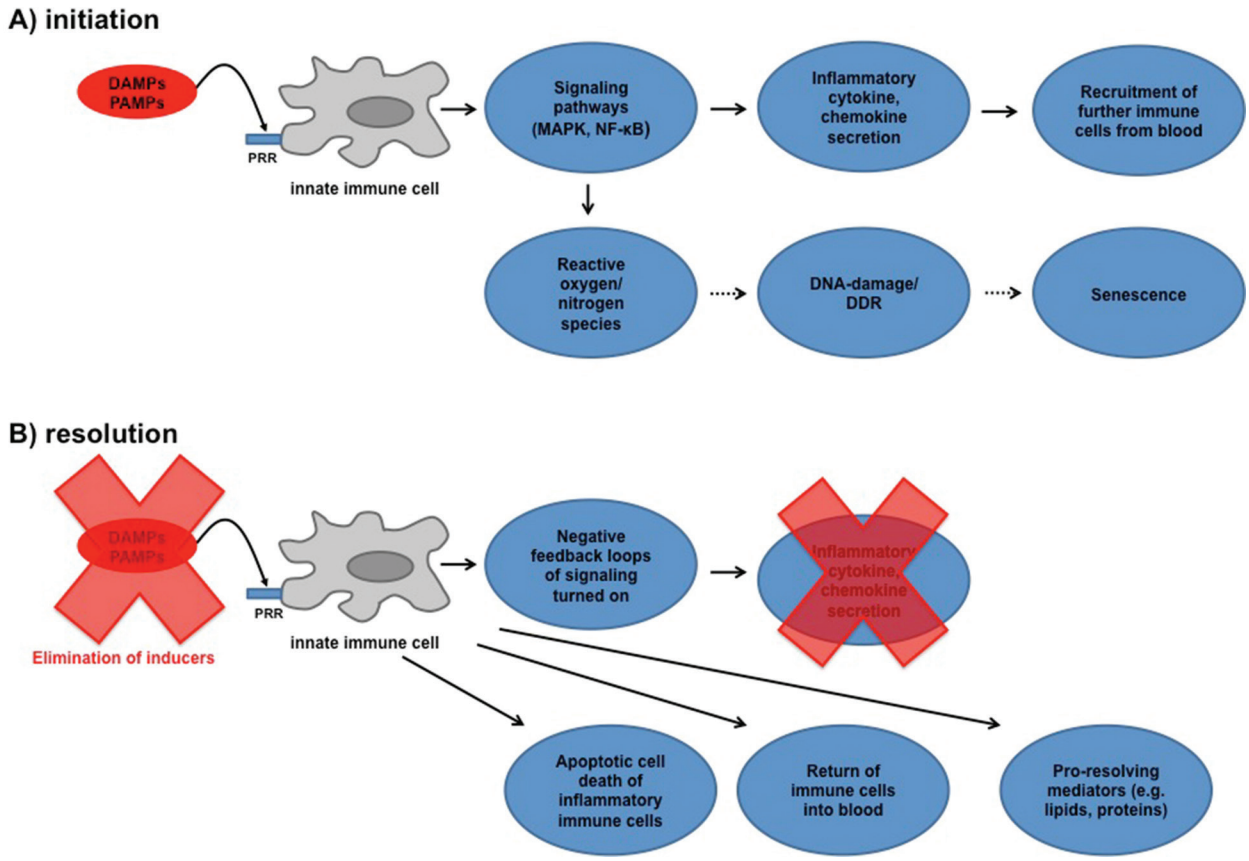

Figure 2. Features of inflammation: initiation and resolution. A) Initiation: Inflammatory reactions are triggered upon the binding of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) to their respective pattern recognition receptor (PRR) on innate immune cells. Upon binding, several pathways, like mitogen-activated protein kinase (MAPK) or nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) induce the expression/release of inflammatory cytokines, chemokines and subsequent the recruitment of additional immune cells from the blood to the site of inflammation. In addition, reactive oxygen/nitrogen species are produced as defense mechanism by immune cells, which could provoke collateral DNA damage/DNA damage response (DDR) in bystander cells and consecutively senescence. B) Resolution: A hallmark for the resolution of inflammation is the elimination of the inducer(s). The missing binding of DAMPs and PAMPs, the subsequent shutdown of related signaling pathways, and the induction of negative feedback loops stop the expression/secretion of inflammatory cytokines or chemokines. In addition, pro-resolving mediators such as lipids (e.g. lipoxins, resolvins, protectin, maresins) and proteins (e.g. annexin A1, prostaglandins) are released by immune cells. Further the overall amount of (inflammatory) immune cells within the site of inflammation is reduced. The latter is accomplished by the return of effete immune cells into the blood or apoptotic cell death of inflammatory immune cells.

type into regulatory M2 macrophages or an intermediate subset suppressing the inflammatory response and initiating tissue repair (84-88).

Nevertheless, some studies suggest not only an involvement of the innate immune system in the resolution of an inflammation, but also a participation of residential tissue lymphocyte populations that control the innate immune response (89, 90). It is important to note that most signaling pathways have negative feedback loops (91-93). Eventually, the resolution is accompanied by tissue repair that allows restoration of tissue homeostasis (88).

To summarize, the initiation of an inflammatory reaction as well as its resolution are tightly regulated processes (Figure 2) to protect the host and, as outlined in the following, are interconnected with other key processes such as cellular senescence.

6. SENESCENCE, INFLAMMATION, AND DISEASE

Senescence and its related SASP foster the recruitment of immune cells. Therefore, the SASP is supposed to act as a danger signal for the immune system aiming to eradicate potentially transformed or damaged cells (94-96). Nevertheless, senescent cells also harbor the potential to induce diseases or accelerate their progression, including cancer, atherosclerosis or neurodegeneration, even they lack replicative capacity (97-107).

Consequently, one burning question is how senescent cells influence the induction or progression of diseases, although they should act as activators of the immune system and thus should, in turn, be cleared.

One possible explanation is an accumulation of senescent cells with increasing age, possibly through inefficient clearance mechanisms or an overload of the clearance capacity of the immune system (44, 108-110). As a consequence, accruing senescent cells and the increased release of SASP factors, could enforce the immunological responses leading to sustained inflammation and potentially induction of inflammation-related diseases. In addition, liberated SASP factors could lead to alterations in surrounding or distal tissue/cells (bystander effects) stimulating growth and angiogenesis (111-114). In conclusion, cellular senescence primarily represses malignant transformation by suppressing division of damaged cells and stimulating their subsequent clearance by the immune system. Nonetheless, secondary senescence harbors harmful potential by inducing bystander effects in neighboring cells via the SASP if the clearance process is ineffective. Hence, cellular senescence has an impact on both, acute and chronic inflammation.

7. INTERCONNECTION OF SENESCENCE, INFLAMMATION, AND DNA DAMAGE

As described above, inflammatory events mediated by immune cells are induced upon the recognition of PAMPs or DAMPs and often involve a NF-κB pathway activation, subsequently leading to the release of pro-inflammatory cytokines/chemokines followed by a recruitment of additional immune cells (59, 65, 68). Further, ROS and RNS are produced by immune cells as defense mechanisms in the context of inflammation (76).

Nonetheless, despite their initial positive relevance as a defense mechanism, the secretion/production of cytokines, ROS, RNS and chemokines can have unwanted side effects. Especially the release of the mentioned factors could have a harmful influence on cells being in near proximity to the effector immune cells. Those possible harmful effects include inflammation-mediated DNA damage. ROS/RNS in particular induce collateral (DNA) damage during inflammatory conditions, as already outlined above (5). ROS/RNS are produced directly by immune cells upon TLR stimulation. Further, cytokines like TNFα could lead to the production of ROS/RNS in non-immune cells upon binding to their respective receptors (115, 116).

Thus, a persistent inflammation could either represent the starting point or the consequence of cellular senescence. It further potentially leads to DNA damage by the mentioned mediators. The inflammation-induced DNA damage could serve as the initial step in mediating cellular senescence and the related SASP. Nonetheless, the SASP could also either be an initiator or a promoter of inflammation. Cellular senescence and particularly the released SASP components lead to the recruitment of immune cells. The resulting inflammatory responses could further initiate DNA damage and activate the DDR, which in turn contribute to sustain the inflammation. This is a classical chicken-and-egg dilemma. Who comes first?

Regarding inflammatory diseases, senescence could also act in a supportive manner as an inflammation related DDR induces the SASP and further manifests inflammation. Nevertheless, as senescent cells accumulate during the life span and given that many inflammatory diseases are age-related, an inducing role cannot be ruled out (117-119).

It is feasible to assume that in cancerous diseases the initial step for senescence is oncogenic rather than an inflammatory event, as the induction of oncogenes or the loss of tumor suppressor genes can end in cellular senescence. In case of the negative effects of senescence, the SASP factors stimulate the recruitment of immune cells, resulting in augmented secretion of inflammatory cytokines/chemokines, induction of DNA damage, and finally sustained or even spreading senescence (120). In both conditions, either inflammation or oncogenic transformation, a vicious cycle results from several mediators and inducers with cellular senescence being a key element. Nevertheless, the described recruitment of immune cells could also lead to the clearance and subsequent eradication of malignant cells.

It is widely accepted that a sustained inflammation can induce and promote malignant transformation. Therefore it is being defined as an enabling characteristic of cancer by Hanahan and Weinberg (121). Patients suffering for example from inflammatory diseases of the intestinal tract like Colitis ulcerosa or Crohn´s disease display a strikingly increased risk to develop intestinal cancers (122, 123). One could assume that sustained inflammatory processes may also induce senescence in cells proximal to the inflammatory region. This could culminate in the release of SASP-associated tumor promoting factors for angiogenesis and/or epidermal-to-mesenchymal transition (EMT), all supporting tumor progression. Nevertheless, one has to keep in mind that tumor promoting and inhibiting events are in part mediated by the same factors. Therefore, the SASP-related immune cell recruitment could also lead to the elimination of cancerous cells and potentially prime anti-tumor-immunity (124).

The interconnection of inflammation and DNA damage events is fortified by recent findings. Figuereido *et al.* demonstrated that an anthracycline-mediated DNA damage and the related activation of the DDR lead to increased autophagy and consecutively reduced sepsis in a monocytic cell line (125). Further, our studies of peritoneal macrophages isolated from mice with

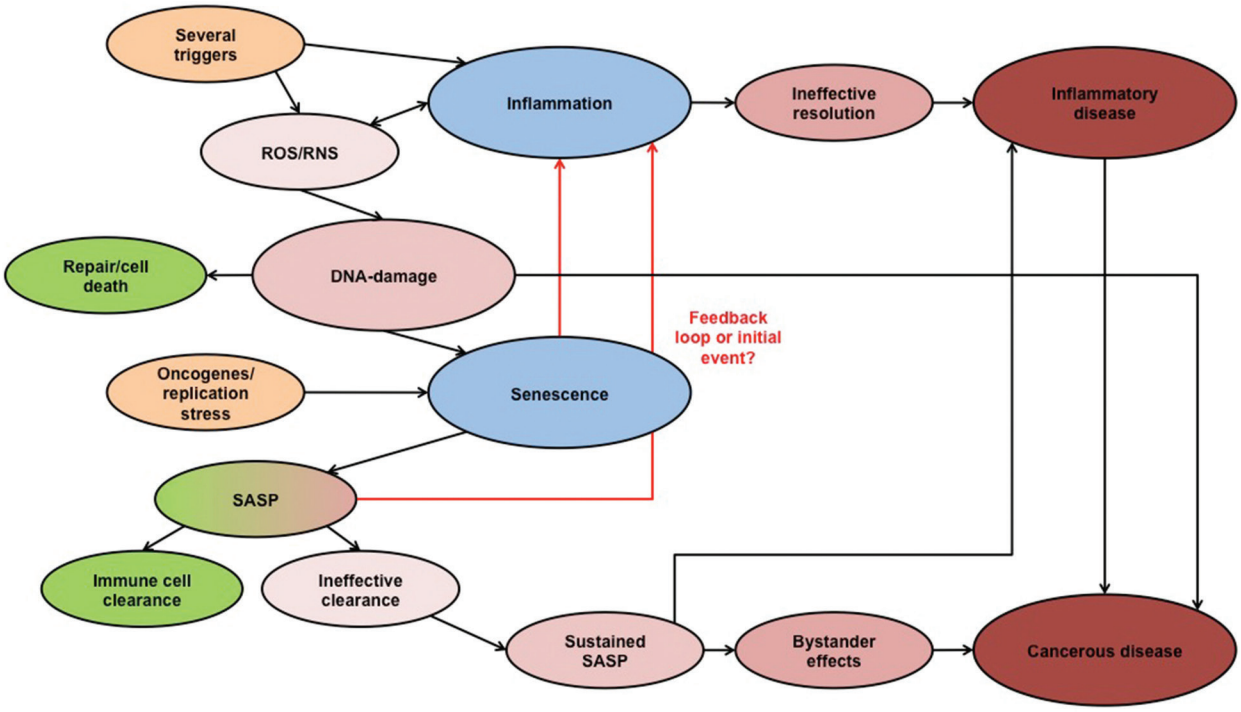

Figure 3. Interconnection of DNA damage, senescence, inflammation, and disease. The starting points which connect/trigger the cascade of DNA damage, senescence, and inflammation in terms of disease induction is multifactorial. First, several triggers induce reactive oxygen/nitrogen species (ROS/RNS) or an inflammation. The inflammation itself triggers ROS/RNS or *vice versa*. The produced ROS/RNS further can lead to DNA damage what either culminates in repair mechanism or cell death, but also potentially generates senescent cells. Senescence represents also one starting point within the network being in addition induced by oncogenes or replication stress. Senescent cells express/secrete the senescence-associated secretory phenotype (SASP) inducing on the one hand the clearance of senescent cells by the immune system and on the other hand can be an inducer of inflammation. If the clearance of senescent cells is not effective, a sustained SASP could lead to bystander effects in nearby cells, which could result in cancerous disease. Nevertheless, the sustained SASP could also induce inflammatory diseases, which also harbor the potential to generate cancerous diseases. Of note is that an ineffective resolution of inflammation could lead to inflammatory disease and probably downstream of the latter to cancer. If senescence or better to say the SASP can act as initial event or a (fueling) feedback loop is probably dependent on the induction mechanism (red arrows). Color code: Inducing factors are shown in orange. Blue color indicates inflammation and senescence as the hallmark centers within the cascades. Positive or non-disease related consequences are shown in green and the disease related consequences are marked in shades of red according to their severity.

deficiencies in DNA repair capacity (more radiosensitive) showed a reduced secretion of the pro-inflammatory cytokine IL-1ß after irradiation in comparison to macrophages form more radioresistant mice (126). Figure 3 illustrates the complex interconnections between inflammation, senescence, SASP, DNA damage and the impact on inflammatory and malignant diseases.

8. RADIOTHERAPY, INFLAMMATION, AND SENESCENCE

Radiotherapy (RT) is widely used in the treatment of cancer, but also for the attenuation of benign, inflammatory diseases. A variety of irradiation regimes with different single and cumulative doses are employed. For the treatment of benign, inflammatory diseases usually a single dose of 0.5 Gy up to a cumulative dose of 3.0 - 6.0 Gy are clinical standard. RT hereby results in reduced inflammation translating into long lasting pain reduction for several years (127-133). The attenuation of inflammation by RT can be attributed to a reduced overall immune cell count in the site of inflammation and further by the induction of an anti-inflammatory immune cell phenotype. Macrophages in particular are rendered more anti-inflammatory by low dose RT (126, 134-137).

For cancer therapy, a higher single dose of approximately 2.0 Gy is used in distinct fractionation schemes. The main goal of RT in cancer treatment is to eradicate the tumor and, if complete eradication cannot be accomplished, to locally control the tumor. This is achieved by suppressing the proliferation of aberrant cells and by stimulating distinct cell death pathways. Local irradiation of the tumor has to fulfill two main requirements: on the one hand the tumor control probability (TCP) must be as high as possible, but on the other hand the normal tissue complication probability (NTCP) has to be as small as possible (summarized in (138)). Therefore, the applied dose is finely balanced between minimal, justifiable NTCP matched with a maximal therapeutic success.

High dose irradiation could, despite primarily inducing cell proliferation stop and cell death as consequence of DNA damage induction, also have consecutive effects for tumor eradication by rendering the malignant cells immunogenic (139). From this point of view radiation is also associated with inflammatory processes (140).

Nonetheless, despite killing tumor cells and mediating anti-tumor immune responses, inducing senescence can also lead to RT-mediated tumor control. If the induction of senescence in 100% of the malignant cells could be achieved, this should result in a complete stop of tumor growth. Nonetheless, this raises the question of the potential of senescent cells to regain dividing potential. Although there is no direct prove of this fact *in vivo*, some *in vitro* studies showed that formerly senescent tumor cells or at least a subset of these, are able to recover from senescence. Jones *et al.* showed that MCF-7 breast cancer cells, regain proliferative capacity in a p53-dependent manner after irradiation with 10 Gy (141). Such a recovery was also detected after the treatment of a human lung cancer cell line with chemotherapeutic agents (142, 143). However, as 50% of human cancers are mutated in p53, this is not relevant for all tumor entities (144). Despite the potential to re-enter the cell cycle and induce tumor progression, senescent (tumor) cells or especially their related SASP could induce malignancy in surrounding cells as described or protect nearby (tumor) cells from damaging therapeutically agents. These so called bystander effects were described for irradiated fibroblast that were co-incubated with untreated breast cancer cells: an increased proliferation rate in the breast cancer cell line was observed (145). Although irradiation was performed in a fractionated manner with 0.5 Gy every 12 h until reaching a cumulative dose of 10 Gy instead of using clinical relevant single doses of ~ 2.0 Gy, the experiments clearly show the tumor promoting potential of senescent cells after irradiation. Clinical studies also demonstrated a senescence-associated reduced overall survival of patients suffering from advanced non-small cell lung cancer after neo-adjuvant chemotherapy or radiochemotherapy (146). Especially a high expression of the senescence-associated biomarkers in tumors was related to a poor outcome (146). Further, Sidi and colleagues showed that senescence is correlated with impaired clinical outcome for patients with pleural mesotheliomas after neo-adjuvant chemotherapy (147).

Nevertheless, one should also mention the tumor-suppressing role of senescence. A mouse model with sustained p53 activity and thereby a high induction of senescence showed less tumor formation, but premature aging (148). This supports the hypothesis that senescence also bears cancer-suppressing functions and the key role of p53 in this scenario (148). Further, accelerated senescence in human advanced colon cancer induced by chemotherapy was related to improved clinical outcome (149).

Apparently, the use of senescence as a biomarker for therapeutic outcome/success reveals controversial results and might be related to the different tumor entities. Further, the same judgement has to be made for the induction/repression of senescence and related inflammatory reactions in a therapeutic context.

9. TARGETING SENESCENCE AND/OR INFLAMMATION AS THERAPEUTIC OPTION

DNA damage, cellular senescence, related SASP, and inflammation can be induced by various ways and stimuli. Especially senescence comprises tumor suppressing as well as tumor-promoting properties (see above). These characteristics render senescence and the SASP potential targets to further improve prevention and/or therapy of cancer.

The different senescence-inducing pathways could act as single or additional targets to support or to improve existing therapies for malignant diseases. Nevertheless, senescence induction is mainly dependent on the p53 pathway, meaning that for malignancies deficient in p53 the induction of senescence could be of minor relevance. Therefore, those malignancies primarily require reactivation/enhancement of the p53 pathway to make senescence an option to stop tumor progression. The enhancement/restoration was first described for small molecules such as nutlins (150). Moreover, restoring the p53 activity has been shown to induce senescence, immune response and also to support tumor regression (94, 151). In addition to nutlins also reactivation of mutant p53 function by new drugs such as PRIMA^{MET} and ellipticine, which lead to structural changes of p53, was described (152, 153). Moreover, RITA a small molecule for reactivation of p53 and induction of tumor cell apoptosis has been shown to induce senescence in head and neck squamous cell carcinoma (HNSCC) cell lines by the induction of CHK2 and subsequent inhibition of Silent information regulator T1 (SIRT) regardless of the p53-status (154).

Further, also targeting other pathways of senescence seem to be promising strategies for cancer therapy. One of those approaches is to use inhibitors of telomerase activity in tumor cells to induce senescence. GRN163L limits the life span of pancreatic cancer cells, reduces tumor growth in a xenograft breast cancer mouse model, and is currently tested in Phase II clinical trials for hematological malignancies and prostate cancer (155, 156). Likewise MDM2 inhibitors (Serdemetan or RO5045337; Johnson & Johnson Pharmaceutical Research & Development, Hoffmann-La Roche respectively) disrupting the MDM2-p53 interaction and thereby enhancing senescence are under clinical

investigation. Even an adenoviral-based vector (Gendicine™, Shenzhen SiBiono GeneTech) gene transfer of human p53 into HNSCC is used in China since 2003 to reinitiate the p53 pathway and senescence.

Another recently described group of modulators of senescence are Mucins (MUC). Those surface proteins, which are heavily O-glycosylated, display high expression levels on tumor cells. For example MUC4 has been shown to be overexpressed on the surfaces of breast, thyroid and pancreatic cancers (157). The regulatory role for senescence has been shown by Macha *et al.* linking a reduced/deficient MUC4 expression to an induced senescence via the p16/pRb pathway in HNSCC cell lines (158). Senescence-induction accomplished by MUC4-knockdown has been further related to tumor growth reduction in animal models (158).

The potential of senescence induction by blocking DNA repair mechanisms in combination with RT as a combinatorial treatment for malignancies has been shown by Meng *et al*. (159). Therapy-induced senescence in tumors led to an increased adaptive immune response through the recruitment and proliferation of tumor specific cytotoxic $CD8^+$ T-lymphocytes mediated by an immunostimulatory phenotype of the SASP. Additionally, these treated, senescent cells were able to act as a vaccine and conferred protection to subsequent inoculation with living tumor cells (159).

Nonetheless, as it is widely accepted, the induction of tumor cell death and subsequent eradication should be the main goal of cancer therapy. However, senescence induction could represent a failsafe mechanism to stop tumor progression in first line if the aforementioned therapy targets (cell death and eradication) cannot be achieved.

The fact that chronic, inflammatory diseases harbor the risk of malignant transformation (Figure 3), offers further possibilities to prevent cancer induction. In this scenario, the initial role of inflammation leading to malignancy could be eradicated in line with an improvement of the clinical symptoms of the inflammatory disease. As inflammation and senescence are connected, the modulation (induction/suppression) of senescence in order to influence the pathology of inflammatory diseases could be a central idea. The choice to either induce or repress senescence has to be based on the phase of disease during which therapy is intended to be applied. Therefore a precise assessment of the disease phase before treatment is required.

As described, senescence cells, or precisely the ineffective clearance of the latter, and related sustained inflammation by the SASP have the potential to induce inflammatory diseases. Therefore, a decrease in the number of senescent cells in the very beginning of the inflammatory disease could reduce the SASP-mediated recruitment of immune cells and further acceleration of inflammation, thereby slowing down the progression of disease. If such a concept could be used during disease onset, the initiating step of senescence-associated inflammatory diseases could be eliminated or reduced, and a downstream malignant transformation could perhaps be suppressed. The elimination of senescent cells could be achieved by enhancing the clearance of those cells or by repressing or bypassing the induction of senescence.

Nonetheless, inflammatory diseases such as rheumatoid arthritis display highly proliferative fibroblasts leading to pannus formation and destruction of the cartilage and bone (reviewed in (160)). The high proliferation rate of the synovial tissue could represent a basic concept for senescence therapy. Taniguchi *et al.* have shown that the induction of the p16 senescence gene by adenoviral gene transfer into rheumatoid arthritis affected tissue was able to target the proliferation of the synovial cells and to induce senescence-associated markers. This led to improved disease associated parameters including joint swelling, pannus formation or cartilage destruction (161). An advantage of senescence induction by p16 would be that the SASP as well as above outlined influences of the latter could be prevented, as the SASP is not initiated upon p16- and p21-mediated senescence induction (162).

In summary, targeting senescence in the context of inflammatory disease has the potential to improve the patients' situation, and in addition inflammation-related carcinogenesis might be prevented.

Despite the induction or repression of senescence, the modulation of its consequences, namely the SASP, could represent another therapeutic option for cancer treatment. The expression and secretion of inflammatory molecules such as cytokines and chemokines is highly dependent on the cellular signaling pathways NF-κB or p38 MAPK, rendering the latter two potential and promising targets to therapeutically influence the SASP production (72, 73, 163). Laberge *et al.* showed that the NF-κB-related secretion of SASP factors such as IL-6 can be targeted by glucocorticoids (especially corticosterone) without reversing the inhibition of proliferation (164). In addition, disruption of the IL-1α/IL-R signaling cascade, which is essential for SASP-induction, by IL-1Ra for instance, could represent a target for therapeutic intervention at the level of SASP production. Another promising drug for the treatment of cancerous diseases is the antidiabetic metformin. The usage of metformin in combination with RT was first described to induce senescence, especially in a p53-independent manner, by increasing ROS levels in HNSCC cell lines *in vitro* (165). Along the same line, the combinatorial treatment has been shown to suppress

tumor growth in animal models and has been related to beneficial outcome for patients in clinical settings (165). In addition it has been shown, that metformin inhibits the NF-κB related induction of the SASP and an antineoplastic activity was detected in laboratory-based and epidemiological studies (166-170). Besides its role in modulating the SASP, the NF-κB pathway is known to be involved in anti-apoptotic gene expression (163). Repression of the NF-κB pathway could thereby further block the increased cellular survival, e.g. in tumor cells. Thus, targeting NF-κB signaling could induce a double positive effect, especially in tumor cells by suppressing related anti-apoptotic effects and the SASP production. However, as the pathway is also related to the induction of beneficial reactions (e.g. inflammation to fight invading pathogens) the application has to be restricted to the site, where its therapeutic action is needed (171, 172). Sehnert *et al.* developed a sophisticated solution to block the NF-κB pathway in a cell type specific manner (173). They introduced a "sneaking ligand construct" (SLC), which selectively binds to endothelia cells and represses intracellular NF-κB activation (173). This concept could also be used to inactivate the NF-κB pathway e.g. in tumor cells by developing a construct, which binds specifically to tumor cells and represses NF-κB activity.

In addition to the local application, also the stage of the cancerous disease and time point of treatment could influence the usage of NF-κB inhibition and therefore need to be considered. Suppressing the pathway in an early phase of tumorigenesis could have adverse effects, as during the elimination phase of the tumor by the immune system a suppressed immune action could be contraindicative. However, during RT an increased DNA binding of NF-κB in myeloid leukemia cells was detected, potentially reducing efficacy of the therapy (174). Opposing the initiation of NF-κB activation for instance by the inhibitor DHMEQ has been shown to increase RT effectiveness in mouse and human studies with different tumor entities (175-178). Tilstra *et al.* recently recognized another feature of NF-κB modulation. They showed that NF-κB inhibition leads to delayed DNA damage-induced senescence (179). This postulates that NF-κB not only is a consequence of senescence but can also be an initiator of the latter.

Furthermore, also the direct targeting of secreted factors of inflammatory reactions or the SASP could represent interesting therapeutic approaches for the therapy of inflammatory and cancerous diseases. Especially in case of inflammatory diseases (such as rheumatoid arthritis) the usage of therapeutic antibodies that e.g. target cytokines such as IL-6 are under investigation, used in clinical trials and lead to clinical improvements (160). As IL-6 is part of the SASP, one could assume that those therapeutic concepts adapted from rheumatology could also be used to target inflammatory reactions in SASP-related cancer therapy.

Additionally, other approaches namely the alteration of miRNAs (miRNA-31) or inhibitors of the Heat Shock Protein 90 (AT13387) seem to alter senescence programs and carcinogenesis making them possible supportive therapy regimes, as both, miRNA-31 and AT13387, are involved in senescence induction and in the case of AT13387 also reduces tumor formation (180-182).

As outlined above, senescence modulation could represent a target of future therapeutic approaches for cancerous diseases. Furthermore, as senescence could be modified at a plethora of levels, all of them should be taken into account for therapy purposes. Nonetheless, the SASP-related negative influences such as induction of bystander tumor cell proliferation, EMT-transition or angiogenesis cannot be neglected. Therefore, these unwanted effects need to be kept in mind when designing new therapies.

10. CONCLUSION

DNA damage, senescence, and inflammation are cellular events that are highly interlinked with each other. They initiate and sustain several pathological situations, especially inflammation and malignant transformation. Keeping this in mind, each of them represents a single key target, which could be used to accomplish therapeutic success or even better to prevent full-blown inflammatory and cancerous diseases. For sure, targeting one component could lead to improved disease outcome or prevention, but modulating them in a synchronized manner might be the way of future treatments in concerted action with the classical therapies. An exciting field of future research represents the timing of these supportive therapies, as some interventions could be promising only in one specific phase of the certain disease, but could have completely opposing effects in later or earlier time points.

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Abbreviations: 53BP1: p53-binding protein 1; ATM: ataxia telangiectasia mutated; ATR: ATM and rad3-related; BER: base excision repair; CDKI: cyclin-dependant kinase inhibitors; CLR: C-type lectin receptors; CXCR: chemokine receptor; DDR: DNA damage response; DNA-SCARS: DNA segments with chromatin alterations reinforcing senescence; DSB: double strand breaks; EMT: epidermal-to-mesenchymal transition; HMGB1: High-Mobility-Group-Protein B1; HNSCC: head and neck squamous cell carcinoma; HR: homologue recombination; IL: interleukin; MAPK: mitogen-activated protein kinase; MMR: mismatch repair; MRN: MRE11-RAD50-NBS1; MUC: Mucin; NER: nucleotide excision repair; NFκB: nuclear factor kappa-light-chain-enhancer of activated B-cells; NHEJ: non-homologous end-joining; NLR: nucleotide-binding oligomerization domain like receptor; NTCP: normal tissue complication probability; OIS: oncogene-induced senescence; PAMP: pathogen-associated molecular pattern; PRR: pattern recognition receptor; RLR: retinoic acid inducible gene-I-like receptors; RNS: reactive nitrogen species; ROS: reactive oxygen species; RPA: replication protein A; RT: radiotherapy; SA-ßGal: senescence-associated ß-galactosidase; SASP: senescence-associated secretory phenotype; SIRT: Silent information regulator T1; SSB single strand break; TCP: tumor control probability; TIF: telomere-dysfunction foci; TLR: Toll-like receptor; TNF: tumor necrosis factor; TopB1: topoisomerase-binding protein 1; TRIF: Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta; VEGF: vascular endothelia growth factor

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