J Food Sci Nutr
Vol 16, p 394~407 (2011)
http://dx.doi.org/10.3746/jfn.2011.16.4.394

J Food Science and Nutrition

Pre-Natal Epigenetic Influences on Acute and Chronic Diseases Later in Life, such as Cancer: Global Health Crises Resulting from a Collision of Biological and Cultural Evolution

- Review -

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Abstract

Better understanding of the complex factors leading to human diseases will be necessary for both long term prevention and for managing short and long-term health problems. The underlying causes, leading to a global health crisis in both acute and chronic diseases, include finite global health care resources for sustained healthy human survival, the population explosion, increased environmental pollution, decreased clean air, water, food distribution, diminishing opportunities for human self-esteem, increased median life span, and the interconnection of infectious and chronic diseases. The transition of our pre-human nutritional requirements for survival to our current culturally-shaped diet has created a biologically-mismatched human dietary experience. While individual genetic, gender, and developmental stage factors contribute to human diseases, various environmental and culturally-determined factors are now contributing to both acute and chronic diseases. The transition from the hunter-gatherer to an agricultural-dependent human being has brought about a global crisis in human health. Initially, early humans ate seasonally-dependent and calorically-restricted foods, during the day, in a "feast or famine" manner. Today, modern humans eat diets of caloric abundance, at all times of the day, with foods of all seasons and from all parts of the world, that have been processed and which have been contaminated by all kinds of factors. No longer can one view, as distinct, infectious agent-related human acute diseases from chronic diseases. Moreover, while dietary and environmental chemicals could, in principle, cause disease pathogenesis by mutagenic and cytotoxic mechanisms, the primary cause is via "epigenetic", or altered gene expression, modifications in the three types of cells (e.g., adult stem; progenitor and terminally-differentiated cells of each organ) during all stages of human development. Even more significantly, alteration in the quantity of adult stem cells during early development by epigenetic chemicals could either increase or decrease the risk to various stem cell-based diseases, such as cancer, later in life. A new concept, the Barker hypothesis, has emerged that indicates pre-natal maternal dietary exposures can now affect diseases later in life. Examples from the studies of the atomic bomb survivors should illustrate this insight.

Key words: barker hypothesis, adult stem cells, epigenetic, metabolic diseases, cell communication

INTRODUCTION

The changing paleochemistry of the Earth's oceans shaped the evolution of energy metabolism for life and the genes required for the emergence of metazoans (1). Early pre-human health and survival depended on complex interactions biological factors, derived through the millions of years of selection of genetic factors that were adaptive to the changing physical and social environment (2,3). Temperature, gravity, changing seasons, diurnal cycles of light and ambient gases worked on biological evolutionary mechanisms that led to the generation of energy for life. This, of course, included the link to the origin of life in the oceans that gave rise to the single cell organisms that metabolized sugars via glycolysis in an anaerobic environment (4). During the alteration of the paleochemistry of the oceans via the appearance of phyto-organisms to produce oxygen and the symbiotic union of mitochondria with the first multi-cellular organism, did a dramatic change occur that resulted in new adaptive features for survival, including the synthesis of collagen, extracellular matrix, and the cellular niche to sequester the unique cell, the germinal and somatic stem cells (1,5-7). Given a new means to generate energy for life for multi-cellular organisms (oxidative phosphorylation), new genes appeared to cope with one of the negative side-products of oxidative phosphorylation, namely, the generation of a number of reactive oxygen species (ROS)/ reactive nitrogen species (RNS) (8), and utilize them to act as adaptive signal transducers and gene regu-

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lators (9-11). Because biological evolution depended on nucleic acid to encode the genetic information for the individual organism and its species, their protection from these highly reactive ROS's became paramount. Therefore, genes that had to be co-evolved (a) to protect the nucleic acid codes from ROS-induced macromolecular damage (anti-oxidants) and (b) to repair the inevitable damage that might occur (DNA repair mechanisms).

That balance to protect the genomic and mitochondria DNA and to repair any damage to the genomic and mitochondrial DNA was, and is, a critical one. For if the protection of the DNA from any genomic damage was close to perfect, the chance for survival of the species would be very small, as the inevitable changes in the environment would create non-adaptive conditions for a non-adaptive genome of the species. Without the generation of adaptive mutations in the genome of a few individuals of the species population, on the other hand, if the genetic coded protective mechanisms and DNA repair mechanisms were very inefficient, too many mutations would result, causing non-adaptive functioning at both the individual and species levels. Clearly, biological evolution led to the selection of both protective and repair systems in the early multi-cellular organisms, which allowed the frequency of germinal and somatic mutations to be sufficient for the individual organism to survive long enough to reproduce and to allow for the offspring to survive to reproduction in an ever-changing physical environment. If the frequency was too high, the individual would accrue mutation-related diseases that would jeopardize its ability to reproduce and to maintain the survival of the species.

To understand that transition from a single cell organism to a multi-cellar organism, several new genetic-based phenotypes appeared. The first was a means to regulate un-controlled cell proliferation. Single cell organisms survived changing environments by cell proliferation, only regulated by the presence of nutrients, temperature, appropriate atmospheres, radiation levels, etc. Without another means of regulating cell proliferation, a colony of cells in the multi-cell organism would ostensibly be a tumor. *"Contact inhibition"* (12) was incorporated in that early metazoan as one means to control the growth of somatic cells. The second phenotype that appeared was via the specialization of some cells carrying the same genomic information but having the ability to express only those genes needed to generate unique functions (muscles, neurons, hepatocytes, germ cells). This phenotype was *differentiation.* The third new phenotype was that of selective removal of damaged or non-adaptive differentiated cells during specific periods of development (*apoptosis*). The fourth critical new phenotype was that of *senescence* of cells/tissues and organs that led to the finite life span of the organism. While the life span of each species is different, it was critical that the life span was long enough for the individual to be sexually mature and long enough to allow survival to sexual maturity of the offspring. A unique fifth phenotype for the metazoan was the formation of both *germinal and somatic stem cells*.(13) This new type of cell is characterized by its ability to proliferate either symmetrically to form two identical offspring and identical to the mother cell or asymmetrically to produce one daughter identical to the mother with stem cell potential and the other daughter to be destined to differentiate into a specialized cell. However, these germinal and somatic stem cells were selected to pass on the to the species those genes that allowed the individual to survive the prevailing environment, so that it could reach reproduction and protection of the offspring (germinal stem cells) and to provide cells for growth, wound repair and differentiation of specific tissue to reach reproductive age. Clearly, the micro-environment in the multi-cellular organism that helped to maintain the "stemness" phenotype was the appearance of a critical sixth phenotype, namely, the *stem cell* "*niche*" (14,15). The major reason for a very specialized micro-environment for both the germinal and somatic adult stem cells is the need for differential oxygen environments in situ (more will be discussed on this point, later) (16).

FROM PRE-HUMANS TO HUMANS: CULTURAL EVOLUTIONS IMPACT ON HUMAN BIOLOGICAL EVOLUTION

While millions of years worth of biological evolution's trial and error took place from the first microorganism's appearance to the metazoans, it was the transition of the human evolutionary ancestors to modern day human that created the major factor that has caused many of the current global health-related problems (17). That factor was the creation of "*culture*", those transmitted learned techniques, behaviors and knowledge that caused rapid environmental (physical, chemical, biological, and psycho-social) on the slowly accumulated biological factors needed for survival. With the assumed Diaspora of early humankind from Africa to all corners of the globe, the genome-influenced phenotypes were shaped by the physical environments in which these early humans had to survive, namely via food-derived energy. With global environmental differences, the nature of the genes needed for survival had to generate, efficiently, energy via gly-

colysis, as well other genes to cope with other environmental stresses (temperature; solar radiation or lack of; vitamin and mineral differences, etc.). However, there seemed to be some near universal requirements, shaped by the food supply. First, humans had to adapt to the famine-feast-need for genes that could cope with long periods of absence of food, followed by gorging oneself, once food was found, since they did not know when their next meal would be found. Second, they ate what was within walking distance. Third, the food they ate was seasonal. Forth, In general, early humans ate only during the day. There were no MacDonalds restaurants open all day long. Fifth, until fire, agriculture and domestication of animals for meat & dairy products, or knowledge of fermentation for preservation of foods and production of alcohol, reliance of grain, fruits, infrequent animal/fish meats, obtained by hunting and gathering, were the source of foods that shaped the human genome. Sixth, probably, the major was "*culture*" that was shaped by the emergence of those human attributes of (a) the ability to abstract, (b) to communicate with language, (c) to translate the abstractions into things via technology, and (d) to value (make choices as to use or not use knowledge or technology) (18). Therefore, while it took millions of years for specific adaptive nutrition-related genes to accrue in the human genome in populations in different regions of the globe, culture started to change very rapidly. In cultural evolutionary time, compared to biological evolutionary time that generated our current genome, it has only been in the last 100 or so years that this cultural change has made its major impact on the biologically-generated genome.

Major cultural changes that have occurred in the last 100 or so years point to the fact that, prior to the invention of the first car, humankind traversed primarily by foot or by horse, on a daily basis. Food, itself, had a limited range. Today, we have access to foods, all year around and all day long, that are processed, packaged and preserved in a manner never seen before. We are eating foods that have become "globalized", such that, in the West, we now eat sushi and sashimi, while the Japanese eat pizzas and hamburgers. Two of the "best diets" of the world, the Mediterranean and the Japanese diets, are linked in a global fashion. The Sicilians, who caught and ate the blue-finned tuna with their olive oil-drenched anti-pasta and sea food pasta, with red wine and a citrus-almond dessert, supplied the Japanese with the blue fin tuna. Today with the pollution and depletion of the blue-fin tuna, the Sicilians, if they catch the few remaining tuna, they ship them to Japan for tens of thousands of dollars and now eat red meat, not the tuna. In Japan, because the blue-fin tuna cost so much, the general consumption of the sashimi and sushi is down and the increase of the Western diet is up. Today, the cultural inter-connectedness is associated with obesity and obesity-related metabolic syndrome of chronic diseases in these previously-best diet countries (19-21).

On another level, this collision of biological and cultural evolution is taking place, namely, human nutritional health is based not only on pristine food being digested by the alimental tract, but it is being influenced by the complex gut microbiome (22-31). In other words, the symbiotic relationship of the populations of microorganisms in a population of human beings' GI tract helps to support the digestion of foods we eat. Given thousands of years in a common physical environment, eating a selected variety of local/seasonal foods, the biology of the GI cells were selected to have the genotype/phenotypes that could cope with, or take advantage of, specific populations of these microbes. Once human migration occurred, plus the cultural changes in our diets/nutrition, stresses have been placed on the symbiotic gut microbiome, altering not only the kinds of new microbiomes, but their consequences on the GI tract cells. This understanding should also include the fact that, not only must one consider how modern foods and food ingestion patterns impact on the gut microbiome, but how the potential pollution in/on the foods, and how the foods are processed or prepared (grilled, marinated, raw, microwaved, etc.) affect the biochemical reaction of the microbiome and of the direct effect on the cells of the GI tract itself. The impact of this alteration of this complex cultural change also affects the immune system, which means, the various immune cells now secrete various cytokines, chemokines, etc., which, in turn, affect the GI cells, which, also, can be directly affected by the chemistry of the foods. Therefore, these triggered immune cells' secreted factors are now interacting on "primed" epithelial GI cells, which would behave differently, if these cells were not primed directly (Fig. 1).

Clearly, the global nutritional/dietary problems are even more complicated, when we see how cultural changes have affected population increases, water and food shortages and pollution, global warming, population diaspora, social-economic imbalances, inexpensive foods being the least nutritious, and caloric restriction in one global area and caloric gluttony and lack of exercise in another area. Consequently, in order to try to sort out how nutrition and diets might increase or decrease risks to various human diseases, the task is to understand how chemicals, in and on food, can affect human toxicity and how these mechanisms of toxicities can affect the pathogenesis of human chronic diseases.

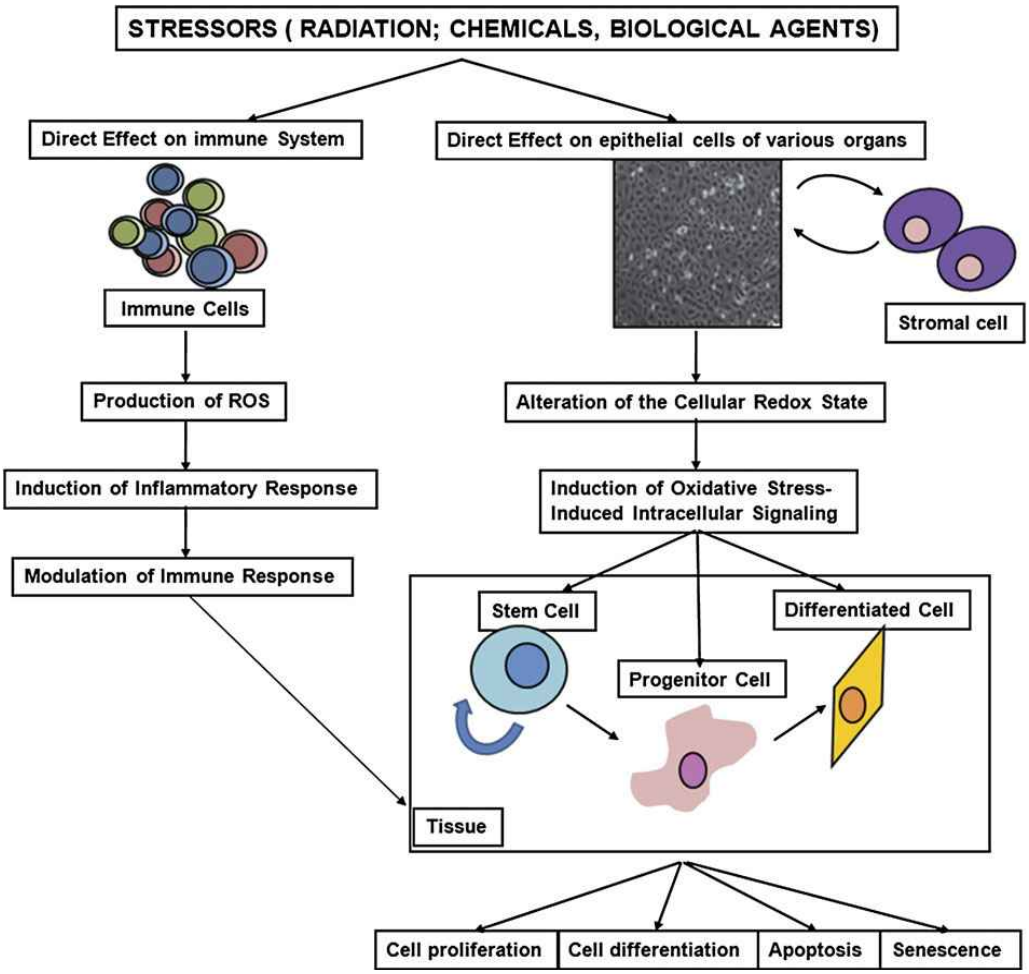

Fig. 1. The diagram tries to incorporate a "systems" aspect of how a physical, chemical, or biological agent could affect a multicellular organism. At noncytotoxic concentrations or doses, an agent could simultaneously trigger oxidative stress in both the cells of the immune tissues and the epithelial/endothelial/stromal cells in various organs. Upon induction of reactive oxygen species and of oxidative stress and induction of intracellular signaling in various cell types of the complex immune system, various cytokines would interact on tissues, containing the three fundamental cell types (adult stem cells, progenitor, and terminally differentiated cells). Given that these cells would have been exposed to the toxic agent and that they, also, would have reacted to the agent deferentially because of their different physiological/phenotypic state, the interaction of all three types could be very different (e.g., the normal stem cells might be induced to proliferate asymmetrically, any initiated pre-cancerous stem cell might proliferate symmetrically, the progenitor cells might be induced to proliferate symmetrically and to migrate, as in wound healing, and the terminally differentiated cell might adaptively respond or to apoptose) in response to the inflammatory signal. In summary, each cell type of the immune system and of the various organ tissues, with their different expressed genes and cellular physiology, will respond differently to sub-lethal exposure to agents inducing oxidative stress–triggered intracellular signaling and epigenetic alterations. The interaction of inflammatory agents on pre-exposed organ cells could be an additive effect, *Permission granted Toxicology Sciences, Oxford University Press, 3/1/2011.*

MECHANISMS OF TOXICITIES AND THEIR ROLES IN THE PATHOGENESIS OF CANCER

Nutrition and diets play roles in many chronic diseases, such as birth defects, cancer, cardiovascular diseases, diabetes, reproductive- and neurological-dysfunctions. Many of the genetic predispositions to one of these diseases can also predispose the individual to other chronic diseases (i.e., Downs syndrome is predisposed to birth defects, leukemia, premature aging, atherosclerosis, Alzheimer's disease, etc.). Also, the physical and chemical agents, that can be associated with one disease, can also be associated with several others. The classic example of the "metabolic syndrome" has shown a link between obesity and diabetes, cardiovascular disease and cancer (32,33). Moreover, for all the nutritional and dietary factors that are associated with an increased risk to chronic diseases, there are nutrient and dietary factors associated with the reduction of risks to the chronic diseases. Therefore, it is important, first, to understand the basic mechanisms by which physical, chemical and biological agents cause toxicities to cells which can contribute to chronic

diseases, as well as to understand how these *mechanisms of toxicities* interface with the *pathogenesis of the various chronic diseases*.

Since it would be impossible to examine each chronic disease in this "*Commentary*", the pathogenesis of cancer will be used to illustrate how all the risk factors of cancer can be positively or negatively influenced by nutrition and diet, causing a synergistic response or possibly, even an antagonistic effect. This could explain the wide range of diseases in which the inflammatory process seems to play a prominent role.

When a physical (X ray or UV light), chemical (poly aromatic hydrocarbons) or biological (virus) agent interacts with a cell in the human body, three possible consequences could result, namely, (a) a mutation in a gene of the cell's genome-mutagenesis; (b) cell death or cytotoxicity, via necrosis, apoptosis, anoikis; or (c) altered gene expression or an epigenetic change. One must remember that, when a mutation is formed in a cell, it could be the result of an error in DNA repair or it could be the result of an error in DNA replication of a non-damaged DNA. In addition, the mutation will only be produced in dividing cells, such as a stem cell or a progenitor or tissue-amplifying cell, but not in a terminally differentiated cell. Some agents, when interacting with cells, will kill cells. These cells will never, directly, give rise to a cancer. However, cell death, can act indirectly to cause a pre-existing pre-tumor cell to proliferate, because that cell death can stimulate compensatory hyperplasia (34). Cell death can be the result of agents damaging genomic DNA, such that, either a lethal mutation is formed, or that, while genomic DNA damage is induced, non-DNA damage is the primary cause of cell death. Other cytotoxic agents might be specific inhibitors of vital enzymes or disruptors of membrane integrity. Lastly, other cytotoxicants could alter gene expression, epigenetically, to induce programmed cell death or apoptosis. Lastly, agents can alter gene expression without mutating the cell or causing cell death, i.e., epigenetic agents can cause the cell to proliferate, differentiate, apoptose or senesce, depending on whether it is a stem, progenitor or terminally differentiated cell. It is the opinion of this author that chemicals, while they could induce ROS and induce oxidative stress, are not carcinogenic mutagens to genomic DNA (35). They might, in fact, induce mitochondrial DNA damage and mitochondria mutations, they do not induce genomic mutations (36).

The pathogenesis of carcinogenesis is extremely complex, in that it is well known that genetic, gender, developmental, dietary/nutrient, exercise, environmental, drug and psycho-social factors can contribute to the ultimate formation of teratomas, carcinomas and sarcomas. One of the most powerful concepts, but often ignored in modern carcinogenesis studies, is the multi-stage, multi-mechanism process involved in carcinogenesis (37,38). Specifically, in both experimental and human epidemiological studies, it is known that there exist three distinct phases of this multi-stage, multi-mechanism process. The first step to start the carcinogenic process is for a single normal cell, after to exposure to an agent, to be prevented from senescing. The fact has been established that the cells within a tumor, albeit, being either or both genotypically and phenotypically different, are clonally-derived from a single cell (39,40). This step is apparently irreversible; It is referred to as being "initiated". Agents that are true genomic DNA-damaging agents or point mutagenic potential, such as UV light, are initiating agents (41). It should also be noted that "initiated" cells can result from a cell that created a spontaneous mutation after an error of replication of a non-DNA damaged genomic DNA. The question now is, "What is the target cell to be initiated? Is it any cell of the body or is it a special cell? To answer those questions will be addressed later.

With a single initiated cell, which has not accrued all of the genotypic or phenotypic "hallmarks of cancers" (42,43), in any given organ, it now can be subject to the next phase of carcinogenesis. That phase is referred to as the "promotion" stage. The operational definition of this promotion phase is the clonal expansion of the single initiated cell. The process by which one can expand this single initiated cell is by a mitogenic event and by the inhibition of programmed cell death or apoptosis (44). As a result of these two biological processes, a net increase of initiated cells occurs because the initiated cells can proliferate without terminally differentiation or without dying by apoptotic cell death. While the underlying mechanisms for both mitogenesis and apoptosis can be varied, mitogenesis can be stimulated by growth factors, hormones, inflammatory factors, chemical mitogens, compensatory hyperplasia, caused by cell removal or cell death (Fig. 2).

One must recognize that that the promotion process has been shown experimentally to require regular, sustained exposure to the promoting agents, given at threshold or above levels, for long periods of time, and in the absence of anti-promoters (45). These are the characteristics of the promotion process that suggests that it is the most efficacious stage to which strategies for cancer prevention must be focused. In addition, this is where one of the significant phases in which nutrition and diet can play a major role in either increasing or decreasing the risk to get cancer. The promotion phase can be interrupted and, possibly, even reversed (46), such as has

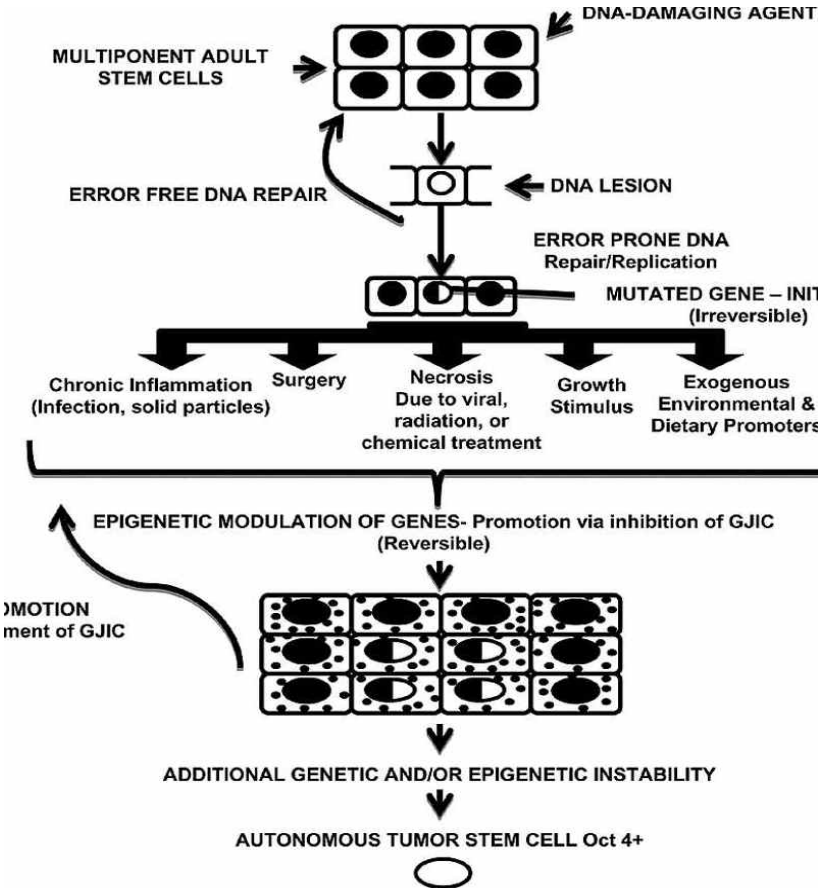

Fig. 2. A diagrammatic scheme to depict the postulated mechanisms of the initiation and promotion phases of carcinogenesis. DNA lesions, induced by physical mutagens or by errors in DNA replication, are substrates in adult stem cells (Oct-4 positive) that can be fixed if they are not removed in an error-free manner prior to DNA replication. Promotion includes those conditions in which a pluripotent-initiated, but surviving, adult stem cell (Oct-4 positive) can escape the nonproliferative state. The build up of initiated cells allows them to "resist" the antimitotic influence of neighboring non-initiated cells. In addition, the changing microenvironment within the growing benign tumor will cause some of the initiated adult stem cells to partially differentiate into cancer nonstem cells. This, together with either additional mutations or stable epigenetic changes, might allow a given initiated adult stem cell to have autonomous, invasive properties of a malignant cell. *Permission granted from Karger AG.*

been seen when the skin promoting agent, phorbol ester, or the rat liver promoting agent, phenobarbital, are stopped after initiation of the skin and papillomas or the initiation of enzyme altered foci in the liver are formed (37,47).

During the promotion process, this, in the case of most human cancers, takes decades to occur, other multiple genetic and epigenetic alterations must occur to generate a phenotype of one of these initiated cells to be invasive and to metastasize. That process, the "Progression" phase, also, seems to be irreversible to date (48).

WHAT ARE THE TARGET CELLS TO INITIATE THE CARCINOGENIC PROCESS?

Since it is well known that nutrients, vitamins and minerals (e.g., retinoids, calcium, selenium, fatty acids, etc.) can affect the carcinogenic process, it will be critical to understand at which stage of the multi-step process and which cells are affected by nutrition and diet. Multiple hypotheses have been proposed to explain the origin of cancer cells, such as the stem cell hypothesis, the "de-differentiation or re-programmed" hypothesis, the oncogene-tumor suppressor gene hypothesis; the mutation-epigenetic hypothesis, monoclonal origin of cancer hypothesis, etc. (49). Each of these hypotheses still carries much theoretical value because each is based on sufficient experimental evidence, on which to support it. However, none of these hypotheses can explain all that is known or necessary to cover the whole cancer process. Basically, each hypothesis is incomplete. In an effort to integrate these different views of the complex cancer process so that on can understand how nutrition and diet might either increase or decrease the risk to cancer (and other chronic diseases that share similar risk factors), an examination will be done of some interesting experiments and concepts, derived from these results.

One of the earliest observations that was made was that cancer cells seemed to be "immortal", while normal cells were "mortal" or had a finite life span or proliferative ability (the Hayflick phenomenon) (50). In addition, cancer cells appeared to lose "contact inhibition", while normal cells contacted inhibited on touching each other (51). Another critical phenotype seen by Loewenstein and Kanno (52) was the fact that cancer cells, which had no growth control, did not contact inhibit or did not senesce or terminally differentiate had no functional gap junctional intercellular communication (GJIC), either because the connexin genes (required for gap junctions) were either not expressed as in HeLa or MCF-7 cells (53,54) or were rendered non-functional by oncogenes or muta-

tions (55). Interesting, it was later shown that growth control or contact-inhibition, differentiation and apoptosis were dependent on functional gap junctional intercellular communication (44). In addition, while all the cells of a tumor seemed to have different genotypes and phenotypes, they were shown to have been derived from a single cell (39,40). Most recently, the re-emergence of the concept of "cancer stem cells" has appeared when it was shown that only a subset of cells in any tumor could sustain the immortal growth of the tumor (56,57).

Within recent exciting reports that embryonic-like cells could be isolated from somatic differentiated fibroblasts and other primary cells, but using a variety of techniques, these "induced pluripotent stem cells ("iPS") were interpreted as having been "re-programmed" from a "mortal state to that of an "immortal" state (58). As one of the definitions of these "iPS's", they had to form termatomas when placed back into an appropriate adult animal. To put these observations into perspective, one needs to examine the definitions and characterizations of stem cells. One of the persistent definitions of a "toti-potent" stem cell is a cell with unlimited proliferative capacity or being "immortal", that could divide either by symmetrical or asymmetrical division, depending on external factors and to give rise to all the cell types of the adult organism. It does so by a series of limiting capacity togive rise to all the cells of the adult body, by the production of pluri-potent, multi-potent organ-specific, bipolar organ-specific and uni- organ-specific stem cells. Once these various stem cells are induced to differentiate into lineage specific cell types, they have become "mortalized".

Weinberg (59) has provided some of the earliest experimental evidence, not only for the functions of oncogenes and tumor suppressor genes in carcinogenesis, but for providing evidence that one must first "immortalize" a normal population of primary fibroblast, and then subsequently, neoplastically transforming these immortalized cells (60). This paradigm has shaped the general thinking and experimental approach and interpretation of cancer studies for decades. However, there is another interpretation of this paradigm to explain the same experiments and those using "immortalizing" viruses, such as SV40 or human papilloma viruses (61,62). Briefly, it now well established that adult organ specific stem cells exist in the skin, liver, breast, intestine, retina, etc. (63). In addition, those adult organ-specific stem cells that have been examined, have been shown to divide both symmetrically or asymmetrically, to have no expressed connexins or functional GJIC (55), until induced to differentiate or become "mortal" (62). Equally important was the demonstration that these human breast adult stem cells are excellent targets for carcinogenesis, while their differentiated daughters are not (64-66).

Before further examination of these observations, the interpretation of "immortalizing" viruses" has to be considered. When immortalizing viruses are introduced to a population of primary human cells, most of the treated cells go through "crisis" phase, where most die. Only a few survive and are characterized by being "immortalized", with the large T-antigen rending the p53 and retinoblastomas proteins non-functional. These "immortalized" cells are not yet tumorigenic. They can be treated with oncogenes, radiation or various chemicals and can eventually become neoplastically transformed. This is the prevailing interpretation. However, an alternative interpretation is that in the original normal primary culture of human cells, exist a few adult stem cells. The immortaling viruses infect all the cells of the population. However, only in the adult stem cells, which by definition os immortal" until it is induced to differentiate or "mortalize", does the large T of the SV40 virus or E6-E-7 proteins of the HPV block differentiation of the adult stem cells. These "immortalizing" viruses do not "immortalize" immortal adult stem cells, but rather they "block" the "mortalization" of normal "immortal" adult stem cells (62,65). In effect, immortalizing viruses are a mis-nommer. They should be called, "blocking mortalizing" viruses, not "inducing immortalization" viruses.

Most recently, the amazing results of the production of "iPS" cells (67) has been re-interpreted (68-71). Basically, when one treats primary cells in vitro with these so-called embryonic stem cell genes or "iPS" cell -inducing factors or conditions, another interpretation of these results is that only the pre-existing adult stem cells are selected to become the "iPS" cells (13,49,62,68-71). Now, putting this new interpretation of the origin of "ips" cells or the concept of "re-programming" into the *in vivo* cancingenesis field, there seems to be an interesting conundrum that relates to possibly resolving the stem cell or "de-differentiation" theory. When an animal is exposed to an initiating or point mutagenic agent, such as UV light, on skin, one knows that a few cells in the skin have been "immortalized" using the classic paradigm. That is, a few normal mortal differentiated fibroblasts have been "de-differentiated" or "re-programmed" to be an "embryonic-like" or pluripotent stem cell. If that is the correct interpretation, why has there not been reported, in all these initiation/promotion skin studies, the eventual appearance of teratomas? In all cases reported, only squamous and basal cell carcinomas have been seen. The other interpretation of what happens in carcino-

genesis for all organs is that the target cell is the ORGAN –SPECIFIC ADULT STEM CELL. These adult stem cells can be mutated by an initiating agent (or by errors in DNA replication), such that they cannot divide asymmetrically to differentiate, but can divide symmetrically to produce two daughters that also are unable to asymmetrically divide. On further stimulation by growth factors, inflammatory factors, hormones, chemical mitogens, and wound-signals, these initiated cells accumulate because they also cannot apoptose.

HOW NUTRIENT AND DIETS CAN AFFECT THE INITIATION AND PROMOTION PHASES OF CARCINOGENESIS

Whether the observations that (a) caloric restriction could reduce the risk to cancer (72), (b) retinoids can either increase or decrease the risk (72-74), (c) calcium might reduce the risk to colon cancer (75); how fatty diets might increase or decrease the risks to certain cancers (76-78) or (d) polyphenols and phytochemicals in the diet could modulate cancer risk (79-80), and (e) scores of other nutrient and dietary exposures reported affects on cancer frequencies (81-83), the underlying mechanisms are still being actively examined since there is a plethora of contradictory reports. The contradictory evidence that has been published might be due to many factors, including poor experimental design, inappropriate models, sampling errors, mis-interpretation of data, etc. However, more importantly, unless basic mechanistic understanding of underlying mechanisms of carcinogenesis are known for the three phases of carcinogenesis, (initiation/promotion/progression) and how dietary factors might influence each phase, empirical studies on either experimental models or epidemiological approaches will always be open to the complexities of the carcinogenic process. Dietary chemoprevention of cancer, post cancer treatment nutrient strategies, "functional foods" to prevent cancers, and whole food versus bio-active components of foods as supplements are being suggested without much detailed mechanistic understanding as to how they might interfere with the complex carcinogenic process. This, then, begs the question, "Where and how can nutrition and dietary behaviors affect the initiation, promotion, and progression phases of carcinogenesis?".

One such mechanism has been proposed that chemopreventive and chemotherapeutic agents must, ultimately, reverse the universal phenotype of all cancer cells, namely the absence of gap junctional intercellular communication. Cancer cell are characterized by the lack of functional gap junctional intercellular communication either by no transcriptional expression of the connexin genes or by the non-functioning of the connexin proteins by epigenetic chemicals (tumor promoters), oncogenes or mutations (55). Clearly, the detailed mechanisms of how a nutrient metal, like selenium or Ca^{++}, a nutrient, like retinoids/carotenoids (84,85), a compound, such as beta-sitosterol in olive oil or psyllium fiber (86), green tea components (87), reservatrol (88), caffeic acid (89), and Quercetin (90) must be very different. Yet, if can be shown that they affect the cancer process at a specific phase (e.g., anti-initiators; anti-promoters, etc.) and if it can be shown what the biological basis of each phase is (initiation due to mutations in adult stem cells or the "re-programming" of somatic differentiated cells), then, possibly, better specific strategies for nutritional and dietary strategies could be designed. However, given unique individual genetic, gender and developmental stage differences, there will probably be no universal, "one fits all"－intervention strategy for cancer prevention/ treatment (86).

To try to start a understanding of this complex problem, we must start with the key event, the "initiation" of a single normal cell that could eventually lead to a human invasive and metastatic cancer cells. As previously indicated, the question is: "Is the normal adult "immortal" organ-specific stem cell that 'target' cell?" or "Is the somatic differentiated "mortal" cell "de-differentiated or "re-programmed" to become "embryonic-like" and restored to an immortal state. Since "initiation" is operationally-defined as a the process that blocks a single cell from terminally-differentiating and having unlimited proliferation capacity, one has to assume that the cell, with "one hit" by a stable irreversible mechanism (i.e., mutational event), the initiated cell either remains immortal if it was an adult stem cell or that it was "reprogrammed" from the "mortal" differentiated state to become "immortal", such as an embryonic-like or "induced pluripotent stem cell ("iPS"). While the recent ability to produce "iPS" cells from primary *in vitro* cultures, it has been assumed that the interpretation that they arose via "reprogramming" has been almost universally accepted. However, an alterative explanation was offered that these "iPS" cells arose via adult stem cells in all organs (62,64,68-71), and recently demonstrated that a small subpopulation of cells in a primary culture or tissue is the target population from which the "iPS' cells can be derived (91). In addition, a direct experiment, using normal human breast stem cells, demonstrated clearly, that they, not their differentiated epithelial breast epithelial descendants, could be prevented from asymmetric cell division to be prevented from differentiated into breast epithelial cells (or to remain "immortal", not to be induced to become "immortal") (49,62). These ini-

tiated cells were not tumorigenic, but could be induced to become weakly and highly tumorigenic via subsequent X ray treatment and transfection with an oncogenic ERB-2 gene. Therefore, for the purposes of this *Commentary*, it will be assumed that the adult normal organ-specific stem cells of all organs are the "target cells" to start the carcinogenic initiation process.

Several points must be emphasized here. First, if the "initiated" cell was derived from the differentiated somatic cells via "re-programming", it should conform to the accepted definition of an "iPS" cell, namely, the cell must be able to give rise to the three germ layers when placed back into an adult animal. If an initiated cell was the result of "re-programming", then, *in vivo*, these "iPS" cells should give rise to teratomas, not carcinomas or sarcomas. Since, that is not the case in adult human cancers, one might assume that "re-programming" did not occur *in vivo* during the initiation event. Second, if mutagenesis is the mechanism underlying the "initiation" of a adult stem cell, one has to recognize that a mutation in the gene (s) that block asymmetric cell division of an adult stem cell could arise via either an error of DNA repair of DNA damage or via an error of replication off a normal DNA template. The latter, could be viewed as a normal, spontaneous mutation, which do occur. It might be assumed that all humans have "initiated" cells in all organs due to this rare, but finite, mechanism of mutation formation. One might also assume that in tissues with more stem cell replication, the risk for more spontaneous "errors of replication" to occur, and also, the older we get, the more "initiated" cells to occur via "errors of replication". Third, nutrients and diets that could increase or decrease the numbers of organ-specific adult stem cells would increase or decrease the risk to the initiation process (more on this in the Barker hypothesis, later).

Now, since it would be impossible to reduce the probability of initiation events to zero (albeit, one can reduce the risk, such as reducing exposures to sunlight-light ultraviolet rays), and since the promotion phase, in the case of adult cancers must take decades, it would seem obvious that impacting this phase of carcinogenesis that has the greatest chance of reducing the risk to cancer. Therefore, it behooves one to understand the biological mechanisms underlying the promotion process.

In the literature, promotion of initiated cells can be produced by growth, wound repair, solid particles, inflammatory stimulation, cell death by necrosis (not by apoptosis), hormones, and mitogenic epigenetic chemicals (92,93). While there still has been no universal acceptance of the mechanism(s) of tumor promotion, one mechanism seems to be supported by more experimental reports that any other suggested mechanism. That cellular mechanism is the inhibition of gap junctional intercellular communication (44). This conclusion is based on the original observation that cancer cells lacked GJIC, either because they never expressed their connexin genes or that the connexin genes were expressed but rendered non-functional by activated oncogenes or by mutations. In addition, classic tumor promoters, such as phorbol esters or phenobarbital, reversibly inhibited GJIC after a threshold level was achieved, were effective when applied regularly, for long periods of time, in the absence of anti-tumor promoters. Anti-sense connexins treatment of normal cells caused them to exhibit a cancer phenotype, while treatment of cancer cells with various connexin genes restored normal growth control. In addition, supporting the idea that adult stem cells could be targets for cancer stem cells is the observation that these tested normal human adult stem cells (kidney, breast, pancreas, liver, intestine) lacked functional gap junctional intercellular communication (63). It also has to be emphasized that these tumor promoters work via different molecular mechanisms to inhibit GJIC. Phorbol esters work via activation of protein kinase C and hyperphosphorylation of connexin proteins to inhibit gap junction function (94). DDT, phenobarbital, PBB's, phthalates, epidermal growth factor, tumor growth factor alpha, estrogens, etc. all work via other intra-cellular signaling mechanisms, such as oxidative-stress induced signaling (10). Therefore, there will not be universal chemopreventive agents to restore all tumor promoting- or chemotherapeutic-inhibition of GJIC (86,95). In addition, in the case of cancer cells that original directly from adult stem cells that never expressed their connexin genes to give rise to "cancer stem cells", such as HeLa or MCF-7, chemopreventive or chemotherapeutic agents that transcriptionally turn on the connexin genes will have to be found, such as Suberoylanilide hydroxamic acid (95).

Finally, to link these observations to nutrients and diet, many bioactive compounds, that have been experimentally or epidemiologically associated with cancer chemoprevention or chemotherapy, have been shown to either prevent tumor promoters or oncogenes from down regulating GJIC or to restore GJIC in tumor cells (45,82-84,94-96). This list, which is not all-inclusive, includes retinoid, carotenoids, green tea components, resveratrol, caffeic acid phenyl ester, and beta sitosterol.

Given the opposite biological effects of a given bioactive compound, such as the genistein in soy products (79,98-100), resveratrol (101,102) and retinoids (73,103-106), the failure of the CARET (Beta-Carotene and Retinol Efficiency Trial) and A BTC (Alph-Tocopherol Beta Carotene Cancer Prevention), due to possible con-

centration effects and the reversal of the antioxidant to the prooxidant state of chemicals (107-110), it might not be surprised that the infamous CARET and ATBC Study trials results in unexpected negative or harmful effects (111,112). It raises the question of whether isolated bioactive compounds from foods will behave differently that when injected within the whole food stuff. The unknown solution to food component supplements versus "functional" whole foods remains to solved. However, both the experimental, *in vitro* and *in vivo*, as well as selected epidemiological studies strongly suggests that dietary behavior and selected nutrients can be both tumor promoters and anti-tumor promoting chemopreventive/chemotherapeutic agents. Equally, whether an individual receiving these nutrient supplements was either deficient or proficient in these nutrients during treatment could alter the results (19,86).

MODULATION OF ADULT STEM CELLS DURING DEVELOPMENT BY NUTRIENTS AND DIET: THE POSSIBLE EXPLANATION OF THE BARKER HYPOTHESIS

While it is well-known that, during, pregnancy, the developing embryo and fetus is a captive in a very unique microenvironment. Although the unique genetic "blue print" of this embryo will impart specific genetic instructions, the environment surrounding that embryo/fetus will influence those genetic instructions. From the oxygen tension, nutrient/dietary factors, medications, maternal stress and behaviors environmental physical, biological and chemical exposures, this prenatal exposure could not only affect normal development seen directly at birth (birth defects or teratogenesis) or it could alter the risk to diseases later in life (i.e., the Barker hypothesis) (113,114). This Barker hypothesis has been defined as pre-natal and early post-natal exposures to certain environmental/dietary factors could alter diseases later in life.

That prenatal exposures to environmental chemicals could affect human cancer is dramatically illustrated with the DES event (115). Pregnant women, who took this drug, during critical periods of fetal development, were predisposed to vaginal cancer when the daughters reached puberty. In animal experiments, pregnant rats exposed to the endocrine disruptor, bisphenol A, gave rise to males that were predisposed to prostate cancers. However, when these pregnant rats were co-exposed to bis-phenol A and a soy diet, but not after birth to either, the risk to prostate cancer was eliminated (116). This clearly implied that dietary factors could, in this case reduce the risk a specific cancer. So the question is, "Could prenatal nutrition and dietary factors, including caloric restriction/caloric abuse, nutrient depredation/overexposure, dietary behavior (eating patterns/daytime/nighttime), whole foods/bioactive food component supplements, specific microbiome microenvironment of the pregnant mother, etc.

One potential human example could illustrate how this prenatal dietary exposure could dramatically affect specific cancer risks. In the atomic bomb survivor studies, it has been shown that breast cancers in the female survivors, who had mothers who eat the traditional Japanese diet (caloric-restricted diets, soy products, raw fish, vegetables, green tea), and were calorically restricted (117) had detectable breast cancers attributed to their radiation exposure at a young age. The background frequency of breast cancers in non-atomic bomb exposure women was very low at that time. Therefore, any increase that might had been attributed to the atomic bomb radiation could be see above the low background. One explanation for this comes indirectly from the epidemiological studies that clearly rules out genetic factors, because of the diaspora of Japanese to other cultures (Hawaii, Brazil, United States). Here the frequency of breast cancer of these displaced Japanese women mimicked the frequency of the non-Japanese women of these new countries. This clearly implied that the new cultural environment and diets had an impact on raising the customary low Japan-influenced breast cancer frequency. One potential influential factor responsible for these observations might be the role of the soy products, including genistein (but also Bowen-Birk inhibitor) (19,118).

The hypothesis to pull all these observations together is the assumption that merely increasing or decreasing the organ-specific stem cell pool (in this case normal human breast adult stem cells) would increase or decrease the "target-size" for the carcinogenic initiation event to occur. If during the development of the female fetus's breast stem cell pool, the pregnant woman's caloric intake was low (as were the Japanese women during and immediately after the atomic bomb exposures) and soy products were predominant in the diet, the numbers of the breast adult stem cells were reduced. Since it has been shown that, *in vitro*, when adult human breast stem cells were exposed to genistein, they differentiated (119). This could suggest that *in vivo*, under these conditions, the female fetus would be born with few adult breast stem cells. On reaching puberty, there would be few stem cells to produce breast tissue and few adult stem cells in small breast to be "targets" for the initiation of the breast carcinogenic process. Today, with the Western diet influencing the pregnant Japanese women, the frequency of breast cancers are increasing.

If one could extrapolate from this example, it would seem that nutrition and diet can influence the "initiation" phase of cancer by either increasing or decreasing the organ-specific adult stem cell pools. After birth, exposures to various nutrients and dietary factors, as well as cultural behavior, could increase (tumor promotion) or decrease (chemoprevention or anti-tumor promotion) of any initiated stem cell. Here, the experimental and epidemiological literature is too large to review to illustrate both increases or decreases of cancer risks. The major reason so many contradictory epidemiological studies have been reported is because the promotion phase.

CONCLUSION

With a better understanding of the disease of cancer, in that it appears to involve multi-steps (initiation/promotion/progress), each of which could involve multi- mechanisms (mutagenesis, cytotoxicity and epigenetic alteration of the expression of genes), and that adult stem cells might be the targets to start the carcinogenic process, it now seems clear that nutrition and diets can influence each of these steps. In addition, from a human perspective, it is becoming clear that our recent cultural evolution, from the discovery of fire, agriculture, diasporas of humans to vary different geo-physical areas to live and obtain foods, our early diets played a major role in selecting genes that allowed each human culture to survive. However, with the explosion of knowledge and technologies in the relative recent centuries, the ability to produce in abundance, distribute both people and foods around the world, process the foods in ways very different than in the past, to eat foods at all hours of the day, and to live longer than before, has created a major challenge to our biology (13,19). It took millions of years in different regions of the globe for individual human groups to select those genes that allowed them to survive the foods available to them (120-124). Yet it has taken only a few generations for unbelievable cultural evolution to impact on those unique genetic backgrounds. Since cultural, as it affects foods, changes in a matter of a few years, our genetic background cannot adapt fast enough to cope with the changing nutritional/dietary habits. Our genes were not selected to cope with the "McDonalization" or the "Coke Colazation" of the world.

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(Received November 1, 2011; Accepted December 2, 2011)