

A Potential Connection between Activation of Transient Receptor Potential Channels by Electromagnetic Wave Radiation and Induction of Heat Shock Proteins in Living Cells

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ABSTRACT

A number of stimuli have been shown to both induce heat shock proteins and activate transient receptor potential channels. However, the direct and indirect relations (i.e., causes and results) between the production of heat shock proteins and the activation of transient receptor potential channels have not yet been clarified. In the present review, we propose that a key phenomenon binding these two molecular events together is the production of reactive oxygen species by environmental electromagnetic-wave radiation. We then hypothesize a signaling cascade from environmental electromagnetic-wave radiation to heat shock protein production via transient receptor potential channel opening. In addition, the roles of the intracellular Ca^{2+} influx through transient receptor potential channels are shown to include the activation of mitochondria and synthesis of new proteins. These two molecular mechanisms are thought to result in heat shock protein production and autophagy activation in order to achieve cellular recognition of the oxidized proteins and replace damaged proteins, respectively. It is hoped that this hypothesis will inspire research into the various beneficial effects of environmental electromagnetic waves on the human body.

Key words: Electromagnetic wave; Heat shock protein; Infrared radiation; Reactive oxygen species; TRP channel

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INTRODUCTION

A wide variety of stimuli have been shown to induce molecular chaperones (i.e., heat shock proteins: Hsps), including temperature, ethanol, oxygen radicals, nitric oxide, ionizing radiation, microwaves (i.e., electromagnetic waves: EMW), exercise, anoxia, endotoxins, and bacteria^[1]. Although, at a glance, these phenomena would be expected to exert completely different types of stimulation, they all share an ability to induce reactive oxygen species (ROS)^[2-4]. In addition, most of these stimuli can activate transient receptor potential (TRP) channels simultaneously^[5,6]. That is, the inducers of Hsps and the activators of TRP channels largely overlap^[7].

This raises the question: between Hsp production and TRP channel activation, which is the cause, and which is the effect? In terms of their temporal order, it is clear that the activation of TRP channels occurs first, followed by the production of Hsps^[8], but a direct signaling pathway connecting TRP channels and Hsps has not yet been revealed. This is because these two proteins have been independently examined in different fields, with the studies on TRP channels being performed mainly in biophysics and cell biology, and those on Hsps in the area of biochemistry and the medical sciences^[9-12]. However, as described above, our studies on aging/senescence have indicated that the stimuli inducing Hsps are often also the stimuli that activate the TRP channels^[7]. In the present review, therefore, we will attempt to clarify the signaling pathways

from TRP channels to Hsps and then apply this pathway to a new hypothesis involving environmental EMW, which is one of the stimuli mentioned above. It is hoped that this hypothesis will inspire research into the various beneficial effects of environmental EMW on the human body.

Studies on the molecular mechanisms of cellular damages induced by EMW have been performed in clinical medicine and electric engineering, resulting in an effective clinical treatment called “focal hyperthermia”^[13]. Almost all the research into focal hyperthermia has been centered on the challenge of focusing microwaves onto an affected part of the human body to kill cancer cells, but little attention has been paid to the question of why high temperature treatment at 43°C can specifically kill cancer cells but not normal cells^[14]. Nonetheless, there have been a few studies that may suggest an answer to this question, including one showing that the transient receptor potential vanilloid receptor 1 (TRPV1) channel opens at 43°C^[15], and another showing that the TRPV1 channel could induce cancer cell death via activation of the Ca²⁺ influx signal^[16]. In support of this mechanism, treatment with the TRPV1-antagonist AMG9810 promotes tumorigenesis in mouse skin^[17].

In addition to hyperthermia, it is worth paying attention to fertilization, which may also be involved in TRP channels. The fact that successful fertilization requires the activation of inositol triphosphate receptors (IP3Rs) in the endoplasmic reticulum (ER) by sperm will also be explained if we assume that IP3-sensitive TRP channels exist in the ER membrane of oocyte cells^[18,19].

Thus, the purpose of the present review is to provide examples of the involvement of TRP channels in the plasma membrane and ER membrane in a variety of unresolved signaling cascades. The final goal of these signaling flows is to produce a suitable number of Hsps to slow the rate of aging and reduce age-related diseases. Thus, we discuss the roles of TRP channels in producing Hsps in living cells, and the possible use of carefully controlled EMW to stimulate this process.

ACTIVATION OF TRP CHANNELS BY EMW AS AN INITIAL STEP

Environmental EMW can produce numerous ROS in living tissues in a wavelength-dependent manner^[20]. According to the Planck-Einstein relation for EMW energy, the following equation holds even in living tissues: $E = h\nu = hc/\lambda$. Here, E is the energy of EMW; h is Planck's constant; ν is the frequency of EMW; c is the speed of light; and λ is the wavelength of EMW.

This equation demonstrates that radiation with short wavelength has high energy that can permeate the whole human body easily. EMW ranging from UV rays to infrared (IR) rays can reach a depth of only several mm below the skin surface, but microwaves can reach a depth of several cm^[21]. Figure 1 shows various types of environmental EMW.

These categories (gamma-rays to microwaves) of EMW produce ROS in different ways, as described below.

In this context, it is important to note that environmental EMW has three different effects on molecules, i.e., ionization, excitation and heat response (Figure 2)^[22]. Ionization is the process by which a molecule acquires a negative or positive charge by gaining or losing electrons to form ions. In the present review, we mainly focus on ionization resulting from the interaction with environmental EMW, in which the inner-shell electrons are ejected. Excitation is an elevation in energy that is often associated with an atom being raised to an excited state. An excited state of a system, such as an atom or a molecule, is any quantum state of the system that has a higher energy than the ground state. After

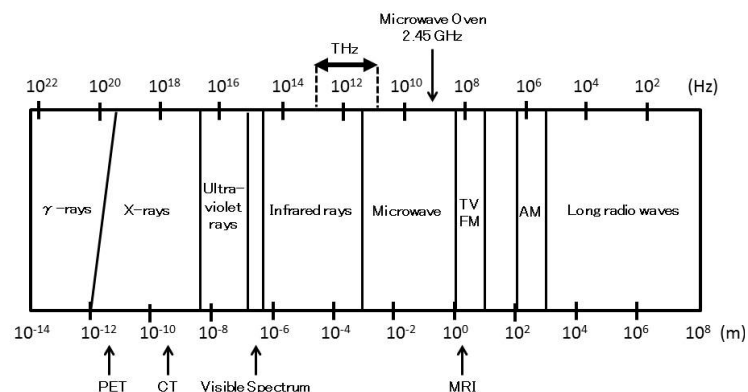


Figure 1 Frequency and wavelength of environmental EMW.

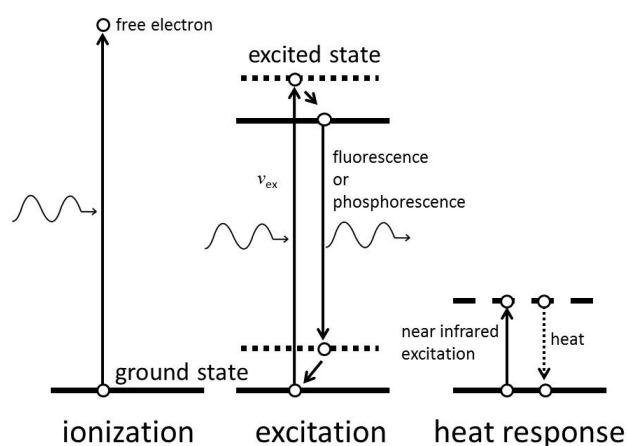


Figure 2 Three different effects of EMW on molecules.

the system has absorbed energy to go to an excited state, radiation is emitted by the system in a process called luminescence, which can take the form of either fluorescence or phosphorescence. Near infrared (NIR) energy is absorbed by a molecule, and the molecule changes its rotational-vibrational movements. NIR energy excites vibrational modes in a molecule through a change in the dipole moment (see also the description of “microwaves” below).

(1) Direct ROS production in living tissues by high-energy radiation

As shown in Figure 1, high-energy gamma-rays and X-rays, which have very short wavelength, can permeate the human body easily, because their energy loss is very small in living tissues^[23]. When high-energy radiation enters into tissues, it ionizes the water molecules in the tissues to form e^{-*} and H₂O⁺* simultaneously. Here, * denotes the excited state. e^{-*} is captured by many water molecules and then changes to e_{aq}⁻, which is known as a hydrated electron. In the presence of oxygen molecules, this e_{aq}⁻ forms super oxide (O₂⁻), while H₂O⁺* results in a hydroxyl radical (·OH). Super oxide (O₂⁻) and hydroxyl radical (·OH) are members of the ROS family (note that hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂) are the other members of the ROS family)^[24]. Exposure to high-energy gamma-rays or X-rays does not usually happen except during imaging or treatment in a hospital, but astronauts are frequently exposed to intense cosmic rays, including gamma-rays and X-rays.

(2) ROS production by middle-range energy EMW (UV, visible and NIR rays)

UV rays do not have sufficient energy to ionize water molecules. However, UV, visible light and NIR rays can generate ROS, including

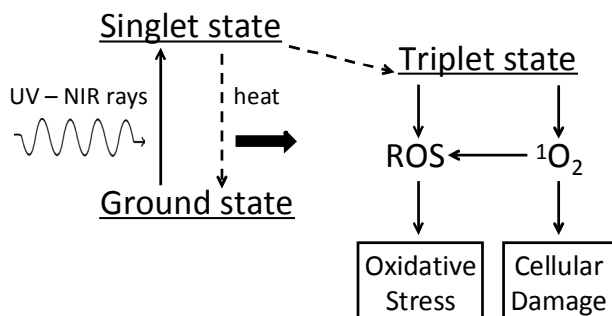


Figure 3 Singlet oxygen production by EMW (UV - NIR rays).

hydroxyl radicals ($\cdot\text{OH}$), and singlet oxygen ($^1\text{O}_2$) by excitation, as shown in Figure 3.

The production of singlet oxygen ($^1\text{O}_2$) involves several complicated processes. As shown in Figure 3, we expect that dissolved organic matter (DOM) exists in a triplet excited state in the water of tissues. The radiation absorption by DOM generates DOM in the singlet excited state ($^1\text{DOM}^*$), and then $^1\text{DOM}^*$ nonradiatively passes into the triplet state ($^3\text{DOM}^*$) via intersystem crossing. $^3\text{DOM}^*$ binds O_2 , resulting in DOM and singlet oxygen ($^1\text{O}_2$)^[25].

Singlet oxygen ($^1\text{O}_2$) is a very popular topic of investigation in dermatology research, due to its harmful effects on the cell membrane^[26]. These harmful effects have been attributed, at least in part, to the transformation of $^1\text{O}_2$ to radicals and other ROS in tissues^[27]. It must be kept in mind that hydroxyl radicals ($\cdot\text{OH}$) are also made in the mitochondria (Mt) of liver or other tissues having high levels of blood pigments, because visible light and NIR rays are absorbed at high levels in these tissues^[28,29].

(3) Indirect ROS production by heat (high temperature)

Hsps themselves cannot be directly induced by ROS in tissues^[30]. However, heat can induce Hsps in the cells of tissues, and independently, heat (or temperature stimulation) can also activate TRP channels, as shown in Figure 4. Here, we will take as an example the heating mechanism by microwave radiation. Microwave ovens are often utilized to thaw frozen cells and tissues, and thus they can be applied to the activation of TRP channels in the thawing process. The frequency of a microwave oven is usually 2.45 GHz. This oscillation frequency moderately changes the electric field that determines the direction of the permanent dipoles of polarized water molecules. Thus, the directional changes of the permanent dipoles lead water molecules to effectively absorb the energy of microwaves and generate heat. Such directional changes in the permanent dipoles of polarized water molecules may affect the hydrophilic amino acids in the *N*- or *C*-terminals of thermo-sensitive TRP channels. In the near future, it will be important to clarify the thermo-sensitivity of TRP channels by investigating the interactions between water molecules and a number of hydrophilic amino acids of the long *N*-terminals or *C*-terminals.

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Thermo-sensitive TRP channels

- Barrier diseases V1, V4, A1, M8
- Skin inflammation V1, V3, A1
- Hair growth V1, V3
- Skin aging and UV-induced diseases V1

Other TRP channels

- Skin cancer
- Skin differentiation

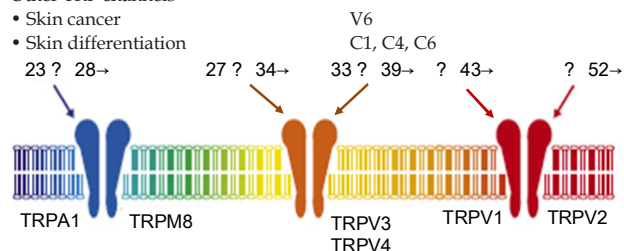


Figure 4 List of TRP channels in the skin.

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Hsps are expressed when the body temperature is kept at more than 41°C^[7]. The most important point to consider in the present context is whether or not “high temperature” can produce ROS in living cells. To answer to this question, we must consider the function of TRP channels. There are several types of thermo-sensitive TRP channels in the skin, and when any of these channel types open, extracellular Ca^{2+} and Na^+ enter into the cell abruptly, and the intracellular Ca^{2+} concentration is transiently elevated^[31], resulting in the activation of Ca^{2+} -uniporters of Mt and the production of ATP^[32]. A transient increase in intramitochondrial Ca^{2+} triggers activation of the electron transport system of Mt, and then ROS are formed in Complex I and Complex III with ATP in Mt, and thereby released into the cytosol^[33]. This is the proposed signaling pathway underlying the increase of Ca^{2+} -induced ROS in cells via Mt.

If the above scenario is accurate, then IR rays would be a strong candidate for intracellular Ca^{2+} production, because IR can carry heat into biological tissues^[34]. The basic laws of IR radiation were established in the field of thermodynamics (physics) in the beginning of the 20th century (e.g., in 1911, Wilhelm Wien was awarded the Nobel Prize in Physics “for his discoveries regarding the laws governing the radiation of heat”). In the 1970s, IR was established as an effective heat conductor for thawing frozen materials, but at present it is more often used for food processing operations such as drying, hydration, enzyme inactivation and pathogen inactivation^[35], and quite recently, in the medical sciences to treat hyperthermia^[36]. In addition, the development of diode lasers for the study of hyperthermia and thermo-sensitive TRP channels has also progressed^[37,38], and this should also encourage further research into TRP channels.

(4) Uncertain effects of radiofrequency on living cells

Mobile phones are now distributed across the planet. In association with this ubiquitous distribution, a toxic effect of EMW in the radiofrequency (RF) region has been proposed^[39]. It was initially conjectured that this toxic effect may arise due to DNA damage caused by RF-induced

disturbance of the proton current on DNA^[40,41]. In this model, the double-strand structure of DNA would act as a semiconductor with free protons. The wavelength of RF-EMW is much longer than that of IR, and the frequency exceeds the order of gigahertz, which is enough to vigorously oscillate protons on the DNA surface^[42]. However, DNA damages were shown to occur selectively at the G:C pair in the DNA double-strand^[43], which suggested another possibility. Namely, the DNA damage by RF radiation could be a form of ROS-induced chemical damage^[2,44,47]. If ROS are produced directly by RF-EMW, the molecular mechanism of ROS production would be quite different from that by heat or radicals, because the energy of RF is far too low to produce heat in living tissues^[4].

ROLES OF TRANSIENT Ca^{2+} ELEVATION IN NORMAL CELLS

Once TRP channels open, the level of cytosolic calcium is elevated transiently and triggers various Ca^{2+} signaling pathways. In regard to ROS signaling, the most interesting aspect of these Ca^{2+} signaling pathways is their mutual interaction with Mt responses and ROS production. Indeed, this interaction is closely related to the aging process^[48]. Intracellular Ca^{2+} elevation triggers the function of mitochondrial Ca^{2+} -uniporters, and Ca^{2+} increase in Mt activates the tricarboxylic acid (TCA) cycle and produces ATP, which is followed by activation of the electron transport system^[49]. Complex I is known to play a major role in ROS production in the electron transport system, and the molecular mechanism of ROS production in the activated state of the respiratory chain has been established^[50]. Another significant ROS production apparatus is Complex III, and although it is not known how Ca^{2+} is involved in ROS production in Complex III, it is clear that both these processes are involved in the Ca^{2+} -activated TCA cycle^[51].

Given the above facts, the important point would be determining how cytosolic Ca^{2+} triggers the TCA cycle. In the 1990s, a process called RaM (rapid uptake mode) was discovered and shown to drive the rapid Ca^{2+} uptake into mitochondria from the cytosol^[52]. A rapid and transient increase of Ca^{2+} is more efficient for the production of ATP in Mt than a slower or longer-term uptake. Although several types of experiments were previously performed using Mt isolated from cells, recent advances in Ca^{2+} indicators have allowed us to measure intra-mitochondrial and cytosolic calcium dynamics simultaneously in living cells^[53,54]. In particular, a gene-encoded fluorescent Ca^{2+} indicator, Pericam, was established as a powerful Ca^{2+} -sensitive dye to measure Ca^{2+} concentration change in the organelles of living cells^[55]. The rate of Ca^{2+} uptake via the uniporter/RaM was estimated to be two orders of magnitude higher than that of Ca^{2+} uptake under the same conditions but via the uniporter alone^[56]. Consequently, Ca^{2+} uptake via RaM may be more effective in activating Ca^{2+} -sensitive processes in the matrix than Ca^{2+} uptake via the Ca^{2+} -uniporter.

The next question we have to consider is whether TRP canonical (TRPC) channels evoke a transient increase in Ca^{2+} intracellularly. A fraction of TRPC3 channels are localized to Mt. A significant fraction of Mt Ca^{2+} uptake that relies on extramitochondrial Ca^{2+} concentration is TRPC3-dependent, and the up- and down-regulation of TRPC3 expression in the cell influences the Mt membrane potential^[57]. If TRPC channels are activated by long-lasting ROS produced by Mt, the role of TRPC channels would be changed. The accumulated data suggest that TRPC channels are activated by ROS^[58,59]. In these cases, Ca^{2+} -uniporters are involved in this process,

because the Ca^{2+} -uniporter activity is regulated by mitochondrial membrane potential.

FUNCTION OF DELAYED PRODUCTION OF ROS BY MITOCHONDRIA

Here, we consider the production of ROS in Mt. Although the molecular process of this production has already been established^[60], we should note the following fact. When living cells are exposed to UV radiation, ROS production is long-lasting, generally continuing for more than 24 h, even though the lifespan of ROS is estimated to be less than several msec^[61]. This long-lasting ROS elevation by UV radiation can be explained if ATP production is continued for a long time after the TCA cycle is triggered by rapid Ca^{2+} entry into Mt. Alternatively, long-lasting intracellular Ca^{2+} elevation may occur, once a transient Ca^{2+} activates a continuous Ca^{2+} elevation in Mt using Ca^{2+} -uniporters.

At present, the latter is more likely, because a long-lasting intracellular Ca^{2+} elevation always requires ATP for activating Ca-ATPase in the plasma membrane as well as in the ER membrane. This mechanism is referred to as the sarco(endo)plasmic reticulum calcium ATPase system (SERCA)^[62]. The protein oxidation and lipid peroxidation may support the fact that living cells are destroyed in the presence of long-lasting Ca^{2+} elevation^[63]. However, to confirm this hypothesis, further experiments should be performed. In fact, it is not especially important how intracellular ROS/ Ca^{2+} are elevated; the cells can survive if Hsps are produced in proportion to the number of systems for reducing ROS in living cells. That is, the function of chaperones is the most desirable for the viability of cells in this context. When a protein is oxidized, two cysteine residues form a single S-S bond, which distorts the tertiary structure of the protein^[64]. The subsequent reduction of oxidized proteins cannot recover their structure, and therefore the chaperoning function by Hsps is required. A large number of chaperoning functions of Hsps have been proposed^[65], but almost all are still at the hypothesis stage. In the following sections, we will propose a new hypothesis for the function of Hsps.

INDUCTION OF HSPS BY Ca^{2+} AND ROS RELEASED FROM MITOCHONDRIA

With respect to the relation between Hsps and ROS, Hsps are produced directly by ROS, and are produced indirectly in response to recognition of the oxidized state of cysteine residues—i.e., the recognition of S-S bonds—in proteins by Hsps themselves^[66,67]. On the other hand, Ca^{2+} activates protein synthetic signals^[68]. Therefore, it is reasonable to assume that ROS induce intracellular Ca^{2+} signaling via the activation of TRP channels, as described in the previous sections, resulting in the production of Hsps. Jorquera *et al.* reported that membrane depolarization can induce Ca^{2+} -dependent up-regulation of Hsp70 and HO-1/Hsp32, called Hmox-1, in skeletal muscle cells^[69]. This was quite an important discovery, because all living cells have voltage-dependent Ca^{2+} channels and K^{+} channels. That is, not only muscle cells but also non-excitabile cells can show a transient Ca^{2+} increase by electrical stimulation or by high K^{+} treatment. Thus, all cells have the potential to up-regulate Hsps in a Ca^{2+} -dependent manner.

What is the role of Mt in this case? As pointed out by Barret *et al.*, Mt-derived oxidative stress induces Hsp responses^[70], a finding which lends further support to our hypothesis. Taken together, the

results suggest that all processes of ROS-induced Hsp formation are described time-sequentially from EMW exposure to Hsp production in the blood, as shown in Figure 5.

When cells in living tissues are exposed to EMW for a short time, and especially to terahertz radiation, ROS are transiently produced both inside and outside of cells^[71]. The externally produced ROS transiently open the TRP channels of the cells, allowing external Ca^{2+} to enter^[6]. On the other hand, internally produced ROS form S-S bonds in functional proteins, which are easily recognized by Hsps. The transiently increased Ca^{2+} in the cytosol triggers the function of Ca^{2+} -uniporters of Mt, and intra-mitochondrial Ca^{2+} increases to produce ATP. Simultaneously, Complexes I and III in Mt induce the delayed production of ROS, which will also form additional S-S bonds in the functional proteins^[48]. In proportion to the increase in the number of S-S bonds, the up-regulated Hsps recognize the oxidized proteins and proceed to repair them or break them down. The residual Ca^{2+} in living cells is pumped out by Ca-ATPase in the cell membrane.

A NOVEL HYPOTHESIS ON THE ROLES OF HSPTS IN LIVING CELLS

Hsps are found not only within cells but also outside cells in the blood^[72-75], which raises the question of the roles of the external Hsps. Previously, it was thought that Hsps could distinguish between oxidized and normal proteins, and then repair the damaged proteins with the aid of Ca^{2+} ^[76]. Because oxidized proteins have more S-S bonds than normal proteins, they also have more distorted structures and different distribution of negative charges compared to their normal counterparts^[77]. Thus, Hsps can distinguish oxidized proteins from normal ones with the aid of attached ATP^[78]. In other words, Hsps have the ability to recognize all “native” proteins from others that enter the body from the outside^[79].

Because protein repair is more difficult than mere protein recognition, the “autophagy” system in cells selectively destroys the damaged proteins among huge numbers of normal proteins^[80]. Hsps acting as repairmen must first loosen the folded parts of the protein and then return the thread of amino acids to the original tertiary structure. However, given the ability of Hsps to perform such repair, we must ask why living cells have an autophagy system for removing damaged proteins. In answer to this question, we now assume that Hsps play a role only in recognition, and that the autophagy system in cells removes damaged proteins and sends orders to the nucleus to synthesize new and flawless proteins to take their place in the original positions. This “self and non-self” recognition function of Hsps is perhaps the most valuable characteristic of the immune system^[79]. Recently, the roles of Hsps in the immune system have been demonstrated in the context of various diseases, such as chronic

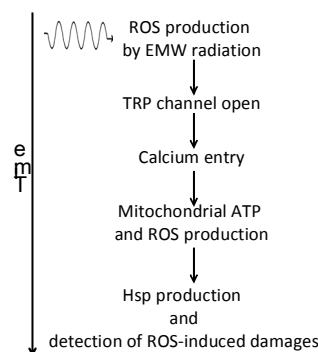


Figure 5 Time-sequential events from EMW radiation to Hsp production via ROS production.

A. General roles of Hsps in normal cells

- Protection of cells
- Repair of denatured proteins / Recognition and removal of damaged proteins
- Production by activation of transcription factor HSF1 and promoter HSE by Ca^{2+}
- Transport of functional proteins with the aid of actin

B. Resistant roles of Hsps for cell survival

- Hsp27 ----- resistance to H_2O_2 and ischemia
- Hsp60 ----- resistance to ischemia
- Hsp70 ----- resistance to ischemia, heat shock and cell cycle arrest
- Hsp90 ----- resistance to hyperthermia, ischemia, apoptosis and cell cycle control

Figure 6 Roles of Hsps for cells survival.

inflammatory diseases, autoimmune disorders, and cancer^[81]. Our new model for the roles of Hsps in cells is shown in Figure 6.

According to Kalmar and Greensmith (2009), both Hsp32 and Hsp70 act as redox sensors in eukaryotic cells by detecting intramolecular S-S bond formation^[65]. On the other hand, in the case of the repair of damaged proteins, Hsp90, Hsp70, Hop (p60), Hip (p48) and p23 must act together on the surface of oxidized proteins^[65]. Here, Hop, Hip and p23 are the cochaperones for the Hsp90 dynamic heterocomplex^[82]. Therefore, it is thought to be easier for Hsps to recognize oxidized proteins than to repair them.

SUGGESTED PATHWAYS FROM EMW STIMULATION TO HSP PRODUCTION

Finally, we propose the most probable pathways from EMW stimulation to Hsp production as follows.

(A) When animal (or human) bodies are exposed to EMW, ROS including free radicals are produced in the body in a wavelength-dependent manner: high-energy EMW (cosmic ray, gamma-ray and X-ray) initially produces hydrated electrons, and then free radicals and other ROS. Intermediate-energy EMW (UV ray, visible light and NIR ray) first generates a singlet oxygen in the body, and then the singlet oxygen is changed to a free radical. The lowest-energy microwave and radiofrequency waves generate heat in the body, which induces ROS production in the cellular Mt.

(B) Both heat and the generated ROS can transiently activate various types of TRP channels and increase the Ca^{2+} levels in cells.

(C) The increased Ca^{2+} plays a dual role. First, it activates the TCA cycle in Mt and generates ROS production with ATP. Second, the residual Ca^{2+} is used as a signal molecule to synthesize new proteins to replace oxidized (damaged) proteins.

(D) The increased Ca^{2+} triggers switches to increase the production of Hsps and protect against cellular damages.

In the near future, it might be possible to control the production of Hsps in cells/tissues by carefully exposing them to limited amounts of EMW. For this purpose, the Raman spectrometer could help to detect the oxidized proteins (S-S bonds) in cells of the whole body in real time.

CONFLICT OF INTERESTS

The Authors have no conflicts of interest to declare.

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