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Stem Cells and Cell-Cell Communication in the Understanding of the Role of Diet and Nutrients in Human Diseases

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ABSTRACT – The term, "food safety", has traditionally been viewed as a practical science aimed at assuring the prevention acute illnesses caused by biological microorganisms, and only to a minor extent, chronic diseases cause by chronic low level exposures to natural and synthetic chemicals or pollutants. "Food safety" meant to prevent microbiological agents/toxins in/on foods, due to contamination any where from "farm to Fork", from causing acute health effects, especially to the young, immune-compromised, genetically-predisposed and elderly. However, today a broader view must also include the fact that diet, perse (nutrients, vitamins/minerals, calories), as well as low level toxins and pollutant or supplemented synthetic chemicals, can alter gene expressions of stem/progenitor/terminally-differentiated cells, leading to chronic inflammation and other mal-functions that could lead to diseases such as cancer, diabetes, atherogenesis and possibly reproductive and neurological disorders. Understanding of the mechanisms by which natural or synthetic chemical toxins/toxicants, in/on food, interact with the pathogenesis of acute and chronic diseases, should lead to a "systems" approach to "food safety". Clearly, the interactions of diet/food with the genetic background, gender, and developmental state of the individual, together with (a) interactions of other endogenous/exogenous chemicals/drugs; (b) the specific biology of the cells being affected; (c) the mechanisms by which the presence or absence of toxins/toxicants and nutrients work to cause toxicities; and (d) how those mechanisms affect the pathogenesis of acute and/or chronic diseases, must be integrated into a "system" approach. Mechanisms of how toxins/toxicants cause cellular toxicities, such as mutagenesis; cytotoxicity and altered gene expression, must take into account (a) irreversible or reversal changes caused by these toxins or toxicants; (b)concepts of thresholds or no-thresholds of action; and (c) concepts of differential effects on stem cells, progenitor cells and terminally differentiated cells in different organs. This brief *Commentary* tries to illustrate this complex interaction between what is on/in foods with one disease, namely cancer. Since the understanding of cancer, while still incomplete, can shed light on the multiple ways that toxins/toxicants, as well as dietary modulation of nutrients/vitamins/metals/ calories, can either enhance or reduce the risk to cancer. In particular, diets that alter the embryo-fetal micro-environment might dramatically alter disease formation later in life. In effect "food safety" can not be assessed without understanding how food could be "toxic", or how that mechanism of toxicity interacts with the pathogenesis of any disease.

Key words: Food safety, human adult stem cells, gap junctional communication, epigenetic toxicity, fetal micro-environment, systems nutrition

"For, make no mistake, there will be no reward for a program of cancer prevention. We can not hope to sell it, and in fact, I expect we will have a difficulty in giving it away." [1] V. P. Potter

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Introduction: What is Meant by Food Safety? Should we Eat Our Hamburger Rare or Well-done?

While the answer might seem self explanatory, when one reviews the literature, not only does one see many very different definitions, but also a bias, depending on one's discipline. The "causes" affecting the safety of foods might be seen to be microbial-derived, environmental chemically-derived, genetically-, developmentally-or culturally-associated". This "*Commentary*" assumes that to recognize a food's "safety", one must understand its potential "toxicity", in the context of the individual exposed to the food. Food safety must be done in the context of a complex integration of information. Given that foods are composed of microbes, thousands of natural chemicals (shaped by the genes of the plants/animals); the environment to which the plant/animal was exposed; how the plant/animal was handled, processed, packaged, transported, cooked, eaten; who ate the food; and what else was present/absent when food was eaten, food safety was determined by the discipline charged to make that assessment.

Microbiologists consider food safety as devoid of microbial contamination. Immunologist focused in on allergies. Toxicologists assessed the health effects of environmental contaminants. Nutritionists study the caloric requirements, micro-nutrients, vitamins, and macromolecular requirements. Physicians, particularly Ob-Gyn physicians, neonatologists, pediatricians, geriatric specialists, have focused in on individual developmental stage requirement. Geneticists concern themselves with specific genetic predispositions to various dietary-related diseases. Biochemists interested in the mechanisms of dietary/nutrient-dependent reactions needed for health. Psychologists on individual behavior related to food's effect on health. Epidemiologist on population effects of food on various markers of acute and chronic diseases. Sociologists view political and economical policies affecting populations.

Using the metaphor of the hamburger, when grilled to be rare, it increases one's risk to food poisoning by microbial contamination. To grill the hamburger to become a "charcoal"-encrusted piece of protein increases the risk to cancer because of the poly aromatic hydrocarbons (PAH's) and other toxic products of pyrolysis. Is there a "middle" way to deal with this dilemma? Is there a temperature where the microbial causes of food poisoning are eliminated but where no risk of toxic chemicals is produced by that process?

Food safety is, then, more than the determination and elimination of microbial toxins or the determination of environmental toxicants, drugs, nutraceuticals, food additives or supplements. It must be an interdisciplinary approach to identify as many elements that can reduce specific individual's or population's risks to either or both acute and chronic diseases to human beings (at all stages of development, with various genetic backgrounds). Understanding [a] basic mechanisms by which the food toxins/toxicants work; [b] basic mechanisms by which various diseases are generated; [c] how the mechanisms of toxicity might affect the pathogenesis of acute and chronic diseases; and [d] how to apply that knowledge to create various intervention strategies (dietary/behavioral/cultural) can help reduce the risk to foodborne diseases. Making individual or population decisions to promote "food safety" involves the use of state of the science- "facts" and our "values". At best, our current knowledge of food safety is, at best, incomplete, and in some cases, dead wrong (on the individual and society levels). In our pluralistic and democratic societies, ones' values can span the whole range, from being health-oriented or health-destructive.

Safety is defined as the "quality of averting or not causing injury or loss" or the "freedom from the occurrence or risk of injury or loss". Therefore, "food safety" might be defined as the quality or not causing injury or loss by the complex interaction of chemicals (natural and/or synthetic) on/in foods with the genetic, developmental state, gender, and mixtures of other chemicals in the individual. Many terms within the definition of "food safety", also, need to be defined. The "quality of averting" could mean the application of basic knowledge and technology, derived from the natural, mathematical, psychological, behavioral and social sciences. "Causing injury" might be interpreted as the contribution of the natural/synthetic chemicals to the induction of acute [e.g., hallucinations, allergies, diarrhea] and chronic diseases [e.g., cardiovascular, diabetes, cancer, neurological] human diseases. "Causing loss" might refer to the loss of "quality of function" of any organ or of the "quality of life".

Basic knowledge (science) of food safety refers to chemicals on/in food stuffs that can contribute to the induction of acute and chronic diseases by understanding the molecular/biochemical/cellular/physiological/ neurological/ behavioral/cultural mechanisms of these chemicals with the interactions of the factors of genetic/ developmental/gender/ and mixtures of other endogenous/exogenous chemicals. It will involve the understanding of the mechanisms underlying the pathogenesis of acute and chronic diseases. Via a number of mechanisms, chemicals can, in principle, cause cells to [a] mutate (i.e., they might be mutagens); [b] die (i.e., are inducers of either necrosis or apoptosis) or [c] are capable of altering gene expression (i.e., they are epigenetic toxins or toxicants). Technology is the application of knowledge for practical ends. Food safety involves multiple disciplines (engineering, communication arts, behavioral sciences, etc.) to prevent chemicals in/on foods in the field, during transport, processing, packaging and on the fork/spoon/chop sticks/fingers from contributing to the "cause of injury".

In order to prevent, ameliorate or treat diseases, understanding mechanisms, contributing to the pathogenesis, is required. One should not apply what one does not know. Determining the "causes" of hazards ("hazard identification") must occur before "risk assessment"/ "risk communication"/"risk management" be translated into public policy. History is replete with examples of confusion and real harm by the application of "incomplete or erroneous information" about either the "cause" or the recommended prevention of dietary/nutritional-related acute or chronic diseases.[2)]

The Complex Biology of Human Development and Health

From the moment of fertilization to the moment of death of an individual human being, the single fertilized egg (zygote) proliferates, differentiates into approximately 200 different cell types in multiple tissues, organs and organ systems to form a human being consisting of approximately 100 trillion cells. This delicate regulation of homeostatic control of cell division, cell differentiation, apoptosis and adaptive responses of the terminally-differentiated cells, is mediated by an integrated system of **"extra"-, "intra"- and gap junctional "inter"- cellular communication** mechanisms.[3)] The fertilized egg is viewed as a "toti-potent" stem cell. As the embryo starts to develop, these toti-potent stem cells are partially differentiated to "pluri-potent" stem cells. Upon further commitment to specific differentiated tissues, these pluri-potent stem cells give rise to "multi-potent" stem cells, which, upon further commitment, gives rise to "bi-polar" stem cells. The stem cells can divide, both **symmetrically** to produce two daughter stem cells or **asymmetrically** to produce one daughter that differentiates (or "progenitor" cell) and another daughter that maintains stem cell abilities. The progenitor cell, which divides only symmetrically, can be considered the "transit" cells which divides until it senesces. The terminally differentiated cells (e.g., red blood cells, neurons, lens cells) lose their ability to proliferate, while gaining their highly specialized function.

Clearly, the cellular functions of cell proliferation, differentiation and apoptosis require energy and both highly specialized micro-nutrients/vitamins/metals/, as well as basic macro-molecular substrates for nucleic acids, proteins/carbohydrates, and lipids. During embryogenesis, fetal development, neonatal transition, adolescent maturation, adult and geriatric stages, the dietary/nutrient requirement vary for each genetic individual and gender.

One must also consider the biological/cultural evolutionary factors that influence food safety.[4-8)] While our knowledge of the early diet/nutritional habits is scanty, clearly the genetic ability to cope with the dietary/food habits had to permit survival until the individual to reproduce and take care of their young until they reached sexual maturity. [9)] This close interaction of genetic backgrounds/ available food sources/ to allow sexual maturation and continuance of the species allowed us to reach this point of our biological/cultural evolution. Whereas until recent decades, where the median life span was a bit more than 40 years, cultural advances reduced infectious diseases due to better sanitation (including food safety), increase food production, better means to transport and preserve foods influence the median life span around the world. Now a large proportion of human beings can enjoy the diseases caused by "effluence of our affluence". One of the major influences on human health, globally, is too many calories rather than too little food.[10)]

This latter view is more than a bit misleading because while obesity is correlated with many chronic diseases,[11] too much food or too little is not only a matter of calories for life, but the quality of the nutrients needed for a healthy life. When one tries to look at the big picture, in time's past, ones diet was determined by what was hunted or grown within walking distance. The nutrient/ caloric composition of that food and dietary patterns of those that survived implies these were adequate to allow development and health sufficient for child bearing and rearing. For those whose genes were incapable with the local conditions did not survive to leave offspring. Today, in such a short span of history, not only can one travel great distances from the environmental shaped inherited genotype of the individual's ethic origins (Japanese who moved to South America, Hawaii, or North America), but foods, can be preserved and transported to places where people who, in the past, never had their genomes exposed to foods they had never seen or eaten before. This new mis-match of genotypes and foods might be having a dramatic effect on human health (positive or negative).

Food-associated Toxins and Toxicants: Is there a Mechanistic Difference?

Usually these two terms, "toxins" and "toxicants" are used interchangeably. However, in the context of this *Commentary,* toxins are defined as chemicals produced by microbial organisms, (bacteria, algae, fungi, viruses) and plants, while toxicants are chemicals produced by the actions of humans (synthetically; during food preparation, etc.) The major point to be made is that the human body doesn't make a distinction in its response to toxins or toxicants. Toxins and toxicants are both chemicals. They will, in principle, cause the cell to respond by a **mutagenic, cytotoxic or epigenetic** response. Chemicals can induce DNA damage [genomic or mitochondrial] [more will be discussed on this point later], induce necrotic or apoptotic death or induce an epigenetic alteration in gene expression. A bacterial toxin or synthetic toxicant can cause cell death by necrosis. Food related toxins, such as ochratoxin or vomatoxin, can induce an epigenetic change in cells without mutating or killing them. [12,13] By a mutagenic effect , one normally refers to an irreversible change in the quality [(point mutations) / quantity (gene duplication/ chromosome numbers such as aneuploidy, polyploidy) of the genomic sequence].

Normally **mutagenic** events are irreversible and they usually are associated with cell killing (many DNA lesions are not repaired or not repaired correctly and some mutations are lethal). **Cytotoxic** mechanisms do not have to involve DNA damage but could be the result of massive membrane damage or inhibition of vital essential enzymatic function.[14] In fact, some cell death can be the result of an epigenetic alteration of gene expression, such as the induction of caspase enzymes needed for **apoptosis. Epigenetic** mechanisms refer to alterations in the expression of genes at the transcriptional, translational or posttranslational levels. One must also recognized that, any toxic mechanism, that could alter the quality or quantity (mutations, cell death or epigenetic alteration) of stem cells, their committed progenitor or terminally differentiated cells, would lead to profound pathological consequence.

Recently, in spite of the historical assumption and the literature supporting the idea that toxins/toxicants can induce various pathological endpoints via genotoxic or mutagenic mechanisms, a recent challenge to this paradigm has been made.[15] The demonstrations, that [a] toxic chemicals can induce DNA lesions, [b] that mutations found in oncogenes of tumor associated with the chemicals, [c] germ-line mutations are associated with cancers and other chronic diseases, and [d] chemicals can induce phenotypes interpreted as mutations found in vitro assays, do not necessary prove that the chemical induced genomic DNA and altered phenotype.[16]

In terms of the pathogenesis of cancer [or maybe even atherosclerosis[17]], there is clear evidence of a multistage, multi-mechanism process.[18] A single normal cell, once **"initiated"**, is prevented from mortalizing or terminally differentiation.[19] This is not a malignant cell. If is suppressed by surrounding and communicating cells, either by a secreted negative growth regulator or by signals through the gap junction. If this growth suppressing effect is blocked by endogenous (hormones, cytokines, growth factors) or exogenous (nutrients, drugs, pollutants, bacterial toxins), then these initiated cells can have clonal expansion due to both mitogenesis [20] or inhibition of apoptosis.[21,22] This is the **"promotion"** process. Promoters seem to be species, cell type, gender specific.[23] **Initiated cells in a given tissue must be exposed to**

specific promoters at "threshold" levels for a regular and chronic period of time and in the absence of anti-promoters.[24]

It has been generally assumed that the initiation phase of cancer is caused by DNA damage, leading to mutations which confers "immortality" on the normal mortal cell.[25] Promotion is now thought to involve "epigenetic processes by either and/or both mitogenesis and inhibition of apoptosis.[26] Initiated cells have been speculated to be adult stem cells.[27] Since normal adult stem cells are thought to be immortal until they are induced to terminally different, "initiation" actually prevents "mortalization" or terminal differentiation.

To use diet and nutrition to prevent or treat cancers, efficaciously, the mechanisms of carcinogenesis must be considered.[28] Clearly, to prevent the "initiation" phase by reducing exposures to true mutagens, i.e. UV-light,[29] is wise. However, we can never reduce the initiation process to zero since every time a cell proliferates there is a finite chance of an error-of-replication. **There, considering that the tumor promotion phase must take decades of regular, sustained threshold-type exposures, the tumor promotion phase would seem to be the most efficaciously phase to prevent carcinogenesis**. Promotion can be interrupted and in some cases, even reversed.[30]

Cell Communication: Homeostatic Regulation of Cell Proliferation, Cell Differentiation and Apoptosis

To go from that single fertilized egg to a human being, containing 100 trillion or so cells, which consists of over 200 cell types in a number of organs/organ systems, a very complex homeostatic positive/negative feedback system of communication mechanisms has been developed through evolution to control whether a cell proliferates, differentiates, apoptoses, if terminally differentiated, adaptively responses or senesces.[31] Of the major cell types that exist in tissues, the adult stem cell, which can proliferate symmetrically or asymmetrically, the progenitor cell, derived from the adult stem cell which can divide symmetrically for a finite period and the terminally-differentiated cell are all communicating with each other via different mechanisms. The adult stem cell seems to exist in its niche [32] without the ability to perform GJIC with its progenitor daughter cells,[33] although it is receiving communication signals from both its niche substrate and soluble negative growth regulators from either or both the progenitor or differentiated daughter cell. In other words, both intracellular signals from the specific substrate to which a cell is adhered, and from extra-cellular signals from hormones, growth factors, nutrients, cytokines, etc combine to determine whether the stem cell divides symmetrically or asymmetrically. On the other hand, progenitor cells do express one or more of the 20 connexin genes.[34] These different connexin genes seem not to only have a role in contact inhibition or growth control,[35] but also in determining the specific differentiated phenotypes of different tissues.[36] In addition, for solid tissues, the apoptotic "death signal" does seem to require functional gap junctions.[22]

Putting this into a larger perspective, the gap junction, a unique channel structure which allows direct transfer of ions and small molecular weight molecules to coupled cells, is the evolutionary-related cellular structure, coded by 20 genes[34] that appeared at the time when a multicellular metazoan appeared.[37] It seems, to this author, it is the consummate family of genes that allowed the appearance of the metazoan. With the transition from a single cell organism, which does not have the connexin gene, nor the functions it represents for survival of the multi-celled metazoan, which now attained the new phenotypes of growth control, ability to differentiate and to apoptose, and have its terminally differentiated cells adaptively respond, at the cost of losing "immortality" of the whole organism. Yet the metazoan maintained immortality for its germ cell line and apparently its adult stem cells for growth and wound repair of tissues. To view the appearance of these gap junction genes and the gap functions of synchronized growth control, electrotonic signaling between cells and possible of non-gap junction-related intra-cellular functions[38] is to see the gap junction as the ultimate down stream organelle that allows the hierarchical nature of a multi-celled organism to be a true organic living "system".[39] Without the gap junction, a society of cells that had no growth control, no ability to differentiate and apoptose, would be similar to a tumor. Cells of a tumor, particularly the "metastatic cancer stem" cells,[19] would be immortal, unable to terminally differentiate or apoptose. These cancer stem

cells almost resemble a collection of bacteria. In other words, a cancer cell could be viewed as an evolutionary reversal to a single celled organism.

To put the gap junction's role an exalted category of cellular organelles, one must recognize that these structures must have been evolutionarily designed to be very sensitive to intracellular perturbations caused by extracellular signals. In other words, these gap junctions were not designed to be "permanent" channels. These gap junction channels had to respond to external signals that triggered either quick responses to posttranslational modifications of inactive/active proteins, such as protein kinases, that could either enhance or inhibit coupling and cell-cell communication of ions/small molecules or to trigger signal transduction leading to transcriptional regulation of connexin genes. For example, if a cell's membrane was breached, and a lethal influx of Ca++ came into the cell, not only would that cell die but so would all the coupled neighboring cells if the gap junction wasn't sensitive to calcium-regulation of junction channel closure. Therefore, if a cell receives a mitogenic signal and the signal only triggered transcriptional regulation of mitogenic genes but did not close the gap junctions, the cell would diffuse these mitogenic- triggered signals to the other neighboring cells. Therefore, the cell receiving the mitogenic signal would not reach the "critical mass" of signal needed to escape the ground state (G_0) of the contact-inhibited cells. So as not to lose sight of how this explanation relates to "food safety", one needs only to remember these intracellular signals are triggered or prevented by the chemical nature of foods and dietary ingredients.

Supporting evidence of the critical roles gap junctions play in embryogenesis, development and adolescent/mature functions comes from a wide variety of both animal genetically-modified mice and inherited human mutants of various connexin genes.[40-42] Since gap junctions are expressed in all tissues and they are vital for the electrotonic and metabolic synchronization in these tissues/organs, any genetic or endogenous/exogenous abnormal modulation (increased or decreased) function has the potential to lead to acute and chronic pathologies.[43] Altered GJIC during embryogenesis and fetal development can lead to either embryo lethality or teratogenesis. Altered GJIC in the reproductive and neurological organs could lead to reproductive and neurological disorders. Cardiovascular problems could be caused by abnormal modulation of the specific connexin proteins and gap junction functions. While these clinical pathologies are very different, they share a common underlying mechanism, namely the **abnormal modulation of GJIC**.[43]

The insight to this unifying mechanism came from the cancer field. After Loewenstein's initial hypothesis that cancer cells differed from normal cells because they lacked gap junction function,[44] the observation that powerful tumor promoting chemicals (and conditions such as wounding and compensatory hyperplasia after cell death), which were not mutagenic, could reversibly inhibit gap junctional intercellular communication.[26] This led to the generation of data showing that many natural chemicals (i.e., phorbol esters), microbial toxins (i.e.,vomatoxin; ochratoxin), nutrients (i.e., unsaturated fatty acids); drugs (i.e., phenobarbital); synthetic chemicals (i.e., DDT, polybrominated biphenyls); food additives (i.e., saccharin); and growth factors (i.e. EGF) could be tumor promoters and inhibit GJIC without killing cells or causing mutations.[12]

These observations, then, lead to the conclusion that **food safety** has to be assess only after it can be shown that the chemical components of the food, either natural components or created or added due to the growth, harvesting, processing, packaging, and preparation, will not inhibit GJIC in the human after consumption. Clearly, the concentration and sustained exposure will have to exceed threshold levels needed to inhibit GJIC and time needed to bring about the clonal expansion of any initiated cell of that affected organ.

Equally important is the fact that these food-borne tumor promoting chemicals are never present in the body alone, in the absence of a class of endogenous and exogenous chemicals which can increase GJIC or prevent the inhibition of GJIC. This logical insight led to the hypothesis that there exists "anti-tumor promoters" (or anti- down regulatory inhibitors of gap junctions in other organs, leading to other type of diseases, such as atherogenesis).[29]

It has been assumed that aflatoxin, polyaromatic hydrocarbons, and estrogens and endocrine disruptors are mutagenic in their ability to induce cancers. How-

ever, there is another interpretation, in that there exists in all organisms, spontaneously - or pre-existing "initiated" cells. If a chemical induces cell death, released cytokines and inflammatory agents from the dying cells could cause any pre-existing initiate cell to proliferate and not to apoptose.[46]

Most normal cells express their connexin genes (of which there are 20 highly evolutionally-conserved genes).[34] Only those cells needing their freedom to migrate in the body (red blood cells, neutrophils, macrophages) do not express the connexin genes or have functional gap junctional intercellular communication. Gap junctions, the membrane-associated channel, allow ions and small molecular weight, freely, to equilibrate between coupled cells. Gap junctions permit synchronized electrotonic and metabolic processes to occur. Gap junctions have been associated with "contact" inhibition or growth control[35] with apoptosis in solid tissues[22] and with differentiation.[47,48]

It is interesting to note that cancer cells, which have lost growth control or contact inhibition, cannot terminally differentiate or apoptose under normal conditions, due not to having functional gap junctional intercellular communication.[49] There are two reasons these cancer cells do not have functional GJIC, namely, their connexin genes are not actively transcribed as in HeLa and MCF-7 cells[50,51] or their connexin genes are expressed but are rendered non-functional by either activated oncogenes, such as ras or src, or by mutations.[52] This observation has two major implications for chemoprevention of cancer and therapy of cancer.[49] First, there will never be a single anti-cancer drug of dietary chemopreventive agent that will prevent or treat all cancers. Second, dietary factors or drugs as chemopreventive agents to the former type of initiated, pre-cancerous cells will have to be some factor that triggers signaling to inhibit soluble mitogenic factors against stem cells. For example since breast stem cells and their initiated (as well as malignant cancer stem cells) express the estrogen receptor, estrogen could be a mitogen to induce proliferation and prevent apoptosis in acting as a tumor promoter.[19] Any dietary factor that would interfere with the estrogen binding to the estrogen receptor or block estrogen receptor signaling would be a chemopreventive agent to this class of pre-malignant cancer cells. In addition, any dietary agent that could induce differentiation of the stem cell or initiated stem cell could be a chemopreventive agent.[53] Genistein has been shown to induce differentiation of normal human breast stem cells.[54] One possible explanation of the low frequency of breast cancers in past generations of Japanese women[55] might be that they were exposed to large quantities of soy products, containing genistein. Genistein has been shown to modulate methylation of critical genes associated with the carcinogeneic process.[56] Another possibility is another dietary factor that is in the soy products, namely, Bowman-Birk inhibitor.[57-59] If the breast stem cell pool is reduced by dietary soy products, not only would there be less breast tissue but also breast stem cells, which might be the "target" cells for breast cancer. In fact, epidemiologically, the role of early pregnancy and a reduced risk to breast cancer might be that pregnancy hormones force differentiation of the breast stem cell pool. If stem cells ate the "target" cells for initiating the carcinogenic process, fewer stem cells would reduce the risk for cancer.

Food Factors as Chemopreventive/ Chemotherapeutic Agents via their Ability to Modulate Gap Junction

Given that it is a fact that gap junctions exist in all human organs and contributes to a dynamic functional role in the regulation of cell proliferation , differentiation and apoptosis in solid tissues, it should not be surprising that disruption of GJIC by endogenous or exogenous agents, including those in and on food stuffs, would lead to disruption of homeostatic control of these vital cellular processes.[60] Moreover, many of these food-related chemicals, which are not mutagenic , that inhibit GJIC at non-cytotoxic concentrations, can act as tumor promoters when there is a sustained exposure of a tissue that has been initiated (containing a spontaneously- or experimentally- mutated stem cell).

It therefore would seem very logical that agents, including chemicals, in and on foods, which either interfere with chemicals that inhibit GJIC or enhance GJIC in non-GJIC-communicating cells, could prevent tumor promotion and act as chemopreventive agents or chemotherapeutic agents.[29] For those tumor promoters that enhance the growth of initiated stem cells that do not express their connexin genes, anti-tumor promoters or

chemopreventive agents would act either to ameliorate the inhibitory effects of endogenous suppressors of stem cells' mitogens. In addition, if these food chemopreventive agents induce the stem cells to terminally differentiate, they could reduce the risk for initiation, as might be the case when Asian women, who ingest large quantities of soy products, and young women, who have full term pregnancies, seem to - ve reduced risk for breast cancer.[61]

Not all of these food factors that prevent GJIC from being inhibited need to be related to cancer, since inhibitors to GJIC could affect both acute and other chronic diseases, such as atherogenesis [which has been characterized as a multi-stage process such as carcinogenesis.[17]] The recent demonstration that an anti-oxidant in red wine, resveratrol could dramatically reduce the risk to life span shortening, and chronic disease in obese rats is a perfect example of a food-related anti-oxidant which can influence multiple disease processes and can affect gap junctional intercellular communication.[62] In this case, the compound was given to these experimental animals as a pure food supplement at much higher concentrations than normally found in the usual consumption of red wine. This chemical has also been shown to prevent the down regulation of GJIC by a tumor promoting chemical.[63] Care should be noted that drinking a large amount of red wine (which would contain varying amounts of resveratrol and other chemicals) is also accompanied by the alcohol, which can inhibit GJIC.[64]

Other examples of food-related agents that, by either blocking the effect of a tumor promoter on a normal or initiated cell or restoring GJIC in a non-communicating tumor cell, would be β-Sitosterol and green tea components. In the green tea case, the tumor promoting agent used in an experimental animal model was pentachlorophenol (PCP). This agent is non-mutagen even though it induces oxidative stress. The reactive oxygen species (ROS) formed triggers a change in the redox state of the cell and this activates a redox-regulated signal transduction mechanism that alters both gene expression and blocks GJIC.[65] Pre-treatment of these PCP-exposed cells with components of green tea prevents the down regulation of GJIC by PCP and prevents the promotion of tumors in the mice.[66]

Good News/Bad News of Dietary "Functional" Foods or Components of Functional Foods

Epidemiology, while not being a precision science that can identify mechanisms of disease causation, can identify, if designed based on understanding the mechanisms of the pathogenesis of any disease process, such as carcinogenesis, interesting associations between a particular agent and a disease. In the case of carcinogenesis being a multi-stage, multi-mechanism process, and that the promotion stage is the most like rate- limiting stage for either appearance or prevention of cancer,[29] dietary foods, that can clonally expand or prevent a single initiated cell from accruing all the "hallmarks of cancer,[67] might be identified as tumor promoters or chemopreventive/chemotherapeutic chemicals. Therefore, in a culture that believes in the idea that a "if a little bit is good, more must be better", it is not surprising that identification of the factor in these "functional foods"[68] and their use as purified chemicals as dietary supplements or as chemopreventive agents in large amounts would be deemed as the efficacious manner in some intervention measure to reduce the risk to cancer (or other disease). Unfortunately, the example of the "CARET" trial only illustrates that this strategy will not necessarily work. (2) When the dietary supplements were given to heavy cigarette smokers, the trial had to be terminated after a number of years because the experimental group receiving the chemopreventive agent actually had more lung tumors than the placebo group. Here the trial failed to note that, while these agents had been shown to prevent the down regulation of GJIC by tumor promoters, it could also inhibit GJIC[69] and act as a tumor promoter when applied at pharmacological concentrations.[70-73]

This issue of a chemical acting as an anti-oxidant under one set of circumstances, yet be a prooxidant under another set, has been well known, including in the field of tumor promotion.[74] Another observation that is rarely considered when there are opposite effects of a given chemical might relate to the fact that, when a chemical works via a receptor-dependent fashion, it usually works at low "physiological" concentrations in the body. This receptor-ligand reaction triggers various signaling- gene expression changes after initial posttranslational modifications within the cytoplasm of the cell. At "pharmacological" concentrations, the same chemical

can also induce receptor-independent reactions, including different signaling and posttranslational modification of cytoplastic components, in addition to the receptor-dependent effects. It then should not be surprising that a concentration –dependent biological response of opposite kinds could happen. In addition, one should not forget, in tissues, the three different kinds of cells, with their three different phenotypes. Stem cells, progenitor and differentiated cells, in a 3-dimensional context[75] (caused by differential gene expression patterns), react differently to the same chemical. Further, within this tissue, containing the few normal stem cells, might also contain a few "initiated stem cells". These, also, would react differently to the same chemical as do the normal stem cells. The point being made is that that initiated cells appear to resist apoptosis and terminal differentiation by tumor promoters, whereas the normal cell would not.

Another interest lesson to be made concerning the use of dietary chemopreventive agents is that the same chemical could be both a chemopreventive agent for one organ or the body while being a tumor promoter for another organ in the same organism. Examples exists, such as butylated hydroxytoluene (BHT) and butylated hydroxytoluene (BHA).[76,77] Another is polybrominated biphenyl is a liver tumor promoter in the rat, yet seems to be a chemopreventive agent against spontaneous mammary cancers in the rat.[78] TCDD, a power tumor promoter has also been shown to be a an anti-carcinogen.[79] Even DDT, a tumor promoting pesticide, has been shown, at low doses, to inhibit male rat hepatocarcinogenesis initiated by diethylnitrosamine, while at higher doses it can promote liver tumors.[80] An interesting example of phenobarbital as a liver tumor promoter in post-weaned rats is a chemopreventive agent in pre-weaned rats.[81] One should not ignore the strain or species differences to a given tumor promoter, as has been shown with phenobarbital and different strains of mice,[82] which correlates with its ability to inhibit GJIC in those strains. The fact that genistein seems to be a dietary chemopreventive agent for breast cancer,[83] yet have tumor promoting activity in rat uterus.[84,85] Even Vitamin C has been shown to inhibit benzo(a) pyrene induced fibrosarcomas in rats, but increased the carcinogenic potency of benzo(a) pyrene-induced undifferentiated sarcomas in the same rats.[86] Although cruciferous vegetables, such as cabbage, has been epidemiologically linked to reduced cancer risks, it has also been shown to enhance pancreatic and skin tumorigenesis in hamsters and mice.[87]

This rather confusing set of observations could lead to a "good news-bad news" view of food-related risk hazard assessment and risk assessment, let alone risk communication and risk management policies. These rather contradictory effects of any given food related toxin/toxicant might be explained by bad experimental design, use of non-comparable model assay systems, species differences, dose-related differences, or real biological/mechanistic differences for which there should be a great deal of attention.[88-90]

Embryo, Fetal, Neonatal Exposures to Dietarey Modulators of Stem Cell Quality/Quantity

While the science of teratology has shown that exposures to both dietary conditions (i.e., folate and vitamin A deficiencies), as well as exposures to alcohol or drugs (thalidomide) can lead to birth defects, only recently has there been attention paid to the possibility that long term chronic disease consequences can be altered by in utero exposures.[91,92] This has been dramatically demonstrated with alterations in the gene expression coding for mice coat color.[93] Even more recently, it has been shown that when pregnant rats were exposed to bisphenyl-A, male offspring developed prostate tumors, whereas pregnant rats exposed to both bisphenyl-A and genistein, had reduced risks to prostate tumors.[94] This observation might related to past history of Japanese low breast cancer risks when pregnant women consumed large quantities of soy products. The fact that human breast stem cells have been shown to be induced to differentiate after exposures to genistein,[95] the lower risk to breast cancer later in life might be due to reduction of the "targets" for carcinogenic initiation. (Using the same logic, in today's changing dietary patterns, which includes new types/amounts of chemicals, embryonic/fetal and neonatal exposures to agents that either induce proliferation or differentiation of the stem cell pool, could either increase or decrease certain chronic diseases.

To make a regulatory agency's responsibilities to protect the public even more complex and difficult, the example of, β-sitosterol, the active chemical derived

from the pyllium fiber, can serve to make the point there will never be a "silver bullet" to prevent all cancers. Epidemiologists, while not always agreeing on the role of dietary fiber as a cancer preventive agent, seem to claim, based on the "weight of the evidence" that dietary fiber seems to associated with reduced health consequences associated with the G.I. Tract, including reduced colon cancer. After extensive testing of various aqueous and alcohol extractions of the psyllium fiber, the compound, β-sitosterol, was found to restore GJIC in human colon cancer cells with the activated ras oncogene.[96] However, when β-sitosterol was given to cancer cells transfected with the src, neu or myc-ras oncogenes, it was totally none effective. The lesson to be learned here is that certain oncogenes are associated with particular organ tumors. Therefore, a dietary supplement of either psyllium fibers or the pure compound, β-sitesterol, might prevent colon cancers expressing the ras oncogene but it have little or no effect on any tumor with another oncogene or with tumor stem cells that do not express an connexin gene and do not have any activated oncogene in the cell.

Evolution, Functional Foods, Dietary Supplements

Back to the beginning, the evolution of humans dependent on a complex interaction of the genes inherited by those that had access to sufficient amounts of calories, required essential nutrients. Those who survived the early years of "feast and famine" episodes developed genetically-controlled metabolic systems to cope with these extreme conditions.[97,98] With the explosion in cultural changes due to increased food production, population explosions, and changes in individual life styles, both foods and people are being moved from their original adaptive environments. The collision of biological evolution, which is slow, with cultural evolution, which is occurring at unbelievable speeds, is now occurring with health consequences that are only now being recognized.

Today, our diets (for more than half the world's population) provide more calories than we need.[99] In addition, the diets are not necessarily compatible with the nutrient requirements for either the population or the specific individuals. With scientific information, narrowly identifying effects of individual food factors (both positive and negative), public policies and individual choices to use this information has not been wise. Clearly one must take into account the extremely complex interactions of genetic, gender, developmental state, dose, duration of exposures, interactions of additive, synergistic or antagonistic elements in foods, pollutants in/on foods in order to maintain health and to prevent controllable diseases.[100-102] Just because [a] a food stuff, such as spinach might be viewed as a healthy food to most, to those who have oxalemia it could lead to kidney stones; [b] peanuts seem to be an excellent source of required vitamins/oils, it could be deadly to an individual with a specific allergy to nuts; [c] vitamins/anti-oxidants are needed by all, however, **if a person is sufficient in their required vitamins, anti-oxidants and essential minerals, additional amounts might do nothing to improve health and might even cause harm. Therefore, even the introduction of the concept, "Functional foods", must be understood within a "systems" context as it applies to the individual person at each developmental stage.**[103]

The concept of functional foods, while possibly having some abstract usefulness for a population as a whole, means very little for the individual whose reaction to any given recommended dietary which be predicated by the aforementioned genetic, gender, developmental stage and other factors that man modify the influence of that diet on his/her health.

Summary:

Given that all life requires energy, and in the case of the human being, our dependence on our food/diet being compatible with our specific, as well as general needs, as shaped by our unique genes, our gender, our developmental stage of life and the physical activities, it seems a wonder that any of us makes it to death as healthy individuals. Moreover, living in our modern world, where the composition of our foods, shaped by genetic engineering, agricultural techniques, world-wide disposition of pollutants in the soil/water and air, processing and preparation of foods, means of storage of foods, consumption of too much-, too little- or wrong-kinds of foods, have set the stage where both acute and chronic diseases are taking its toll on human health. In the specific example of the disease of cancer, these factors do play a major role in both the induction of cancers

(i.e., promotion phase of carcinogenesis] and the prevention of cancers (interference of the promotion phase of carcinogenesis). While it might be viewed as a totally hopeless task of the lay person to integrate the incomplete scientific information into a health diet strategy, take heart in the age- old wise advice: "Too much or too little of anything will be harmful; eat in moderation, and use the middle way or by the "Golden mean" to shape our dietary strategy.

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