Review

Consolidation of Carcinogenic Factors and the Development of Precancerous Cells

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Consolidation of Carcinogenic Factors and the Development of Precancerous Cells

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Abstract: Many disparate factors have been associated with causing, or somehow contributing to, the formation of malignancies. There does not seem to be a universal mechanism of action among them, or, in some cases, the mechanism of action is not known. Some carcinogens are directly toxic or mutagenic, while others appear to be somewhat indirect contributors, and none cause neoplasia 100% of the time. Before the sequence of events that leads to malignant transformation may be discussed, we must first establish a relationship among all identified genetic, environmental, dietary, viral, or other factors as to their roles in the formation of precancerous cells. Ultimately, when viewing all of these elements as a whole, it may be posited that any factor, or combination of factors, that contributes to damage, affects a cell's ability to protect or recover from damage, and/or encourages a cell's survival after damage, has the ability to promote — directly or indirectly — the formation of a precancerous cell. This premise is then examined in a model for the development of a precancerous cell from a normal cell undergoing repetitive stress and recovery over time.

Keywords: carcinogens; cancer biology; etiology; precancer; damage response; chaperones; transformation; senescence; unifying theory

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Significance Statement

Any conversation concerning the etiology of cancer must begin with addressing how the constantly expanding list of actual and suspected carcinogens — each of which has a different mode of action or effect on cells — works to create precancerous cells. From there, we may begin to explore the conditions necessary to trigger transformation, as carcinogenesis is more common among so-called precancerous cells.

Introduction

What, ultimately, is cancer? What does it take to cause a cell to malfunction to the point of seeming like a new organism with its own agenda and survival skills? Despite the enormous mass of data accumulated over more than a century of observations and study, the biology of cancer eludes us.[1,2] Cancers arise from many different tissues, often after exposure to a variety of factors ranging from smoking, radiation, asbestos, heavy metals, viruses, and more, with increasing risk associated with inherited genetic traits, chronic inflammation, poor diet, and more.[3–5] The result of all these variables is that no two cancers are the same. To date, no single feature, be it a specific genetic flaw or any other defect, is shared by all kinds of cancers.[2,3,6–8] Each cancer is unique, to the point where "personalized medicine" — designed to more accurately target each patient's version of cancer — is a growing field of treatment options.[7,8]

Despite that variability, the 200 or so types of cancers are connected into a family of diseases because the outward manifestations of the malignancies are similar: uncontrolled proliferation and spread to distant tissues, evasion of the body's defenses, resiliency, angiogenesis, and a metabolic rewiring, among others.[9,10] What is not known is how the vast variety of carcinogenic factors leads to these characteristics, as there is no generally recognized mechanism. Most efforts to describe the origins of cancer depict a normal cell gone haywire, where control is lost, or sometimes increasingly

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convoluted descriptions are provided of random mutation events, followed by Darwinism, followed by more random mutations, leading to these specific shared behaviors.[10,11]

Rather than risking the temptation to form fit data into preconceived notions of mutation as the driving force, it seems more plausible to presume that the assorted carcinogens somehow start a sequence of events that eventually leads to a common pathway or genetic program, revealing an underlying order to the chaos.[12,13] In other words, although the causes of cancers are varied, the process must be the same. What those assorted carcinogens do to a healthy cell to drive it toward malignancy must involve a similar pathway or process. It would follow, then, that post-transformational idiosyncracies would result from such a program playing out in various microenvironments, and influenced by mutations and more.

Most investigators agree that cancer evolves via a multistep process through a so-called precancerous stage, as carcinogenesis often begins among such anomalous cells.[14,15] The first step in the sequence of events leading to the malignant transformation of cells requires that we establish a relationship among all identified genetic, environmental, dietary, viral, or other factors, and/or their indirect contributors, as to their roles in the creation of precancerous cells. To test this premise, we will utilize the concept in a model of the development of a precancerous cell.

Consolidation of Carcinogenic Factors

Many disparate factors have been associated with causing, or somehow contributing to, the formation of malignancies. There does not seem to be a universal mechanism of action among them, and, in some cases, the mechanism of action is not known.[14–16] Some carcinogens are directly toxic or mutagenic, while others appear to be somewhat indirect contributors, and none cause neoplasia 100% of the time. If there is one common feature among the assorted carcinogens, it may be the condition of long-term or repetitive exposure versus a single exposure.

Many of the identified carcinogens are genotoxic, which has led to the general consensus that cancer is the result of any genetic mutation that causes too much growth or not enough death in a cell (i.e. uncontrolled cell proliferation). This “mutated gene hypothesis of cancer” adequately describes the rampant overgrowth of virtually any tumor, benign or malignant. However, that general description often neglects the most common, shared traits of malignancies, the existence of which makes it statistically improbable that cancer is merely the haphazard loss of control in a cell.[9,11] A single gene mutation, or even a dozen of them, does not a cancer make, as it is also interesting to note that transformation to malignancy isn’t immediate, but often follows a latency period of months to decades in humans after carcinogen-mediated mutation occurs, if transformation occurs at all.[3,6,11,13,17,18] The mutated gene hypothesis has been reworked over time to describe a gradual development through a progressive series of alterations, followed by a Darwinian selection for survival and growth, which could explain the long latency period. However, despite the innumerable faulty genes that have been identified in cancers, no single causative gene, or gene sequence, has been discovered that links all malignancies.[1,3,6–8,18] Complicating matters further is that even the most commonly observed genetic mutations don’t occur in every malignancy.[6,11,17,18] Other exceptions are the roles of nonmutagenic agents in carcinogenesis.[13,14,19]

The widely accepted “multiple hit hypothesis” — which assumes a single cell must experience a series of gene-altering events in a stepwise, gradual transformation to full malignancy — applies to a multitude of carcinogenic factors when one broadens the definition. Since many carcinogens lack direct genotoxicity, it might be preferable to rework the concept to mean repeated stress or damage to a single cell over time. The term damage is defined here as any physical injury to a cell, including that which harms genes, proteins, membranes, electron transport system, or other processes within a cell. Using this broad definition, a single factor or a combination may contribute toward the malignant transformation of cells. The most common characteristic among the abundant environmental carcinogens appears to be chronic, intermittent, sublethal cellular stress or injury. Intermittence, which allows cells to recover somewhat between challenges, is an important factor, as too much damage over too short a period of time leads more often to cell death (via necrosis, or

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apoptosis, or variations thereof), thus decreasing the chances for the development of abnormal cells. The chronic nature of the stresses is necessary as too much time between abuses allows for full cellular repair or degradation, again precluding the development of abnormal cells. Repetitive stresses and limited recovery times seems necessary for the appearance of aberrations.[20,21]

Non-damaging events, such as exposures to electromagnetic frequencies (EMF), perhaps should be included in this version of the "multiple hit hypothesis" as well, as intermittent cellular stresses (i.e. anything that triggers checkpoint kinases and cell cycle arrest) can induce greater-than-constitutive levels of protective proteins within a cell.[22,23] Some lines of evidence suggest that increasing levels of heat shock proteins (HSP), also known as molecular chaperones or stress proteins, not only protect, repair, and transport proteins, but also inhibit cell death, therefore, cells repeatedly stressed over time gradually build up both extra protection and the inability to die, further encouraging the development of aberrant cells.[23,24] This will be explored further in the next section.

An individual's susceptibility to cancers often takes the shape of inherited genetic abnormalities which inhibit a cell's ability to repair itself after injury and/or its ability to commit organized suicide when damage is too great to repair.[4,25,26] In effect, this decreases the "contact time" necessary for chronic, intermittent, sublethal stresses to promote precancerous development. A diet low in antioxidants (which help defend against cellular damage) contributes indirectly to the formation of anomalous cells via a decrease in protection from both intracellular and extracellular insults. Short term inflammatory responses defend the body against pathogens, often using peroxides and other reactive oxygen or nitrogen species to destroy the foreign bodies. Chronic inflammation exposes cells to long term contact with reactive oxygen or nitrogen species, which may eventually outpace a cell's ability to protect and repair itself.[27] Chronic inflammation turns short term protection into near constant stress, adding to the "hits" a cell takes over its lifetime.

But this version of the "multiple hit hypothesis" does not at first glance seem to explain the role of virus in carcinogenesis. Rather than inflicting physical injuries, viruses insert genes into a cell that usurp control over its normal behaviors. Viral genes can alter apoptotic mechanisms, overexpress mitotic signals, and/or increase a cell's normal life span (for example, with the addition of genes that code for telomerase), among other effects.[28,29] Indirectly, oncogenic viruses may contribute to chronic inflammation or immunosuppression.[29] Some viruses have been noted for their tendency to influence the development of specific cancers, however, viral carcinogens merely increase the probability of developing certain types of cancers, as infection by a virus alone does not guarantee cancer.[28,30] Viruses may be more of a susceptibility factor in the development of specific malignancies.[28,29]

One way that viruses may contribute to the formation of cancers, or perhaps add to a future cancer's individuality, is via the insertion of genes that increase the rate of mitosis of a cell. Although the induction of cell proliferation per se may not be sufficient to induce carcinogenesis, it creates a favorable environment for tumor development as a rapidly dividing cell is at greater risk of acquiring further mutations via the weakening of checkpoints or error correction. Or, through the insertion of genes that inhibit apoptosis, a virus promotes the survival of a cell that might normally eliminate itself. The inability to commit suicide forces a cell to make alterations to exist, encouraging the development of further aberrations, especially if the infected cell's microenvironment includes other stressors. Viruses may not cause direct physical damage to a cell — the "hits" of this version of the multiple hit hypothesis — but if their genetic alterations are viewed as "hits" that assist in the creation of abnormal cells, then perhaps it does apply.

The basic concept of "repeated damage to a single cell over time" ultimately holds up, but perhaps this version of the multiple hit hypothesis should be reworked to include other, more indirect contributors to cancer emergence. When viewing all of these elements as a whole, it may be posited that any factor, or combination of factors, that contributes to damage, affects a cell's ability to protect or recover from damage, and/or encourages a cell's survival after damage, has the ability to promote — directly or carcinogenic factors. *[see Figure 1]* To test this premise, let us devise and examine a

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model for the development of a precancerous cell from a normal cell undergoing repetitive stress and recovery over time.

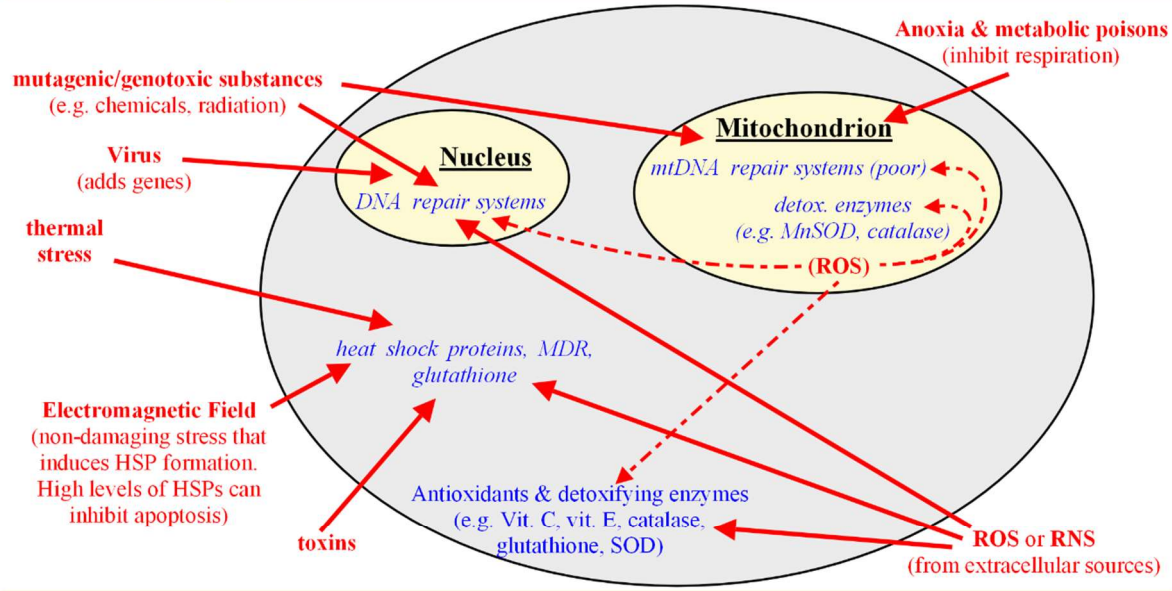
Figure 1: Simplified Cellular Attacks and Defenses (other organelles omitted for clarity)

Red (Bold) denotes various cellular stressors **(factors that contribute directly to cell damage)**. Most cellular stresses are from extracellular sources. Internal sources of reactive oxygen species (ROS) are also produced as a by-product of mitochondrial production of ATP.

Blue *(italics)* denotes cellular defenses. Specific defenses are arranged where they are most needed against particular types of cellular insults. **Any factor that inhibits these defenses contributes indirectly to cell damage.**

A balance (homeostasis) is required. If cellular stresses outnumber or outpace cellular defenses, damage ensues.

Apoptosis [not pictured] takes place if the cell sustains large amounts of damage. **Any factor that inhibits apoptosis contributes indirectly to aberrant cell formation, as a cell that cannot suicide will be forced to make alterations to continue.** (Factors that inhibit apoptosis may include mitochondrial impairment, alterations to p53 or other apoptotic genes, & high levels of HSPs, among others.)



Consolidation of Carcinogenic Factors

When attempting to establish a relationship among all identified genetic, environmental, dietary, or viral factors as to their roles in carcinogenesis, it may be posited that ***any factor that contributes to cellular damage, affects a cell's ability to recover from damage, and/or encourages a cell's survival after damage, has the ability to promote -- directly or indirectly -- the formation of an aberrant (precancerous) cell.***

Abbreviations: HSP = Heat shock proteins, ROS= reactive oxygen species, RNS= reactive nitrogen species , MDR = multidrug resistance transporters, SOD = superoxide dismutase.

Figure 1. Simplified Cellular Attacks and Defenses.

Chaperones as Protectors, Regulators, and Autonomic Decision-Makers

One point mentioned earlier is the role of protective proteins in cellular responses before, during, and after stresses and injuries, which requires some additional information. Originally named for the discovery that they protect cells from temperature extremes — so long as a cell receives a sublethal advance notice of future dangers — heat shock proteins, also called molecular chaperones or stress proteins, have since been found to do quite a lot more, which must be superficially addressed here. They are a diverse family that collectively stabilize proteins to prevent denaturing, assist in the folding of newly synthesized or corrupted proteins, or transport flawed proteins for controlled destruction.[24] Chaperone occupancy, and/or overload, governs various aspects of cellular stress responses as well as protein interactions that take place during routine cellular activities.[31,32] Chaperones are constitutively expressed at all times in a healthy cell to maintain proteostasis (known as Heat Shock Cognates, HSC), with more induced as needed (inducible forms are classified as HSP to distinguish them from constitutive versions), and although the holding or folding of defective proteins takes precedence over all other functions, they also sequester or chaperone many other

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proteins, and therefore help to regulate many important signal transduction pathways [24,31,32]. Molecular chaperones also play a role in shuttling cyclins into and out of the nucleus, which places chaperone occupancy into a primary position for controlling cell proliferation or arrest during periods of stress.[33,34] HSPs themselves repress transcription factor Heat Shock Factor 1 (HSF1) during non-stressful conditions, holding them in the cytoplasm and only releasing the transcription factors during proteotoxic challenge, thus functioning as "stress detectors" for the stress-induced production of supplemental HSPs when overburdened. [35,36] *[see Figure 2A,B]*

The ratio of misfolded or aggregated proteins to chaperone availability guides cellular outcomes during and after damaging events. Denatured proteins that greatly outnumber protective chaperones lead to a rapid death via necrosis. The sudden release of cellular contents to the extracellular space during necrosis incites an inflammatory reaction which can stress or damage neighboring cells, so whenever possible a more controlled, less inflammatory, method of removing permanently injured or defective cells is preferred.[37] Increasing levels of intracellular HSPs, in response to recurring environmental challenges, can delay and convert potential necrotic cell death into programmed suicide or senescence, much like deploying a drogue chute behind an incapacitated airplane to change a wild tumble into a more controlled crash.[24] *[see Figure 2C]* The chaperone occupancy hypothesis can be reviewed more thoroughly in [24,31]

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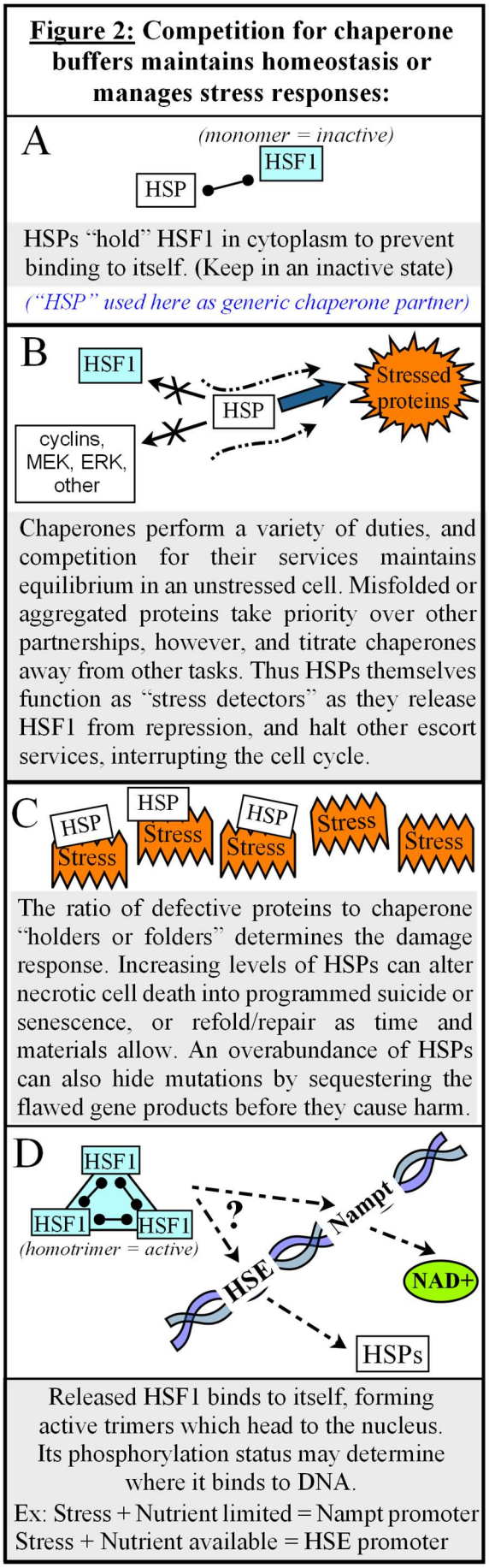

Figure 2. Competition for chaperone buffers maintains homeostasis or manages stress responses.

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The competition for finite chaperone partners may also dampen the DNA Damage Response (DDR), if chaperone buffers are overburdened.[38–42] As an example, damaged DNA must be sensed by MRE11-RAD50-NBN/NBS1 (MRN complex), and it has been found that HSP90 interacts with NBN, and although its role has not been fully elucidated, it may be involved in either stabilization of NBN protein, or responsible for its nuclear translocation, or both.[38–42] Inhibition, or overloading, of HSP90 leads to defective DNA damage signaling and impaired repair pathways.[38,39]

While protective, an overabundance of stress proteins — resulting from a frequently stressful environment — can become something of a double-edged sword, as they can inadvertently hide gene mutations by sequestering, refolding, or transporting flawed gene products for destruction before they cause harm. Only when additional stress and damage occurs, overloading all available chaperones, will the mutated phenotype be revealed.[24,31] It's also plausible that "chaperone overload" may occur less often in cells subjected to frequent bouts of stress, to the point that, rather than cell cycle arrest or other stress responses, proliferation could continue despite defects, with adjustments to metabolism and/or shunting to alternate cellular pathways to compensate, if the defects aren't immediately lethal. This will be discussed further in another section.

Another activity of note is the discovery of how HSF1 and metabolic sensor AMP-activated protein kinase (AMPK) antagonize each other, both directly and indirectly. Metabolic stress suppresses the proteotoxic stress response and vice versa.[43–45] HSF1 induces conformational changes to limit LKB1 binding and activation of AMPK, while AMPK phosphorylates HSF1 to eject it from the nucleus, or to prevent, or possibly alter, transcription once DNA bound.[43–45] Perhaps even more interesting is the observation that HSF1 is not only a regulator of the canonical Heat Shock Response, but also coordinates bioenergetics as well. When glucose is limited, HSF1 can bind directly with the Nampt promoter (nicotinamide phosphoribosyltransferase, a rate limiting enzyme for recycling NAD^+ from nicotinamide), perhaps due to AMPK phosphorylation.[45–47] *[see Figure 2D]* NAD^+ levels affect the sirtuin family of enzymes, and SIRT1/3 play critical roles in mitochondrial "quality control", among other effects.[46–48] Thus a high population of molecular chaperones, induced by recurring environmental challenges, could artificially create low levels of activated HSF1 because they can simultaneously restrain HSF1 and manage aggregated and misfolded proteins.[35,46] Low levels of activated HSF1 during nutrient deprivation affects Nampt levels, which decreases available NAD^+, which in turn decreases SIRT1/3 activity, whicheventually could lead to an accumulation of dysfunctional mitochondria via a reduction in mitochondrial fission and quality control.[46–48] In a similar manner, artificially low levels of activated HSF1, induced by an overabundance of stress proteins, might also affect NAD^+dependent PARP1 (Poly(ADP-ribose) polymerase 1) activity, thus also affecting nuclear DNA break repair.[49,50]

Also of note is how both HSF1 and AMPK competitively interact with p53. HSF1 appears to be responsible for the enhanced import of p53 into the nucleus during stress, while p53 may help HSF1 with its binding and transcriptional activation.[51–53] Activated AMPK on the other hand, phosphorylates and stabilizes p53, indirectly inhibiting HSF1 via a downregulation of mTORC1.[54–56]

Much of the subcellular localization, timing, and activities of these molecules are still being worked out. This subject is too broad to adequately cover in this space and remains to be explored at a later date, but it appears that competition for chaperone partners lies at the core of the interactions among protein homeostasis, energy metabolism, and the initiating sequences of the DDR, as well as determining cellular survival or the method of cell death after damage.[24,38,39,41] *[See Figure 3]*

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Figure 3: Cell Stress Response Axis

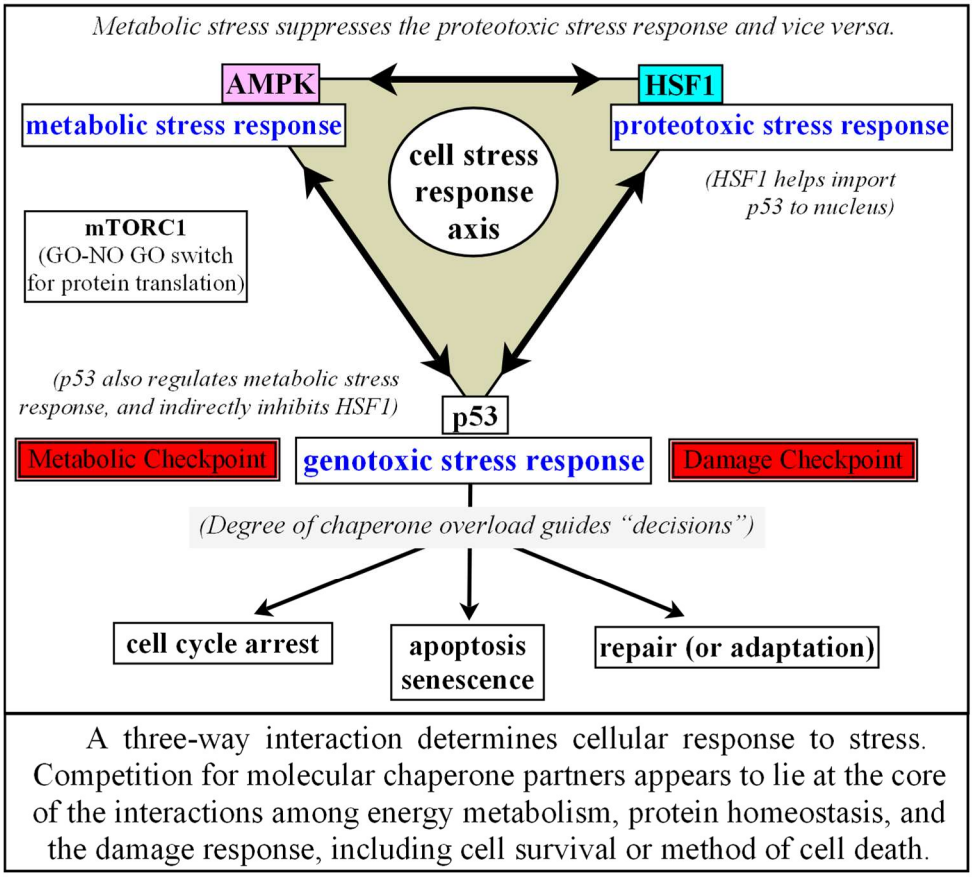

Figure 3. Cell Stress Response Axis.

The Role of Senescence

Another point that requires clarification before we may begin discussion of the evolution of cancer is the role of senescence in cell biology. The exact details are beyond the scope of this work, but some basic points can be made. It must also be mentioned that the vast majority of senescence research is conducted with fibroblasts, which are among the most common cells in the human body, while investigation into senescence of epithelial/endothelial cells is limited.[49] The relevance of this will be addressed in a separate work. For the majority of this discussion, however, we shall group general characteristics under the category of "senescence", without distinguishing among cell types, unless specifically mentioned.

Senescence was originally thought to be an age-related permanent cessation of the cell cycle. The enzyme telomerase is not active in most adult human cells and as a result, with every cell division, the telomeres shorten. Telomere length was once considered as a sort of "timing device" for the absolute number of cell divisions allowed in a cell's lifetime. After a finite number of cell divisions, referred to as Hayflick's limit, the cell stops dividing and enters the permanent state of cell cycle arrest termed replicative senescence.[57] More recently, however, it has been discovered that senescence is more than simply the "retirement" of an older cell. Senescent cells can be found in culture and *in vivo* without telomere shortening.[58–60]

Senescence is activated in response to double-stranded DNA breaks or other events that activate checkpoint kinases.[59–61] Such "premature senescence" has been termed Stress-Induced Premature Senescence, or SIPS, and is virtually indistinguishable from replicative senescence. Unraveling telomeres are perceived by the cell as double stranded DNA breaks, so it appears that replicative senescence actually occurs in response to severe damage to a cell, rather than the advanced age of the cell.[59] It would then be no coincidence that senescence occurs most often in older cells. As a cell ages, the number of injuries it sustains over time accumulates, and the ability to repair itself

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declines.[62] The most important variable to the induction of senescence appears to be the inhibition of apoptosis.[63] Cellular senescence is controlled by many of the same tumor suppressor genes as apoptosis, and both apoptosis and senescence work to prevent the growth of cells at risk for malignant transformation. Whereas apoptosis eliminates damaged cells, cellular senescence permanently arrests their proliferation.[60,64] Stress-induced senescence is often considered to be a "failsafe" mechanism to prevent neoplastic formation in the event a severely damaged cell cannot undergo programmed cell death.[65,66]

Although arrested, senescent cells remain metabolically active, and become secretory cells, releasing chemokines, inflammatory cytokines, proteases, collagenases, and growth factors into the extracellular space — many of which are associated with wound healing.[66,67] The secretory products of senescent cells may, in fact, be a resolution stage of the wound response, where degradation of the extracellular matrix prevents fibrosis, scarring, and the loss of tissue function.[68] As populations of senescent cells accumulate over time in an aging organism, however, benefits become detriments. The secretory products of senescent cells, perhaps meant only as a short-term resolution of wound healing, begin to adversely alter the tissue microenvironment, affecting neighboring cells in myriad ways and contributing to both age-related pathologies and the inadvertent progression of mutant cells.[64,66,67] A growing body of evidence also suggests that precancerous cells must somehow escape senescence in order to progress to malignancy.[49,69,70] However, artificially forcing a cell past senescence (via viral telomerase, or bypassing checkpoint kinases, for instance) causes it to reenter mitosis regardless of damage, yet, without the transformation to cancer, the cell will continue only until crisis.[70,71] Merely bypassing senescence provides a kind of "extended life", but does not initiate immortalization.[69,70] How a precancerous cell might not only escape senescence but transform into malignancy will be discussed in a separate article.

Model for the Development of a Senescent Cell with Malignant Potential

[Please refer to Figure 4 for the following discussion]

For ease of description, Figure 4 depicts a simplified model for a generalized single cell undergoing stress and then recovery in a repetitive cycle, as chronic, intermittent stresses appear to be significant to the formation of precancerous cells. A *stress event* could be exposure to toxins, anoxia, heat, radiation, ROS, and more, although detectable cell injury may not always occur (as appears to be the case with EMF).[23] A stress event is merely anything that triggers a pause to assess and repair or degrade the cell. The term *damage* will again be defined here as any physical injury to a cell, including that which harms genes, proteins, membranes, electron transport system, or other processes within a cell. The concentric circles represent the various pathways a cell could take following each stress event. The innermost circle **(black pathway)** represents the "normal" cell, and this discussion begins there. Each successive circle is akin to the expanding electron orbitals of atoms, wherein each jump to a higher orbital indicates less stability in the system. In the case of the cell, each progressively wider circle represents increasing impairment and/or genetic instability. At the starting point of our generic "normal" cell, molecular chaperones, which perform essential roles in the daily cellular housekeeping and repair, are constitutively expressed at a level to accommodate the balance of the competing duties from intrinsic processes.

Immediately following a *stress event*, there are two possible outcomes: either the cell undergoes a rapid death via necrosis or it doesn't **(A)**. If the damage greatly exceeds the available stress proteins to mitigate, the cell dies abruptly, releasing its cellular contents to the extracellular space, resulting in an inflammatory reaction which can stress or damage neighboring cells.[37] If a cell possesses enough chaperones to buffer the damage somewhat so that it does not die outright following a stress event, then it temporarily halts its cell cycle **(B).** Cell cycle arrest allows time for assessment of damage, and then the controlled elimination **(C)** or recovery **(D)** of the cell.[72]

If our simplified cell sustains an injury too great to repair, it will be shunted to an "elimination" pathway **(C)** where it will undergo programmed suicide or, if restricted from that option, senescence. The dominant method of removing a defective cell from operation is apoptosis **(C_1)**, a process by

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which membrane-bound cell fragments, marked for disposal, are quietly eliminated by macrophages without disturbing nearby cells.[37,72] However, there are multiple ways to inhibit apoptosis, including inherited or acquired genetic mutations, the insertion of viral genes, high levels of molecular chaperones, and/or mitochondrial defects, among others. A cell that has sustained severe damage, but cannot undergo apoptosis, follows the "failsafe" tumor suppressor pathway and enters a state of permanent cell cycle arrest known as senescence **(C_2)**.[60,66,73] Although this model uses a cycling cell to describe events that ensue during recurring stresses, senescence can also occur in post-mitotic cells.[74] The most consistent feature of senescence — aside from the inability to commit suicide — is a persistent, unresolved DNA Damage Response (DDR).[74]

Figure 4 – Simplified Diagram of a Cell under Chronic, Intermittent Stress

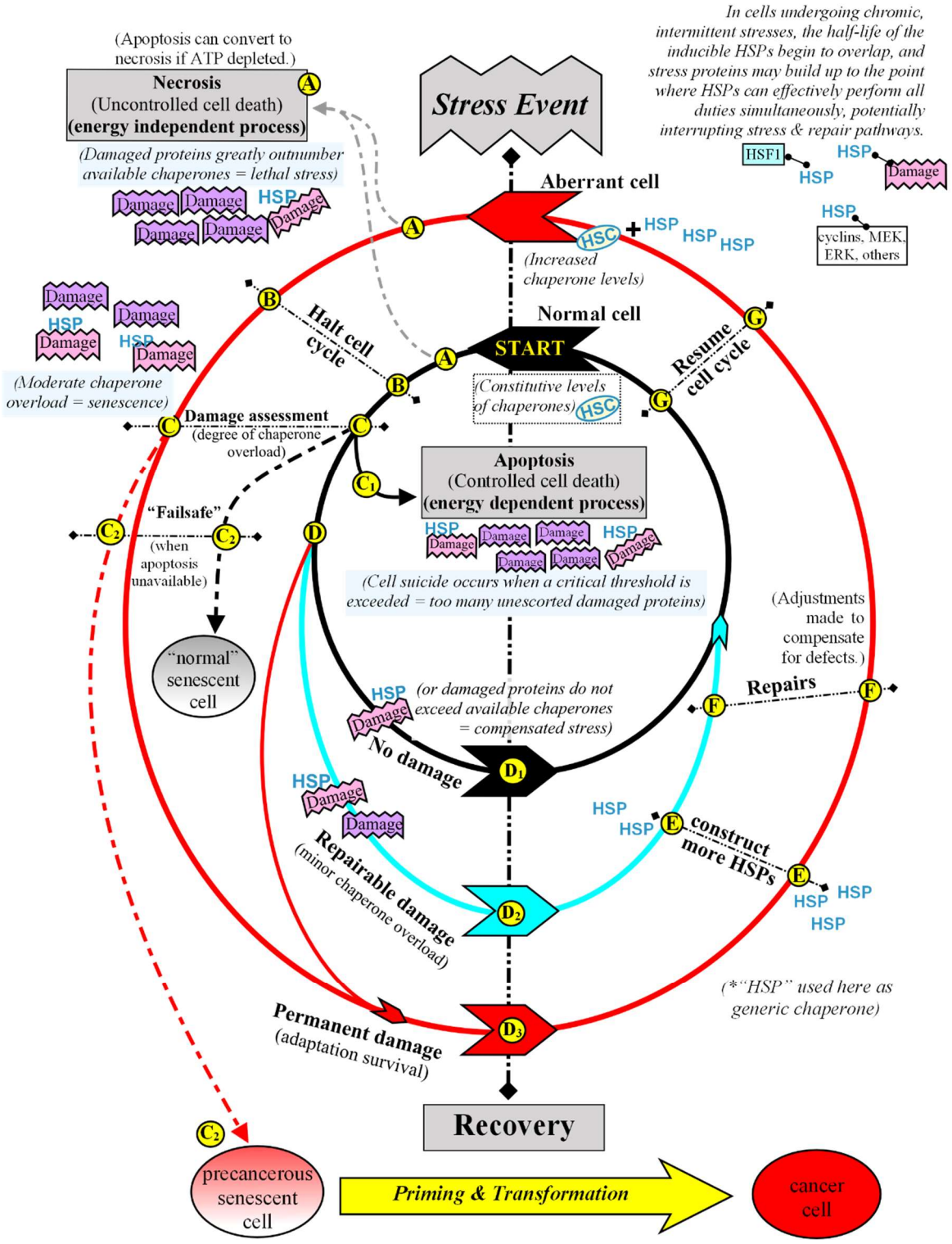

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Figure 4. Simplified Diagram of a Cell under Chronic Intermittent Stress.

Once the cell has cleared the initial damage appraisal, there are three possible recovery pathways for a cell to take **(D)**, depending on the level of injury sustained:

If no damage occurred during the *stress event*, or rather, if the available chaperones were capable of handling all tasks of protein transport, degradation, or refolding (compensated stress), then the cell will continue on the **black pathway (D_1)**.

If minor, repairable damage occurred during the *stress event* (a low level of chaperone overload), the cell will begin to follow the **blue pathway (D_2)**. Note that the blue pathway is only a temporary detour and once repairs are completed the cell will eventually return to the black (fully restored) pathway. HSF1 is normally held latent in unstressed cells, however, as chaperones become fully occupied with damage control duties, HSF1 is released, allowing it to trimerize and translocate to the nucleus, where it can begin the stress-induced transcription of extra HSPs to compensate for future stresses **(E).**[24,35,36] In this way, moderate stress can prepare a cell for more severe stress in the immediate future. Stresses need not cause cellular injury for HSP induction to occur, for example, electromagnetic fields can induce HSPs without obvious cell damage.[23,75] In this simplified model, the generalized term "repair" **(F)** takes in several methods of restoring order in an injured cell, and so "repairs" will merely be mentioned, rather than going into detail. Once the cell has recovered from its stress, it can resume its activities **(G)**.

If intermittent stresses occur close enough together, the half-life of the inducible HSPs can begin to overlap, and the population of stress proteins increases.[22] This can be something of a double-edged sword, as the extra HSPs simultaneously protect a cell from a repetitive damaging environment, yet also prevent cell death, and even mask gene mutations by sequestering or degrading flawed gene products, as already mentioned previously.[24,31] Some lines of evidence suggest that increasing molecular chaperones above constituent levels may be an important event which steers a cell away from apoptosis and toward adaptation-survival or senescence when it receives irreparable injury.[24,73] If our model injured cell is able to bypass various forms of elimination via its higher HSP ratio, the cell will begin to follow the **red pathway (D_3)**. Note that the deviation to the red pathway is a permanent one, as a cell that has irreparable damage can never resume completely normal operation. The permanently injured cell following the red pathway must make whatever repairs it can and adapt to its disabilities, allowing it to continue functioning on some new level. Those adjustments may directly or indirectly cause other changes due to extensive crosstalk among the organelles. For example, one of the most easily damaged organelles of a cell are its mitochondria. As a cell's metabolism changes, the expression of nuclear genes may be altered to compensate.[72,76–78] The converse is also true, as there is crosstalk between the nuclear and mitochondrial genomes, and any changes to nuclear gene expression (through mutation or other means) may cause even undamaged mitochondria to alter their functions to accommodate.[76,77] Cells following the red pathway are identified during biopsies of suspicious growths as "altered", "abnormal", or "mutant" cells, or benign neoplasias, among other terms. Once repairs or adaptations are completed, the cell may then resume its activities **(G)**.

This model, as depicted in Figure 4, predicts two potential subtypes of senescent cells: "normal" versus precancerous. "Normal" senescent cells would be those derived from cells which were following the black pathway that, for whatever reason, were unable to undergo programmed cell death after a serious injury. "Precancerous" senescent cells would be those derived from the aberrant cells which were already following the red pathway prior to the stress event, thereby taking their mutations and other alterations into the senescent state. At present, actual evidence for such a distinction is lacking, although the heterogeneity of senescent cell phenotypes *in vivo* has been noted. It has even been suggested that there exists at least two subtypes of senescence: acute and chronic, depending upon the stressors that created them.[65,79] If, in the future, it is found that the two potential senescent cell subtypes do not exist, it is unlikely to greatly alter this theoretical discourse.

The result of a cell's journey through repetitive injury and healing over time (in this model) is a senescent cell with malignant potential, awaiting priming and a stimulus to escape senescence barriers and transform to malignancy.

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Results and Discussion

Any conversation concerning the etiology of cancers must begin with discovering how the constantly expanding list of actual and suspected carcinogens — each of which has a different mode of action or effect on cells — work to create precancerous cells. From that starting point, we may begin to explore the conditions necessary to trigger malignancy, as carcinogenesis is more common among such anomalous cells.

The most common characteristic among the huge variety of carcinogenic factors, on a cellular level, is repetitive stress and recovery over time, not necessarily genotoxicity, leading us to revise and expand the generally accepted "multiple hit hypothesis" to include general cellular damage, i.e. that which causes any physical injury to a cell, including that which harms genes, proteins, membranes, electron transport system, or other cellular processes. Repetitive stress and recovery factors could also include non-damaging stresses, such as EMF, as they can encourage an increase in protective proteins, i.e. heat shock proteins or molecular chaperones. Evidence shows that competition for chaperone partners lies at the core of the interactions among protein homeostasis, energy metabolism, and the initiating sequences of the DDR, as well as determining cellular survival or the method of cell death, or permanent arrest, after damage. The overload or overabundance of molecular chaperones is influenced by the frequency of cellular stresses.

Other carcinogenic factors appear to be more indirect contributors to cancer emergence, not causing direct physical damage or stress themselves, but more often via a decrease in cellular defenses, or encouraging survival over elimination of damaged cells. If we include the more indirect contributors to the revised "multiple hit hypothesis", then it may be posited that any factor, or combination of factors, that contributes to damage, affects a cell's ability to protect or recover from damage, and/or promotes a cell's survival after damage, has the ability to promote — directly or indirectly — the formation of a precancerous cell. In this way, we consolidate the huge variety of carcinogenic factors.

Damaged cells that have been encouraged to survive even past a certain elimination point undergo a permanent cell cycle arrest termed senescence, which is often considered a "failsafe" mechanism to prevent carcinogenesis in such cells. A growing body of evidence suggests that precancerous cells must somehow escape senescence in order to transform to malignancy. As the population of senescent cells increases in an aging organism, the arrested cells begin to alter the microenvironment, as they remain metabolically active even after arrest. The inflammatory environment produced by their secretory products paradoxically promotes carcinogenesis and other age-related pathologies, in what has been described as "antagonistic pleiotropy".[66] The latency period between carcinogen exposure and the development of cancers may be due to the local senescent cell population, but this subject remains to be explored in another article.

The models developed here indicate that a cell's lifelong contact with various carcinogenic factors — and the associated damage responses — results in a senescent cell with malignant potential, awaiting priming and a stimulus to escape senescence barriers and transform to malignancy.

Methods and Materials

The data generated in this study are publicly available in repositories such as PubMed (RRID:SCR_004846), Scopus (RRID:SCR_022559), Web of Science (RRID:SCR_022706), and others.

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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