ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 1 & 2, 2005

Forum Review

New Molecular Target for Modulation of Aging Process

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ABSTRACT

Despite many endeavors, no satisfactory strategy has emerged for modulating the aging process, most probably because they were based on faulty rationales. In an extension of the "gate theory of aging" that we proposed recently, we propose here that caveolin, an essential component of caveolae structure, may offer a potential target for modulating the aging process. According to the gate theory, certain biomolecules such as caveolins, amphiphysins, G proteins, and integrins play decisive roles in determining the senescent phenotype and thus provide targets for modulating the aging process. Among these molecules, we chose caveolin, because it can associate with a variety of regulatory and structural molecules via their scaffolding domains and thereby influence a broad spectrum of biological phenomena including both the physiology and morphology of the senescent cells. This is an attempt to review the vast body of evidence available in the literature, both direct and indirect, supporting the accord of this pivotal role to the caveolin in the background of the gate theory for the aging process. *Antioxid. Redox Signal.* 8, 620–627.

INTRODUCTION

A HORDE OF HYPOTHESES and theories have been proposed to explain the aging phenomenon, but none of them have totally answered all the fundamental questions on aging. Whether the age-related functional changes are responsible for the structural alterations in aging or vice versa, awaits the elucidation of the molecular mode of crosstalk between structural and functional motifs. Based on the prevailing concept that the age-related molecular and cellular changes are a one-way phenomenon, "replace principle" has been proposed as a solution to aging process at a variety of levels such as gene, cell, organ, and organism (7). Nonetheless, there have been few studies that have satisfactorily addressed the question whether the so-called "irreversible and inevitable" senescent process can be modulated at the molecular level. Some relevant issues in this regard include, among others: deterministic nature of aging, age-related crosstalk between structure and function motif's, and molecular basis of age-related diseases. These issues are reviewed here in the background of the gate theory of aging (53) with specific reference to the role of caveolin as a model prime modulator.

NOTES ON MOLECULAR TARGETS OF AGING MODULATION

Up to now, most of the endeavors to modulate the aging process, except calorie restriction, have largely failed. The apparent failure of "antioxidants" as modulators of the aging process, and of the clinical trials based on the oxidative stress hypothesis of aging, has been an especial disappointment. It is now clear that oxidative radicals are not always damaging but may actually be required sometimes for maintenance of life. In consequence, neutralizing the oxidant stress, by use of antioxidants, will not necessarily constitute an approach to favorably modulating the aging process. What are some of the other molecules that might serve as potential target molecules for aging modulation?

A prime requirement for a potential modulator of the aging process is that it should be both efficacious and safe. To be "efficacious", the aging modulating molecule should have a double-edged effect both on structure and function of critical cells, since aging causes both functional deterioration and morphological alteration. To be "safe", it should have no side effects in long-term use for several decades.

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The aging modulation approach with antioxidants failed because it relied on the broad "oxidative stress hypothesis of aging" rather than on specific molecular targets, and was solely dependent on general increase of overall antioxidative power. We feel that an approach at modulation of aging, involving targeting a rationally chosen specific molecule, rather than on a class of compounds chosen on the bias of an overly general hypothesis, is more like to succeed.

ON THE DETERMINISTIC NATURE OF AGING

The biological characteristics of senescent cells can be represented as the result of an irreversible and inevitable process, which in turn impose deterministic characteristics on aging which apply not only to the functional aspects but also to the structural alterations.

The obvious question is; can the functional efficiency of the senescent cells be improved, and aging process thereby modified? Senescence-associated functional changes in cells clearly involve altered (deteriorating, in some cases) metabolic responses, stress responses, and mitogenic responses. These responses need to be readily divided into normal and age-related changes. In the case of metabolic responses, there must be no significant age-dependent changes, otherwise maintenance of life would not be possible. As illustrated in glucose homeostasis, the blood glucose level should be kept strictly stable, if not, the devastating pathologic diabetic condition would be induced. Age-dependent changes in other metabolic systems are minimized but the basal responses maintained. Age-dependent alterations in responses to various stresses have been reported between young and old cells and organisms, but it is clear that the organism responds to a variety of stresses despite aging only with some quantitative differences from the young animals. But the basal mechanism can operate without major alterations to save the organism. However, in the case of mitogenic responses, it is evident that there is a drastic difference in response to growth-stimulating agonists between young and old, almost in an all-or-none basis. This is an intriguing contrast from metabolic or stress response differences between young and old.

In order to study the age-dependent differences in mitogenic response, special interest needs to be paid to age-dependent alteration in cellular responsiveness. Cellular responsiveness depends mainly on numerous levels of signal transduction cascades such as ligand supply, receptor status, ligand-receptor interaction, downstream signaling events, intracellular signal networks, signal translocation, transcriptional status, chromatin remodeling, and posttranscriptional apparatus, among others. These changes can be mainly traced to differences in receptor systems. When the effect of growth stimulating agents are analyzed, three different receptor routes should be taken into consideration; receptor tyrosine kinase (RTK) system, G protein coupled receptor (GPCR) system, and nuclear receptor system. Age-dependent differences in those receptor systems may provide clues to the possibility for aging modulation via defining the differences and restoring the defective steps (31–33, 52).

What are the prospects for restoring or delivering the mitogenic signaling in the senescent cells? Whether there are changes in levels of receptors and ligands between young and old cells, is controversial, but there is no controversy regarding altered responses of senescent cells to the mitogenic agonists. These facts led to conjectures on the presence of an aging- specific, unique defective signaling system, although it is still controversial. In order to illustrate the senescence-specific signaling problem, the defective three-dimensional and temporal system of the intracellular trafficking system such as receptor-mediated endocytosis has been invoked instead of the planar interaction of ligands and receptors, or the complicated downstream flux of the signals (7, 9, 31, 32). In this system, endosome formation is required right after the ligand and receptor interaction, after which the translocation of the endosome to the specific target site including the nucleus should be verified for the functional efficiency of the ligands. This entire pathway of intracellular trafficking should be ensured for the full activity in addition to the intact ligand and receptor interaction and the subsequent downstream signal apparatus. Therefore, any problem in any part of this system may affect the efficiency of signaling in the senescent cells. Actually, defects of the receptor-mediated endocytosis system in the senescent cells could be identified not only in the caveolae-dependent endocytosis but also in the clathrin-mediated endocytosis as well (31, 33).

In the case of epidermal growth factor, senescent cells show hyporesponsiveness. Among many levels of the signal system, downregulation of receptor-mediated endocytosis, especially of the caveolae-dependent type, is mainly responsible for the hyporesponsiveness without any significant changes in quantity of signals or receptors, significantly related to age-dependent upregulation of caveolin (31, 33). One might assume therefore that it may be possible to induce the recovery of the functional mitogenic response of senescent cells simply by restoring the receptor-mediated endocytosis through modulation of caveolin status, as confirmed by induction of BrdU incorporation in the senescent cells by EGF in caveolin-siRNA treated cells (32). By restoring endocytosis, not only the growth factor response but also the nutrient uptake system could be restored in the senescent cells, as shown in clathrin-mediated endocytosis by modulating the amphiphysin status (9, 31). Therefore, the question of feasibility of functional restoration of the senescent cells by adjusting the specific defined target is answered affirmatively. In addition to the RTK system, GPCR systems are also altered during the aging process in a variety of organs, (53) in which cAMP status plays the pivotal role, suggesting another mode of mitogenic modulation in the senescent cells (2).

In addition to functional modulation, the tight molecular linkage of regulatory molecules such as caveolins with molecules governing cytoskeletal structure, offers another means of correcting age-dependent morphological alterations (8).

A related basic question concerns the deterministic nature of morphological alterations accompanying aging, and whether these changes can reverse senescent cells? In this regard, the crosstalk between the structure motif and the function motif would become important. This will be discussed in detail in a later section. Based on these considerations, it is reasonable to assume that aging is not a totally deterministic

process, but is subject to modulation of a variety of related molecules.

ROLE OF CAVEOLIN IN COMPLEX SIGNAL SYSTEMS

Receptor-mediated endocytosis, either clathrin-dependent or clathrin-independent (typically caveolae-mediated) is downregulated in the senescent state (31, 33). The role of caveolae-mediated endocytosis in determining the functional decay and structural alterations of the senescent phenotype will be further discussed here.

Caveolae have been implicated in many important dynamic and regulatory events at the plasma membrane, and are abundant in terminally differentiated cell types, such as adipocytes, endothelial cells, and muscle cells (1, 4, 13, 27). Since signaling molecules are enriched in caveolae structure (39), caveolae may function as subcellular compartments for storage of signaling molecules, in the regulation of their activities and mediating the cross-talk between distinct signaling cascades. The principal components of caveolae are caveolins, 21–24 kDa integral membrane proteins. The mammalian caveolin gene family consists of caveolin-1, -2, and -3. Caveolin-1 and -2 are coexpressed, forming a hetero-oligomeric complex in many cell types, particularly high levels in adipocytes, in contrast to caveolin-3 expression of muscle-specific manner (34, 40, 41, 44, 46).

The interaction between caveolin-1 and signaling molecules is mediated via a membrane-proximal region of caveolin, termed the "caveolin-scaffolding domain" (residues 82–101) (11, 42). It is through the "caveolin-scaffolding domain" that caveolin-1 interacts with G-protein alpha-subunits, H-Ras, Src-family tyrosine kinases, PKC isoforms, EGF-R, Neu, and eNOS (5, 10, 13, 25, 44). The targeted downregulation of caveolin-1 induces cellular transformation with hyperactivated Erk kinase cascade (13). Moreover, co-expression of EGFR, Raf, MEK-1, or Erk-2 with caveolin-1 inhibits the signaling translocation from the cytoplasm to the nucleus *in vivo* (15). Senescent cells have an increased level of caveolin, which co-localizes with EGFR; also overexpression of caveolin-1 in young cells suppresses the activation of Erk-1/2 upon EGF stimulation (33). Premature senescence was observed in primary cultures of fibroblasts from caveolin-expressing transgenic mice (48). These findings clearly demonstrate the central role of caveolin-1 in suppression of mitogenic signaling, resulting in tumor suppression and promotion of cellular senescence.

CAVEOLIN AS A DETERMINANT OF SENESCENT PHENOTYPE

Caveolin as the mitogenic modulator of the senescent cells

Caveolin levels seem to influence mitogenic signaling efficiency and cell cycling. Downregulation of caveolin-1 by antisense-oligonucleotide or siRNA treatment leads to restoration of the basal p-Erk level and Erk activation upon EGF stimulation, followed by p-Elk activation in the nucleus. A simple reduction in the caveolin level of senescent cell can restore the Erk signaling system upon EGF stimulation not only in terms of its phosphorylation but also its translocation into and activation of transcriptional factors in the nuclei (9).

Recently it was reported that caveolin-1 mediated cell cycle arrest occurs through a p53/p21^{Waf1}-dependent pathway (18). Downregulation of caveolin-1 in senescent cells also reduces the level of the cell cycle inhibitors, p53 and p21 (9). The cell cycle reentry of senescent cells by BrdU incorporation in response to EGF after caveolin reduction suggested that the adjustment of the caveolin level in senescent cells can profoundly influence the aging phenotype of mitogenic insensitivity. Moreover, the role of caveolin as a tumor suppressor gene in cancer cells has been well documented (37). Thus, it appears that caveolin plays an important role in regulation of mitogenic system in both aging and cancer. Therefore, modulation of caveolin status may be an approach for restoring the mitogenic activity of the senescent cell, although the traditional deterministic view considers loss of mitogenic activity to be an irreversible process (Fig. 1).

Caveolin as the morphological determinant of the senescent cells

Caveolin-1 is associated with focal adhesion complex through integrin in the membrane (6, 51), and activation of focal adhesion kinase (FAK) is significantly decreased by downregulation of caveolin-1 (47). These findings indicate that caveolin plays an important role in focal adhesion, and participates in the adhesion complex, which might result in regulation of cellular morphologic and dynamic system. Moreover, formation of focal adhesion and actin stress fiber increase and they become anchored in the membrane via interaction with caveolin-1 in senescent cells (8). Recently, it was suggested that caveolin-1 plays an important role in cell motility function with its anterior-posterior polarization during cell migration. Caveolin-1 is accumulated at the leading edge of cultured fibroblasts (26, 38). Phosphorylation of Tyr14 of caveolin-1 is required for polarization of the protein during transmigration (29).

Rho GTPases participate in the regulation of polarity, microtubule dynamics, and cell shape. They regulate cell shape by altering the actin cytoskeleton organization: Cdc42 induces filopodia, Rac induces lamellipodia, and Rho induces focal adhesion and associates with stress fibers (22). In endothelial cells, caveolin-1 is directly bound with RhoA but not with Cdc42 (20), and integrins regulate targeting of Rac and Rho GTPases to the plasma membrane via lipid raft or caveolae and their coupling to downstream effector molecules (28). In senescent HDFs, the activities of Rac1 and Cdc42 are significantly increased, and overexpression of active Rac1 and Cdc42 in young HDFs results in the senescence-like morphological changes. The active forms of Rac1 and Cdc42 are localized in caveolae, directly interacting with caveolin-1 (8). These findings suggest the involvement of caveolin in determining cell shape and migration through polarity arrangement and regulation of Rho GTPases activities.

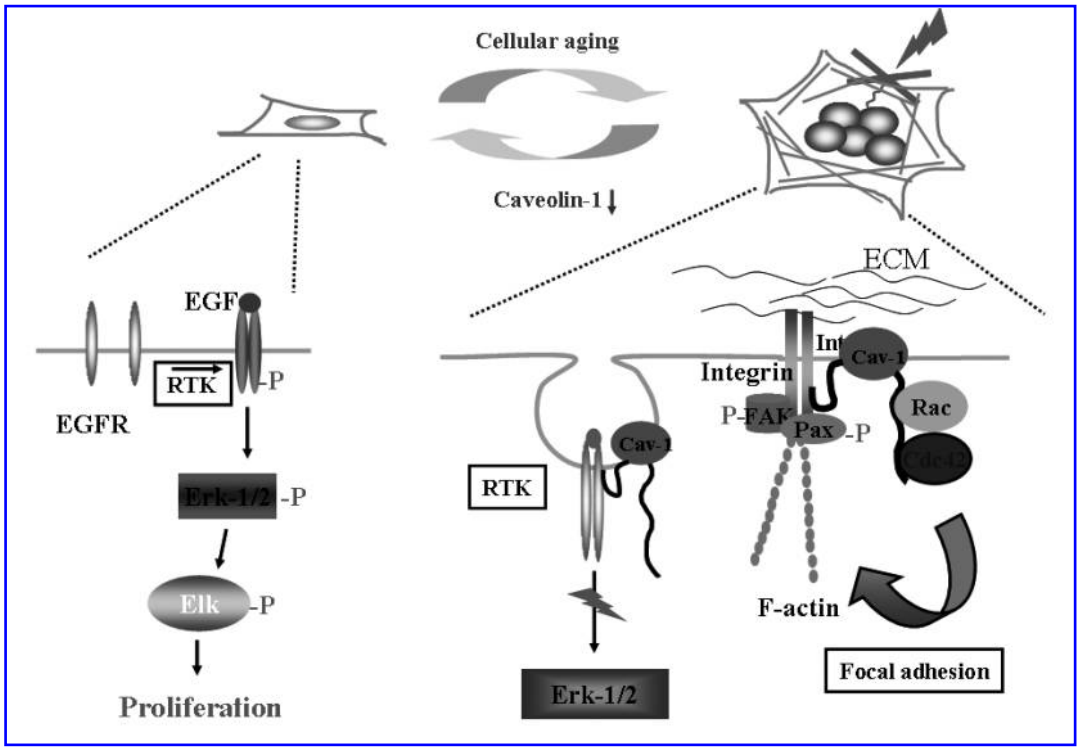

FIG. 1. Summary of caveolin function for mitogenic signaling. In young cells, EGF signaling can operate for the proliferation, whereas in old cells, the signaling is inhibited by caveolin. Caveolin can also interact with focal adhesion complex.

Integrins at the tip of filopodia bind to the extracellular matrix (ECM) and initiate the formation of focal adhesions. Actin-rich lamellipodia are then generated as the cell spreads on the ECM (19). The reorganization of actin filaments into larger stress fibers in turn causes more clustering of integrins, thus enhancing the matrix binding and organization by integrins in a positive feedback system. In consequence, ECM proteins, integrins and cytoskeletal proteins aggregate in each side of the membrane. The inhibition of caveolin expression results in suppression of focal adhesion and actin stress fiber formation, but the biochemical nature of this interaction is not yet clear (8, 49). Integrins activate protein tyrosine kinases, such as focal adhesion kinase (FAK), followed by phosphorylation of other cytosketetal proteins. Also, β1 and αv integrins activate the tyrosine kinase Fyn and the adapter protein Shc. Caveolin-1 appears to function in this pathway as a membrane adaptor, coupling the integrin α-subunit to Fyn (50). The reduction of caveolin-1 expression may lead to disruption of focal adhesion and actin stress fiber, probably via dephosphorylation of FAK in the senescent cells (8). Therefore, the age-related morphological alteration could be restored to some extent by simple adjustment of the cellular status of the specific molecule, caveolin, in this case (Fig. 2).

Caveolin in the internalization process

Caveolae-mediated endocytosis has been implicated in internalization of certain membrane components, extracellular ligands, bacterial toxins, and several nonenveloped viruses (35, 36). Simian virus 40 (SV40) entry into host cells occurs by endocytosis through caveolae (2, 45). After binding to caveolae, virus particles induce the transient breakdown of actin stress fibers. Actin is then recruited to virus-loaded caveolae as actin patches (actin "tail" formation). Latrunculin A, an actin monomer sequestering drug, and jasplakinolide, an actin polymer-stabilizing drug, reduce virus internalization by 60%–65% in later steps of the entry process (16, 35). These events are dependent on the presence of cholesterol and activation of tyrosine kinases, which might phosphorylate proteins in caveolae. Since SV40 particles induce tyrosine phosphorylation of proteins at virus-loaded caveolae, the inhibition of tyrosine kinases reduces the caveolae-mediated internalization of the virus. These findings indicate that phosphorylation of caveolae proteins is required for formation of caveolae-derived endocytic vesicles and for viral infection into cell (35). Moreover, downregulation of caveolin 1 gene in senescent cells influences the intracellular transport system profoundly in a variety of transport-associated systems (manuscript in preparation). These data seem to provide a novel mechanism for the age-related increase of the infectious diseases as well as a new therapeutic strategy for the control of the age-related proneness to infection.

REGULATION OF CAVEOLIN GENE EXPRESSION

The relationship between cholesterol and caveolae function has been the subject of a number of studies. Caveolin-1

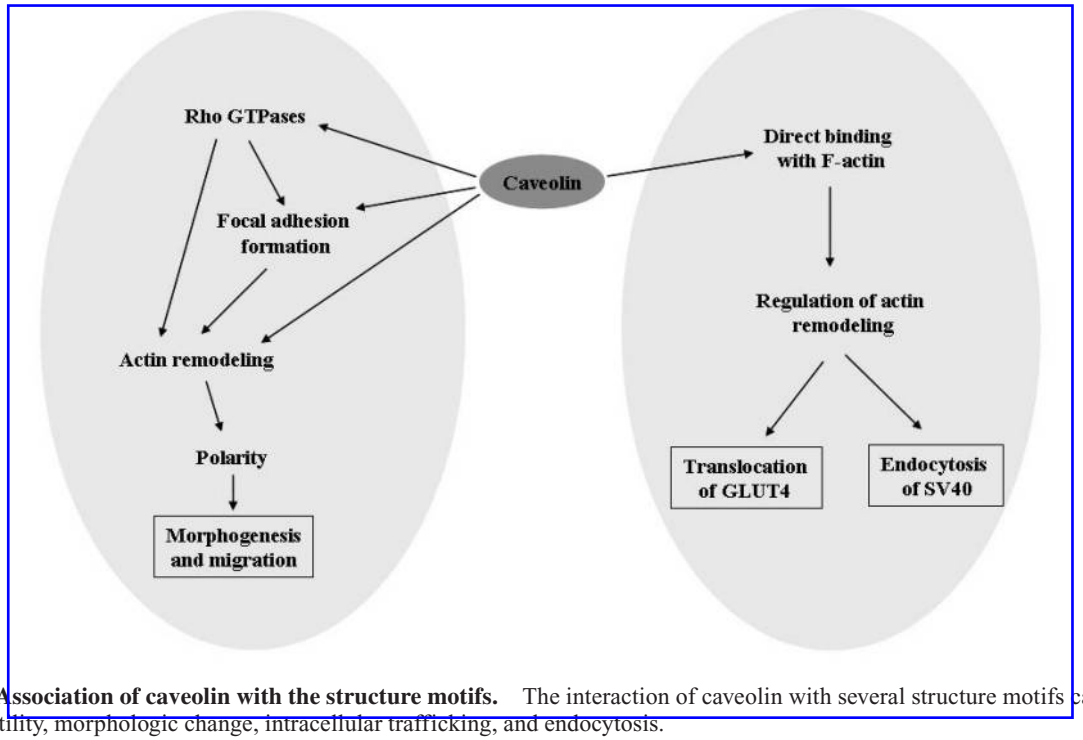

FIG. 2. Association of caveolin with the structure motifs. The interaction of caveolin with several structure motifs can influence motility, morphologic change, intracellular trafficking, and endocytosis.

appears to constitutively transport cholesterol consistently present in the cell to the cell surface (21, 43). Free cholesterol is selectively transferred from LDL to cells, which appears first in clathrin-coated pits and is eventually transferred to caveolae via the *trans*-Golgi network. Moreover, the amount of caveolae is regulated by the cellular cholesterol level (17). Cholesterol depleting drugs can reduce the caveolae level (21, 38) and LDL can induce an increase of caveolae in cells (17). Cholesterol appears to modulate caveolin-1 expression through a steroid regulatory binding element (SRE) present in the caveolin-1 promoter and SRE binding protein 1 (SREBP-1) (3). Interestingly, the senescent cells contain higher levels of cholesterol than young cells (33). Thus by modulation of cellular cholesterol levels, either by nutritional intervention or by using cholesterol depleting drugs, one may alter caveolin levels in the cells, which might influence the senescent phenotype.

Caveolin-1 is also known to be a tumor suppressor, and is mapped to human chromosome 7q31.1 (14). A number of studies have revealed that caveolin gene expression in various tumors is regulated by methylation of its promoter region (23). CpG sites at the 5′ promoter of caveolin-1 are methylated to a greater extent in tumors than in adjacent normal prostate cells (12). Moreover, 5-aza-2′deoxycytidine, a demethylating drug, can induce caveolin-1 expression in young cells, and this provides a novel approach to modulate the caveolin gene expression, suggesting that methylation of the promoter region of caveolin during the aging process might activate caveolae formation (unpublished observations). Therefore, caveolin, operating at the transcriptional level, seems to determine the cellular fate in cancer and aging.

"RESTORE PRINCIPLE" FOR MODULATING THE AGING PROCESS

The functional deterioration of senescent cells is closely related with dysregulation or imbalance of the signal transduction network in an agonist-specific or a pathway-specific manner. Senescence-associated functional decay leads to hyporesponsiveness to growth factors and susceptibility to disease. In the senescent-dependent mitogenic response, the RTK system is more damaged than the GPCR system (52). Major age-related defects in the RTK system of receptor-mediated endocytosis are due, at least partly, to age-related increases in caveolin for caveolae-dependent receptor-mediated endocytosis, and age-related decreases in amphiphysin-1 for clathrin-mediated endocytosis (31, 33). Interestingly, functional recovery of receptor-mediated endocytosis could be readily achieved in senescent cell by a simple adjustment of the levels of caveolin or amphiphysin-1 (31–33).

This plausibility of functional adjustment of receptor-mediated endocytosis, either caveolae-dependent or clathrin-dependent, has opened a new horizon in aging research with respect to the emergence of "restore principle" for the senescent phenotype. The adjustment of receptor-mediated endocytosis in senescent cells resulted in not only the improvement of their functional sensitivity to external stimuli, but also in the induction of resumption of their morphological appearance as well (8).

The possibility of converting the senescent phenotype into the functionally active and structurally normalized state simply by restoring the membrane signaling apparatus points to the significance of the membrane adjusting system in the

aging phenomenon. Based on these results, we proposed the gate theory of aging, which emphasizes the fundamental role of the general membrane on/off switch system for a variety of signals (53). Several different molecules are candidates for the possible role of aging modulators; to illustrate some, caveolins for the caveolae system, amphiphysin-1 for the clathrin system, G proteins for the GPCR systems, and integrins for extracellular communications.

This possibility can lead to a new challenging program of "Restore principle" for modulating the aging process in place of the traditional "Replace principle." The conventional replace principle toward aging intervention is inadequate in a variety of aspects. For example, replacement of the gerontogenes, the causative genes for aging, can not be carried out, because of lack of information on the identity of the genes; replacement of aged cells by stem cells would not be practical because of uncertainties of problems due to differentiation adequacy and malignant transformation; replacement of tissues or organs will not be available in many cases due to the paucity of the donor materials and the ethical problems. Also, hormone replenishment or cosmetic therapeutic approaches are not suitable for prolonged use due to potential side effects and efficacy loss. But the novel concept of aging as an adaptive and responsive process will allow for the restore principle as a potential approach for aging modulation. As an example, simple adjustment of caveolin level can profoundly influence the senescent phenotype not only of mitogenic response but also of morphology and dynamics. Thus modulation of aging process may be possible by adjustment of a specific defined molecule without resorting to complicated approach of substitution manipulation. Moreover, activity of caveolin as a tumor suppressor and its participation in regulation of stem cell differentiation (30) ensure its safety in long-term use.

Based on these considerations, the concept of aging as a deterministic process can be discarded and substituted with an adaptive and responsive view. The emerging new concept will contribute to the development of strategies to improve the functions and restore the morphological alterations by adjusting the level of the defined target molecules, such as caveolins. This defined nature of the target molecule might ensure the feasibility of aging modulation in contrast to the ambiguity of the conventional approach to modulate aging processes such as oxidative stress modification or calorie restriction. We feel that this novel approach of defined target approach, to modulating the aging process, which impacts on both the structure and function of the senescent cells, without side effects in long-term use, will satisfy the requirements of efficacy and safety.

CONCLUSION

The gate theory of aging was proposed previously to emphasize the role of the prime modulator at the membrane level in determining the senescent phenotype. This hypothesis suggests the possibility of restoring the senescent phenotype simply by adjusting the status of the defined target molecules. In this context, caveolin is a strong candidate modulator of aging that can influence not only the signaling functional responses but also the structural alterations through its association with signal cascade molecules, the cytoskeletal adhesion complex, and other related molecules. Further studies are clearly needed to verify this hypothesis that restoration of age-related changes in the senescent cells can be brought about by manipulating these modulators in responsive manner and put the deterministic theory of aging to rest.

ACKNOWLEDGMENTS

This study was supported by the grants from the Korea Science and Engineering Foundation (RII 2002–097–05001–0 and RII 2002–097–00001–0) and the Korea Research Foundation of Health Science.

ABBREVIATIONS

ECM, extracellular matrix; EGF-R, epidermal growth factor receptor; FAK, focal adhesion kinase; GPCR, G protein coupled receptor; LDL, low density lipoprotein; PKC, protein kinase C; RTK, receptor tyrosine kinase; siRNA, small interfering RNA; SRE, steroid responsive element.

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Received after revision October 11, 2005; accepted October 11, 2005.

This article has been cited by:

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