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Review article

Heat-induced inflammation and its role in esophageal cancer

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Esophageal cancer, the sixth most common cause of death from cancer worldwide, consists of different histological types and displays various patterns of incidence. Esophageal adenocarcinoma and esophageal squamous cell carcinoma are the most prevalent types. As epidemiological studies report that ingesting hot substances is one major risk factor for squamous cell carcinoma, evaluating the effect of this external stress on esophagus cells seems desirable. This specific kind of stress brings about cellular changes and stabilizes them by affecting different cellular features such as genetic stability, membrane integrity and the regulation of signaling pathways. It also causes tissue injury by affecting the extracellular matrix and cell viability. Thus, one of the main consequences of thermal injury is the activation of the immune system, which can result in chronic inflammation. The genetic alteration that has occurred during thermal injury and the consequent reduction in the function of repair systems is further strengthened by chronic inflammation, thereby increasing the probability that mutated cell lines may appear. The molecules that present in this circumstance, such as heat shock proteins, cytokines, chemokines and other inflammatory factors, affect intercellular signaling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells, signal transducer activator of transcription-3 and hypoxia-inducible factor 1α in supporting the survival and emergence of mutant phenotypes and the consequent malignant progression in altered cell lines. This investigation of these effective factors and their probable role in the tumorigenic path may improve current understanding.

KEY WORDS: esophageal neoplasms, genetic alteration, heat shock proteins, heat stress, inflammation.

INTRODUCTION

Esophageal cancer is the eighth most common cancer and the sixth most common cause of cancer death in the world.[1,2] A geographical area called the Asian belt of esophageal cancer, which stretches from northern Iran to north-central China, has the highest prevalence rate of esophageal cancer. Southeast Africa, parts of South America and western Europe are intermediate-risk areas for esophageal cancer. Other parts of the world have low rates of incidence. Esophageal cancer is more common in men, but in the Asian belt of esophageal cancer the ratio between men and women is equal.[1,3,4]

Most esophageal cancers (90%) are either esophageal squamous cell carcinoma (ESCC) or esophageal adenocarcinoma (EAC). The varying prevalence rate for distinct subtypes of esophageal cancer means that, although recent studies have shown that EAC has increased and ESCC has decreased in Western countries,[5,6] ESCC still comprises more than 90% of all esophageal cancers in the developing world.[7,8] As

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more than 80% of cases still occur in the developing countries,[8] surveying the risk factors for ESCC and their possible molecular and cellular foundations is of great interest.

A complex set of risk factors (environmental, genetic and cultural) may increase an individual's chance of developing esophageal cancer. These factors can increase irritation, inflammation and mutation in the esophagus.[9] Environmental factors are most often discussed in the origin of ESCC, including the consumption of very hot beverages and food,[9] a low intake of fresh fruit and vegetables, alcohol consumption, cigarette smoking and low socioeconomic status[1,10]. Recently, two meta-analyses showed that the consumption of hot beverages and food were significantly associated with ESCC.[11,12]

These data and previous findings on the altered expression profile of cells under heat stress persuaded us to focus on the effect of heat shock on this pathway.[13] It is conceivable that hot beverages and food may cause thermal injury to the esophageal mucosa, and there are various biological mechanisms by which thermal injury could increase the risk of esophageal cancer. It appears that chronic heat stress can activate inflammatory processes through chronic irritation of esophageal mucosa. Therefore, in this review we attempted to discern the relationship between heat stress and the role inflammation plays in the tumorigenesis of esophageal cancer.

HEAT STRESS

Hot substances can significantly affect the esophageal epithelia due to their temperature on ingestion,[11] although it is modulated by the oral cavity so that esophagus itself is exposed to temperatures that are well below that of the ingested liquid. Hot beverages at temperatures ranging 55–65°C increase distal esophageal temperatures by an average of 5–12°C; therefore, beverages that are up to 80°C may expose the esophageal epithelium to temperatures as high as 58°C (range 48–58°C). The data extracted from experiments that were performed in regions with a high prevalence of ESCC support the view that consuming substances that are hotter than 65°C have the potential to damage the tissues of upper gastrointestinal (GI) tract.[14–16] This effect of food temperature can be separated from its consequences on the cells and tissues of esophageal epithelia.

Physiological response

Many physiological processes and structural components involved in a living cell are affected by increased environmental temperatures. Heat changes both the fluidity and permeability of membranes, which are important properties of plasma membrane. These changes result in an increased flux of ions and a decreased binding rate of the membrane's proteins.[17,18] The permeability of other intracellular membranes is also altered. Among these changes, the increased proton permeability of mitochondrial membranes may contribute to adenosine triphosphate (ATP) depletion, which inhibits ATP-driven processes, including the cell cycle and cytoskeleton stability.[18] Other properties of the membrane affected by heat are the structure of rafts and distribution of membrane proteins, leading to the modified activity of potential signaling proteins that sense stress in these subdomains.[17]

Heat shock factor (HSF)-1 is a main known transactivator of heat shock response, integrating signals derived from plasma membrane and organizing the expressions of different heat shock proteins (HSP).[17] HSP are generally expressed at low levels,[19] but during a period of hyperthermia and shortly thereafter they become the predominant proteins synthesized by the cells[20]. All HSP families share a chaperoning function and are present in the cytosol, mitochondria, endoplasmic reticulum and nucleus.[21,22] Intracellular HSP, including HSP27, HSP70 and HSP90 play critical roles in preventing protein aggregation, preserving receptor interactions, cellular rescue and the induction of cell death pathways.[23] During heat shock response these molecules inhibit DNA synthesis and alter transcription and RNA processing and translation. Moreover, by conserving the stability of vital proteins, HSP increase the degradation of other proteins through both proteasomal and lysosomal pathways, thus conducting the resulting energy to the production of requisite proteins.[20] Consequently, these different processes, which are managed by HSP, lead to cell rescue and cell cycle arrest. However, acute heat shock and chronic heat treatment may lead to type-specific forms of cell death.[18]

Genetic and epigenetic responses

Heat shock conditions can completely change the regulation of different genes in cells. HSF-1 plays an important role in activating heat shock response and managing gene regulation. Although this highly conserved molecule is present in both the cytoplasm and

nucleus in monomeric form, it has no DNA-binding activity.[24] In response to heat shock it assembles into a homotrimer structure that binds to the major groove of cis-acting DNA promoter elements called heat shock elements.[25] It can then initiate the assembly of the transcription machinery and increase the concentration of HSP.

At the translational level, the inhibition of protein synthesis has been found to be mediated by reversible changes in the formation of eukaryotic initiation factor subunit complex and the phosphorylation of proteins involved. These processes reduce the energy dedicated to the production of domestic proteins, thus increasing critical messenger-RNA (mRNA) transcription and permitting a rapid change in the complement of proteins synthesized in the cells.[26] HSP mRNA is translated during heat shock because of their low recruitment to eukaryotic initiation factors or other alternative mechanisms that require sequence elements in their 5′-untranslated region (UTR).[27]

A high temperature can also affect double-stranded DNA directly. Its impact has been discerned through an examination of micronuclei, which is a marker of DNA double strand breaks that increases not only in heated cells but also in surrounding and co-cultured unheated cells.[28] Moreover, heat stress can induce the mutagenesis of long trinucleotide repeats in human cells via the DNA re-replication mechanism.[29] The point here is that in normal cells repair systems fix DNA damage, but in heated cells the repair system function is inhibited or impaired, leaving the damage in the genetic material of the cells. This reduced function is a result of the decreased expression or degradation of the proteins that are involved in different repair processes such as the base excision repair (ogg1), nucleotide excision repair (xpg), double-strand break repair system (RAD54, RAD51, BRCA2) and other proteins that are involved in final stages of the repair process (DNA polymerase β, DNA ligase III).[30,31]

When these DNA repair systems operate inappropriately, the accumulation of endogenous DNA damage can open a window for more permanent cellular changes in the future.[28,32] Severe damage to the esophagus makes the stem cells of this tissue divide to produce new cells and to repair the damage. The more often hot drinks are ingested, the more stem cell division and more DNA alterations occur.[33]

Epigenetic regulation is another factor that changes during heat shock, at least transiently, and it can affect genomic stability by modifying chromatin configurations.[34] Heat shock can trigger a transient alteration of the epigenetic program and the assembly of the higher order structure of specific heterochromatic regions, which leads to the transcription of silent portions of the genome. These changes may lead to the assembly of new nucleolar districts called stress bodies, which are formed by the heterochromatic regions of chromosomes 9, 12 and 15. These bodies are the location of accumulation of HSF-1 and may be involved in modulating epigenetic changes during heat shock.[35]

Another epigenetic alteration is nucleolar perturbation and the disruption of Cajal bodies. It has been shown that the multifunctional nucleolus senses stress as a central hub for coordinating the stress response. The disruption of the nucleolus in response to increased temperature leads to a stabilization of the level of p53, which is a key molecule in cellular arrest or apoptotic pathways. Furthermore, alteration in Cajal bodies that are closely linked to the nucleolus leads to a decrease in RNA processing and cellular metabolism. It seems that at this stage these stress bodies are involved in transcription and splicing regulation. Hence, according to the seriousness of the stress, cells will face different destinies, from the arrest of the cell cycle to the p53-dependent apoptotic pathway.[36–38]

Histological modifications

Epithelia in the GI tract are classified into three groups: leaky, moderately leaky and tight.[39] The esophageal epithelia in the third group constitute a multilayered, non-keratinized stratified squamous epithelium with different layers: the stratum corneum, which constitutes of several layers of flat cells; the stratum spinosum, which constitutes of several more layers of actively transporting cells and the stratum (basalis) germinativum, including a single or double cell layer of mitotically active cells. The cells of the luminal layers, the stratum corneum, provide defense in the form of a permeability barrier.[40]

The paracellular barrier is mainly formed of tight junctions. A mild increase in temperature (37–41°C) creates a significant disturbance to these junctions, leading to increased epithelial permeability.[41] A deficient barrier caused by heat increases the risk of esophageal damage by subsequent contact with refluxed gastric acid,[14] intraluminal carcinogens,[11] bacterial translocation[42] and other noxious substances[43]. Heat also affects other types of intercellular

junctions, including Gap junctions and transporter proteins.[44] For example, an experiment performed on the rabbit esophagus found that heat reduced short-circuit current (*I*sc), which reflects the active transportation of ions across the epithelium predominantly due to the sensitivity of active sodium-positive transporters to temperature.[14]

Extracellular matrix (ECM) components such as collagen are also affected by heat, which can rupture the stabilizing intramolecular and molecular cross-links of their fibers.[45] It has been observed that under moderate heat stress most of these structures, both the junctions and the esophageal cancer component, are overexpressed by the mediation of HSP and HSF molecules.[41,46,47] These changes take place to protect the tissues and increase tolerance to the heat. However, under severe heat shock (up to 56°C) widespread cellular injury and necrotic cell death have been noted.[48,49] Necrosis, as a key player in the activation of the immune system, is capable of producing extensive inflammation[50] through different mechanisms, such as activating dendritic cells and eosinophils[49] and releasing inflammation mediators like HSP[48] and high mobility group box 1 molecules[51]. In animal experiments that when tissue is exposed to heat injury, some carcinogens have been shown to interfere with epithelial regeneration and cause the recurrence of thermal injury and inflammation.[52]

Overall, it seems that the exposure of the esophagus to heat stress can lead to mutations, weaken the performance of repair systems and increase cell division, which can eventually lead to the appearance of mutated cell lines. Moreover, heat alters the components of ECM, hence making it susceptible to more damage from other causes. Finally, this damage, along with the necrotic response of the tissue, recalls the immune system to the injured region and makes the situation more complicated.

INFLAMMATION

Inflammation is the host response to tissue injury with a complex network of cellular reactions and chemical signals. Inflammation is normally self-limiting but under certain circumstances, such as the existence of chronic external danger signals, it can become chronic.[53]

Innate and adaptive immune systems strike out to eliminate the damage although these systems themselves will also be affected by hyperthermia; therefore, the response and alteration of these two systems through cellular and humoral branches act with and upon each other, boosting the effect of each.

Humoral innate immune system components and the humoral branch of adaptive immunity are involved in recognizing danger and are one of the first-line systems to deal with tissue injury.[54] This system can detect immunological danger in form of damage-associated molecular patterns (DAMPs), which have general metabolic consequences of inflammation, such as the recruitment of leukocytes and plasma proteins from the blood and activating leukocytes and plasma proteins. DAMPs are upregulated and released during the cell lysis and tissue damage. Well-characterized DAMPs include the high mobility group box 1 (HMGB1) protein, HSP and uric acid;[55] which has already been mentioned regarding extracellular presence after heat injury.

Another function of this system is to secrete cytokines, which are critical for the acute inflammatory response. Three of most important pro-inflammatory cytokines of innate immune system are tumor necrosis factor (TNF), interleukin (IL)-l and IL-6. These cytokines are mainly produced by tissue macrophages and mast cells, which are parts of the cellular innate immune response. Furthermore, other cell types, including endothelial and epithelial cells, can also participate in this process.[54] HSP themselves, especially HSP60, HSP70, HSP90 and gp96, can activate the secretion of proinflammatory cytokines (IL-1b, IL-6, TNF-α) from monocytes, macrophages and dendritic cells via the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) pathway.[56,57]

Thermal injury disturbs the internal milieu, which leads to the release of endogenous danger signals and consequently leads to a defensive response of immune cells. In the affected area, two groups of cells are involved in inflammatory response: the epithelial cells that line the GI tract and the immune cells that are present at the injured tissues.[55] The first group of cells can activate the second and in addition cooperate with them to develop a suitable reaction. Immune cells involved in inflammation include macrophages, dendritic cells, mast cells, neutrophils, eosinophils, natural killer cells, natural killer T cells and lymphocytes.[54] Among the cells that are involved in the immunological response, the cells of the innate immune system play a more prominent role against injury than adaptive immune cells. In this regard, the focus is on the cellular components

reacting in the case of thermal injury and tissue damage in the esophageal epidermis.

Neutrophils are the first cells that emerge at sites of infection or injury and play a key role in the inflammatory response.[58] In non-resolving chronic inflammation, as in this situation, the recruitment of neutrophils may continue over months or years.[59]

Monocytes, which are always present in blood flow, upregulate due to thermal injury as a result of a rise in the chemokines and chemoattractant proteins in the spleen, resulted in increased levels of inflammatory monocytes.[60] Consequently, macrophages, which are differentiated form of monocytes, accumulate at the site of inflammation to clear cellular debris after tissue necrosis. In this case, the debris from necrosis is filled with endogenous danger signals, such as HSP, nuclear proteins (including HMGB1), histones, DNA and other nucleotides and components of ECM. The phagocytosis of these molecules by macrophages leads to considerable changes in their physiology, including changes in the expression of surface proteins and production of more cytokines and proinflammatory mediators in this region.[61]

Dendritic cells are the most potent antigen-presenting cells and have an important role in initiating innate and adaptive immune responses. They are matured and activated indirectly by inflammatory mediators as well as the HSP that are released in the extracellular milieu.[62,63] The activation of dendritic cells results in the increased expression of co-stimulatory molecules and leads to the production of immunomodulatory cytokines. All these alterations have a profound effect on T-cell priming and differentiation.[64] Studies have shown that it is not only necrotic cells that can activate dendritic cells, stressed apoptotic cells also have a stimulatory effect on them.[62]

Beside the effects that heat has on the innate immune system, it temporarily suppresses the adaptive immune response. The most prominent alteration, as has been shown in numerous studies and clinical observations, is a reduction of the function and cytokine production of T helper 1 after thermal injury, which is one of the initiators of the adaptive response and leads to a reduced performance of the adaptive immune system.[65,66] As the most efficient response against abnormal cells is mediated mainly by T lymphocytes,[54] their reduced function may help mutated and transformed cells that arise during heat shock to survive.

The immune system is totally involved in fighting to heal–injured tissues and eliminate danger signals. In this way, it may even cause some collateral damage. The continual exposure of tissue to heat shock can lead to chronic inflammation that makes an inflammatory microenviroment together with the enhanced function of innate immunity and the reduced performance of adaptive immunity, which provide opportunities for the survival and growth of transformed cells in the injured tissues.

MALIGNANT TRANSFORMATION

During recent decades, accumulating evidence has firmly established the relationship between inflammation and cancer. The underlying role of inflammation in carcinogenesis is due to its mutagenesis-enhancing capacity that can increase the risk of tumor initiation. Moreover, a micro-inflammatory environment can assist tumor promotion and progression with its complicated signaling network.[67,68]

Moreover, heat shock contributes to the process of tumorigenesis even without considering its role in inflammation initiation. It can damage DNA and affect epigenesis and structural molecular defects. The HSP family members, which are the major proteins during heat shock, play essential and overlapping roles in both the promotion of autonomous cell proliferation and the inhibition of death pathways.[69] Thus, the roles of inflammation and heat shock are discussed separately and in relation to each other in different stages of tumorigenesis.

Tumor initiation

In the process of tumor initiation, normal cells acquire the first mutational hits that steer them to the tumorigenic path by providing them with both growth and survival advantages over their neighbors. To do so each cell must transmit these mutations to its offspring and, in cancers within rapidly renewed epithelia, mutations must occur in long-lived stem cells or in transient amplifying cells instead of differentiated cells that are rapidly eliminated before next mutation.[70]

As stated, heat exposure can damage DNA and impair repair systems, thus leading cells towards undesirable changes. Inflammation can also play part in tumor initiation by activating phagocytes (neutrophils, monocytes and macrophages), which produce a large number of reactive oxygen species (ROS) and reactive nitrogen intermediates.[71] ROS can induce different

forms of DNA damage, including strand breaks, base modifications and cross-links that result in replication errors and consequently genomic instability.[72,73] The affected cells endeavor to repair this damage but with the interference of inflammation repair remains defective and damage to the cellular line is established.

Inflammation disturbs DNA repair systems through different mechanisms, predominant among which is the increase in free radicals and cytokines that alter the expression and function of the proteins involved. Free radicals enhance global methylation, which may in part lead to the silencing of mismatch repair and nucleotide excision repair pathway proteins. Furthermore, these radicals contribute to the degradation of enzymes involved at the protein level. Cytokines (e.g., IL-6) also decrease the expressions of repair pathway proteins by increasing the amount of methylation.

The production of inflammatory factors and the hypoxic condition in the microenvironment causes an elevated expression of hypoxia-inducible factor 1α (HIF-1α), which is the primary sensor of tumor cell hypoxia. This transcription factor can also affect repair pathway proteins. HIF-1α downregulates these proteins via inducing the expression of microRNA (miRNA) that destroy transcribed mRNA.[74]

The expression of mismatch repair proteins decreases by the displacement of c-Myc from its promoters by HIF-1α and prostaglandin E2.[75] Moreover, free radicals, which are produced by inflammatory cells, can affect mismatch repair proteins by silencing their promoters and damaging them at the protein level.[76,77] Another pathway that appears to be affected is the nucleotide excision repair pathway. It has been noted that IL-6, which induces hypermethylation, can defeat the function of key nucleotide excision repair components. Alternatively, HIF-1α can downregulate the expression of nucleotide excision repair components by inducing miRNA. Base excision repair enzymes may also be implicated, as has been reported in colon cancer.[75]

Along with these changes, HSP, especially HSP90, stabilize the conformation of transformed proteins that emerge during mutations such as Bcr-Abl, v-Src and p53. By allowing transformed proteins to accumulate HSP new phenotypes are able to arise.[69]

In general, esophageal cells exposed to heat shock and consequent inflammation are prone to chromosomal mutations under the influence of both factors. As heat stress and inflammation attenuate the repair systems through different mechanisms, the mutations become stabilized and when tissue regeneration begins, the cells that maintain DNA damage survive, proliferate and produce mutated cell lines.[53]

Tumor promotion and progression

Tumor promotion can be defined as the process of tumor growth from a single original cell to a larger population of cells that risk accumulating more genetic changes. In the progressive phase the primary cancerous cells proliferate increasingly and block the inhibitory signals. To do this HSP molecules and different inflammatory cytokines and chemokines affect transcription factors and commence the promotion of the tumor.[70] Tumor progression is characterized by the emergence of malignant phenotypes and invasive tumor cells. Angiogenesis, invasion and metastasis are the key processes in this step and they are all are closely associated with the inflammatory microenvironment. Inflammatory cells, cytokines and involved transcription factors can augment angiogenesis, the degradation of ECM and epithelial-mesenchymal transition which is the crucial step of metastasis.[78–82]

Any of the effector molecules can play a role in the different stages of the promotion and progression of cancer, and their impacts are briefly reviewed in the following sections.

HSP

Heat shock due to the increased expression of HSP can effectively worsen malignant features in a primary mass. HSP can reinforce the autonomy of the cell in growth, which is the first hallmark of cancer.[83] As a major molecular chaperone HSP90 generally stabilizes the structure of proteins and acts as subversive agent during tumorigenesis, allowing malignant transformation by maintaining the active conformation of defective proteins. The stabilization of overexpressed and mutated proteins such as protein kinases, receptors and transcription factors thus contributes to aberrant signaling cascades and facilitates rapid growth in transformed cells.[84,85] HSP also help the limitless proliferation of tumor cells by stabilizing the telomerase enzyme that elongates telomere, which is a key regulator of the number of divisions.[86,87]

HSP help transformed cells to evade both fast (programmed cell death) and slow (senescence) cell death

pathways. HSP70 and HSP27 inhibit caspase-dependent apoptosis by affecting mediator molecules.[88,89] In another way, HSP70 blocks another cell death pathway that is mediated by autophagy.[90] These blockages in precancerous cells lead to a proportion of such cells dying by necrosis, which not only decreases the efficacy of death pathways but also leads to the initiation of secondary inflammation.[91,92]

HSP also play an indirect role in the accumulation of key factors in angiogenesis.[69,89] In the cells that lead to cancer progression, HSP stabilize HIF-1α, thus inducing downstream molecules in this pathway. Moreover, HSP90 is capable of stabilizing downstream molecules (i.e., vascular endothelial growth factor [VEGF] and nitric oxide synthase) which augment the cascade of signaling.[93,94]

Furthermore, it has been shown that HSP contribute during the final stages of cancer as well. Although their functional mechanisms during these processes are not completely known, it has been reported that the increase during their number is related to the enhanced capacity of invasion and metastasis[95,96] (Fig. 1). In brief, it can be stated that overexpressed HSP molecules during heat shock can encourage nearly all the features of cancer cells.

Cytokines and chemokines

Inflammatory cytokines, which are mainly produced by immune cells, provide exogenous survival factors for primary and fully established cancer cells.[97] These molecules are capable of inducing growth signals in peripheral cells and with this ability they also affect malignant cells and activate oncogenic transcription factors.[98] TNF-α and IL are among the major cytokines secreted by inflammatory cells. TNF-α secretion, which is induced by a wide range of danger signals, acts as a stimulator for other inflammatory mediators at the site of the lesion.[99,100] Moreover, it has been reported to induce the generation of angiogenic and lymphangiogenic growth factors.[53,101] So far, TNF-α polymorphism has been shown to increase the risk of ESCC, suggesting that this molecule plays a role in

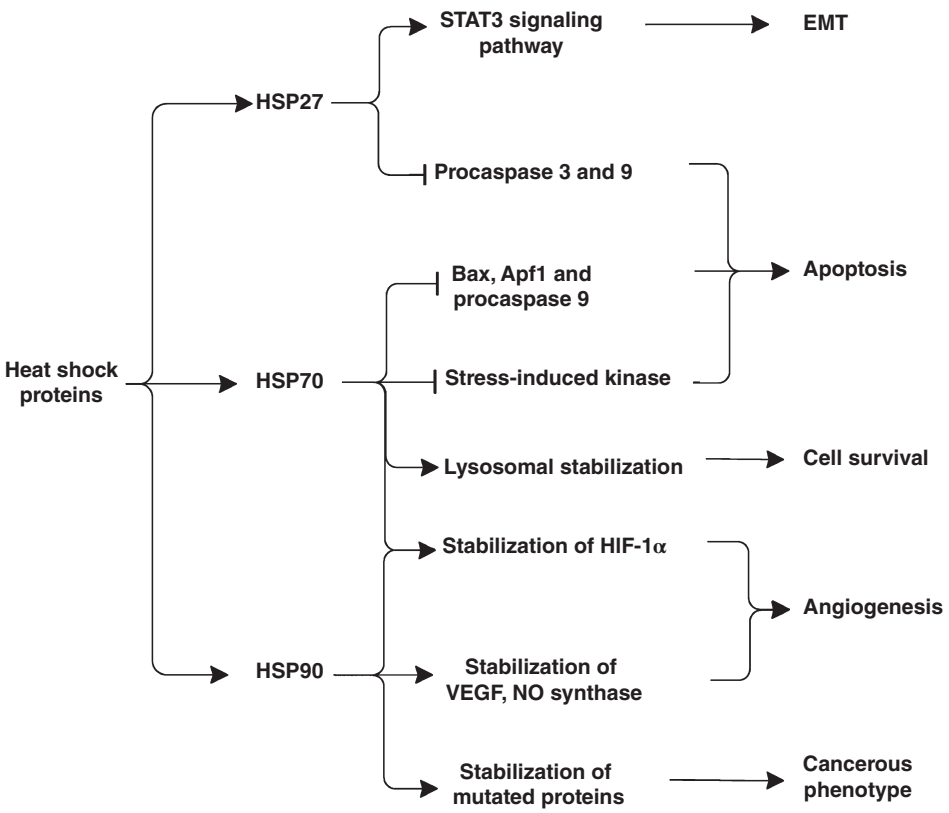

Figure 1. Alongside released heat shock proteins (HSP) from a necrotic cell that can augment secondary inflammation, increased HSP in cells can provide the features required for cancer cell progression. Cell survival and the exhibition of cancerous phenotypes can assist the first steps of tumorigenesis while apoptosis inhibition, epidermal-mesenchymal transition (EMT) and angiogenesis are the characteristics of cancer that has progressed. HIF, hypoxia-inducible factor; NO, nitric oxide; STAT3, signal transducer activator of transcription-3; VEGF, vascular endothelial growth factor.

developing cancer.[102] ILs are another large subset of cytokines that regulate cell cycle, differentiation and cellular motility. Among them, IL-6 and IL-8 inhibit apoptosis and promote angiogenesis by affecting the intercellular signaling pathway and the induction of matrix metalloproteinase (MMP) synthesis, respectively. In addition, the elevation of these two ILs has been observed in ESCC tissues.[103–105] Finally, HSP molecules, which are released in the injured tissues during heat stress, are also capable of cytokine induction.[106,107] Therefore, inflammatory stimulators on one side and continuous heat stress on the other lead to the production of cytokines at the site of injury. Ultimately, these molecules mediate promotion, angiogenesis and invasion in the incipient tumor colonies.

Chemokines are soluble chemotactic cytokines that play a central role in the recruitment of leukocytes at the site of inflammation. Several studies have noted the involvement of chemokines and chemokine receptors in cell proliferation and preneoplastic transformation.[101,108] For example, CXC chemokine ligand 12 (CXCL12), which is secreted by stromal cells, can promote angiogenesis through the activation of MMP-9.[109] They can also directly stimulate the migration of malignant cells towards blood vessels.[80] Furthermore, one report declared that some important chemokine receptors, such as CXCR1, C-C chemokine receptor type 9 (CCR9) and CCR1, which are evolved in metastasis, show high expression in esophageal cancer cells.[110] In total, a connected network of cytokines and chemokines affect precancerous cells and support their progression. However, more evaluations are needed to clarify their complicated role in ESCC.

Transcription factors

Transcription factors, which are the main target of environmental stimulators, play an important role as endogenous promoters in chronic inflammation cascades. Among them, NF-κB, signal transducer activator of transcription-3 (STAT3) and HIF-1α are the most extensively investigated.[111] These factors are interrelated, thus creating a complex regulatory network within cells.

NF-κB is a dimeric transcription factor with an essential role in the inducible expression of cytokines such as TNF, ILs and chemokines, in addition to cyclooxygenase (COX)-2 and MMP-9.[101,112] The constitutive activation of NF-κB has also been found in cancers, suggesting that it has a crucial role in boosting the development of inflammation in cancer.[113] Its activation leads to transcription of the target genes coding for cytokines, enzymes and adhesion molecules that are involved in apoptosis, proliferation, inflammation and tumorigenesis.[114] Cytokines from cancerous cells further stimulate the transcription of proinflammatory genes via NF-κB in tumor cells and surrounding cells, thereby creating a sustained chronic inflammatory situation within the tumor microenvironment.[108]

STAT3, a member of the STAT family, participates in normal cellular responses to growth factors and cytokines. This molecule lodges in the nucleus as a response to cytokines and targets different genes that contribute to cell cycle regulation (cyclin D1 and D2), cell survival and angiogenesis (VEGF).[80,115]

HIF-1α is a transcription factor that belongs to the basic helix-loop-helix family and regulates a wide range of genes.[116] It has previously been reported that at sites of inflammatory lesions, hypoxia commonly occurs as the result of metabolic shifts within the inflammatory microenvironment.[108] Inducible nitric oxide synthase, COX-2, glucose transporters and some glycolytic enzymes and chemokines are stimulated by HIF-1α and play role in promoting inflammatory reactions.[71,117] However, the most important feature of HIF-1α is its major regulatory role in the activation and consequent neovascularization of VEGF.[118] As a final point, remarkably increased expression of HIF-1α participates in Barrett's metaplasia–dysplasia–adenocarcinoma sequence, which may be mediated by inflammation[119] (Fig. 2). While all these major signaling pathways interact with each other and make a network that leads to the progression of cancer cells it would be very desirable to identify the most effective pathway.

Other relevant factors include the fact that MMPs are a family of enzymes that degrade components of the ECM and basement membrane. Heat, cytokines and chemokines induce stromal and inflammatory cells to produce these enzymes, which have a low expression in normal tissue, at the site of injury. MMP acts as a double-edged sword that can both promote and attenuate tumor progression. They can recruit inflammatory cells by releasing chemoattractants and generating growth-promoting and cytostatic signals. They also have both apoptotic and anti-apoptotic actions. Likewise, they can both activate angiogenesis and produce angiogenesis inhibitors.[120,121] However, the overexpression of some MMPs such as MMP2, MMP7 and MMP9, suggests that their role in the development of

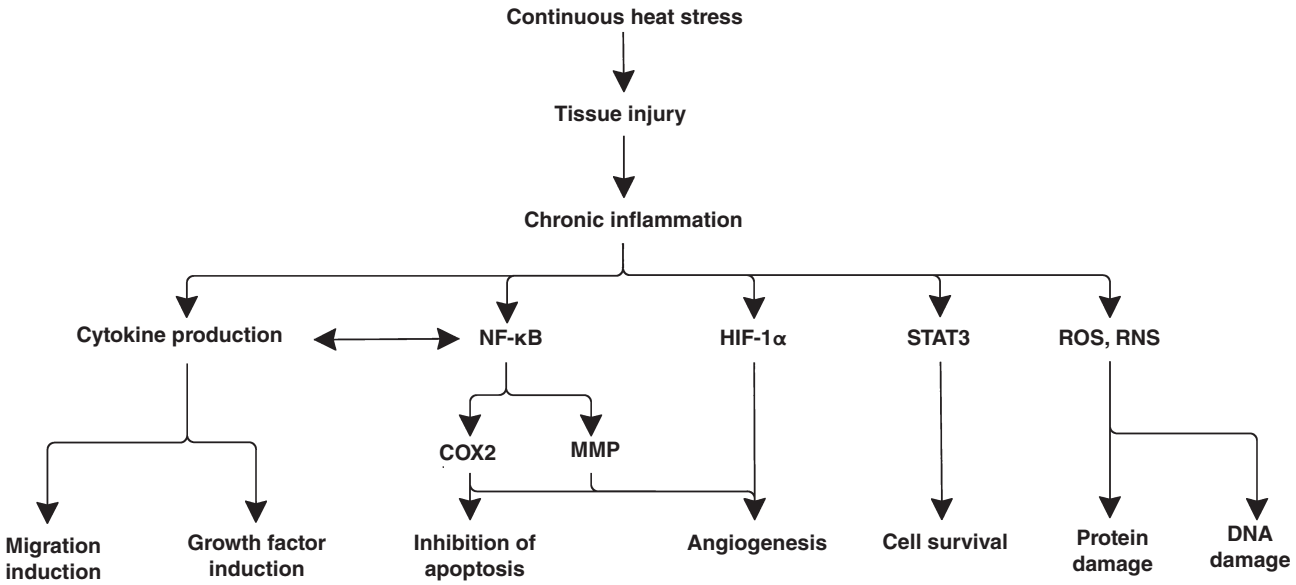

Figure 2. Chronic inflammation created by consecutive heat stress provides the conditions for the initiation and progression of a cancerous mass. COX-2, cyclooxygenase 2; HIF-1α, hypoxia-inducible factor 1α; MMP, matrix metalloproteinase; NF-κB, the nuclear factor kappa-light-chain-enhancer of activated B cell; ROS, reactive oxygen species; RNS, reactive nitrogen intermediates; STAT3, signal transducer activator of transcription-3.

ESCC may be more prominent than hitherto suspected.[122,123]

Prostaglandins such as prostaglandin E2 are lipid mediators of the inflammatory immune response derived from the oxidative metabolism of arachidonic acid. They can contribute to many mechanisms, including stimulating cell proliferation, producing cytokines (e.g., IL-6), forming mutagenic by-products (e.g., malondialdehyde), inducing angiogenesis, inhibiting apoptosis through the expression of anti-apoptotic oncogenes (e.g., bcl-2) or removing pro-apoptotic factors (e.g., arachidonic acid).[124] It has also been shown that the production of prostaglandin E plays a crucial role in regulating HIF-1 during hypoxia.[125]

Two different COX enzymes catalyze the rate-limiting first step in prostaglandin synthesis.[126] Among them, COX-2 is expressed in inflammation[124] and it is thought to be the target downstream protein in most chronic inflammation pathways. Proinflammatory cytokines, among which are IL-1, TNF and growth factors such as epidermal growth factor, induce COX-2 expression.[113] COX-2 facilitates some mechanisms including the inhibition of apoptosis, the modulation of motility and cellular adhesion and the progression of angiogenesis and immunosuppression.[118]

It has been experimentally shown that COX-2 expression can be upregulated during the early stages of ESCC and its inhibition may be effective in the prevention of proliferation and tumorigenesis in cancerous tissue.[127] However, another experiment showed that COX-2 expression was significantly higher in adenocarcinoma than in squamous cell carcinoma.[128]

MiRNA

In the field of epigenetics, miRNAs have emerged as a newly identified class of gene expression regulators. They constitute a large family of non-coding, small-size (19–22 oligonucleotides) and gene-silencing RNA, which negatively regulate gene expression via mRNA degradation and translational repression. Experiments have shown that being exposed to recurring high temperatures alters the expression pattern of miRNAs.[129] However, due to the context-dependent expression of miRNA there have been few studies in this field. On the other hand, the presence of cytokines and inflammatory mediators in the milieu can also affect the expression of miRNA.[130] As these molecules are intracellular regulators that affect cellular balance, growth and differentiation, changes in their expressions can cause unwanted consequences.

Although the relationship between the alteration of miRNA expression and tumorigenesis is just starting to be explored,[67] a growing body of evidence

suggests that these molecules may play a significant role in cancer progression.[131,132] Reviewing studies in the field of miRNA suggests that some of the miRNA that are upregulated due to heat stress and inflammation are involved in squamous cell carcinoma, which may indicate a relationship between these factors and the development of cancer. For example, it has been shown in esophageal cancer that miR-21 expression, which is regulated by IL-6 and is also upregulated under heat stress, acts as an oncogene by inducing cell proliferation and invasion.[133–135] MiR-9, miR-155, miR-125b and miR-34b are also among the group that is involved in squamous cell carcinoma and show deregulation under heat shock and inflammatory conditions. However, given the complicated nature of miRNAs, their function requires further investigation for better understanding.[130,136,137] This limited evidence piecing together the role of miRNA in linking heat shock, chronic inflammation and cancer points to a novel and promising field for research.

In conclusion, heat stress is considered to be one of the risk factors in the etiology of esophageal cancer. In May 2016 a working group of scientists from 10 countries meeting at the International Agency for Research on Cancer to evaluate the carcinogenicity of ingesting very hot beverages noted that there is a positive association between high temperatures (65°C or above) and ESCC and this factor was classified as being probably carcinogenic to humans, although the mechanisms have not yet been identified.[138] According to the evidence collected, heat stress contributes to significant tissue damage by affecting intercellular junctions and the ECM, by altering signaling systems, disrupting the ion balance and, finally, imposing genome damage and impairing repair systems.[41,45,139] This injury also permits other chemical carcinogens to have a marked effect and to expose tissue to recurrent inflammation.[52] The chronic exposure of tissues to heat, while bringing about the abovementioned effects, can also ultimately lead to the appearance of cells with genomic instability and also cause other cells, which suffer unbearable injuries, to die by necrosis. This kind of cell death on a considerable scale can trigger inflammatory responses.

Although the inflammatory reactions themselves can result in anti-tumor activities, the mechanisms that trigger a clear inflammatory response against the protumor actions of the dark side remain to be determined.[75] Although there is limited evidence, the inflammation induced by consistent danger signals can be converted to chronic form, causing the development of mutagenic substances such as ROS, reactive nitrogen intermediates, cytokines and chemokines. These inflammatory mediators lead to genomic alterations and render repair systems in damaged cells ineffective and, together with the detrimental effect of heat, take the first steps toward tumorigenesis. The recruitment of signaling pathways such as NF-κB, under the influence of inflammatory mediators, finally disrupts the gene expression balance in favor of tumor development and progression.

It has been shown that inflammation plays a role in every stage of cancer development including its initiation, promotion, invasion and metastasis.[70] The parallel effect of heat shock and HSP in this process suggests there is a positive feedback loop among heat, inflammation and cancer.[67] However, more experiments are needed to determine the exact mechanisms and direct relations involved due to complex nature of both the heat shock response and inflammation.[111] The identification of the effective agents in this mechanism can lead to improvements in diagnostic and treatment strategies. This pattern may also be useful for other cancers in the upper GI tract that are also exposed to hot substances and show a positive links to thermal injury.[140]

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