INTRODUCTION

The word ‘senescence’ originated from the Latin word ‘senex’ meaning ‘old age’ or ‘advanced in age’ (Gordon and Nelson, 2012). The term “cellular senescence” represents a permanent loss of proliferative capacity even in the presence of mitogenic stimuli, regardless of its sustained viability and metabolism. The initial observations from primary in vitro cultures of human tissue explants pointed towards existence of “replicative senescence”, a phenomenon brought about by the attrition of telomeric DNA due to the inability of DNA polymerase to completely replicate the lagging strands, resulting in the “end-replication problem” (Kuilman et al., 2010), and consequently limiting the replicative
lifespan of normal cells (Wu et al., 2012). Besides telomere attrition, a variety of stress signals can also induce senescence in cells, such as oxidative stress, stress induced by oncogene expression, loss of tumour-suppressors such as NF1, VHL, etc. (Kuilman et al., 2010). Senescence thus serves as a tumour-suppressor mechanism that can be exploited for anti-cancer therapeutics.

Therapy-induced senescence also known as accelerated cellular senescence (ACS) is a state of cell cycle arrest following administration of genotoxic anti-cancer therapeutic agents. Most of the solid tumours undergo TIS in response to anti-cancer therapies, resulting in disease remission during the early stages of treatment (Wu et al., 2012). In tumour cells, senescence can be a normal cellular response to DNA damage induced by various cytotoxic agents, which results in stabilization of the disease rather than tumour regression. Indeed, presence of senescent cells has been observed in clinical tumour samples following administration of genotoxic therapies. For e.g., post neoadjuvant therapy with cyclophosphamide, doxorubicin and 5-FU in breast cancer, about 41% (15/36) of the tumours were found to be positive for senescence markers such as SA-β-galactosidase activity and p16INK4A, as compared to 10% (2/20) in the untreated tumours, illustrating the induction of senescence as an outcome of therapy (te Poele et al., 2002; Ewald et al., 2010).

**Therapeutic agents inducing senescence in cancer cells**

Anti-neoplastic chemotherapeutic drugs and ionizing radiation are therapeutic modalities in cancer inducing cell death via apoptosis; however, genotoxic agents induce senescence in a subset of tumour cells and stable growth arrest. Thus, senescence serves as an alternative mechanism for curbing proliferation of tumour cells with impaired apoptotic pathways. In contrast to apoptosis, which is rapidly activated within 24 hours, establishment of the senescence phenotype requires several days, often 3–7 days. Cellular decision between apoptosis and senescence is partly governed by the magnitude of stress generated within cancer cells, which in turn depends on the type and dose of the chemotherapeutic agent (Ewald et al., 2010).

Doxorubicin is a chemotherapeutic agent and the anti-tumor activity is via DNA intercalation, inhibition of
macromolecule synthesis (Momparler et al., 1976) and interfering with Topoisomerase II (Fornari et al., 1994). Treatment with 500 nM doxorubicin induced senescence in synovial carcinoma (FU-SY-1SS) cells, eventually leading to cell death (Joyner et al., 2006). Similarly, MCF7 cells with functional p53 underwent senescence upon treatment with 1µM of doxorubicin due to preferential DNA damage at the telomeric regions of chromosomes, resulting in cyogenetic abnormalities (Elmore et al., 2002). Combination treatment of human neuroblastoma cell line SKN-SH with Q-VD-OPH (pan-caspase inhibitor) and 0.1 µM doxorubicin induced senescence via upregulation of p21 (Rebbaa et al., 2003).

Several chemotherapeutic agents induce senescence, 5-Fluorouracil (5-FU) inhibits Thymidine synthase and causes cytotoxicity (Longley et al., 2003). However, treatment with 5µM of 5-FU induced senescence in HCT116 cells (Tato-Costa et al., 2016). Similarly, 5-FU induced senescence in HUVEC and HCM (cardiomyocytes) cell lines at a range of 10 to 1000 µM concentration (Focaccetti et al., 2015).

Etoposide causes DNA double strand breaks by binding to both, DNA and topoisomerase II (Pommier et al., 2010). Besides, it causes formation of free radicals and reactive oxygen species (ROS), causing increased levels of lipid peroxidation. The whole process leads to induction of senescence as shown in B16F10 cells at a concentration of 2 µM (Flor et al., 2016). Treatment of A549 cells at concentrations from 0.75–6 µM etoposide induced a senescent-like phenotype, as evident from the enhanced SA-β-galactosidase activity. However, the phenotype was not correlated with other senescence markers such as stable cell cycle arrest, formation of SAHF and induction of p21. The cells underwent polyploidization with an increasing G2/M population, suggestive of endoreplication post mitotic slippage. The heterogeneous response was possibly due to homozygous deletion of the Ink4b/Arf/Ink4a locus in the cell line (Litwiniec et al., 2013).

Cisplatin interferes with DNA replication by formation of intrastrand crosslink adducts with platinum ions, eventually culminating in apoptosis of tumour cells (Siddik, 2003). Recently it was demonstrated that cisplatin induces senescence in A2780 ovarian cancer cells by negatively regulating the expression.
of GRP78 a major stress induced chaperone. Similarly, treatment with 1–5 μg/ml of cisplatin induced G1 arrest and senescence in HepG2 liver cancer cells via upregulation of p53 and p21 (Qu et al., 2013).

Ionizing Radiation (IR) is one of the most common modality for cancer treatment, primarily inducing senescence in various cancer cells. However, the response is highly context dependent in tumour cells. The status of PTEN tumour suppressor modulates the response of glioma cells to ionizing radiation. PTEN expressing glioma cells undergo apoptosis; whereas PTEN deficient cells underwent senescence in response to ionizing radiation through activation of AKT/ROS/p53/p21 pathway (Lee et al., 2011). Similarly securin-null HCT116 cells underwent senescence, whereas cells with wild-type securin failed to do so in response to radiation (Chen et al., 2010). The percentage of senescent cells increased to 100% with increasing doses of radiation (up to 25 Gy) in B16F10 cells (Flor et al., 2016).

Besides, small molecule inhibitors such as MLN8054 (Aurora kinase A inhibitor) caused senescence induction via upregulation of p53 and p21, at low concentrations ranging from 0.25–4 μM in HCT116 cell line (Huck et al., 2010). Similarly, AKI603, a novel small molecule inhibitor of Aurora kinase A induced senescence in chronic myeloid leukemia cells expressing wild type or T315I mutant BCR-ABL at concentrations from 0.16 μM and above (Wang et al., 2016). Administration of aurora kinase A inhibitors MLN8054 (60 mg/kg) or MLN8237 (30 mg/kg) induced senescence in orthotopically implanted metastatic melanoma tumours in mice, mediated via the ATM/Chk2 pathway (Liu et al., 2013).

The other chemotherapeutic agents known to induce senescence in various cancers are compiled in table 1 (Ewald et al., 2010; Perez-Mancera et al., 2014).

Mechanisms of senescence induction in response to therapy
Therapy induced senescence is not associated with the conventional shortening of telomeres, and is not prevented or reversed by overexpression of telomerase. Generally, the central players of the senescence machinery remain the same in replicative or therapy-induced senescence, viz. p53, p21 and p16. However, senescence is also induced in the absence of these proteins, indicating presence of
<table>
<thead>
<tr>
<th>Therapeutic agent</th>
<th>Concentration</th>
<th>Cell line(s)</th>
<th>Mutation status of TP53/ RB1/ CDKN1A/ CDKN2A</th>
<th>Origin/Cancer Type</th>
<th>Mechanism of action</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Aphidocolin</td>
<td>200 nM</td>
<td>REF52</td>
<td>P53+/+/+/+</td>
<td>Rat embryonic</td>
<td>DNA polymerase inhibitor</td>
<td>Marusyk et al., 2007</td>
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<td>fibroblast cells</td>
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<td>Bleomycin</td>
<td>50 mU/ml</td>
<td>A549</td>
<td>+/-/-/+</td>
<td>Human lung</td>
<td>p53/p21 activation</td>
<td>Linge et al., 2007</td>
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<td>carcinoma</td>
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<tr>
<td>Hydroxyurea</td>
<td>100–150 µM</td>
<td>REF52</td>
<td>P53+/+/+/+</td>
<td>Rat embryo</td>
<td>Ribonucleotide</td>
<td>Marusyk et al., 2007</td>
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<td></td>
<td>fibroblast cells</td>
<td>reductase inhibitor</td>
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<tr>
<td>Camptothecin</td>
<td>20 nM</td>
<td>HCT116, RKO</td>
<td>--</td>
<td>Human colorectal</td>
<td>ATM-Chk2-p53-p21 pathway</td>
<td>Han et al., 2002; Zhang et al., 2014</td>
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<td>cancer cells</td>
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<td>Diaziquone</td>
<td>250 nM</td>
<td>DU145</td>
<td>pRB+/p53+/+p16*</td>
<td>Human prostate</td>
<td>DNA alkylation</td>
<td>Ewald et al., 2012</td>
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<td>cancer cells</td>
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<td>Resveratrol</td>
<td>10–50 µM</td>
<td>BJ</td>
<td>P53+/+/+/+</td>
<td>Human foreskin</td>
<td>P53/p16INK4A activation</td>
<td>Kilic Eren et al., 2015</td>
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<td>fibroblast cells</td>
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<tr>
<td>Lovastatin</td>
<td>0.3 µM</td>
<td>PC3</td>
<td>P53+/+/+/+</td>
<td>Human prostate</td>
<td>HMG-CoA reductase</td>
<td>Lee et al., 2006</td>
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<td>cancer cells</td>
<td>inhibitor</td>
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<td>Nocodazole</td>
<td>50 ng/ml</td>
<td>MEF</td>
<td>+/-/-/+</td>
<td>Mouse embryonic</td>
<td>Via miR290</td>
<td>Pitt et al., 2009</td>
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<td>fibroblast cells</td>
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<tr>
<td>Colchicin</td>
<td>2.5 nM</td>
<td>A549</td>
<td>+/-/-/+</td>
<td>Human lung</td>
<td>Inhibition of spindle microtubule</td>
<td>Bhattacharya et al., 2016</td>
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<td>carcinoma cells</td>
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* Mutant; + Wild Type; – Not Available
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<thead>
<tr>
<th>Therapeutic agent</th>
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<th>Mechanism of action</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>10 μM</td>
<td>TIG7</td>
<td>--</td>
<td>Human fibroblast cells</td>
<td>Activation of MAP kinase</td>
<td>Palaniyappan, 2009</td>
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<tr>
<td>MLN4924</td>
<td>30 nM, 100 nM</td>
<td>AGS, SGC7901</td>
<td>+/+/+/+</td>
<td>Human gastric cancer cells</td>
<td>Neddylation inhibitor</td>
<td>Lan et al., 2016</td>
</tr>
<tr>
<td>K858</td>
<td>1.3 μM</td>
<td>HCT116, ARPE-19</td>
<td>+/+/+/+</td>
<td>Human colon cancer cells, Human retinal pigment epithelial cells</td>
<td>Eg5 ATPase inhibitor</td>
<td>Nakai et al., 2009</td>
</tr>
<tr>
<td>VO-OHpic</td>
<td>500 nM</td>
<td>MEF</td>
<td>+/+/+/+</td>
<td>Mouse embryonic fibroblast cells</td>
<td>PTEN inhibitor</td>
<td>Alimonti et al., 2010</td>
</tr>
<tr>
<td>Diosmin citrus fruit flavonoid</td>
<td>5 and 10 μM</td>
<td>MCF-7, MDA-MB-231, SK-BR-3</td>
<td>+/+/+/+</td>
<td>Breast cancer cell lines</td>
<td>Stimulate oxidative and nitrosative stress, DNA damage and changes in global DNA methylation patterns, elevation in p53, p21 and p27 levels</td>
<td>Lewinska et al., 2017</td>
</tr>
<tr>
<td>Triptonide (diterpene triepoxide)</td>
<td>7.5 and 12 nM, respectively</td>
<td>U937, HL-60</td>
<td>P53+/+/+</td>
<td>Human leukemic cell lines</td>
<td>Suppressing transcription of TERT and oncogenic c-Myc, and promoting transcription of senescence-promoting genes p16 and p21</td>
<td>Pan et al., 2017</td>
</tr>
</tbody>
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* Mutant; + Wild Type; – Not Available
alternative mechanisms of senescence induction (Ewald et al., 2010; Shay and Roninson, 2004). Evidence also suggests that heterochromatinization near the promoters of cell-cycle related genes may play a role in the maintenance of irreversible growth arrest in senescence (Kahlem et al., 2004).

As outlined in figure 1, induction of senescence requires activation of two major tumour suppressor proteins – p53 and Rb. Cellular senescence pathways regulated by the tumour suppressors are reviewed by Dimri (2005). It is evident that the senescence pathways do not act in isolation, rather, a considerable crosstalk exists between the two pathways, which collectively act to establish a senescent phenotype.

**p53-p21 pathway:** Prolonged activation of DNA damage response (DDR) is the major event involved in the onset of senescence, wherein ATM phosphorylates and activates the major DDR proteins such as H2AX, 53BP1,
NBS1, MDC1, finally converging to activation of checkpoint kinases CHK1 and CHK2 (Wu et al., 2012; Ben-Porath and Weinberg, 2005). This leads to activation and stabilization of p53 via phosphorylation (Ben-Porath and Weinberg, 2005), leading to upregulation of p21 (CDKN1A) and inhibition of CDK2 activity, causing G1 arrest (Sabin and Anderson, 2011).

Besides the DDR proteins, products of INK4a locus act upstream of p53 and Rb to mediate senescence (Ben-Porath and Weinberg, 2005). The INK4A gene locus contains a set of overlapping genes with independent promoters. p16INK4A transcript consists of exons 1α-2-3, whereas p19ARF (in mouse) or p14ARF (in humans) transcript is made of exons 1β-2-3. Despite the presence of common exons 2 and 3, the amino acid sequences of p16 and p19 differ due to the alternative reading frames (Serrano, 1997; Evan and di Fagagna, 2009). p16INK4A was initially identified as a CDK4-binding protein which binds to and inhibits CDK4 and CDK6 in competition with cyclin D, subsequently preventing the phosphorylation of Rb (te Poele et al., 2002). Expression of p16INK4A is low in normal proliferating cells, and is significantly increased during cellular senescence. Expression of p16INK4A is regulated by extrinsic stress signalling pathways (Ohtani et al., 2004) mediated by upstream activators of p38-MAPK pathway (Ben-Porath and Weinberg, 2005). Ras/MEK signal transduction pathway is essential for Ras-induced cellular senescence. Transcription factors Ets1/2 are the downstream mediators of the Ras/Raf/MEK pathway, which control expression of p16INK4A (Ohtani et al., 2004).

Similar to p16, ARF is induced in response to ionizing radiation, oncprotein induced senescence and other stress. ARF sequesters MDM2, E3 ubiquitin ligase in the nucleolus, and prevents proteolytic degradation of p53, resulting in activation. However, the function of p16 is prominent in human cells; whereas ARF response is enhanced in mouse cells. Several regulators of INK4A locus include Bmi1 protein, a member of the polycomb repressive complex which causes transcriptional repression of p16 and ARF, Tbx2, Tbx3, JunB; Twist and E2F3 act as transcriptional repressors of ARF and Ets transcription factor family that serve as activators of p16 (Ben-Porath and Weinberg, 2005).
**p16-Rb pathway:** Regulation of pRb activity occurs by various post-translational modifications such as phosphorylation, acetylation and ubiquitination. Mitogenic signals induce the expression of CDK2, CDK4 and CDK6 (cyclin D kinases), which are the crucial mediators of Rb phosphorylation. Phosphorylated Rb releases E2F family of transcription factors (E2F1-3) causing cell cycle progression into the S-phase (Serrano, 1997) and initiation of DNA replication (Ohtani et al., 2004).

As mentioned previously, p16INK4A inhibits phosphorylation of Rb by inhibiting CDK4 and CDK6 (Takahashi et al., 2007). Active, hypophosphorylated Rb and related proteins p107 and p130 bind to and inhibit transcription factors required for cellular proliferation (Serrano, 1997) such as E2F, and prevents G1 progression. Inhibition of cyclin E/CDK2 by p21 also activates Rb, and Rb regulates the activity of MDM2, thereby controlling the stability of p53, illustrating crosstalk between the two major senescence inducing pathways. Thus, p53 and p21 are the common mediators of DDR, apoptosis and senescence pathways (te Poele et al., 2002; Takahashi et al., 2007). Inactivation of Rb or p53 also upregulates expression of p16INK4A and induces senescence (Serrano, 1997). The existence of feedback loop between activation status of Rb and transcription of p16 has been demonstrated. Inactive pRb causes active transcription of p16, which binds to and inhibits cyclin D-CDK4/6 complex, causing increased phosphorylation of Rb, and decreasing expression of p16 by transcriptional repression (Li et al., 1994).

In mortal fibroblasts, DNA damage immediately induces p53 leading to activation of p21; however, after several days, expression of p53 and p21 decreases while p16INK4A is increased. Thus, p53 and p21 act to initiate cellular senescence in response to DNA damage, while p16INK4A is responsible for maintaining the cell cycle arrest in senescence (Robles and Adami, 1998). Similarly, topoisomerase inhibitors on colon, ovarian and breast adenocarcinoma cell lines expressing wild-type p53, expression of p53 and p21 increased for the onset of senescence, but subsequent p16INK4A is required for maintenance. Administration of topoisomerase inhibitors to p53-null or mutant ovarian and colon cancer cell lines resulted in a transient S-G2 arrest, followed by apoptosis (te Poele et al.,...
Thus, although the p53-p21 and p16-Rb pathways are discussed separately in this review, we emphasize that these are not two distinct pathways, but rather act together to initiate and maintain a senescent state.

Inactivation of Rb after p16 overexpression inhibits cellular proliferation, but stimulates DNA replication, implying that initial activation of Rb is sufficient to induce irreversible cell cycle arrest by mechanisms other than prevention of DNA replication (Beauséjor et al., 2003). Since subsequent inactivation of p53 resulted in increased number of multinucleated cells, abrogation of cytokinesis was hypothesized to be an alternative mechanism for maintenance of cell cycle arrest in senescent cells. Subsequent studies have shown that p16-Rb pathway unites with mitogenic signals to block cytokinesis by posing a stimulatory effect on ROS production. Active E2F regulates the genes involved in ROS production and decreases ROS levels. However, under conditions of cellular stress, p16-Rb pathway inhibits E2F activity, thereby increasing the levels of ROS and eventually resulting in sustained activation of sustained ROS-PKCδ signalling through a positive feedback loop. The net outcome is an irreversible arrest of cytokinesis through downregulation of LATS1 – a mitotic exit network (MEN) kinase required for cytokinesis. The mechanism serves as a second barrier to prevent proliferation of senescent cells upon inadvertent activation of p53 or Rb in senescent cells (Takahashi et al., 2007).

**Reversal of therapy induced senescence**

Although the initial in vitro studies revealed that cellular senescence is essentially an irreversible process, ensuing studies have shown the existence of mechanisms mediating senescence reversal (Kuilman et al., 2010). Tumour cells are endowed with the ability to overcome the pro-senescence signals due to mutations in their tumour suppressor pathways (Sabin and Anderson, 2011). The selection and clonal expansion of cells possessing active senescence abrogating mechanisms represents a major rate limiting step in tumour progression (Kuilman et al., 2010).

The therapeutic efficacy of anticancer regimen depends upon the fate of the cells undergoing ACS. Very rarely, about 1 in 10^6 cancer cells may escape senescence upon termination of therapy and resume proliferation, eventually
repopulating the tumours. Importantly, these 'senescence escapers' maintain viability and overcome cell cycle arrest for reversal of senescence. Although the phenomenon has been demonstrated in various cancers, a detailed comprehension of the underlying mechanisms is pending, a crucial event for upholding the development of pro-senescence therapies in cancer (Wu et al., 2012). Treatment with chemotherapeutic drug etoposide induced senescence in Non-Small Cell Lung Cancer cells, the senescent cells were capable of escaping from arrest and recover the proliferative capacity, thereby validating the role of dormant tumour cells in cancer recurrence (Saleh et al., 2017). Similarly, MCF-7 breast cancer cells exposed to single or fractionated doses of 10 Gy radiation (2 Gy × 5 days) undergoes senescence; however, the cells were capable of giving rise to proliferating population within 10–12 days post radiation (Murray and Mirzayans, 2013). Studies from our laboratory showed that in glioblastoma, a small percentage of the bulk tumour cells when treated with radio-chemotherapy survive and undergo senescence; however the senescence phenotype is transient and soon the cells overcome senescence and resume growth giving rise to aggressive recurrent tumour (Kaur et al., 2015).

Several essential components playing a role in senescence reversal include (Wu et al., 2012):

**Cdk1:** Cells require activated cyclin B1/Cdk1 complex for entry into mitosis. During G2, inhibitory phosphorylation at 14T and 15Y of Cdk1 by Wee1/Mik1 family of protein kinases retains the molecule in an inactive state. The removal of inhibitory phosphates by Cdc25C phosphatase activate Cdk1. The Cdc25C is rendered inactive by Chk1/2-mediated phosphorylation in response to DNA damage. Moreover, p21 and p27 also downregulate Cdk1 kinase activity by binding to the cyclin B1/Cdk1 complex (Roberson et al., 2005). Treatment with moderate doses of various chemotherapeutic agents such as cisplatin, camptothecin, etoposide, paclitaxel and vindesine induces senescence and G2-M arrest in non-small cell lung cancer. In the senescent cells, downregulation of Cdk1 is required for maintaining the cell cycle arrest and upregulation is essential for escape from senescence and maintenance of viability of the 'escapers' (Roberson et al., 2005).
senescence and escape induces polyploidization and mediates survival by abrogating apoptosis, enabling the senescent cells to enter cell cycle (Wang et al., 2013).

**Survivin:** A nuclear protein with a molecular weight of 16.5 kDa, survivin is the smallest member of the human inhibitor of apoptotic protein (IAP) family, and is stabilized by phosphorylation of T34 by Cdk1, thereby functioning in Cdk1-mediated survival. It interacts with the mitotic spindle and inhibits apoptosis by caspase-9 (Wu et al., 2012). In an extension to the study on chemotherapy-induced senescence in non-small cell lung cancer (Roberson et al., 2005), survivin was the downstream mediator of Cdc2/Cdk1 survival signal, which co-operates with the latter in deciding the fate and reversibility of therapy-induced senescence. Moreover, phosphorylated survivin was the critical downstream effector maintaining viability of 'senescence escapers' (Wang et al., 2011).

**CDK inhibitors – p16, p21 and p27:** Besides activating Chk1/2, p21 exerts anti-apoptotic effects by interacting with SAPKs, apoptosis signal-regulating kinase 1, procaspase-3 or release from Cdk2 in the nucleus. p27 prevents premature entry into S-phase, whereas p21 suppresses Cdk1-mediated apoptosis leading to tolerance of genotoxic stress (Wu et al., 2012). p16, the regulator of pRb plays a vital role in mediating irreversibility of senescence, by acting as a barrier to proliferation. Thus, abrogation of p53 was effective in inducing cell proliferation in senescent cells expressing low-levels of p16, but failed to do so in senescent cells expressing high levels of p16. Rb inactivation was also able to restore cellular proliferation in cells ectopically expressing p16, when the inactivation occurred prior to p16 expression. Interestingly, when the inactivation occurred post p16 expression, cellular proliferation was hampered, although the cells underwent DNA replication (Beauséjour et al., 2003). Thus, the order of expression of p16 and Rb inactivation essentially determines reversibility of senescence induced by p16. The transcription factors Twist1 and Twist2 involved in EMT, mediates escape from senescence by transcriptional repression of p21 and p16 (Evan and di Fagagna, 2009).
**mTOR:** Mammalian target of rapamycin (mTOR), a member of the PI3K-related kinase family is a master regulator of cellular senescence (Chitikova et al., 2014) and functions by integration of multiple environmental signals into cellular response, in terms of cell growth, proliferation, survival, motility and protein synthesis (Walters et al., 2016). mTORC1 and mTORC2 are the complexes formed by mTOR, which serve as negative regulators of autophagy (Chitikova et al., 2014); mTORC1 causes phosphorylation and inactivation of ULK1, the autophagy-initiating kinase (Walters et al., 2016). Development and maintenance of senescence phenotype requires activation of autophagy and increase in mTORC1 activity is required for establishing irreversible cellular senescence from a quiescent state (geroconversion). Following exposure to 6 Gy X-irradiation, apoptosis resistant rat embryonic fibroblasts transformed by E1A + E1B undergoes G2/M arrest and forms polyploid giant and multinucleated cells, along with persistent activation of DDR signalling due to delayed DNA repair, leading to the induction of senescence. However, a downregulation of mTORC1 and mTORC2 is observed in the cells, along with a consequent decrease in geroconversion. The IR-induced senescent cells are capable of resuming proliferation and give rise to cells of near normal ploidy. Moreover, suppression of mTOR and subsequent induction of autophagy facilitates reprogramming, as evident from the upregulation of stem cell markers Oct 3/4 and Nanog (Chitikova et al., 2014). Acute inhibition of mTORC by treatment with AZD8055 (a pan-mTORC inhibitor) reverses senescence-associated phenotypes in near-senescent human skin fibroblasts (Walters et al., 2016).

**Implications of therapy induced senescence**

Contradictory opinions regarding the consequences of therapy-induced senescence with reference to favourable or adverse therapeutic outcomes have been reported (Gordon and Nelson, 2012). Besides apoptosis and mitotic catastrophe, senescence is a major mechanism to curb tumour cell proliferation, influencing the success of anti-cancer therapy. During the course of neoplastic transformation, cells evolve various genetic and epigenetic mechanisms to escape senescence. The tumour cells are prone to undergo senescence spontaneously or in response...
to chemotherapy or radiotherapy (Shay and Roninson, 2004). Induction of senescence is achieved by p53 activation/stabilization, p27 stabilization or inhibition of CDK2. Pro-senescence therapy essentially relies on the senescent cells being non-proliferative and are ultimately eliminated by the immune system or other mechanisms. However if a cancer cell possesses mechanisms to evade therapy induced senescence and regain proliferative properties, pro-senescence therapy may poses detrimental effects on patient survival (Gordon and Nelson, 2012).

The beneficial effects of therapy induced senescence in cancer are outlined by Ewald et al. (2010), which include indefinite capacity to remain in a stable, non-proliferative state, increased propensity for immune system-mediated clearance of senescent tumour cells, and use of lower concentrations of cytotoxic drugs for inducing senescence, thereby minimizing the therapy-related adverse effects. The likelihood of senescence-reversal in tumour cells is low, as senescent cells proliferate by inactivation of p53 and p16INK4A and a limited capacity to undergo cell divisions prior to apoptosis (Ewald et al., 2010). In sharp contrast to these assumptions, a study in non-small cell lung cancer reported that detection of cellular senescence in vivo post therapy is associated with adverse outcome in patient survival (Wang et al., 2013).

Senescent cells secrete large amounts of secretory factors such as inflammatory cytokines and chemokines, extracellular proteases and growth factors, termed as “senescence-associated secretory phenotype (SASP)”, also known as “senescence messaging syndrome (SMS)” (, ) or DNA Damage associated Secretory Program (Gordon and Nelson, 2012). Various signalling factors included under the SASP family are enlisted and their functions reviewed by Davalos et al. (2010) and Coppe et al. (2010). The phenotype has evolved to signal tissue damage and initiates repair mechanisms (Gordon and Nelson, 2012). SMS creates a complex signalling network with autocrine and as paracrine effects on the microenvironment. The eventual downstream effect is determined by the type of stress, genotype of target cells and stage of tumorigenesis, adding further complexity to the implications of senescence in cancer (Kuilman et al., 2010; Perez-Mancera et al., 2014).
SASPs are regulated at transcriptional and post-transcriptional levels. Apart from the transcription factor activity, chromatin organization plays an important role in modulating gene expression during senescence, as evident from the striking alterations in chromatin architecture in senescent cells. Genes comprising SASPs are physically clustered together and increased activities of NF-κB and C/EBPβ during senescence mediates expression of SASP components. In particular, the expression of pro-inflammatory cytokines such as IL-6 and IL-8 are regulated by C/EBPβ (Perez-Mancera et al., 2014); IL-1, IL-8 and regulators are proposed to be the major upstream regulators of SASPs (Acosta and Gil, 2012).

Some of the SASPs such as PAI-1, IGFBP7, IL-6 or IL-8 contribute to the establishment or reinforcement of cell cycle arrest via various molecular players such as IL-6-mediated induction of p15INK4B, IL-8-mediated increase in ROS production and consequent DNA damage, and PAI-1-mediated increase in GSK3β activity. Some SASPs can also trigger the immune-system mediate clearance of senescent cells. However, SASPs can also mediate the occurrence of 'senescence-escapers' or de novo premalignant cells during tumorigenesis. Thus, persistent secretion of pro-tumorigenic cytokines from senescent cells can result in poor outcome. SASPs such as IL-6, IL-8 and GROα display a context dependent tumour suppressive or protumorigenic effect. The switch governing the final outcome is not clear yet, although the genetic makeup of the neighbouring target cells, in terms of their p53 and Rb status is thought to be the key players (Acosta and Gil, 2012). The current anti-cancer therapy regimen, administered in cycles are conducive for the development of resistant cells through modulation of microenvironment and the resultant generation of SASPs enriched in pro-survival factors during the initial cycle. Thus, the tumour cells surviving the initial treatment regimen are protected against subsequent rounds of treatment (Gordon and Nelson. 2012).

Various studies have examined the role of SASPs in maintenance or progression of tumours. Multiple myeloma cells exposed to genotoxic stress inducing 6 Gy X-irradiation or doxorubicin treatment undergo senescence triggered by persistent DNA damage and activation of Chk2, and secreted chemokines such as CXCL10 and CCL5 (RANTES). The SASPs
released from these senescent cells induces emergence, maintenance and migration of cancer stem-like cells; abrogation of SASPs by downregulation of Chk2 significantly reduces emergence of cancer stem-like cells (Cahu et al., 2012). Similarly, pemetrexed treatment of malignant pleural mesothelioma (MPM) cells induce senescence and secretion of SASPs such as IL-6, IL-8, VEGF-A, IFN-γ, CXCL1 and PIGF1. Treatment of naïve MPM cells with conditioned medium from pemetrexed treated senescent MPM cells favours EMT and emergence of chemoresistant cells with high ALDH activity, which is mediated by downstream activation of STAT3 via SASP-induced cytokine signalling (Canino et al., 2012). Glioma stem cells differentiate into non-stem glioma cells which undergo senescence and secrete pro-angiogenic factors such as VEGFs, SASPs such as IL-6 and IL-8. The combined implantation of glioma stem cells and senescent non-stem cells enhanced tumorigenic potential of the gliome stem cells (Ouchi et al., 2016). Similar results were reported in breast cancer cells, which showed enhanced radioresistance and proliferative capacity upon co-culture with therapy-induced senescent fibroblasts (Murray and Mirzayans, 2013). Besides secretion of SASPs, drug-induced senescent cells evolve into aggressive drug-resistant stem-like cells, with increased expression of stem cell markers such as CD133 and Oct4 (Achuthan et al., 2011). DNA damage-induced polyploidy in cancer cells was reversible, with induction of self-renewal potential (stem cell-like feature) in the descendent surviving cells, implying requirement of senescence in rejuvenation of cancer cells after exposure to genotoxic agents (Erenpreisa and Cragg, 2013).

CONCLUSIONS AND FUTURE ASPECTS

In the current review, we have outlined several major mechanisms involved in establishment of cellular senescence in response to commonly used anti-neoplastic agents. Although senescence was presumed to be the terminal end of cellular proliferative capacity, evidence indicates that tumour cells have evolved various mechanisms to abrogate senescence and resume proliferation, to give rise to recurrent and therapy-resistant tumours. Moreover, the senescent cells are metabolically active and exude secretory proteins with pleiotropic effects on the surrounding...
cells and the microenvironment. The overall effect of the secretory proteins is dependent on nature of the former and biological characteristics of the surrounding tissues. Tumour cells can utilize the microenvironmental cues for promoting malignancy, posing a negative impact on therapy outcome, prognosis and survival of patients. This implies that despite the reported beneficial effects of pro-senescence therapy, such therapeutic interventions must be administered with caution, taking into account potential negative consequences. Along with conventional therapies, co-administration of therapeutic agents that prevent senescence reversal or eliminate senescent cells, appears to be a promising approach towards improving patient survival. Besides, a need for detailed examination of the mechanisms underlying senescence reversal in cancers. Since the senescent cells that survive completion of therapy regimen give rise to aggressive, therapy-resistant tumours, development of novel therapies aimed at preventing the re-emergence of therapy resistant cells by eliminating the 'residual' senescent cells should be considered in cancer therapeutics.

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