REVIEW

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The Role of Cellular Senescence in the Gastrointestinal Mucosa

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Cellular senescence is a biologically irreversible state of cell-growth arrest that occurs following either a replicative or an oncogenic stimulus. This phenomenon occurs as a response to the presence of premalignant cells and appears to be an important anticancer mechanism that keeps these transformed cells at bay. Many exogenous and endogenous triggers for senescence have been recognized to act via genomic or epigenomic pathways. The most common stimulus for senescence is progressive loss of telomeric DNA, which results in the loss of chromosomal stability and eventual unregulated growth and malignancy. Senescence is activated through an interaction between the p16 and p53 tumor-suppressor genes. Senescent cells can be identified *in vitro* because they express senescence-associated β-galactosidase, a marker of increased lysosomal activity. Cellular senescence plays an integral role in the prevention and development of both benign and malignant gastrointestinal diseases. The senescence cascade and the cell-cycle checkpoints that dictate the progression and maintenance of senescence are important in all types of gastrointestinal cancers, including pancreatic, liver, gastric, colon, and esophageal cancers. Understanding the pathogenic mechanisms involved in cellular senescence is important for the development of agents targeted toward the treatment of gastrointestinal tumors. **(Gut Liver 2013;7:270-277)**

Key Words: Cell aging; Gastrointestinal neoplasms; Aging; Gastrointestinal mucosa

INTRODUCTION

Gastrointestinal (GI) malignancies represent one of the most common cancer diagnoses worldwide resulting in a significant burden to both healthcare costs and patient quality of life.[1] There are an estimated 150,000 new cases of colorectal cancer diagnosed in the United States in 2012 with an estimated 50,000 deaths, making this type of cancer the second leading cause of cancer related deaths in the United States.[2] Pancreatic cancer portends a grim prognosis with approximately 40,000 deaths per year due in part to the late stage of diagnosis. The majority of luminal GI cancers follow an adenoma-carcinoma sequence with sequential and progressive genomic instability, loss of heterozygosity, up-regulation of oncogenes and down-regulation of tumor suppressor genes.[3] Cellular behavior becomes altered with loss of control of cell cycle check points, allowing for increased survival, decreased detection by immune-mediated cytotoxic cells and the development of a microenvironment that promotes growth and metastases.[4] However, recent literature is emerging that suggests an adapted, endogenous mechanism known as cellular senescence that may have a major role in altering the neoplastic microenvironment and inhibiting growth of preneoplastic and neoplastic cells.[5]

Cellular senescence refers to a physiologically irreversible state of cell growth arrest following a replicative or oncogenic stimulus.[6] This phenomenon has been found in all organ systems as a response to the presence of premalignant cells and appears to be an important anticancer mechanism that keeps these transformed cells at bay. As its name implies, cellular senescence is also believed to have a number of biologically significant roles and is intimately involved in the process of aging. Similar, although entirely separate from apoptosis, cellular senescence appears to be involved in the response to inflammation and neoplasia. The recent literature has begun to address the importance of this mechanism, however the concept of senescence was first recognized almost 50 years ago.[7,8] Hayflick[7] discovered that normal human embryonic cells in culture could only divide a limited number of times until entering a senescent state. He postulated that unlimited cellular division and escape from senescent-like changes could only be achieved by somatic cells that had undergone neoplastic transformation. However,

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the inciting events that lead to cellular senescence and the mechanism by which these cells prevent progression to malignancy were not fully elucidated until quite recently.

This review summarizes the current experimental and clinical literature as it relates to cellular senescence and its potential anticancer role in the GI tract. In addition, emerging therapies that could be used to enhance cellular senescence as a putative target for GI cancers will also be discussed.

INDUCTION OF CELLULAR SENESCENCE

In order for cells to enter a senescent phenotype, a physiologic trigger or oncologic stress must occur.[9] Many exogenous and endogenous triggers for malignancy and senescence have been recognized and take place via a genomic or epigenomic pathway.[10] The most widely accepted stimulus for induction of senescence is progressive loss of telomeric DNA.[11] Telomeres are repetitive segments of noncoding guanine rich DNA found at the end of chromosomes. These are important as they maintain chromosomal stability within cells. Progressive erosion occurs after many generations of cell division. Foreshortening of telomeres leads to loss of chromosomal stability and eventual unregulated growth and malignancy.[12] This progressive loss can be countered by the addition of telomerase, an enzyme responsible for restoring telomeric DNA which occurs in neoplasia, as does an alternative lengthening of telomeres that does not involve telomerase. However, most cells do not express telomerase and the result leads to dysfunctional telomeres and a potent oncogenic stimuli.[13] This trigger leads to a cascade of cellular events known as DNA damage response. Other oncogenic stimuli that promote senescence include direct DNA damage from exogenous injury, such as radiation or carcinogenic molecules.[14] This leads to intercalation of DNA strands at nontelomeric sites with eventual DNA double strand breaks and disrupted chromatin. The result is a persistent DNA damage response and preservation of the senescent phenotype (Fig. 1).

However, structural DNA damage is not always necessary in inducing a DNA damage response and senescence. Relaxation of chromatin strands by histone deacetylase leads to up-regulation of tumor suppressor genes and proteins that permit the activation of cellular senescence.[15] Loss of specific tumor suppressor genes can also result in activation of this pathway.[16] Thus, one can envision that prolonged exogenous cellular stress, for example in the form of persistent esophageal acid exposure in the case of Barrett's esophagus or chronic inflammation in ulcerative colitis, would lead to an ongoing DNA damage response and eventual malignancy. The proteins that regulate and dictate when and which cells become senescent or neoplastic have not been fully elucidated. The bigger question is which factors and cell cycle markers predict the development of cancer including esophageal adenocarcinoma or colorectal carcinoma. As cellular senescence represents an endogenous antitumor pathway, one would expect a vigorous senescence response in those patients who are less at risk of developing GI malignancies. In other words, one would hypothesize that the degree of cellular senescence is inversely proportional to the risk of GI malignancy.

MEASURING CELLULAR SENESCENCE

In order to quantify cellular senescence within tissues, it is first important to distinguish the properties of a senescent cell from that of a nonsenescent cell. Senescent cells have several different characteristic functional and morphologic phenotypes.

1. Senescence-associated β-galactosidase

Following an oncogenic stimulus, a senescent cell stops dividing although remains metabolically active. This growth arrest is irreversible and occurs via an interplay between p16 and p53

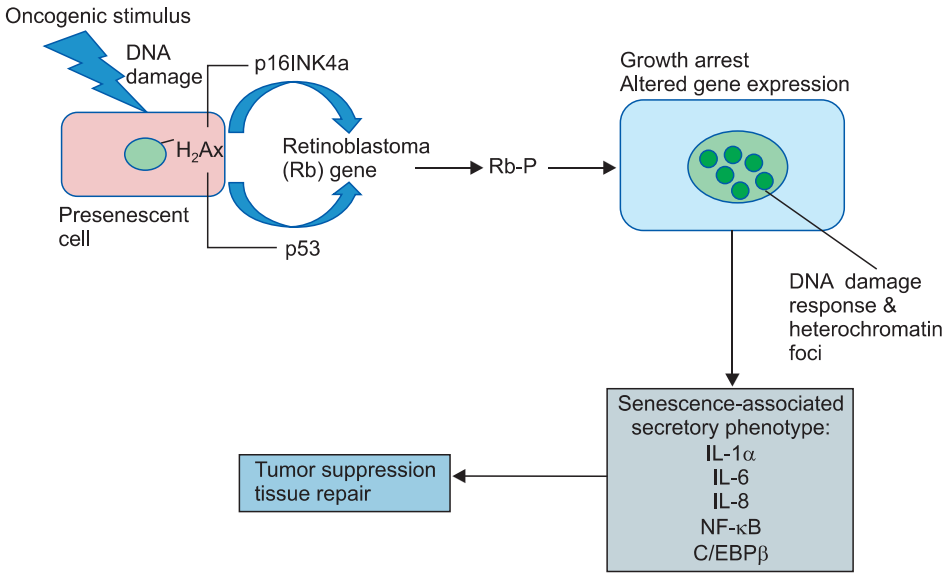

Fig. 1. The cascade of molecular events that comprise cellular senescence. Double-strand DNA breaks from oncogenic exogenous stimuli activating the p16INK4a and p53 tumor suppressor pathways and, resulting in irreversible growth arrest and the secretion of growth factors, cytokines, and proteases. This response leads to suppression of malignant tumorigenesis and tissue repair.
Rb-P, phosphorylated retinoblastoma protein; IL, interleukin; NF-κB, nuclear factor kappa B; EBPβ, enhancer binding protein beta.

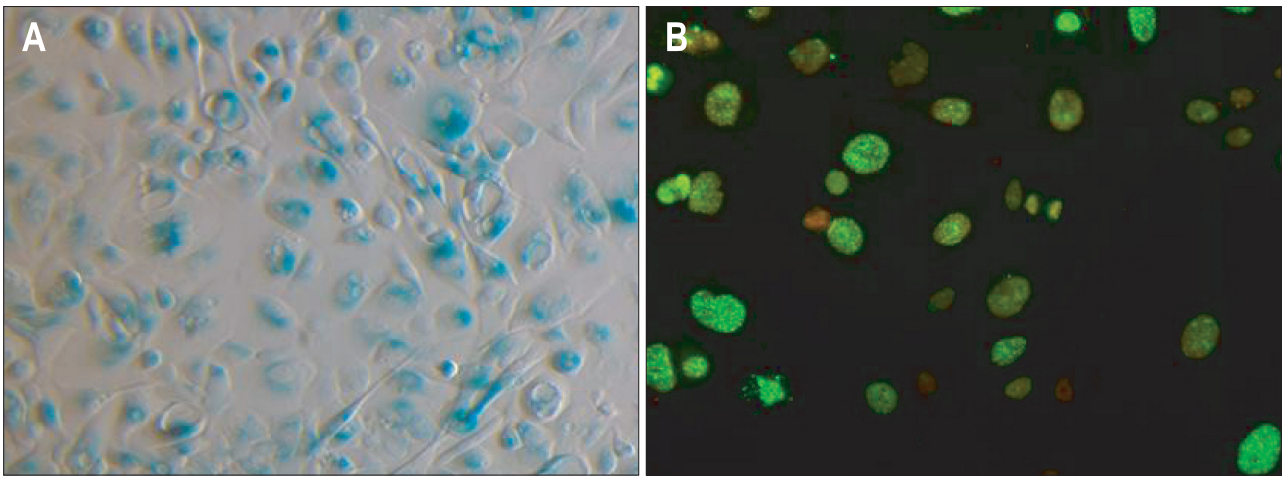

Fig. 2. (A) Senescent cells are identified using senescence-associated proteases, which stain cells blue at pH 6.0. Shown here are nondysplastic hTERT-immortalized Barrette cells using senescence with cisplatin. (B) Immunoreactivity of hTERT-immortalized cells induced to senescence. The cells were treated with propidium iodide, which fluoresces red when bound to nucleic acids. Double-stranded DNA breaks are identified by green foci.

tumor suppressor genes (Fig. 1). There are no physiologic stimuli that can reverse this process once it is activated. Cells *in vitro* that are induced to senesce increase in size by 2-fold. Additionally, senescent cells can be identified *in vitro* as the majority express a senescence-associated β-galactosidase (SA β-gal), a marker of increased lysosomal activity.[17] Commercially available products are available that allow identification of SA β-gal in the cytoplasm of senescent cells, resulting in blue staining cells (Fig. 2). Although SA β-gal is the most widely used marker of senescence, there is no universal or specific marker for cellular senescence.

2. p16INK4a

Most senescent cells also express the tumor suppressor protein p16INK4a.[18-20] This activates the retinoblastoma (RB) gene whose primary function is to repress transcription genes needed for re-entry into the S-phase of the cell cycle. This ultimately results in the formation of heterochromatin products, known as SA heterochromatin foci, which also act by silencing the expression of genes needed for cell cycle progression.[21] Other nuclear foci also form within these cells to perpetuate cellular senescence and sustain its metabolic activity and paracrine effects. The activation of p16INK4a is believed to be a late marker of a sustained DNA damage response and its expression is found to be increased with age.[22]

3. γH_2Ax

Other markers to detect cellular senescence utilize antibodies against the histone changes that take place following and during a DNA damage response. Such markers include γH_2Ax and lamin B1.[23,24] Double-stranded DNA breaks occur as a reaction to cellular damage at the site of histone H2A. DNA double-strand breaks have severe consequences for cell survival and the maintenance of genomic stability.[25] Histone H_2Ax is a genomic care-taker and tumor suppressor. Phosphorylation of H_2Ax to form γH_2Ax in chromatin around DNA breaks is an early event in the induction of cellular senescence and serves as a landing pad for the accumulation and retention of the central components of the signaling cascade in senescence.[26] Immunohistochemistry of γH_2Ax can be used as a biomarker of DNA damage and senescence (Fig. 2).

4. SA secretory phenotype

In addition to the above mentioned morphologic changes, senescent cells also undergo drastic functional changes that alter the surrounding microenvironment. The growth arrest of senescent cells in essence allows a structural barrier to prevent migration and proliferation of neoplastic cells, including those cells at risk for neoplastic transformation.[27] This mechanism effectively walls off and isolates the cancer cells. However, this wall of protective cells is by no means inert. Senescent cells secrete proteases and cytokines that promote inflammation in the local stromal tissue, ultimately leading to the recruitment of lymphocytes and macrophages which can lead to the elimination of premalignant cells.[28] This response is known as the SA secretory phenotype and represents a potent anticancer mechanism.[29,30] Prior studies have confirmed a higher signal of senescence markers in premalignant conditions, such as colon adenomas, compared to adenocarcinomas.[31] Other studies involving mouse models and prostate cancer found that inactivation of p53 led to decreased numbers of senescent cells as well as aggressive growth of cancer cells.[32] The establishment of p53 activity and induction of cellular senescence are important mechanisms in regression of tumor bulk following chemotherapy.

Table 1. Summary of Mechanisms of Cellular Senescence in the Gastrointestinal Tract

	Possible mechanism of senescence	Reference
Benign		
Chronic pancreatitis	Senescence leads to recruitment of immune cells and activation of pancreatic stellate cells leading to inflammation and fibrosis.	28,41
Cirrhosis	Loss of p16, p21, and p53 leads to excessive liver fibrosis and stellate cell proliferation. Reduced production of ECM by senescent cells.	42,47
Malignant		
Pancreatic adenocarcinoma	Loss of Rb gene, p16INK4a and p53 leads to escape from senescence and development of cancer.	21,40
Hepatocellular carcinoma	Activation of p53 in HCC leads to tumor regression by induction of senescence and up-regulation of cytotoxic killing.	43,45,46
Esophageal squamous cell carcinoma	DEC1, a transcription factor that sustains senescence, is increased in precursor lesion intraepithelial neoplasia and decreased in carcinoma.	49

ECM, extracellular matrix; Rb, retinoblastoma; HCC, hepatocellular carcinoma; DEC1, differentiated embryo chondrocyte expressed gene 1.

CELLULAR SENESCENCE AND THE GI TRACT

Cellular senescence plays an integral role in the prevention and development of GI cancers.[33-37] The cascade of events that make up senescence and the cell cycle check points that dictate the progression and maintenance of senescence are important in all types of GI cancers including pancreatic, liver, gastric, colon, and esophageal cancer (Table 1). The aggressive nature of GI cancers is determined by the balance between incendiary oncogenes and tumor suppressor genes.[38]

1. Chronic pancreatitis & pancreatic adenocarcinoma

Up-regulation of the oncogene KRAS occurs with the development of pancreatic cancer.[21] Pancreatic adenocarcinomas arise from premalignant pancreatic intraepithelial neoplasia.[39] Progression from pancreatic intraepithelial neoplasia to adenocarcinoma results in loss of p16INK4a and thus impaired ability to form senescent cells.[40] Carrière *et al*.[21] assessed the degree of senescence in pancreatic intraepithelial neoplasia and adenocarcinoma in an activated oncogenic +Kras/-RB mouse model. RB is an important tumor suppressor gene that is intimately related with the cascade of events that make up the senescent pathway and its loss permits uncontrolled mitosis. The authors found that p16INK4a staining, and to a lesser extent SA β-gal staining, were both increased in all grades of pancreatic intraepithelial neoplasia as compared to pancreatic adenocarcinoma cells in which p16INK4a expression and SA β-gal staining were markedly decreased. It was found that pancreatic intraepithelial neoplastic cells may not demonstrate SA β-gal staining, which highlights the issues of specificity with this particular biomarker. The authors also found that pancreatic intraepithelial neoplastic cells had a high proliferative index, as determined by Ki67 immunoreactivity. This suggests that in these genetically modified mice with up-regulated KRAS and absent RB, the senescent cascade may have been initiated although did not appear to lead to growth arrest. In essence, the loss of RB and subsequent p16INK4a and p53 pathways resulted in escape from cellular senescence.

Cellular senescence also has a role beyond its anticancer properties in the GI epithelium. The SA secretory phenotype results in a robust inflammatory cascade that leads to marked recruitment of immune cells.[28] Fitzner *et al*.[41] hypothesized that senescence plays an integral role in the severity of inflammation and fibrosis seen in chronic pancreatitis. Using a model of dibutyltin dichloride-induced chronic pancreatitis in rats, cellular senescence was closely correlated with activation of pancreatic stellate cells as well as the severity of inflammation and extent of fibrosis. Senescence increased the susceptibility of pancreatic stellate cells to immune-mediated cell destruction.

2. Cirrhosis & hepatocellular carcinoma

Activation of cellular senescence leads to regression of cancer, including hepatocellular carcinomas.[42] This primarily occurs via induction of p53 in murine liver carcinomas.[43] Xue *et al*.[43] used a mouse model of liver carcinomas to show that activation of p53 in p53-deficient liver tumors led to complete tumor regression by induction of a senescent pathway as measured by SA β-gal and immunoblotting of senescence markers. A p53 activation was also associated with up-regulation of cytokines. The subsequent inflammatory cascade led to activation of an innate immune response with cytotoxic killing and tumor clearance of liver carcinomas. Similar data from human thyroid anaplastic carcinoma cells also supports the inhibition of proliferation and cell growth following p53 re-expression.[44] Human studies with hepatocellular carcinoma have found that p53 mutations are commonly associated with poorly differentiated tumors and appear to be an early molecular event.[45,46] Similar to fibrosis in chronic pancreatitis, stellate cells and cellular senescence both have an intimate role in the development of liver fibrosis and cirrhosis. SA β-gal positive cells have been observed in cirrhotic livers in human patients.[47] Krizhanovsky *et al*.[42] used murine models treated with CCl_4 to evaluate the role of cellular senes-

cence in the development of liver fibrosis and cirrhosis. They found that mice lacking key senescence regulators, as measured by SA β-gal, p16 immunohistochemistry and p21 and p53 immunofluorescence, were more likely to have excessive liver fibrosis and stellate cell proliferation compared to mice with an intact and robust senescence response. Senescent stellate cells had reduced production of extracellular matrix components, increased secretion of extracellular matrix degrading enzymes and enhanced local innate immune response within the microenvironment. Cellular senescence facilitated the reversion of fibrosis by cytotoxic means; natural killer cells preferentially killed senescent activated stellate cells leading to resolution of fibrosis.

3. Esophageal and gastric adenocarcinoma

There are few studies that examine the role of cellular senescence in esophageal cancers, including esophageal adenocarcinoma, Barrett's esophagus, and esophageal squamous cell carcinoma. As escape from cellular senescence confers a proliferative advantage, one might expect that the density of senescent cells in Barrett's esophagus decreases as such cells progress from no dysplasia through to high-grade dysplasia. Going *et al*.[48] assessed the presence of cellular senescence in normal and dysplastic epithelium from the upper GI tract by using an intensity-weighted scoring system for SA β-gal. Histologic specimens from 28 patients with Barrett's esophagus and 15 patients with gastric adenocarcinoma were evaluated. Barrett's esophagus with low-grade and high-grade dysplasia did not show a decrease in SA β-gal staining, however reduced activity was seen in gastric and esophageal adenocarcinoma. Our experience confirms the existence of cellular senescence in Barrett's esophagus. We evaluated the presence of senescence in a series of 11 patients who had undergone endoscopic mucosal resection for Barrett's esophagus. Cellular senescence was identified using two methods: SA β-gal staining on Barrett's epithelium and reduced expression of multiple DNA cell cycle associated genes from disrupted Barrett's tissue. Nine out of eleven mucosal resection specimens stained positive for senescence. The intensity-weighted value did not correlate with grade of dysplasia, although the two patients who did not have any senescence detected had nondysplastic Barrett's mucosa while the other mucosal resection specimens had dysplasia. Decreased expression of cell regulatory genes including RB and p16INK4A was also found. Identifying senescent cells, DNA damage response and loss of p16 in both the Barrett's epithelium and underlying esophageal stroma may have a role in predicting whether patients with high-grade dysplasia will respond to endoscopic therapy and radiofrequency ablation.

Recent literature suggests that cellular senescence plays a protective role in esophageal squamous cell carcinoma.[49] Xu *et al*.[49] correlated SA β-gal with the transcription factor human differentiated embryo chondrocyte expressed gene 1 (DEC1) in both esophageal squamous cell carcinoma cell lines and *in vivo* tissue from 241 patients. DEC1 is an important protein transcription factor that induces and sustains cellular senescence. Overexpression *in vitro* induced senescence and inhibited cell growth in cell lines. Compared to normal tissue, DEC1 expression was found to be significantly increased in the precursor lesion intraepithelial neoplasia and significantly decreased in carcinoma tissue. Kaplan-Meier analysis of all 241 patients revealed that DEC1 expression levels were significantly correlated with depth of invasion, lymph node metastasis and survival after surgery. The authors concluded that DEC1 overexpression in precursor lesions acts as a protective mechanism by inducing cellular senescence.[49]

CELLULAR SENESCENCE: A TARGET FOR CHEMOTHERAPY

The relevance and up-regulation of cellular senescence *in vivo* has not been fully elucidated although recent and exciting data suggests that the senescent pathway may be a potential target for the treatment of cancers (Table 2). Oncogenes are the targets for anticancer drugs which inhibit their function as well as that of their transcribed proteins. Specific inhibitors of BCR-ABL tyrosine kinase, epidermal growth factor receptor, and c-KIT have been used with success against cancers including chronic myeloid leukemia, nonsmall cell lung cancer, and GI stromal tumors, respectively.[50-52] Ventura *et al*.[53] found that tumor suppressor genes and their products, specifically those involved with cellular senescence, may provide an additional pharmacologic target for the regression of cancers. They postulated that sustained inactivation of p53, a master cell cycle

Table 2. Potential Therapeutic Approaches to Enhance Cellular Senescence

Therapeutic approach	Comment	Reference
Restoration of p53	p53 restoration has led to senescence induced tumor regression in mice models of sarcoma.	53,54
IGFBP7	Decreases metastatic disease in melanoma xenografts in mice by reactivation p53 and inducing senescence.	55-57
Bevacizumab	Attenuates VEGF and up-regulates senescence.	35
Ki8751	VEGFR2 kinase inhibitor.	35

IGFBP7, insulin-like growth factor binding-protein 7; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

protein that is needed to trigger the senescence pathway, may be required for continued tumor maintenance and survival. A p53 promotes senescence by activating genes that inhibit proliferation, including p21/Cip1/Waf1 cyclin-dependent kinase inhibitor and miR-34 class of microRNAs.[54] The p53 mutations increase cell proliferation, promote genomic instability, and eventually lead to resistance to chemotherapeutic drugs. Using a novel strategy, the group was able to restore p53 expression in mice models. This led to increased apoptosis among mice with lymphoma as demonstrated by increased TUNEL staining. Interestingly, restoration of p53 expression in mice with sarcomas led to cell growth suppression with features of cellular senescence and tumor regression. The authors concluded that the mechanism of tumor regression after p53 restoration is tumor type specific. They concluded that the regression of sarcomas was secondary to either rapid clearance of senescent cells or changes to the stromal microenvironment, leading to decreased angiogenesis. The effect was only confined to the tumor itself and normal appearing cells did not appear to respond to p53 reactivation.

Pharmacologic reactivation of p53 may then be a promising target for the treatment of human cancers.[32,55,56] This could be accomplished by immunomodulating drugs and biological therapies that interact with cell surface receptors to activate senescence. Recent exciting data suggests this goal may not be that far-fetched. Systemic delivery of the senescence inducer IGFBP7 can modulate the progression of melanoma xenografts in mice, decreasing the likelihood of metastatic disease.[57] Recent data indicate that vascular endothelial growth factor (VEGF) can decrease cellular senescence in human endothelial cells.[35] Elucidating the intricate pathway of cellular senescence and determining the exact role and fate of these cells *in vitro* will undoubtedly lead to the development of molecular targets aimed at the treatment of GI tumors.

Cellular senescence has been increasingly recognized as an antitumor mechanism in other GI cancers. As mentioned above, VEGF attenuates cellular senescence in colorectal cancer.[35] VEGF receptors are known to be up-regulated in colorectal cancer. An inhibitor of this receptor, bevacizumab, has improved outcomes in metastatic colorectal cancer, presumably by modulating and enhancing the senescent pathway. Hasan *et al*.[35] evaluated the effect of bevacizumab on cellular senescence in colorectal cancer cell lines. They found that bevacizumab led to a significant increase in cellular senescence in colorectal cancer cells compared to immunoglobulin G-treated controls. Similar results were also obtained from cells treated with a VEGF receptor-2 kinase inhibitor Ki8751. The authors found similar results with *in vivo* studies. Using murine tumor xenografts, 75% of mice treated with bevacizumab were found to have cellular senescence compared to zero xenografts treated with saline. The proportion of senescent cells in colon cancer tissue obtained from patients treated with bevacizumab was 4-fold higher compared to untreated patients.[35] Up-regulation of senescence by VEGF inhibitors represents a novel antitumor activity in colorectal cancer.

CONCLUSIONS

Cellular senescence is emerging as a widespread cellular end point in GI oncology and its importance is becoming increasingly recognized as an essential pathway in the pathogenesis of cancer. The exact mechanism of senescence and it's interaction with the surrounding microenvironment as it relates to premalignancy, dysplasia and cancer needs further study, both from a basic science and clinical standpoint. This process poses new possibilities for chemotherapeutic regimens that may allow up-regulation of cell cycle check points with decreased proliferation and activation of the innate immune system. However, we must take caution in interpreting this endogenous defense mechanism as recent evidence suggests that the cascade of events that constitutes cellular senescence may be a double-edged sword; the chronic inflammation that develops over time may potentiate a more favorable microenvironment for the growth and spread of GI cancer.[6] In particular, the senescence associated secretory phenotype that includes proteases and collagenases may promote cellular invasion. In addition, it has been found that in the process of epithelial mesenchymal transition (EMT), translational factors such as twist not only facilitate EMT but can bypass oncogene stimulated senescence. As the relevance of escape from cellular senescence becomes increasingly clear, so too will the need for more appropriate, effective and reliable means in which to identify this process. Further studies are also needed to help delineate whether senescence can predict clinical outcomes such as those associated with endoscopic therapy for premalignant lesions.

CONFLICTS OF INTEREST

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REFERENCES

1. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States. Part III: liver, biliary tract, and pancreas. Gastroenterology 2009;136:1134-1144.
2. National Cancer Institute. Colon and rectal cancer [Internet]. Bethesda: National Cancer Institute at the National Institutes of Health; c2012 [cited 2012 Sep 20]. Available from: http://www.cancer.gov/cancertopics/types/colon-and-rectal.
3. Goel A, Boland CR. Epigenetics of colorectal cancer. Gastroenterology 2012;143:1442-1460.
4. Vanuytsel T, Senger S, Fasano A, Shea-Donohue T. Major sig-

naling pathways in intestinal stem cells. Biochim Biophys Acta 2012;1830:2410-2426.
5. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 2007;8:729-740.
6. Campisi J. Cellular senescence: putting the paradoxes in perspective. Curr Opin Genet Dev 2011;21:107-112.
7. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 1965;37:614-636.
8. Watts G. Leonard Hayflick and the limits of ageing. Lancet 2011;377:2075.
9. Breivik J, Gaudernack G. Carcinogenesis and natural selection: a new perspective to the genetics and epigenetics of colorectal cancer. Adv Cancer Res 1999;76:187-212.
10. Tollervey JR, Lunyak VV. Epigenetics: judge, jury and executioner of stem cell fate. Epigenetics 2012;7:823-840.
11. Frias C, Pampalona J, Genesca A, Tusell L. Telomere dysfunction and genome instability. Front Biosci 2012;17:2181-2196.
12. Feldser DM, Hackett JA, Greider CW. Telomere dysfunction and the initiation of genome instability. Nat Rev Cancer 2003;3:623-627.
13. Cheung AL, Deng W. Telomere dysfunction, genome instability and cancer. Front Biosci 2008;13:2075-2090.
14. David G. Regulation of oncogene-induced cell cycle exit and senescence by chromatin modifiers. Cancer Biol Ther 2012;13:992-1000.
15. Yao H, Rahman I. Role of histone deacetylase 2 in epigenetics and cellular senescence: implications in lung inflammaging and COPD. Am J Physiol Lung Cell Mol Physiol 2012;303:L557-L566.
16. Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor gene p16. Int J Cancer 2012;130:1715-1725.
17. Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc 2009;4:1798-1806.
18. Wang Y, Zang X, Chen P. High expression of p16INK4a and low expression of Bmi1 are associated with endothelial cellular senescence in the human cornea. Mol Vis 2012;18:803-815.
19. Bazarov AV, Van Sluis M, Hines WC, et al. p16(INK4a)-mediated suppression of telomerase in normal and malignant human breast cells. Aging Cell 2010;9:736-746.
20. Coppé JP, Rodier F, Patil CK, Freund A, Desprez PY, Campisi J. Tumor suppressor and aging biomarker p16(INK4a) induces cellular senescence without the associated inflammatory secretory phenotype. J Biol Chem 2011;286:36396-36403.
21. Carrière C, Gore AJ, Norris AM, et al. Deletion of Rb accelerates pancreatic carcinogenesis by oncogenic Kras and impairs senescence in premalignant lesions. Gastroenterology 2011;141:1091-1101.
22. Waaijer ME, Parish WE, Strongitharm BH, et al. The number of p16INK4a positive cells in human skin reflects biological age. Aging Cell 2012;11:722-725.
23. Mah LJ, El-Osta A, Karagiannis TC. gammaH2AX: a sensitive molecular marker of DNA damage and repair. Leukemia 2010;24:679-686.
24. Freund A, Laberge RM, Demaria M, Campisi J. Lamin B1 loss is a senescence-associated biomarker. Mol Biol Cell 2012;23:2066-2075.
25. Nagaria P, Robert C, Rassool F. DNA double-strand break response in stem cells: mechanisms to maintain genomic integrity. Biochim Biophys Acta 2013;1830:2345-2353.
26. Kuo LJ, Yang LX. Gamma-H2AX: a novel biomarker for DNA double-strand breaks. In Vivo 2008;22:305-309.
27. Davalos AR, Coppe JP, Campisi J, Desprez PY. Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev 2010;29:273-283.
28. Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 2010;16:238-246.
29. Rodier F, Muñoz DP, Teachenor R, et al. DNA-SCARS: distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. J Cell Sci 2011;124(Pt 1):68-81.
30. Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 2011;30:1536-1548.
31. Oikonomou E, Makrodouli E, Evagelidou M, Joyce T, Probert L, Pintzas A. BRAF(V600E) efficient transformation and induction of microsatellite instability versus KRAS(G12V) induction of senescence markers in human colon cancer cells. Neoplasia 2009;11:1116-1131.
32. Chen Z, Trotman LC, Shaffer D, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005;436:725-730.
33. Froeling FE, Feig C, Chelala C, et al. Retinoic acid-induced pancreatic stellate cell quiescence reduces paracrine Wnt-beta-catenin signaling to slow tumor progression. Gastroenterology 2011;141:1486-1497.
34. Garagnani P, Pirazzini C, Franceschi C. Colorectal cancer microenvironment: among nutrition, gut microbiota, inflammation and epigenetics. Curr Pharm Des 2012;19:765-778.
35. Hasan MR, Ho SH, Owen DA, Tai IT. Inhibition of VEGF induces cellular senescence in colorectal cancer cells. Int J Cancer 2011;129:2115-2123.
36. Krizhanovsky V, Xue W, Zender L, Yon M, Hernando E, Lowe SW. Implications of cellular senescence in tissue damage response, tumor suppression, and stem cell biology. Cold Spring Harb Symp Quant Biol 2008;73:513-522.
37. Katz SF, Lechel A, Obenauf AC, et al. Disruption of Trp53 in livers of mice induces formation of carcinomas with bilineal differentiation. Gastroenterology 2012;142:1229-1239.
38. Peltomäki P. Mutations and epimutations in the origin of cancer. Exp Cell Res 2012;318:299-310.
39. Macgregor-Das AM, Iacobuzio-Donahue CA. Molecular pathways

in pancreatic carcinogenesis. J Surg Oncol 2013;107:8-14.
40. Rabien A, Sanchez-Ruderisch H, Schulz P, et al. Tumor suppressor p16INK4a controls oncogenic K-Ras function in human pancreatic cancer cells. Cancer Sci 2012;103:169-175.
41. Fitzner B, Müller S, Walther M, et al. Senescence determines the fate of activated rat pancreatic stellate cells. J Cell Mol Med 2012;16:2620-2630.
42. Krizhanovsky V, Yon M, Dickins RA, et al. Senescence of activated stellate cells limits liver fibrosis. Cell 2008;134:657-667.
43. Xue W, Zender L, Miething C, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007;445:656-660.
44. Moretti F, Nanni S, Farsetti A, et al. Effects of exogenous p53 transduction in thyroid tumor cells with different p53 status. J Clin Endocrinol Metab 2000;85:302-308.
45. Nakano A, Watanabe N, Nishizaki Y, Takashimizu S, Matsuzaki S. Immunohistochemical studies on the expression of P-glycoprotein and p53 in relation to histological differentiation and cell proliferation in hepatocellular carcinoma. Hepatol Res 2003;25:158-165.
46. Woo HG, Wang XW, Budhu A, et al. Association of TP53 mutations with stem cell-like gene expression and survival of patients with hepatocellular carcinoma. Gastroenterology 2011;140:1063-1070.
47. Wiemann SU, Satyanarayana A, Tsahuridu M, et al. Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. FASEB J 2002;16:935-942.
48. Going JJ, Stuart RC, Downie M, Fletcher-Monaghan AJ, Keith WN. 'Senescence-associated' beta-galactosidase activity in the upper gastrointestinal tract. J Pathol 2002;196:394-400.
49. Xu Q, Ma P, Hu C, et al. Overexpression of the DEC1 protein induces senescence in vitro and is related to better survival in esophageal squamous cell carcinoma. PLoS One 2012;7:e41862.
50. Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. Blood 2009;113:1619-1630.
51. Galvani E, Alfieri R, Giovannetti E, et al. Epidermal growth factor receptor tyrosine kinase inhibitors: current status and future perspective in the development of novel irreversible inhibitors for the treatment of mutant non-small cell lung cancer. Curr Pharm Des 2013;19:818-832.
52. Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer 2011;11:865-878.
53. Ventura A, Kirsch DG, McLaughlin ME, et al. Restoration of p53 function leads to tumour regression in vivo. Nature 2007;445:661-665.
54. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. Nature 2007;447:1130-1134.
55. de Keizer PL, Laberge RM, Campisi J. p53: pro-aging or pro-longevity? Aging (Albany NY) 2010;2:377-379.
56. Chiantore MV, Vannucchi S, Accardi R, et al. Interferon-beta induces cellular senescence in cutaneous human papilloma virus-transformed human keratinocytes by affecting p53 transactivating activity. PLoS One 2012;7:e36909.
57. Wajapeyee N, Serra RW, Zhu X, Mahalingam M, Green MR. Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. Cell 2008;132:363-374.