

TARGETING SENESENCE PATHWAYS TO REVERSE DRUG RESISTANCE IN CANCER

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Abstract

Irreversible proliferation arrest (also called senescence) has emerged recently as a drug-responsive program able to influence the outcome of cancer chemotherapy. Since the drug amounts required for induction of proliferation arrest are much lower than those necessitated for induction of cell death, forcing cancer cells to undergo senescence may represent a less aggressive approach to control tumor progression. However, to achieve a long-standing control of proliferation, the ability of cancer cells to escape senescence and become drug resistant must be inhibited. Therefore, a clear understanding of the mechanisms that govern drug-induced senescence is critical and can lead to discovery of novel approaches to suppress drug resistance. The present review discusses the relevance of senescence in response to chemotherapy and the onset of drug resistance development. Particular emphasis is directed toward the utilization of findings from the field of research on aging, than can be applied to induction of senescence in cancer cells and reversal of their drug resistance phenotype. Proof of principle of for this relationship is represented by the identification of inhibitors of aging associated proteases such as the proteasome and cathepsin L as novel and potent cancer drug resistance reversing agents.

1. Introduction

Chemotherapy has been used for many decades as a major cancer treatment modality. However, the lack of cytotoxic drug specificity and the consequent generation of toxic side effects when high doses are administered often limit the use of this treatment modality. Low drug doses are not very effective and their use generally results in patient relapse and development of drug resistance. A rational approach based on better

understanding of the molecular mechanisms of drug action should help determine whether low drug doses can be rendered effective with reduced chances of drug resistance development. Studies using cancer cells in culture have demonstrated that irreversible growth arrest (senescence) may be achieved by using relatively low drug concentrations as compared to those required for apoptotic cell death. A satisfying goal would be to keep the proliferative behavior of cancer cells in check with drug doses that are less toxic for the patient. However, the ability of tumor cells to escape such low level of toxic stress and become drug resistant must be also kept under control. Thus, strategies aiming to force cancer cells into senescence could prove to be very useful in cancer therapy particularly with regard to development of drug resistance. Prior understanding of the underlying mechanisms responsible for drug induction of cellular senescence is however needed to accomplish these goals. The present review summarizes current knowledge about cellular senescence and addresses the possibilities of using this cellular demise in suppression of cancer resistance to chemotherapy.

2. Cellular senescence in normal and cancer cells

Normal somatic cells have a limited life span and cannot proliferate indefinitely in culture [1]. After a certain number of doublings (also called the Hayflick limit), cells irreversibly stop proliferating through what is now recognized as replicative senescence. This suggested that there may be intrinsic mechanisms that count cell divisions, thus, the search for a putative “clock” was undertaken. About a decade ago, it was discovered that telomeres (the end of chromosomes) become shorter after each cell division and that this erosion could be perceived by the cell as a form of DNA damage that results in arrest of cell proliferation. The responsible enzyme, telomerase capable of preventing the loss of telomeres, was believed to be the key modulator of cellular senescence particularly that this enzyme was over-expressed in tumor cells (immortal) and not or at very low level in normal cells [2, 3]. However, there is now compelling evidence that cultured rodent cells do not use the shortening of telomeres to time their cessation of division after 10 to 15 doublings [4], instead the progressive loss of proliferative capacity is thought to result from cumulative trauma imposed by tissue culture *per se* [5]. This reinforced the notion that stress generated by the environment causes cellular senescence in a manner that could be independent of telomerase and telomere shortening. In fact, a number of studies have demonstrated that exogenous stresses such as UV and IR irradiation, H₂O₂, hypoxia, and various chemicals, induce a senescence phenotype that is mainly characterized by enhanced expression of the cell cycle inhibitors p16^{INK}, p21/WAF1, p27 KIP and disruption of lysosomal function through enhanced activity of the senescence associated beta galactosidase (SA-β-Gal).

Contrary to somatic cells, most cancer cells have extended or infinite life span. Escape from senescence in the course of neoplastic transformation has been linked to inactivation of the tumor suppressor p53 and to constitutive activation of telomerase [6, 7]. Although these cells are less sensitive to senescence induced by physiological conditions, they are subject to irreversible growth arrest when exposed to other stimuli such as radiation or chemotherapeutic agents, suggesting that they have retained key molecular elements that facilitate senescence in response to exogenous insults. Similar to

normal cells, exogenous stress-induced senescence in cancer cells is mostly telomerase-independent.

3. Mechanisms of drug-induced senescence

Some early events that mediate chemotherapeutic agent-induction of proliferation arrests are now described but their precise contribution to cellular response and the level of stress required for their activation are either not yet defined or subject to controversy. The most described signaling pathway for drug induced proliferation arrest is initiated by DNA damage. Major cancer drugs such as topoisomerase inhibitors doxorubicin and etoposide, camptothecin, and alkylating agents cisplatin and BCNU have been reported to induce DNA damage and particularly DNA double-strand breaks (DSBs), considered as the most lethal DNA lesions [8,11]. Cell cycle arrest in response to DNA damage is mediated through checkpoints to allow repair before cell cycle progression is resumed, or, if the damage is too extensive, they trigger senescence or even apoptosis. ATM and ATR are members of the phosphatidylinositol 3-kinase-related kinases (PIKK) responsible for sensing DNA damage and propagating the signal through phosphorylation and activation of downstream intermediates including H2AX, NBS1 and BRCA1, which in turn lead to activation of the Chk1 and Chk2. The most fundamental event for drug-induced G1-S checkpoint is the stabilization of p53, leading to enhanced transcription of p21/WAF1 and growth arrest. A number of conflicting findings rendered the mechanisms of DNA damage-induced cell cycle arrest more complicated than initially thought. For instance, activation of the tumor suppressor p53 have been found to be associated with but not required for drug-induced p21/WAF1 expression [12, 13] rendering less evident the role of p53 in drug-induced senescence. Also not clear is the relationship between DSB-mediated activation of H2AX and induction of cell cycle arrest. DSBs are believed to signal cancer cells to slow their proliferation and allow the DNA repair to take place [14-17] and phosphorylation of histone H2AX at serine 139 (γ -H2AX) has been shown to be a very sensitive and reliable marker for DSB formation [18,19]. However, the role of H2AX in mediating cell cycle arrest and its relationship to cell cycle inhibitors such as p21/WAF1 is somewhat confusing as illustrated by the following reports: 1) DSB formation requires DNA replication and active DNA polymerase delta [20, 21] while p21/WAF1 is known to prevent DNA replication by inhibiting PCNA which is a cofactor of this same DNA polymerase [22,23]. 2) p21/WAF1 has been shown to reduce DNA damage repair *in vivo* [24] and *in vitro* [25, 26] suggesting this cell cycle inhibitor may control activation of H2AX and not the contrary. 3) Ubiquitin-dependent down-regulation of p21/WAF1 triggered by UV radiation promotes activation of H2AX and DNA repair [27]. 4) RB, an upstream activator of p21/WAF1, prevents replication-dependent DNA double strand breaks and activation of H2AX following genotoxic insult [28]. It is clear from these studies that further definition of the mechanism underlying drug-induced senescence and particularly the relationship between DSBs, p53 and p21/WAF1 will be critical to the investigation of their role in modulating drug sensitivity.

4. Importance of senescence in cellular response to cancer drugs

The ability of potential anti-cancer agents to inhibit cell proliferation *in vitro* has been always considered as a major criteria for their selection, however, the role of drug-induced senescence in the outcome of cancer chemotherapy has been overlooked and

most of research interest in this field was directed towards understanding the role that apoptosis plays in cellular toxic response to these agents. A well-described signaling pathway initiated by activation of p53 leading to enhanced Fas expression and caspase 8 activation was described as a mediator of cytotoxic drug action [29, 30]. Consequently, it was believed that inhibition of apoptosis (through p53 mutation, over-expression of bcl-2 or caspase inhibition) should render cancer cells resistant to drugs. While these approaches reduced apoptotic cell death in the majority of cases, it did not ameliorate the overall cell survival and cellular ability to resume proliferation [31-33], raising questions about the role of apoptosis in cancer cell survival and chemotherapeutic outcome. In fact, numerous studies have shown that p53 mutation or over-expression either do not affect patient prognosis nor lead to better outcome after treatment [34, 35]. In addition, inhibition of apoptosis by targeting caspases [36] or over-expression of bcl-2 [32] did not protect cancer cells from the toxic effects of chemotherapeutic agents, instead, it switched apoptosis to senescence or other forms of cell death. Based on these findings, apoptosis seems to have only a limited role if any in the prediction of cancer sensitivity to therapy.

Chemotherapeutic drugs generally induce both proliferation arrest and apoptosis [37-41], however, the stress levels required for each one of these toxic responses are very different. Irreversible growth arrest or senescence can be achieved by using relatively lower drug concentrations than those required for apoptosis [36, 42, 43]. This suggests that in order for cancer cells to escape drug toxicity and continue to proliferate, not only apoptosis, but also senescence must be inhibited. Another argument in favor of the key contribution of senescence in response to drugs is that many cancer cell lines with altered apoptotic pathway and reduced susceptibility to drug-induced cell death still can undergo proliferation arrest in response to these drugs [44-46]. Similar response can be found in cells that have intact apoptotic machinery when treated with drugs that do not induce apoptosis. In sum, despite their effect on apoptosis, virtually all cancer agents inhibit cellular proliferation. One can easily imagine that a major roadblock to cancer would be simply prevention of the unlimited proliferation that characterizes this disease. Compelling evidence has recently been obtained suggesting that senescence programs contribute to the outcome of cancer chemotherapy [47-49]. For instance, mice bearing tumors susceptible to drug-induced senescence had better prognosis following chemotherapy than those harboring tumors with senescence defects [47]. Induction of irreversible proliferation arrest and maintenance of cancer cells in this state with less toxic drug concentrations than those required for induction of apoptotic cell death appears to be an attractive concept. However, considering the heterogeneity of tumor cell populations, a possibility exists that proliferation arrest may not be irreversible for every cell and that those with senescence defects may escape drug control and resume proliferation. Solutions to this problem will depend on our understanding of the mechanisms that govern resistance to drug-induced senescence which are up to date not well defined.

5. Relevance of senescence in drug resistance

Resistance to chemotherapy is a common problem encountered in cancer treatment. Whether it is intrinsic as for melanomas, lung and pancreatic cancers [50] or acquired as for leukemias, neuroblastomas, ovarian and breast carcinomas, this phenomenon seems to involve similar major mechanisms. The most described among them is the alteration in

drug efflux mediated by P-glycoprotein [51]. Other mechanisms such as drug inactivation and drug target over-expression have been also reported [52, 53]. More recently, it has been recognized that independent of these factors, the ability of cancer cell to undergo proliferation arrest or cell death could be a key factor in drug resistance [54-57]. For instance, cells with altered apoptotic pathway such as mutation of p53, absence of key caspases, or increased expression of the anti-apoptotic molecule bcl-2 [58, 59], displayed resistance to drug-induced cell death and were considered as drug resistant. However, since cell proliferation is also inhibited under apoptosis inducing drug concentrations [36], inhibiting apoptosis alone in these circumstances does not guarantee reactivation of proliferation. Drug resistant cells not only have to be alive, but able to proliferate. Inhibition of senescence is also required for the cell to resume proliferation. More importantly, if sub-apoptotic drug concentrations were used, inhibition of senescence alone would be sufficient for initiation of drug resistance development.

Relevance of senescence versus apoptosis in the onset of drug resistance can be best appreciated by analyzing studies on generation of drug resistant cancer cells *in vitro*. This is routinely accomplished by cell exposure to stepwise increments in drug concentrations over a period of three to six months. Cells are first exposed to sub-senescent drug concentrations and allowed to adapt to the ensuing stress level before increasing the drug amounts to senescent (sub-apoptotic) ones. Cells that escaped are usually about 2 to 10 times more resistant than their parental counterparts (resistance index 2 to 10). Further exposure to higher apoptotic drug concentrations and allowing cells to adapt to the corresponding stress level could result in a resistance index between 10 to 100 or even more. By following this procedure, cancer cells can survive and proliferate in drug concentrations that normally induce senescence in parental cells or even in higher drug doses that induce apoptosis. The key requirement in this process is that cells must develop resistance to senescence first, then to apoptosis. It is very unlikely that incubation of drug sensitive cells in drug concentrations that induce apoptosis will directly result in development of drug resistance because virtually no cell survives drug action and if some did, they are unable to resume proliferation [36]. Considering the fact that *in vivo*, a resistance index between 1 and 10 represents by itself a significant roadblock to chemotherapy, resistance to apoptosis (index 10 to more than 100) would require that tolerable drug doses be exceeded in any attempt to overcome it. All these considerations point to the fact that the onset of drug resistance is likely to be associated with senescence and not apoptosis.

6. Targeting DNA damage and cell cycle molecules to force cancer cells into senescence and reverse drug resistance

Considering the lack of clear understanding of the molecular mechanism(s) underlying chemotherapeutic agents-induced senescence, it is no surprise that very little is currently known about the role of DNA damage sensors or cell cycle inhibitors in cancer drug resistance. Nevertheless, the assumption would be that when combined with chemotherapeutic agents, these inhibitors act by inducing additional cell cycle perturbations that collectively facilitate and keep senescence in check [60]. Members of the DNA damage sensors and effectors have been reported to be associated with cellular sensitivity to stress. Among those, ATM, a mutated gene in patients with ataxia-

telangiectasia, which is a disorder characterized by chromosomal instability, hypersensitivity to γ -irradiation, predisposition to cancer, and premature senescence [61]. ATM serves a surveillance function that helps maintain genomic integrity by promoting cell cycle arrest and damage repair, possibly by recruiting repair proteins to the site of damage to prevent double-strand break repair from entering an error prone pathway [62]. Although the actual role of ATM in development of drug resistance has not been described, there have been reports that ataxia telangiectasia cells display defective G1/S and G2/M cell cycle checkpoints and hypersensitivity not only to γ -irradiation but also to anti-cancer drugs [63, 64]. A downstream signaling intermediate of ATM, Rad9 is believed to play an important role in promoting chemo resistance, radio resistance and control of cell cycle progression when DNA damage is incurred. However, the relevance of rad9 and related molecules in these processes was described mostly in *Schizosaccharomyces pombe* that served as a model to provided evidence for the requirement of this molecule for promoting resistance to radiation and hydroxyurea [65], and in *saccharomyces servisiae* where it induced resistance to selenite [66]. In mammalian cells, the role of Rad9 and related molecules in the development of drug resistance is not yet understood. A further downstream intermediate of this pathway, Chk1, has been shown to be associated with resistance to topoisomerase inhibitors among other drugs [67, 68]. Targeting the Chk1 pathway was proposed as having the potential of significantly improving the therapeutic potency of DNA damaging agents but this has not been proven yet. However, like many other DNA damage sensors and effectors, the degree of expression and/or activation of ATM, Rad9 or Chk molecules in drug resistant tumors and their targeting for drug resistance reversal has not been described. The evidence presented however makes these molecules compelling targets to facilitate drug induced cancer cell senescence and to prevent their ability to become resistant.

With regard to tumor suppressors and cell cycle inhibitors, although these molecules are key mediators of cancer cell response to genotoxic damage, information on their role in determining treatment efficacy is far from being complete. The frequent occurrence of p53 mutations in human cancer has led to numerous investigations evaluating its role in drug resistance. Over-expression of wild type p53 has been reported to result in increased cell death by apoptosis and enhanced drug sensitivity [69, 70]. Another member of this family, p21/WAF1 appears to have unique properties since approaches leading to both its suppression or over-expression resulted in increased sensitivity of cancer cells to genotoxic stress. Expression of the corresponding gene is regulated by both p53-dependent [71] and independent [72] pathways however, its ability to enhance cellular response to drugs is generally independent of p53 status. For instance, ectopic expression of p21/WAF1 sensitized hepatoma cell line Hep 3B (p53-null) to cisplatin [73]. Similarly p21/WAF1 sensitized the human non-small-cell carcinoma H1299 (p53 null) and colon carcinoma DLD-1 (p53-mutant) cell lines, to all transretinoic acid [74]. In cells with functional p53, this cell cycle inhibitor also facilitated drug-induced cell death [75]. In all instances, p21/WAF1 induction of drug sensitivity was found to be associated with apoptosis, a fact that is at odd with the implication of this molecule in forcing cancer cells to undergo senescence. Most recent studies indicate however that drug-induced expression of p21/WAF1 does not correlate with the induction of apoptosis [36, 76-78]. Taking into account the key role of this molecule in mediating of drug induced senescence, it is easy to imagine that diminished or total

suppression of p21/Waf1 expression will render cancer cells less susceptible to senescence and therefore resistant to drugs; however, a number of studies have demonstrated the contrary. When assayed *in vitro*, loss of this cell cycle inhibitor in human colon cancer cells results in a selective induction of apoptosis [79]. Loss of p21/waf1 also resulted in increased cell sensitivity to killing by ionizing radiation [80] and cisplatin [81], or growth factor withdrawal [78]. These findings are in support of the notion that p21/WAF1 inhibits formation of DNA double strand breaks, a lethal form of DNA damage [20-23]. Although approaches aiming to induce p21/WAF1 expression contribute to improved cellular drug response, reduction of its expression may not be an indicator of drug resistance. Nonetheless, the unique property that both activation and inhibition of p21/WAF1 sensitize cancer cells to chemotherapy provides tremendous potential for exploitation of this molecule in cancer drug discovery. Given the intrinsic ability of cell cycle inhibitors to induce cellular senescence and apoptosis [82-85], the possibility exists that one or more of these actions may lead to circumvent drug resistance. Future investigations are needed to better exploit the opportunity of enhancing cancer cell response to chemotherapy through induction of cell cycle arrest and senescence.

7. What can research on aging teach us about targeting senescence to reverse drug resistance in cancer?

Progressive cellular damage over life span, generally the consequence of environmental insults such as oxidation, radiation and chemicals, is believed to be the major cause of cellular senescence and aging of the organism. Although molecular mediators of aging are not fully identified, most of them share similarities with those implicated in cancer cell response to chemotherapeutic drugs, particularly the ones that play a role in DNA damage and cell cycle arrest. It is therefore conceivable that these two areas research should take advantage from each other's progress. As mentioned above, understanding the mechanism(s) of senescence should uncover critical molecular targets to force drug resistant cells into proliferation arrest and reverse their resistance phenotype. Some information about this mechanism(s) may be acquired from mechanistic studies of drugs and irradiation induced DNA damage and cell cycle arrest. Additional information may be obtained from dissecting the field of aging and particularly some of its hallmarks such as replicative senescence and the progressive cellular degeneration leading to accumulation of lipofuscin material into senescent cells.

Although telomerase and telomere shortening are considered to play a key role in replicative senescence and perhaps in aging of mammalian organisms other than rodents [86, 87], this pathway did not seem to be required in chemotherapy-induced cellular senescence, therefore, its role in drug resistance, if any, would be very limited. Early studies on the potential use of telomerase in cancer have proposed that this enzyme may serve as a marker for tumor cell killing *in vitro* [88] and a potential indicator in chemo-sensitivity assays. In fact, some reports have established a correlation between telomerase activity and chemo-sensitivity in several human cancer lines [89-91] while others have found no association between drug-induced cell killing and inhibition of telomerase activity [92, 93]. A practical difficulty that may accompany the use of this approach in the clinic would be the need to wait for telomeres to shorten in order to observe any effect, a process that usually takes dozens of cell divisions.

Other aspects of senescence that are non-DNA damage related have been largely exploited in aging but surprisingly not or very little in cancer. The best example is impaired protein degradation and clearance of damaged (oxidized, cross linked) proteins in the aged cell [94-96] that generally results in accumulation of lipofuscin. In this process, the proteasome and lysosomes are thought to play major roles since impaired function of their respective protein degradation pathways are hallmarks of aging-mediated cellular senescence. In most tissues of aged organisms and in most aging model systems including nematodes, fruit flies and cultured fibroblasts, overall proteolysis decline with age [97]. This is more evident for long-lived proteins some of which are known to be substrates for either proteasomal or lysosomal pathways of proteolysis.

a) Proteasome

Inhibition of the ubiquitin-proteasome pathway has been reported to facilitate drug resistance reversal in a variety of cancer cell lines [98-102]. PS-341 (Pyrrolylcarbonyl-Phe-Leu-Boronate, VelcadeTM), directed against the 26S proteasome activity [103] holds great promise since it has been shown to overcome classical drug resistance in pre-clinical and in early clinical studies [104-105]. This compound is believed to reverse drug resistance in multiple myelomas by inhibiting degradation of I κ B α , an inhibitory protein constitutively bound to cytosolic NF- κ B, thereby inhibiting its nuclear translocation, binding to DNA and expression of its target genes including cytokines implicated in cell survival [106]. Additional mechanisms to explain the effect of proteasome inhibition on drug resistance have been postulated as mediated by accumulation of p53, p21/WAF1 and induction of apoptosis [107, 108]. However, reintroduction of wild type p53 into p53-null PC3 prostate carcinoma cells did not increase their drug sensitivity. Likewise, comparison of parental and p21/WAF1-deficient cells demonstrated that p21/WAF1 was not required for proteasome inhibitor-induced cytotoxicity [107]. Additional mechanisms may mediate the action of proteasome inhibitors and since apoptosis appears to mediate their action, implication of apoptotic signaling elements in this process is very likely. Inhibition of the drug transporter P-glycoprotein may mediate drug resistance reversal by proteasome inhibitors since it has been reported recently that enhanced ubiquitination of this molecule results in decrease of its function and that the proteasome inhibitor MG-132 induced accumulation of the ubiquitinated P-glycoprotein [109].

b) Lysosomal cathepsins

It is generally accepted that the activity of lysosomal proteases declines with age but the underlying mechanism as well as the consequences have not yet been delineated [97, 110]. Cathepsins are a family of lysosomal proteases of which at least 11 members have been already described [111, 112]. These enzymes cleave a plethora of protein substrates often with overlapping specificities leading to the notion that there may be redundancy in their function. However recent specific targeting in transgenic mice revealed that cathepsins have very distinctive roles in fulfilling specific cell biological functions [113]. For instance, gene knockout for cathepsin L, which is one of the most efficient lysosomal cysteine proteases, revealed that this enzyme could play key a role in cell survival [114, 115], spermatogenesis [116] and hair loss [117]. Of interest with regard to aging, is the report that the activity of cathepsin L decreased by 90% from 2 to

28 months in the neocortex, hippocampus, striatum and cerebellum of the rat [118]. Age related losses do not occur for all lysosomal hydrolases, the activity of cathepsin B for instance is reported to remain relatively constant in brain throughout life span of the rat except in the striatum, where it increases with old age [118]. Other indirect approaches used to determine the implication of lysosomal protease in aging are represented by the infusion of molecules that disrupt lysosomal function and inhibit cathepsin-mediated protein degradation such as the acidotropic agent chloroquine and the cysteine protease inhibitor leupeptin, into the lateral ventricles of young rats, caused the appearance of morphological features of old age in the brain [119, 120]. Infusion of ZPAD, an inhibitor of both cathepsin L and B, into cultured brain slices led to increased lysosomal number and the formation of meganeurites, both of which were reported to occur during brain aging [121]. This clearly demonstrates that disruption of lysosomal function could set in motion a greatly accelerated gerontological sequence. Therefore, it may be tempting to extrapolate these findings to cancer cells and ask the question whether targeting an aging related protease such as cathepsin L could facilitate chemotherapeutic agent-induced senescence or reverse cancer resistance to drugs.

We have tested a series of cysteine protease inhibitors for their ability to affect doxorubicin toxicity in various drug resistant cancer cell lines (43). The compounds tested were Q-VD-OPH (a general cysteine protease inhibitor including caspases), cathepsin B inhibitor [L-3-*trans*-(Propylcarbamoyl)oxirane-2-carbonyl]-L-isoleucyl-L-proline], cathepsin L inhibitor [Z-Phe-Tyr(t-Bu)-diazomethylketone], cathepsin K inhibitor [1,3-Bis(N-CBZ-Leu-NH)-2-propanone 1,3-Di(N-carbobenzoyloxy-L-leucyl)amino acetone], and cathepsin S inhibitor [Z-Phe-Leu-COCHO • H₂O]. Among these molecules, only cathepsin L inhibitor and Q-VD-OPH (which also inhibits cathepsin L) reversed drug resistance to doxorubicin. The finding was validated in several doxorubicin resistant cell lines including human neuroblastoma, SKN-SH/R, murine neuroblastoma, Neuro 2A, leukemia, HL60/R, and osteosarcoma, OSA/R. Further investigation of the underlying mechanism indicated that the combination of cathepsin L inhibitor with very low doxorubicin concentrations (sub-senescent) resulted in induction of p21/WAF1 and induction of the senescence phenotype (cell flattening and increased of SA- β -gal activity) without induction of caspase-3 or apoptosis. Specific targeting of cathepsin L using siRNA to this enzyme completely blocked its expression and reversed resistance to doxorubicin through induction of p21/WAF1. These findings indicated that cathepsin L may have a survival function and that its specific targeting could force cancer cells into senescence and that alone may be sufficient for reversal of drug resistance without induction of apoptosis.

Investigation of the mechanism by which cathepsin L inhibition facilitates doxorubicin-induced senescence led to the discovery that drug amount in the nucleus was significantly increased when this enzyme is inhibited. Considering the fact that weakly basic drugs preferentially accumulate in acidic organelles such as lysosomes [122, 123] and in a manner that is greater in drug resistant cells than their sensitive counterparts [124, 125], it is suggested that lysosomal sequestration may be responsible, at least in part, for development of resistance to these drugs. In fact, we and others have shown that doxorubicin accumulates principally in the lysosomal compartment of drug resistance cells and only in the nucleus of their drug sensitive counterparts [43, 125]. Drug re-localization from lysosomes to the nucleus initiated by cathepsin L inhibition may

represent a likely mechanism for its effect on drug sensitivity. In accordance with this, resistance to drugs that do not accumulate in lysosomes such as cisplatin was not reversed by this approach, however, since resistance to cisplatin often result in cross-resistance to doxorubicin, cathepsin L inhibition was able to reverse resistance to the later drug in cisplatin resistant cells. Forcing cancer cells to become senescent through targeting of proteases associated with aging such as the lysosomal enzyme cathepsin L has proved to be a logical approach for reversal of drug resistance. As compared to other drug resistance reversing agents, cyclosporin A and verapamil, both known to inhibit the drug transporter P-glycoprotein and, in a non-specific manner other ion transporters, specific inhibition of cathepsin L could prove to be more effective *in vivo* where the two other approaches have failed. Numerous questions remain however to be answered particularly concerning the mechanism by which cathepsin L inhibition mediates drug translocation from lysosomes to the nucleus leading to cellular senescence. There is also the possibility that non-lysosomal mechanisms could be implicated since we have found that p21/WAF1 can be a substrate for cathepsin L at least *in vitro* [43] and that the active form of this enzyme has been detected in the nucleus where it activates molecular signaling leading to cellular proliferation [126].

Proteasomal and cathepsin pathways are only examples among other stress response pathways that may play a role in both aging and cancer resistance. Signaling pathways associated with increased life span such as those mediated by *sirt-1*, *daf* or related genes, or premature aging mediated by *wrn* gene, may constitute potential areas of investigation on development of drug resistance in cancer. Further investigation of the relationship between elements of these pathways and drug response in cancer may shed light on novel mechanisms and lead to identification of alternative strategies to address both cancer and aging.

8. Conclusion

With respect to the recent findings that apoptosis may not be required for the onset of drug resistance in cancer, cellular senescence has emerged as an alternative target to overcome or even to prevent drug resistance from occurring. Alterations of senescence pathways without disruption of apoptosis may be sufficient to accomplish these goals and enhance chemotherapy efficacy. Research in the respective areas of drug resistance in cancer and increased life span should take advantage from each other's progress since resistance to stress is their common driving force. The close relationship between these two areas of research is exemplified by the implication of protein degradation pathways in facilitating senescence and circumvention of cancer drug resistance. Proteasomal and cathepsin L inhibition are only some of the signaling pathways that are likely to mediate this process. Further research needs to be directed towards a deep and clear understanding of how chemotherapeutic agents induce cellular senescence, and identification of drug targets that could be used in combination with chemotherapeutic drugs to facilitate irreversible growth arrest. The ultimate goal is to reach satisfactory levels of drug effectiveness with less toxic effects.

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