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Establishing cellular stress response profiles as biomarkers of homeodynamics, health and hormesis

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ABSTRACT

Aging is the progressive shrinkage of the homeodynamic space. A crucial component of the homeodynamic space is the stress response (SR), by virtue of which a living system senses disturbance and initiates a series of events for maintenance, repair, adaptation, remodeling and survival. Here we discuss the main intracellular SR pathways in human cells, and argue for the need to define and establish the immediate and delayed stress response profiles (SRP) during aging. Such SRP are required to be established at several age-points, which can be the molecular biomarkers of homeodynamic space and the health status of cells and organisms. SRP can also be useful for testing potential protectors and stimulators of homeodynamics, and can be a standard for monitoring the efficacy of potential pro-survival, health-promoting and aging-modulating conditions, food components and other compounds. An effective strategy, which makes use of SRP for achieving healthy aging and extending the healthspan, is that of strengthening the homeodynamics through repeated mild stress-induced hormesis by physical, biological and nutritional hormetins. Furthermore, SRP can also be the basis for defining health as a state of having adequate physical and mental independence of activities of daily living, by identifying a set of measurable parameters at the most fundamental level of biological organization.

1. Introduction

A fundamental characteristic of all living system is the ability to respond, counteract, and adapt to internal and external sources of disturbance. This ability is traditionally termed as homeostasis, which is a somewhat misleading term since there is little or no constancy in living systems. Rather it is the dynamic equilibrium of continuous changes and remodeling that determines survival, and for which the term homeodynamics (Yates, 1994) is better suited to expound the continuity of growth, development and aging. The term homeodynamics has been further modified to *homeodynamic space* as indicative of the overall survival ability and the buffering capacity of a biological system (Holliday and Rattan, 2010; Rattan, 2006, 2007, 2011).

One of the crucial components of the homeodynamic space is the stress response (SR), by virtue of which a living system is able to sense disturbance, and initiates a series of events for maintenance, repair, adaptation, remodeling and survival. There are three main aspects of SR: (1) immediate SR involving extra- and intracellular signaling during the period of disturbance and exposure to stressors; (2) delayed SR involving sensors and modulators in the presence of stressors or after the removal of stressors; and (3) downstream effectors for counteracting the effects of disturbance and for re-establishing homeodynamics. At present it is not known how these three steps are maintained interactively in terms of kinetics and intensity, and how these may alter during growth, development and aging. In order to develop novel and effective means of aging modulators and maintainers of homeodynamics, it is crucial to elucidate the nature and effects of immediate and delayed SR in cells and tissues undergoing aging.

In this article, we briefly review the main intracellular SR pathways in human cells, and discuss the urgency of defining and establishing the immediate and delayed stress response profiles (SRP) of normal cells. Such SRP are required to be established at several age-points, and can be used as molecular biomarkers of homeodynamic space and the health status of cells and organisms. SRP can be very useful for testing potential protectors and stimulators of homeodynamics, and can be a kind of "gold-standard" for monitoring the efficacy of potential pro-survival, health-promoting and aging-modulating conditions and compounds.

2. Stress responses (SR)

The term SR is defined as a response by cells, tissues and organisms to any physical, chemical or biological factor(s), which initiates a series of biological events that facilitate and promote counteraction, adaptation and survival. At the intracellular and molecular level, mammalian cellular SR can be categorized into seven main distinct

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pathways which are integral to the organismic property of homeodynamics (Table 1). Based on the involvement of one or more molecular SR, higher order (cellular, organ level and body level) SR is manifested, which include apoptosis, inflammation, thermoregulation and hyper-adrenocorticism (Rattan and Demirovic, 2010a). Not all pathways of SR respond to every stressor, and although there may be some overlap, generally SR pathways are quite specific.

The specificity of SR is mostly determined by the nature of the disturbance or the damage induced by the stressor and the variety of downstream effectors involved. For example, cytoplasmic induction of protein denaturation initiates the so-called heat shock response (HSR) by activating heat shock transcription factors (HSF), and by inducing the transcription and translation of several heat shock protein (HSP) genes (Liberek et al., 2008; Verbeke et al., 2001). However, unfolded proteins in the endoplasmic reticulum (ER) induce the unfolded protein response (UPR), and initiate the induction of synthesis of a totally different set of proteins and their downstream effectors, such as GRP78/BiP, GRP94/gp96, GRP170/ORP150, GRP58/ERp57, PDI, Erp72, calnexin, calretuculin , EDEM, Herp and other co-chaperones (Banhegyi et al., 2007; Lin et al., 2007; Ni and Lee, 2007; Yoshida, 2007). Similarly, a mitochondria-specific SR involves the induction and activation of various other chaperones, such as chaperonin-10 (Cpn10/Hsp10), chaperonin-60 (Cpn60/Hsp60), and mortalin (Kaul et al., 2007; Zhao et al., 2002).

Another well-known SR is the oxidative stress response, in which cells respond to oxidative stressors through the regulation of transcription of several antioxidant genes. The main regulator of this specific antioxidant phenotype is the nuclear factor-erythroid-2 (Nrf2) transcription factor, which regulates the basal and inducible expression of numerous detoxifying and antioxidant genes (Ishii et al., 2002). Under normal conditions Nrf2 is held in the cytoplasm by the specific inhibitory protein KEAP1. Oxidative modification of cysteine residues of KEAP1 induces conformational changes and a loss of Nrf-2 binding, allowing Nrf2 to translocate to the nucleus where it heterodimerizes with specific co-factors, and leads to the transcription of various genes through the regulatory regions of antioxidant response elements (Motohashi and Yamamoto, 2004; Yamamoto et al., 2004). Some of the genes activated by stress-induced activation of Nrf2 are heme-oxygenase1 (HO-1), NAD(P)H-quinone oxidoreductase-1 (NQO1), and GST mRNA and proteins in rodent tissues (Calabrese, 2008; Pearson et al., 2008).

Activation of DNA repair enzymes in response to DNA damage is a SR which is essential for the maintenance of genomic stability (Hakem, 2008; Vijg and Campisi, 2008). DNA damage response (DDR) involves a variety of transcription factors being translocated to the nucleus, binding the double- and single-strand DNA breaks, and activating the transcription of several DNA repair enzymes. Other SR involve activation of sirtuins causing deacetylation of histones and other proteins in response to reduced levels of metabolic energy (Longo, 2009; North and Sinclair, 2007); activation of NFkB-mediated cytokine synthesis (Yeung et al., 2004); and the autophagy response which is the lysosomal-mediated and chaperone-mediated sequestering of damaged membranes and organelles induced during nutritional limitation, starvation, and hypoxia (Markaki and Tavernarakis, 2011; Martinez-Vicente et al., 2005; Terman et al., 2007; Yen and Klionsky, 2008).

However, an induction of a specific SR pathway as the first response (immediate response) does not rule out the induction of one or more other SR pathways later on (delayed response). A complete and successful SR for effective homeodynamics and for the maintenance of the homeodynamic space includes both immediate and delayed SR. It is therefore important that all SR pathways are analysed simultaneously and a complete SRP is established under a given condition, such as age-, health- and disease status, and during and after exposure to single or multiple stressors.

3. Stress response profiles (SRP)

Determining SRP is essential for establishing the nature and extent of the homeodynamic space of cells, tissues and organisms. Furthermore, being able to map the kinetics and amplitude of different SR, and their effects on each other, can form the basis to evaluate the health status of an individual and to develop effective means of aging modulators and maintainers of homeodynamic space.

In Fig. 1, two theoretical SRP are visualized for an idealized healthy young cell exposed to either heat shock (HS) or nutritional deprivation for a short period. Fig. 1A represents the virtual SRP in response to HS, in which HSR is the primary response and is set to reach the maximum in a given period of time. There is a large body of published data for HSR in various biological systems (Richter et al., 2010; Verbeke et al., 2001), but little is known with respect to the other SR pathways during and after HS. For a complete SRP, one needs to study the other six pathways in the same samples during the same duration and beyond. Obviously, not all pathways will respond equally in time and amplitude, and even some pathways may be suppressed due to the limitation of energy resources. For example, it has been proposed that autophagy may be triggered as a late response after exposure to HS (Zhao et al., 2009). Similarly, whereas one can expect to see UPR, inflammatory response and sirtuin response to varying extents as delayed responses to HS, it is unlikely that any significant DDR may occur during or after HS (Richter et al., 2010).

Another example of our theoretical SRP (Fig. 1B), envisages the behavior of the stress pathways after nutritional deprivation, in which case the autophagic response is expected to be the primary response (Rabinowitz and White, 2010). Autophagy is a conserved response initiated by the inhibition of the mammalian target of rapamycin (mTOR) complex, resulting in the formation of a membrane-encapsulated

Table 1
Main molecular level stress response pathways and their respective inducers and effectors in human cells.
Modified from Rattan and Demirovic (2010b).

Response	Stressors	Effectors	Molecular markers
Heat shock response (HSR)	Heat, heavy metals, antibiotics, protein denaturation	Heat shock proteins, proteasome and other proteases	Translocation of HSF-1 into the nucleus; induction of HSP72 (HSPA1A)
Unfolded protein response (UPR)	Unfolded and misfolded proteins in ER	Chaperones and co-chaperones	Activation of transcription factor 6 (ATF-6); CHOP (GADD153)
Autophagic response	Food limitation, hypoxia, damaged organelles	Autophagosomes, Lysosomes	Altered LC3-I and LC3-II ratio; increased number of lysosomes; degradation of damaged mitochondria
DNA damage response (DDR)	Radiation, oxidants, free radicals	DNA repair enzymes	Translocation of ATRIP to the nucleus; increased levels of checkpoint proteins p53, p16, p21, mortalin
Antioxidant response	Free radicals, reactive oxygen species, pro-oxidants	Nrf-2, heme oxygenase, FOXO	HO-1; FOXO protein levels
Sirtuin response	Energy depletion	Sirtuins	Sirtuin-1 protein level; NAD/NADH ratio
Inflammatory response	Pathogens, allergens, damaged macromolecules	Cytokines, nitric oxide synthase, COX-2	Increased level of NF-κB protein and inflammatory interleukins