Guest Editorial

Is (Your Cellular Response to) Stress Killing You?

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Free radicals provide a generally accepted explanation for age-related decline in tissue function. However, the free radical hypothesis does not provide a mechanistic course of action to explain exactly how damage to macromolecules translates into the recognizable pathophysiology of aged organisms. Recent advances in the fields of DNA damage and cellular senescence point towards a substantial role for the DNA damage response, rather than DNA mutations per se, in the genesis of cellular and/or tissue damage. Furthermore, several studies suggest that protein damage can be at least as important as DNA damage in bringing about the aging phenotype. Here we propose that a “protein damage response,” namely the ER/UPR (endoplasmic reticulum/unfolded protein) stress response is likely to play an important role in the aging process.

In 1956, Harman proposed the free radical hypothesis of aging (1). The hypothesis has ebbed and waned, but it has survived fairly intact through almost half a century. Much understanding of the aging process has come from experiments designed to test Harman’s tenets, but a central question still remains: How exactly does random damage to macromolecules translate into the recognizable organismic phenotype we call “aging”? This issue was first raised in 1959 (2), but it still remains unsolved (3). Most often, free radicals or other noxious agents will damage macromolecules in a very stochastic, idiosyncratic fashion. It is difficult to understand how such a random process can lead to the significant and reproducible loss in tissue function observed during aging. Therefore, the premise of this writing is that it is not damage per se that leads to the phenotype of aging, but rather, it is the cellular response to that damage that is at the center of the action.

Thus, we propose that aging ensues only if a cellular response to that damage is activated. Here we propose that the ER or UPR stress response (Endoplasmic Reticulum or Unfolded Protein Response, respectively) (6) could play this role, and thus be central to the development of the aging phenotype. As in the case of the DNA damage response, activation of the ER/UPR stress response by damaged proteins can also lead to both loss of cellular function and, if overwhelmed, apoptosis (Figure 1).

DNA DAMAGE AND AGING

DNA is believed to suffer different types of damage, such as breaks, abasic sites, and base lesions. It has been estimated that at least 2,000–10,000 purine bases are turned over per day per cell (7). This creates an enormous burden, and consequently, a complex and interwoven system of DNA repair enzymatic activities exists within each cell, to reverse such damage. It has been estimated that hundreds of genes in the human genome are directly devoted to DNA repair mechanisms, with many more ancillary factors suspected (8). The genome is kept intact by two major mechanisms: caretakers and gatekeepers (9). The functions of caretakers are to ensure that the DNA is not damaged and, if it is, to get it repaired. Caretakers include enzymes that scavenge reactive oxygen species (ROS) and DNA repair mechanisms. However, if the caretakers fail and DNA gets irreparably damaged, then gatekeepers come into play, and they “decide” the fate of the affected cell as a whole. Classical gatekeepers include the tumor suppressor proteins p53 and pRB. Gatekeepers are not activated by point mutations; therefore, point mutations do not engage a cellular response. However, when gatekeepers sense that the damage is irreparable, they activate a cellular response.
(either apoptosis or senescence), which effectively removes the affected cell from the pool of proliferating cells (4).

There are considerable data indicating that, if a cellular response to damage is not activated, then cells can withstand a significant amount of damage to DNA without the organism showing signs of premature aging (10). Indeed, in most of the mouse models where caretaker functions have been inactivated, an increased level of DNA mutations has been observed as expected, but the mice fail to display an accelerated aging phenotype. For example, Xpc−/− mice accumulate up to 30-fold higher levels of DNA mutations than do their wild-type counterparts, yet there is no effect on their life span (11). A similar, though less dramatic result has been observed in the case of scavenging proteins. For example, Van Remmen and colleagues (12) reported that the SOD2+/− mouse suffers a 3-fold to 4-fold increase in DNA mutations, with no detectable effect in life span. Because these animals indeed display a significant increase in DNA damage, it cannot be argued that the effect of the gene knockout is being counterbalanced by a partly redundant mechanism: There is a considerable accumulation of unrepaired mutations that has no deleterious effect on life span. There are exceptions, including the Ku86−/− mouse (13), the XPD−/− mouse (14), and more recently, a model in which catalase was directed to the mitochondria (15). In these cases, the expected changes in life span were observed (decrease in the two former, increase in the latter). The mechanisms that explain the differences between the different models still need to be unraveled. In contrast, mouse models in which the activity of gatekeepers (including telomerase, Wrn, Blm, ATM, or p53) has been manipulated do generally display an accelerated aging phenotype (16–20). In the cases of telomerase, Wrn, Blm, and others, the gene knockout results in generalized genomic instability, including not only double-stranded breaks, but also telomere shortening and/or stalled replication or transcription complexes, both of which appear to be interpreted by the cell as an unrepaired double-stranded break. As previously mentioned, this type of damage leads to activation of a response that culminates in cellular senescence or apoptosis (4). From these data, we must conclude either that genomic instability (but not mutations) plays a role in aging, or that longevity is related to the cellular response of the cell to such DNA damage.

**CELLULAR RESPONSE TO PROTEIN DAMAGE**

The integrity of the cellular proteome can also be viewed as being dependent on caretakers and gatekeepers. Caretakers would perform all the activities that prevent protein damage (ROS scavengers and chaperones, for example) or take care of the damaged proteins (proteolysis and protein repair mechanisms). In contrast, gatekeepers would function to assess this damage and decide whether it can be repaired. In analogy to genome gatekeepers, the ER/UPR response can be viewed as a gatekeeper function for proteins, because its activation can, under certain circumstances, lead to programmed cell death (6).

The ER/UPR stress response is a cell-protective mechanism that can be triggered by accumulation of damaged or misfolded proteins in any cell compartment, including the nucleus (21). In young organisms at least, accumulation of damaged proteins activates a series of sensors, including PERK, IRE1, and ATF6 (reviewed in 6). Activation of these pathways converges in the activation of ER chaperones, including several glucose-regulated proteins (GRP), primarily GRP78 (22). The overall effects are: (i) to increase the levels of available chaperones, (ii) to reduce the rate of protein synthesis, and (iii) to activate several protein degradation pathways. Altogether, these effects result in an alleviation of the stress produced by the misfolded proteins (23). However, the need for the cell to devote resources to protect itself comes at the price of a partial disregard for its more differentiated functions. This switch is mediated by the overall decrease in the rate of new protein synthesis, which occurs concomitantly with the upregulation of genes encoding chaperones. This temporary change in priorities

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**Figure 1. Stress responses to irreversible damage.** Ordinarily, most damage to nuclear DNA ([left]) is efficiently repaired by a complex set of DNA repair mechanisms. However, when the damage is of a nature or extent that makes its repair impossible or overwhelming (irreversible damage, such as double-strand breaks, for example), then the cell activates the DNA stress response, which allows it further time and capabilities for repair. If repair is still not possible, then this same pathway unfolds a cellular response that takes the cell out of the dangerous path that could lead to cancer, by forbidding its further cell division. This can be accomplished by entering either cell senescence or apoptosis (or any other kind of programmed cell death). We propose that damage to proteins ([right]) can follow a similar course: If the damage cannot be repaired or the damaged proteins cannot be eliminated via degradation (irreversible damage), then the ensuing accumulation of damaged proteins activates the endoplasmic reticulum (ER) stress response, the function of which is to allow the cell further time and capabilities for repair (by inducing chaperones, for example). If the damage becomes overwhelming, then the cell enters into apoptosis, or possibly cell senescence, although this last point has not been proven (dotted line).
results in saving the cell from deleterious environmental conditions, and therefore saving the tissue from permanent loss of function (21). However, this shift also means that the ER/UPR stress response is even better equipped than is the DNA damage response to produce tissue-wide dysfunction, because its activation results in loss of differentiated tissue functions even if damage is not overwhelming enough to activate the apoptosis or senescence pathways.

Because the ER stress response is a transient protective mechanism that leads to the re-establishment of proper homeostasis, under most circumstances, activation of the ER stress response should not lead to the progressive deterioration observed during aging. However, it is possible that during aging, decreased efficiency of the response, or other factors including persistent insults or a decreased capacity to activate proteolytic pathways (24,25) might lead to a sustained ER stress response. This sustained response could be directly responsible for the loss of tissue function, through the persistent diversion of cellular efforts away from differentiated activities, as described above. Some additional facts about aging and the ER stress response become relevant at this point: (i) GRP levels increase with age, and the increase is rapidly reversed by diet restriction (26); (ii) Induction of heat shock protein (hsp) 70 family members by a variety of stressors is diminished in old organisms [reviewed in (27)]; (iii) Overexpression of hsp70 family members extends life span in Caenorhabditis elegans (28,29); (iv) One of the hallmarks of long-lived organisms is their increased resistance to oxidative as well as many other types of stress (30); and (v) Having said that, cells under severe stress do die! We propose that it is this cell death, in conjunction with the loss of functionality of the surviving cells, that is at the root of the age-related decline in tissue mass and function.

The main issue here is that stochastic DNA mutations or random damage to proteins or other macromolecules will not lead to a coordinated cellular response, and therefore, will not lead to the phenotype of accelerated aging. In contrast, activation of a programmed damage response will lead to a decrease in cell and tissue function that does not depend on which molecule was originally affected, and therefore is no longer idiosyncratic. Instead, all the cells subjected to the insult would have a concordant, preestablished response, which includes a decrease in differentiated functions (Figure 2).

In summary, activation of the ER stress response will lead to loss of tissue functionality both by diversion of energy away from differentiated functions and, eventually, by a loss in cell number. It logically follows that a loss in cell numbers driven by a programmed cellular response should proceed via programmed cell death, and not by necrosis. Interestingly, it has been proposed (11) that the reason Xpc−/− mice fail to show a premature aging phenotype is because the mice do not have an increased apoptotic response, in contrast to the XpdTTC model, which is indeed short-lived (14). Finally, it should be pointed out that, in most tissues derived from young healthy organisms, a decrease in the number of differentiated cells can be compensated either by self-duplication (31) or by activation of the stem cell pools, the asymmetric division of which allows for the replenishment of the missing cells, while still retaining the stem cell pool. There are several indications in the literature that suggest that activation of stem cells is suboptimal in aged individuals (32,33). Although this is still a budding area in aging research, data available suggest that aged organisms suffer from a variety of deficits in their stem cells, both quantitative (decreasing numbers of stem cells) and qualitative [decreased proliferation capacity, mobilization, and/or differentiation properties; reviewed in (34)]. It is possible that stem cells suffer the same type of damage as do other cells, and activation of a stress response in these cells also leads to their declining functionality (or in some niches, increased death), just as in other cellular compartments. Thus, stem cells represent a niche where stress-induced loss of either cell function or cell numbers could be of particular relevance to the aging process. Because stem cells divide asymmetrically, it would be interesting to establish whether they (and germline cells) have more sophisticated ways to deal with damage, as has been shown in asymmetrically dividing yeast cells (35). It should be pointed out that, although this proposal invokes a programmed response to cellular damage as the leading cause of aging, it does not contradict evolutionary theory, because the response did not evolve with the purpose of producing aging, but rather, aging is the side effect of a protective mechanism active in younger organisms. In that sense, the ER/UPR stress response could represent a case of antagonistic pleiotropy.

Conclusion

If aging is controlled not by how much damage an organism sustains, but rather, by its ability to respond to such damage, then it is reasonable to conclude that aging and longevity should be controlled genetically, at least in part. This prediction has been proven to be true in the last decade or so. A few more direct, testable hypotheses can be drawn from this proposal. For example, life span should be affected by genetic manipulation of GRP or heat shock proteins (28,29), as well as by manipulations that affect the equilibrium that dictates whether a challenged cell goes into apoptosis or senescence. Precise tuning of either the threshold or the overall activity of cellular responses such as the ER response should also correlate with life span in organisms of different species, or among members of a cohort. Another testable corollary is that apoptosis and/or senescence pathways should be activated in those mouse models which display accelerated senescence, but not in models where damage is increased, but longevity is not affected. Activation of these pathways has been shown in a subset of the models, but more research will be necessary before a general conclusion can be drawn.

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Figure 2. Outcomes of damage to macromolecules. Macromolecules can be damaged by a variety of different agents and mechanisms. Some of the agents most studied by gerontologists are depicted (top). In general, the cell deals with this damage either by activating repair mechanisms or by degrading the affected molecules (except in the case of nuclear DNA, which can be repaired, but not degraded). In most instances, persistence of unrepaired damage to individual molecules, including DNA, will lead to an "idiosyncratic functional loss," meaning that in any given cell, a small fraction of any kind of molecule will be damaged and nonfunctional. However, because this damage is random (idiosyncratic), the tissue as a whole will not be significantly affected because each cell within the tissue will suffer damage to a different macromolecule. By and large, this should not affect tissue functionality (happy face). An exception is that, in a small number of cases, persistent damage to a small handful of genes could lead to a growth advantage for the affected cell, which, if unchecked, could lead to cancer (sad face). As depicted in Figure 1, the inability to repair or eliminate the damage activates a stress response (in the case of lipid damage, this is only proposed, as indicated by the question mark). The stress response allows one more chance for repair (happy face), but the persistence of damage can lead to a redistribution of cellular resources or activation of the cell senescence pathway, both of which will result in a generalized functional loss, and aging (sad face). If, in contrast, the response results in cell death, then tissue function can still be restored by activation of stem cell proliferation and differentiation. If this last resort fails for any reason, then the functional loss will still lead to aging (sad face).

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