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The inflammatory network: bridging senescent stroma and epithelial tumorigenesis

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1. ABSTRACT

Cellular senescence or cellular aging, defined by permanent cell cycle arrest, is well known for its evolutionary advantage in protecting the organism from developing cancer; however, it is also acknowledged that aged stromal cells can significantly expedite epithelial tumorigenesis, although exactly how they function to augment tumor formation remains elusive. Recent evidence suggests that this tumor-promoting effect is likely mediated by diffusible pro-inflammatory molecules synthesized and released by senescent stromal fibroblasts, acting in a paracrine fashion on adjacent tumor epithelium. Mobilization of the inflammatory network by senescent fibroblasts has bifurcated roles on the epithelial and stromal compartments, converging on the promotion of epithelial tumorigenesis. A thorough understanding of the regulatory mechanisms underlying these events may lead to improved approaches in cancer treatment.

2. INTRODUCTION

The incidence of epithelial cancers rises exponentially with age in humans. Aging is by far the most potent carcinogen for humans (1). Beginning from the fifth decade of human life, cancer cases rise exponentially with age, peaking in a risk of 1 to 2 in men and 1 to 3 in women (1, 2). Historically, activation of oncogenes, loss of tumor-suppressor genes or both in the epithelium have long been thought to be the only alterations required for the transformation of epithelial cells to a tumorigenic state (3). However, accumulating evidence suggests that mutations alone are not sufficient for the development of epithelial cancers (3); rather, alterations in the stroma, a heterogeneous group of cells composed of fibroblasts, blood vessels, and inflammatory cells that grow together with cancer cells, are concomitantly needed (4-7). Among the stromal cells, fibroblasts are the most abundant components and have been implicated in facilitating

epithelial tumorigenesis (8). Moreover, recent studies have shown that fibroblasts near epithelial cancer are senescent and can indeed promote tumorigenic growth of the host epithelium (9). Additionally, the inflammatory network has been implicated in mediating the enhancement of tumor formation by senescent fibroblasts, affording a novel angle in the study of stromal-epithelial interactions. Although excellent reviews on cellular senescence are readily available (8, 10, 11), ones that focus on mechanistic studies of senescent stroma-mediated promotion of epithelial cancer development are lacking. Here, we review the current understanding of cellular senescence and epithelial carcinogenesis, with an emphasis on senescence-associated mobilization of the inflammatory network in stroma and its effect on epithelial tumorigenesis.

3. EPITHELIAL TUMORIGENESIS: MUCH MORE THAN MUTATIONS

The majority of age-related human cancers are carcinomas-cancers of an epithelial origin, such as those of the mammary gland, stomach, colon, lung, prostate, and ovary (8, 12, 13). For a long time, the "mutation theory" of carcinogenesis dogmatized the cancer biology field, postulating the sufficiency of malignant transformation by only a few genetic alterations (3). Indeed, aberrant activation of oncogenes and inactivation of tumor suppressors caused by environmentally or virally-derived mutations still play an unequivocally important role in the development of many types of cancers. During the past decade, however, interesting findings from laboratories worldwide have challenged the mutation model of tumorigenesis. For instance, spontaneous mutations rise with an estimated rate of 2×10^{-7} per cell cycle division (14), which should give rise to only a small number of mutations over the entire human life span, well below that required for tumor initiation (15). What underlies the apparent discrepancy between the laboratory-calculated low mutation load and the high cancer incidence in reality? Additionally, inherited deleterious genetic alterations almost always target a single organ for cancer development. If mutations were the only factor required for tumorigenesis, why are most organs exempt from hereditary tumorigenesis, since all the cells in the organism contain identical genetic content (16)? Thirdly, epithelial cancers prevail in the aged population and are rarely encountered among the young; on the other hand, hematological tumors, sarcomas, and other non-epithelial cancers tend to occur more frequently in children and young adults (8). Of course, as some may argue, epithelial cells have a much more rapid turnover and renewal rate than do cells of non-epithelial origins, resulting in significantly better chances to incorporate DNA-damaging mutations. Nevertheless, even if the shorter lapse of time needed to accumulate mutations for the initiation of epithelial versus mesenchymal cancers is taken into account, the incidence at which epithelial malignancies occur still largely exceeds the expected rate (8). What, then, makes epithelial cancers a disease of the old much more than of the young? Questions such as these inspired heated discussions, and it was soon realized that in addition to mutational stress, the cellular microenvironment, which supports and nourishes the epithelial host cells, also plays a pivotal role in epithelial tumorigenesis.

4. STROMAL-EPITHELIAL INTERACTIONS: OPENING PANDORA'S BOX

Epithelium-enclosed organs consist of two fundamental components: the epithelial layer that resides on a specialized basement membrane and the stroma that supports the epithelial cells from below (17). A heterogeneous group of cells, including fibroblasts, fat cells, smooth muscle cells, nerve cells, and inflammatory cells, together with secreted macromolecules compose the stroma (18). The discovery that the genome of evolutionarily advanced animals lacks autonomy dates back to almost a hundred years ago, when Spemann's group demonstrated that areas adjacent to certain frog embryonic cell groups were sufficient in determining the developmental fate of those cells (19, 20), suggesting that communication between cells and their milieu can shape cellular plasticity. Subsequent laboratory findings further substantiated the indispensable role of the cellular microenvironment in specifying cellular behavior (16, 21). Stromal influence on epithelial organization, proliferation, and differentiation begins with embryogenesis and continues throughout adulthood. In the mammary gland, stromal fibroblasts induce formation of the epithelial buds, whereas the fatty stroma induces branching of the epithelial ductile network (22). Similarly, in the prostate, stroma-derived male sex hormones direct epithelial morphogenesis and maintain proper prostatic epithelial functions (23). It is therefore evident that stromal activities have significant influence over epithelial behavior.

Compelling evidence has implicated stromal-epithelial interactions in a broad spectrum of epithelial pathogenesis, foremost of which is epithelial tumorigenesis (1, 24). Illmensee's group was one of the first to demonstrate that manipulation of the stroma could both promote and inhibit epithelial malignancies. In this elegantly-designed study, rodent teratocarcinoma cells formed germ cell tumors when transplanted into unsuppressive tissues but differentiated into multiple normal mouse tissues when injected into the blastocysts of developing embryos (25), directly pointing to factors that resided in the stroma in determining the behavior of epithelial cells. This notion was further supported by reports that exposure of the mammary stroma to irradiation (26) or carcinogenic stimulus (27) considerably enhanced tumor formation in premalignant breast epithelial cells; in contrast, orthotopic expression of a cell adhesion molecule was able to abrogate the malignant phenotype in human bladder cancer cells (28). These observations strongly argued that instead of being an inert mass merely providing physical support for the epithelium, stromal cells actively participated in regulating epithelial tumorigenesis.

Conversely, cancer cells themselves can have a substantial effect on their microenvironment, transforming the stroma from one hostile to tumor formation to one that avidly supports tumor progression (29). Cancer cells produce a broad range of stroma-regulating paracrine molecules that disrupt normal stromal structure and

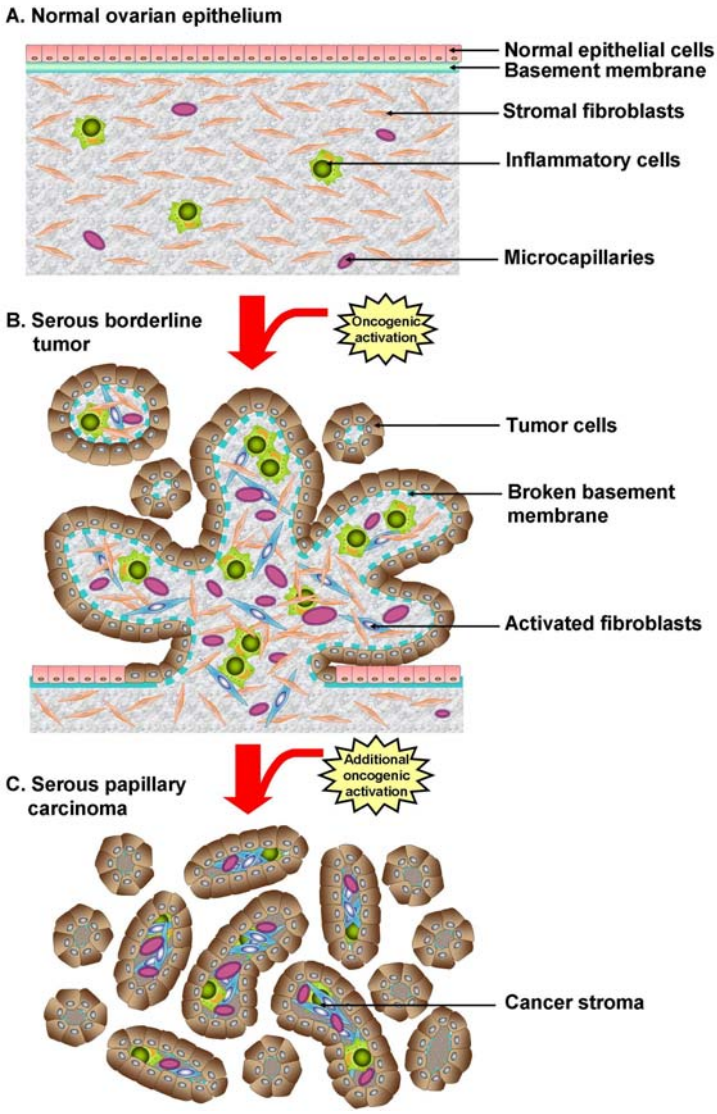

Figure 1. Epithelial-stromal interactions during epithelial ovarian cancer progression. Normal ovarian epithelium is characterized by flat surface epithelial cells, a well-defined basement membrane, and a stroma composed of fibroblasts, immune cells, intercalating capillaries, and matrix molecules (shown in A). As presented in panel B, formation of serous tumor of low malignant potential is typically initiated by oncogenic stimuli affecting the epithelial cells, such as activated KRAS or BRAF expression. Premalignant epithelium thus reprograms the stroma by secreting growth factors, inflammatory mediators and degradative enzymes, transforming the stroma into one that favors tumorigenesis. Simultaneously, activated stroma generates an additional array of signaling molecules capable of modifying epithelial behavior and fostering growth of these epithelial cells into borderline serous tumor. Full-blown malignant transformation to serous papillary carcinoma (low grade), as depicted in C, is further aided by extensive epithelial-stromal communications, resulting in the (partial) devouring of stroma by invasive epithelial cancer cells and the formation of numerous papillae.

homeostasis, including basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), interleukins, and colony-stimulating factors (30). In addition, proteolytic enzymes released by cancer cells compromise the integrity of the basement membrane, paving the way for subsequent cell migration and cancer metastasis (31, 32). Concomitantly, stromal cells such as fibroblasts, adipocytes, and smooth muscle cells are activated by these factors, serving as a reservoir for additional tumorigenic growth factors and proteases (29). Combined, extensive crosstalk between cancer cells and their microenvironment leads to reciprocal activation of both compartments, generating a positive-feedback route that continuously stimulates epithelial tumorigenesis (Figure 1).

5. CELLULAR SENESCENCE: THE TWO-FACED PLAYER

As an organism ages, both the epithelium and the

stroma are involved. However, aging may exert opposing functions in these two compartments. While senescence serves as a powerful barrier for tumorigenesis in epithelial cells, senescent but not normal stromal fibroblasts can significantly promote epithelial cancer development. What, then, is cellular senescence? How does senescence perplex stromal-epithelial communications to coordinate tumor promotion?

5.1. Overview of cellular senescence

The phenomenon 'cellular senescence' was first described by Hayflick and Moorhead more than four decades ago when these authors observed that certain factors were able to induce growth arrest in normal fibroblasts (33). It was later speculated and substantiated by laboratory evidence that cellular senescence could be induced by a variety of internal and external stimuli, including telomere erosion accompanied by each replicative cell cycle (replicative senescence) (34-36), oxidative DNA damage (37, 38), chromosomal instability (39, 40) and exposure to oncogenic insults (oncogene-induced senescence) (41). The tumor-suppressing p53 and p16/pRB pathways execute the senescence program, essentially by implementing a DNA-damage response (DDR) resulting in permanent growth arrest (8, 10, 11, 42). The p53 pathway is generally believed to cause senescence induced by telomere attrition and oxidative DNA stress, whereas the p16/pRB pathway is thought to respond to senescence stimuli such as oncogenes and chromosomal disruption (43-45). However, functional specialization of the p53 and p16/pRB pathways is far from being clear-cut, and extensive crosstalk between these two cascades has been identified, presumably mediated by the p53 downstream effecter p21 (11). More recently, the high-mobility group A (HMGA) proteins have also been shown to facilitate the implementation of senescence. This is believed to occur via physical interactions between HMGAs and chromatin modulators that stabilize and maintain senescence-associated heterochromatic foci (SAHFs), which may be strengthened by cooperation between HMGAs and p16 (46). Aside from the perpetual inability to return to replicative cell cycle, the majority of senescent cells are also characterized by apoptosis resistance, expression of specific senescence markers, and altered gene expression profile (8, 10, 42).

In addition to the canonical senescence mediators discussed above, latest findings have involved microRNAs in regulating cellular senescence and tumor formation. Specifically, multiple research groups have showed that miR-34 is a direct downstream effector of p53, regulating a variety of p53-mediated cellular effects, including senescence, apoptosis and tumor suppression (reviewed in (47, 48)). Detailed molecular mechanisms underlying microRNA-mediated cell cycle arrest are poorly understood. However, identification of these small RNA molecules as potential senescence modulators has opened novel avenues in targeting these molecules for cancer therapies.

5.2. A focus on oncogene-induced senescence

Oncogene-induced senescence (OIS), a concept first introduced by Serrano *et al.*, has evolved as an important evolutionary advantage endowing cancer prevention. As demonstrated by these authors, expression of oncogenic RAS in primary human or rodent cells incurred a G1 cell cycle arrest mediated by p53 and p16/pRB (41). It was hence hypothesized that cellular senescence is registered not only by accumulated cell divisions but by oncogenic stimuli as well. More interestingly, Courtois-Cox and colleagues recently observed that induction of senescence involves a global negative-feedback loop to "shut off" RAS rather than "activating" RAS following initial activation. In this study, RAS, PI3K, AKT, and ERK activities were transiently activated and then quickly suppressed to lower-than-baseline levels in human fibroblasts (49). A similar result was reproduced by Wajapeyee *et al.*, who reported that oncogenic BRAFV600E-induced insulin growth factor binding protein-7 (IGFBP7) acts to accomplish fibroblast senescence by inhibiting RAS-BRAF-MEK-ERK signaling (50). These studies suggest that RAS activation may lead to a strong hyperproliferative phase followed by a senescence response possibly due to activation of the DNA damage response following hyper-replication of DNA, mediated by ataxia telangiectasia mutated (ATM), CHK kinase I (CHK1), and CHK kinase II (CHK2) signaling pathways (51, 52).

Because senescent cells stop dividing, senescence serves as a solid barrier to malignant transformation. In this regard, OIS has an important role in maintaining premalignant cells in a nonaggressive state, and abrogation of RAS-induced senescence has been suggested to be a prerequisite step in advanced tumorigenesis (41). In line with this concept, Michaloglou *et al.* showed that oncogenic BRAF, a downstream kinase in the RAS cascade, induced senescence in melanocytes, a phenomenon commonly seen in growth-arrested benign human melanocytic lesions (nevi) (53); however, blocking of the senescence program rapidly transformed BRAF-baring melanocytes into malignant melanoma cells (50), indicating that cellular senescence restricts cancer development *in vivo*. Ectopic expression of other oncogenes such as MEK and E2F-1 has also been reported to cause cellular senescence in a p53- and/or p16-dependent fashion (54-55). The tumor-preventing role of OIS is further supported by physiologically-relevant observations. Senescent cells were found only in benign but not in malignant lesions in humans (49, 53, 56) and laboratory mice (56-59). Additionally, genetically-engineered mice that harbor mutations in the *TP53* gene, the principal mediator of OIS, were significantly cancer prone (56, 57, 60). Likewise, humans carrying dysfunctional *TP53* or *CHK2* genes develop Li-Fraumeni syndrome characterized by a high incidence of multiple spontaneous cancers (61, 62). These findings imply that it is the circumvention of OIS but not oncogenic activation itself that leads to malignant transformation.

5.3. The concept of antagonistic pleiotropy

Meanwhile, a severe side-effect accompanies the growth-static role of cellular senescence: mounting evidence has involved cellular senescence in causing organismal aging. Senescence-associated β-galatosidase

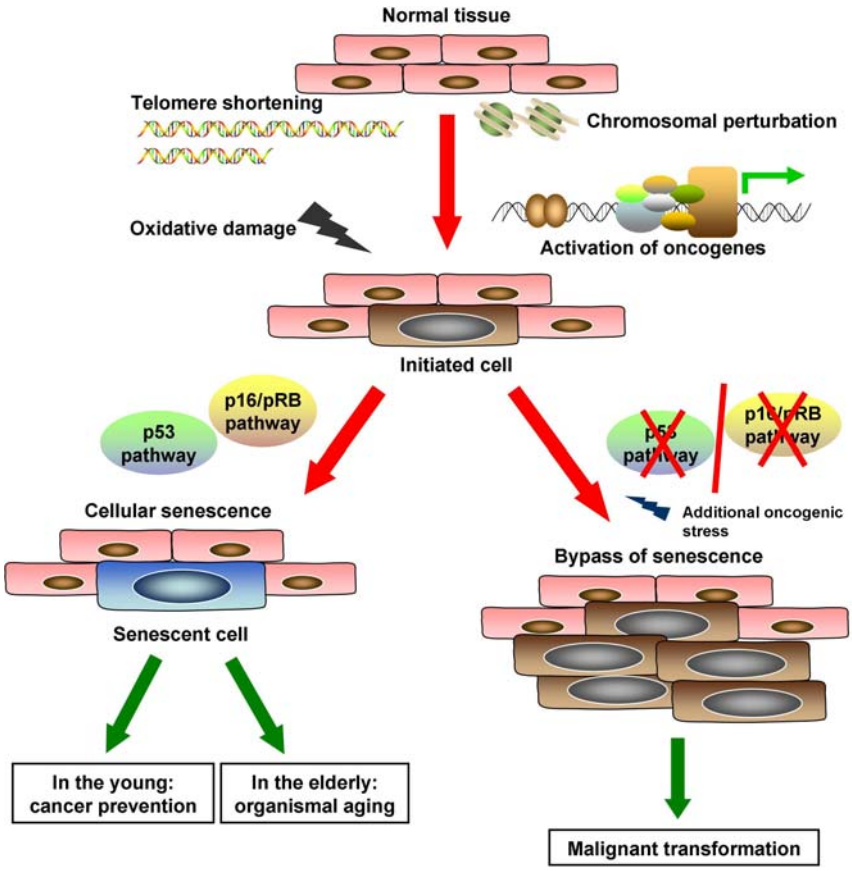

Figure 2. Cellular senescence induced by multiple stimuli. Cellular senescence is triggered by endogenous and exogenous stimuli that potentially induce a DNA-damage response (DDR), including shortening of telomeres, oxidative damage to the DNA, compromise of chromosomal integrity, and inappropriate activation of oncogenes. Classically, as shown on the left, DDR signals are sensed by the p53 and p16/pRB pathways, leading to senescence of the cell. On the other hand, as shown on the right, when signaling cascades in the tumor-suppressing p53 and/or p16/pRB are partially or completely blocked, cells fail to undergo senescence in response to stimulating "Hayflick factors". These cells are at a particularly high risk for developing cancers when additional oncogenic events occur. Of note, senescent cells have been suggested to prevent cancer in young organisms owing to their growth-static nature. However, in the aged population, senescence seems to contribute to aging in the whole organism, a topic still under heated debate.

(SA-β-gal), a commonly-used specific marker for cellular senescence, was reported to exhibit increasingly positive staining with age in the monkey skin (63) and retina (64), rat kidney (65), and human liver (66). Similarly, p16 was found to be upregulated with age in the progenitor cells of the mouse brain (67), bone marrow (68), and pancreas (69), along with age-associated decreases in neurogenesis, hematopoiesis, and pancreatic function, respectively. Importantly, cellular senescence of the stem cell population indicates that a decline in cellular renewal power together with an accumulation of aged cells concertedly results in widespread aging in the entire organism (10). The dual-faced role of cellular senescence-cancer protection in the young and age promotion in the old-reflects the core concept of antagonistic pleiotropy (70) (Figure 2).

6. SENESCENCE OF THE STROMAL FIBROBLASTS: 'BAD NEIGHBORS' DO EXIST

In a manner similar to the establishment of epithelial senescence, aging of the stroma occurs when the mitotically-active stromal cells (i.e., proliferative fibroblasts) undergo irreversible cell cycle arrest in response to DNA-damaging agents. However, it is not until recently that stromal senescence has been demonstrated to promote tumor formation in the neighboring epithelium, providing novel insights for the study of epithelial tumorigenesis.

6.1. Inducers of senescence in stromal fibroblasts

Oncogenic insults remain one of the most frequently-encountered inducers of stromal senescence. Of interest, cytokines downstream of the RAS signaling pathway have recently been involved in mediating senescence of stromal fibroblasts. Results from our laboratory showed that oncogenic RAS-induced chemokine growth-regulated oncogene (Gro-1/Gro-α) was adequate in rendering ovarian stromal fibroblasts senescent (9), suggesting that chemokines downstream in the RAS pathway may participate in the senescence program of

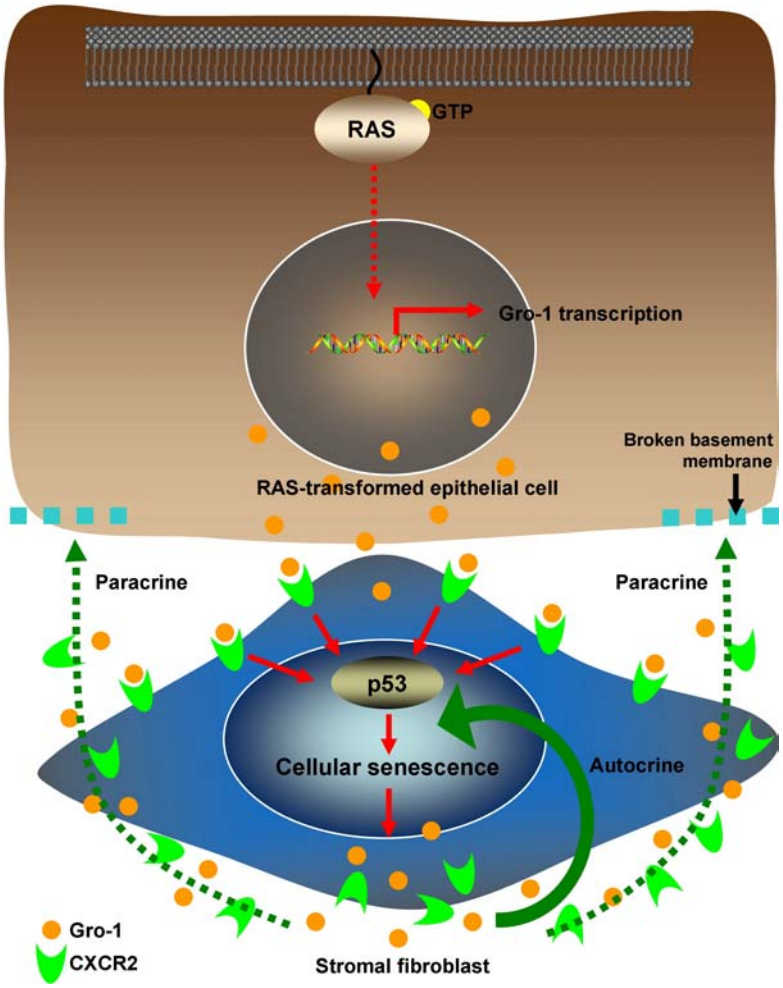

Figure 3. RAS-induced senescence in stromal fibroblasts mediated by Gro-1-CXCR2 signaling. The chemokine Gro-1 has been demonstrated to be a downstream signaler of oncogenic RAS, although detailed regulatory mechanisms mediating this event are unknown. Suggested by recent works, RAS-induced Gro-1 released by transformed epithelial cells can induce senescence in stromal fibroblasts in a paracrine fashion and this effect is likely p53-dependent. Senescent fibroblasts in turn generate secondary Gro-1 and its cognate receptor CXCR2 molecules, completing a positive-feedback loop that strengthens the senescence program (solid green arrow) and a paracrine route by diffusing into epithelial cells (dotted green arrows).

neighboring fibroblasts in a paracrine fashion (9). Subsequent to our findings, Acosta *et al.* reported the induction of senescence in human fibroblasts by CXCR2, a *bona fide* receptor for chemokines including IL-8 and Gro-1, 2, 3/Gro-α, β, γ (71),, further validating the critical role of Gro-1 in implementing senescence. Intriguingly, senescent cells have also been reported to exhibit elevated levels of both Gro-1 and CXCR2, thus completing a positive feedback loop culminating in strengthened cellular senescence (71). A suggested mode of action by which the Gro-1-CXCR2 pathway induces senescence in stromal fibroblasts is presented in Figure 3. As indicated by this model, mutationally-activated RAS leads to marked overexpression of Gro-1 in transformed epithelial cells via undefined pathways, which then diffuses through the basement membrane, is taken up by the stromal fibroblasts, and functions to establish the senescence program in these cells in a p53-dependent manner; meanwhile, committed senescent cells reinforce senescence by overexpressing Gro-1 and CXR2, providing a self-driving force that perpetuates senescence. In this case, cellular senescence of the fibroblastic stroma is attributable to synergism between paracrine (between epithelial cells and the stroma) and autocrine (within stromal fibroblasts) signaling of Gro-1-CXCR2, although detailed regulatory pathways underlying these events remain to be identified. Other recently documented mediators of oncogene-induced fibroblast senescence include the pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) (72), and IGFBP7 (50), all of which were shown to be induced by the oncogenic BRAFV600E protein downstream of RAS. Historically, RAS-induced cytokines such as IL-6 (73, 74) and IL-8 (75, 76) have been widely involved in augmenting tumorigenesis primarily by mobilizing the pro-inflammatory network, but essentially nothing has been reported within the context of cellular senescence. It is now

suggested that small-molecular-weight pro-inflammatory cytokines could also function as key mediators of RAS-triggered cellular senescence in stromal fibroblasts, adding new, exciting chapters to the biology of both cytokine signaling and cancer.

6.2. Senescent fibroblast-mediated promotion of epithelial tumorigenesis

Cancer is, uncontroversially, a disease closely associated with organismal aging (1). However, the anti-proliferative essence of cellular senescence prompts the question of why, as organisms age, profound cellular aging induced by accumulating "Hayflick factors" does not entail stronger protection against tumorigenesis (42)? As discussed previously, the answer resides in the role of an age-associated tumor-promoting microenvironment. Generally, stroma of young adults has been considered anti-proliferative and differentiative (18), supported by the early observation that normal mammary stromal adipocytes suppressed tumorigenesis and induced differentiation in breast epithelial cells (77). Similarly, normal prostatic stromal cells inhibited cancer growth from malignant prostatic epithelial cells (78). Whereas young stroma provides an inhibitory environment for epithelial tumorigenesis, increasing evidence suggests that senescent stroma markedly enhances tumor formation in the host epithelium.

The delicate role played by the 'age' of the stroma in modulating epithelial tumorigenicity was first described in the early 1990s. In this pioneering study, McCullough *et al.* showed that rat liver carcinoma cells were barely tumorigenic when injected into the liver parenchyma of young rats; remarkably, tumorigenicity in these cells rose in parallel with increased age of the host animals (79). Likewise, Rinehart *et al.* later found that whereas early-passage endometrial stromal cells repressed malignant transformation of endometrial epithelial cancer cells, this growth-inhibitory potential of stromal cells was gradually lost with increasing passage numbers (18). Both of these studies demonstrated that, as the stroma aged, the tumor microenvironment was shifted from being suppressive to being permissive of epithelial tumorigenesis. Subsequently, senescence-associated promotion of epithelial tumorigenesis has been reported by a large number of investigators. Krtolica and colleages showed that senescent but not normal human fibroblasts were markedly tumorigenic in premalignant (initiated) human skin epithelial cells both in culture and in immunocompromised mice (80), thus providing the first experimental proof that senescent stromal fibroblasts can promote epithelial tumorigenesis. Similar results were found using aged stromal fibroblasts in human preneoplastic epithelial cell lines derived from diverse origins, including the prostate (81, 82), mammary gland (83, 84), skin (85), and ovary (9), suggesting that the tumor-promoting effect is a consistent theme in senescent stromal fibroblasts. In addition to augmenting tumor formation, senescent fibroblasts have also been implicated in accelerating tumor neovascularization. Coppe *et al.* showed that senescent lung fibroblasts effectively stimulated tumor angiogenesis in premalignant murine epithelial cells injected into nude mice (86). Similarly, Parrinello *et al.* disclosed a crucial role of senescent fibroblasts in inducing branching morphogenesis of breast epithelial cells, a potentially pro-migratory and invasive phenotype in the breast epithelium (84). Collectively, senescent stromal fibroblasts may contribute to epithelial tumorigenesis by concomitantly enhancing cell proliferation and tumor neovascularization, culminating in a considerably exacerbated malignant phenotype.

Despite rising laboratory evidence, how pertinent is this phenomenon during tumor growth *in vivo*? Not surprisingly, results from our laboratory showed that stromal fibroblasts adjacent to human ovarian epithelial cancers are in fact senescent (9). On a related note, Olumi *et al.* demonstrated that cancer-associated fibroblasts (CAFs) isolated from the proximity of prostatic epithelial cancer potently stimulated tumorigenicity in initiated epithelial cells (87). CAFs were also reported to express a broad range of growth factors and cytokines, such as insulin-like growth factor 1 (IGF-1) (which promotes tumor cell survival), hepatocyte growth factor (HGF) (which promotes tumor cell migration and invasion), and stromal-derived factor 1 (SDF-1) (which promotes angiogenesis) (88). Although it was not determined in these studies whether CAFs exhibited signs of senescence or were mixed with senescent fibroblasts, it was proposed that they may share certain features with senescent fibroblasts and hence function similarly in stimulating epithelial tumorigenesis by, for example, secreting macromolecules and matrix remodeling enzymes (87).

6.3. Paracrine signalers as molecular effectors

The tumor-promoting nature of senescent stromal fibroblasts has been progressively recognized as an important factor in the development of epithelial cancer. Accordingly, we question how this effect is mediated by aged fibroblasts. Accumulating studies have suggested that diffusible paracrine signaling molecules secreted by senescent fibroblasts orchestrate the senescence-associated enhancement of tumorigenesis. As early as the beginning of the 1990s, expression of interleukin-1 (IL-1) was found to increase with age in human fibroblasts (89). Later, a comprehensive comparison of the expression profile of normal and senescent fibroblasts revealed a large group of soluble paracrine molecules being noticeably upregulated upon induction of senescence. These paracrine signalers could be categorized into the following groups: matrix remodeling enzymes, including matrix metalloproteinase 10 (MMP10/stromelysin-1) and 3 (MMP3/stromelysin-2), plasminogen activator inhibitors 1 (PAI-1) and 2 (PAI-2), and urokinase plasminogen activator (uPA); inflammatory cytokines, such as interleukin-15 (IL-15), interleukin-1β (IL-1β), and Toll-like receptor 4 (Tlr-4); and chemotactic cytokines, for instance, monocyte chemotactic protein 1 (MCP-1) and Gro-1 (90). Findings from this heralding study suggested that senescent fibroblasts exhibited a radically pro-inflammatory phenotype compared with their normal counterparts. Subsequently, a number of studies focusing on the role of senescent fibroblasts in regulating epithelial tumorigenesis confirmed this hyper-inflammatory trend in aged stromal fibroblasts. Via cDNA microarray

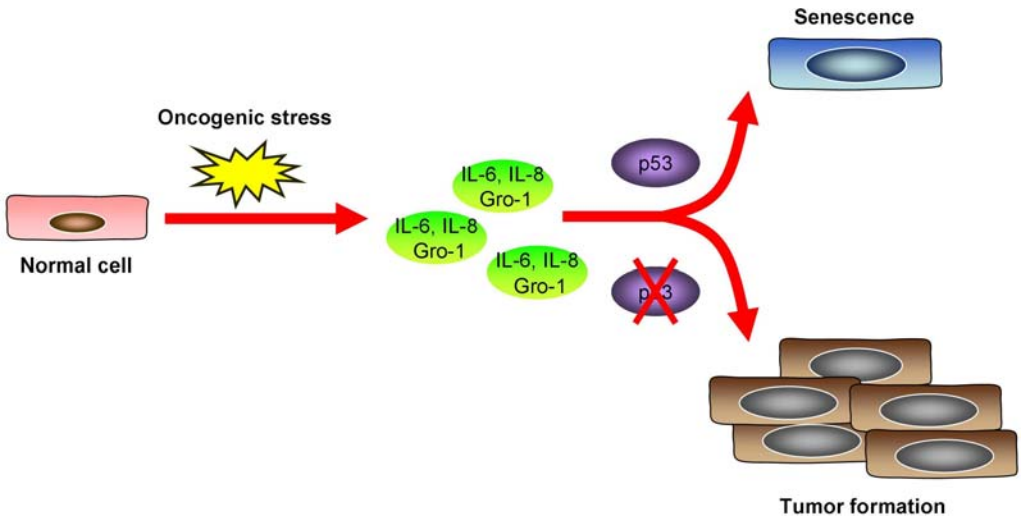

Figure 4. Dualistic functions of cytokines in senescence and tumorigenesis. Certain senescence inducers such as IL-6, IL-8 and Gro-1 exhibit dual functional roles under distinctive physiological settings: they can suppress cellular growth in some cells and increase the proliferative potential in others. The unique genomic composition of the host cells is suggested to underlie this seeming paradox. As depicted in this figure, oncogene-induced cytokines can cause senescence in a p53-dependent manner. Nevertheless, when the senescence-building machinery becomes dysfunctional (in this case, silencing mutations in the *TP53* gene), such cytokine molecules betray the senescence-inducing commission and lead to uncontrolled cell proliferation that eventually results in tumor formation.

analysis, Bavik *et al.* presented a long list of paracrine mediators, including Gro-1, IL-8, and MMP2, be to overexpressed in senescent prostatic stromal fibroblasts compared with their pre-senescent siblings (81). Significantly, a definitive role of the pro-survival and pro-inflammatory molecule amphiregulin (AREG), an epidermal growth factor ligand, in mediating senescence-associated stimulation of tumor growth in prostatic epithelial cells was successfully established (81). In parallel, marked upregulation of MMPs, plasminogen activators, and interleukins has also been observed upon induction of senescence in human fibroblasts (83); one step further, a critical role of MMPs in mediating senescence-triggered acceleration of mammary epithelial tumorigenesis was identified, since broad spectrum anti-MMP antibodies effectively abolished this event (83). Contribution of MMPs to the tumor-enhancing effect of aged fibroblasts is consistent with a previous report, in which senescent fibroblast-secreted MMP3 induced invasiveness of mammary epithelial cells (84). In a related study, vascular endothelial growth factor (VEGF), a potent pro-angiogenic and pro-inflammatory mediator released by aged fibroblasts, was also directly implicated in augmenting epithelial tumor neovascularization and invasiveness ((86) and unpublished data from us).

A systematic study focusing on such an inflammatory network activated along with cellular senescence came very recently, in which Coppe and colleagues utilized protein arrays and disclosed an elevated secretory protein profile upon induction of fibroblast senescence, termed 'senescence-associated secretory phenotype' or SASP (91). In accordance with studies reported previously, myriad cytokines (e.g., IL-6, IL-8, MCP-2), growth factors (e.g., GRO, HGF, IGFBPs) and matrix remodeling molecules (e.g., ICAM, uPAR) constitute the SASP, reflecting an activated pro-inflammatory network upon establishment of senescence in these cells (91). Novel discoveries presented by this work is that SASP induced epithelial-mesenchymal transition (EMT) as well as promoted migratoriness of epithelial (including tumor) cells (91), features commonly seen in more invasive epithelial cancer cells. Coppe's work not only persuasively demonstrated the existence of a senescence-induced secretome composed of pro-inflammatory mediators, but provided concrete support implicating these molecules as key effectors in executing senescent fibroblast-mediated promotion of epithelial tumor growth as well. As suggested by these and other results, aged stromal fibroblasts send the tumor-promoting signals to adjacent epithelium by, at least to a certain extent, creating a hyperactive pro-inflammatory tumor milieu interwoven with secreted paracrine molecules.

7. DUALISTIC FUNCTIONS OF THE PRO-INFLAMMATORY NETWORK

In retrospect, there is a repeatedly-occurring theme central to the studies outlined above: pro-inflammatory cytokines such as Gro-1, IL-6 and IL-8, have essential yet multifaceted functions in modulating cellular senescence and tumor promotion. For decades, strong connections have been built between the inflammatory network and cancer progression. It is generally-agreed that cancer development is fueled by pro-inflammatory mediators and matrix components, which concertedly treat the cancerous lesion as an 'unhealing wound' by stimulating cell growth, inhibiting apoptosis, and creating a favorable tumor extracellular matrix (ECM) (reviewed in (92)). Nevertheless, latest research indicates that how

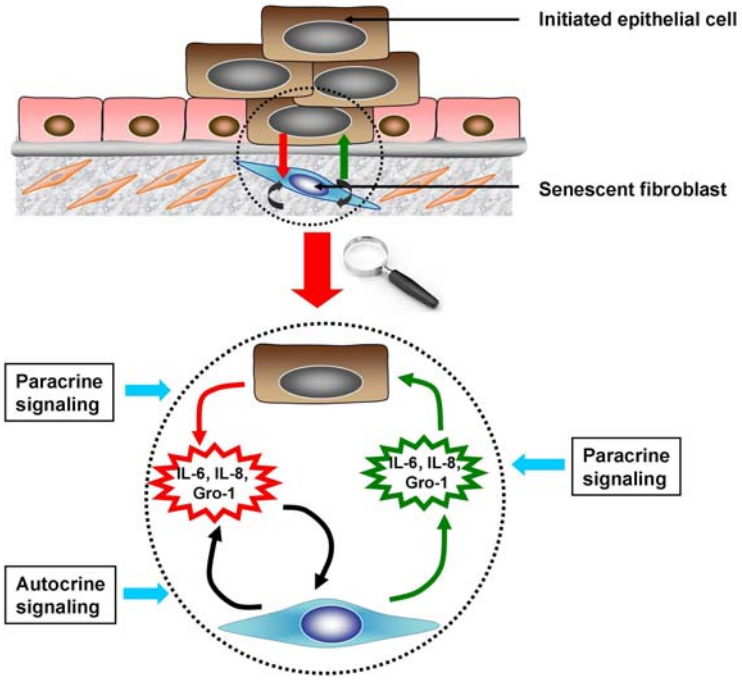

Figure 5. Senescence-associated tumor promotion in the epithelium regulated by the inflammatory network. In response to accumulating "Hayflick factors", both epithelial and stroma cells become initiated (premalignant) and subsequently undergo senescence, provided that the senescence-establishing venues are intact. However, because epithelial cells undergo death and renewal cycles much faster than do stromal fibroblasts, tumorigenic events likely accumulate in the former more readily. In such cases, senescent stromal fibroblasts can promote epithelial tumorigenesis by releasing paracrine-acting molecules, some of which have been identified as inflammatory mediators. Paradoxically, mobilization of the pro-inflammatory network has been involved in the implementation of senescence as well as the senescence-mediated enhancement of epithelial tumor formation, two seemingly conflicting events. Extensive interactions between the epithelium and the stroma, wreathed by paracrine and autocrine signaling pathways, fine-tune the tumor microenvironment and determine the responses of these inflammatory mediators, leading to continuous stimulation of epithelial tumorigenesis.

inflammatory molecules respond to diverse cellular stimuli is strictly context-dependent. Studies on IL-6 (72) and Gro-1 (9, 71, 76) have provided perfectly-suited examples of molecular dualism, as both molecules have been shown to possess cytostatic (senescence-inducing) as well as growth-stimulatory (tumor-boosting) potentials. Particularly, in the case of IL-6, these two seemingly opposing effects were manifested in physically-connected cellular compartments (tumor stroma and the epithelium) (72). Because senescence and tumorigenesis are fundamentally two extremes of cell proliferation, how are they reconciled by a single molecule? Although it remains unsettled precisely what determines the biological responses of such molecules as Gro-1-CXCR2 and IL-6, it is suggested that the genetic composition of the host cells could have a major impact on their plasticity (72). In this respect, cytokines such as these may cause inflammation-associated oxidative DNA damage and/or hyperproliferative cell growth that is processed as cellular senescence in normal cells; in contrast, in transformed cells, particularly the ones that have bypassed the senescence program, activation of inflammatory mediators could foster inflammation-propagated tumorigenesis and malignant transformation (Figure 4).

Integrating cytokine-associated senescence and tumorigenesis, a hypothesized mode of action is hence put forward to describe this complex regulatory network: when the senescence program in epithelial cells is abrogated, further oncogenic activation initiates aberrant proliferation of epithelial cells and upregulates the expression of a unique battery of cytokine molecules, which diffuse into the stroma and cause senescence in the fibroblasts. Concomitantly, senescent fibroblasts gain elevated expression of these molecules, thus constitutively propelling cytokine signaling in fibroblasts to maintain the senescence program in a cell-autonomous manner. Additionally, pro-inflammatory mediators released by senescent fibroblasts can conversely act on neighboring epithelial cells in a paracrine fashion to augment tumorigenic epithelial cell growth. Thus, tumorigenesis in the epithelium and senescence in the stromal fibroblasts are tightly regulated by the inflammatory network, converging on a continuous loop of sustained cell proliferation in the epithelial cancer cells (Figure 5).

8. PERSPECTIVES

In addition to alternations in the genome,

epigenetic changes share equal significance in dictating cancer development. Cancer has been acknowledged as a "community disease", one that is incubated in a nourishing environment created by the stroma. As the stroma ages, cancer incidence sharply increases. Thanks to the continued effort of devoted investigators, a consolidated picture of senescence-mediated enhancement of epithelial tumorigenesis is rapidly emerging. It is now well-believed that senescent fibroblasts promote tumor formation in initiated epithelium by providing a tumor-prone microenvironment to foster epithelial cancer formation. Furthermore, a novel mechanism involving inflammatory molecules underlying this event is starting to be understood. The concept that senescent stromal fibroblasts promote epithelial tumorigenesis offers appropriate answers to questions raised in the beginning of this review regarding the etiology behind the age-associated development of epithelial cancers. This notion is also consistent with the consensus that cancer is, in general, a chronic disease of an inflammatory nature.

Despite the growing body of knowledge generated on this exciting topic, important questions remain. For example, aside from the molecules already identified, what are the other molecular messengers bridging the communication between the tumor epithelium and the stroma? How exactly is the inflammatory network dynamically regulated so that the inflammatory molecules respond differently to anti-proliferative and growth-stimulatory signals within the apposite cellular context? Could the senescence program be eliminated in tumor stromal fibroblasts in order to trigger tumor-stasis and regression? If in fact feasible, will blocking of stromal senescence decrease the incidence of epithelial tumor formation? Hopefully, a thorough understanding of these subjects will not only considerably advance cancer biology but provide new opportunities for the development of anti-cancer approaches.

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