J Membrane Biol (2007) 218:93–100
DOI 10.1007/s00232-007-9072-6

Gap Junctional Intercellular Communication as a Biological "Rosetta Stone" in Understanding, in a Systems Biological Manner, Stem Cell Behavior, Mechanisms of Epigenetic Toxicology, Chemoprevention and Chemotherapy

James E. Trosko

Received: 25 September 2007 / Accepted: 25 September 2007 / Published online: 25 October 2007
© Springer Science+Business Media, LLC 2007

Abstract In spite of the early speculation by Loewenstein that one of the critical distinguishing phenotypes of cancers from normal cells was the dysfunction of gap junctional intercellular communication (GJIC), this hypothesis has not captured the attention of most birth defects and cancer researchers. Moreover, even with later demonstrations that factors that influence normal development and carcinogenesis by modulating GJIC, such as chemical teratogens and tumor-promoting chemicals, inflammatory factors, hormones and growth factors, antisense connexin genes, knockout mouse models, human inherited mutated connexin genes, si-connexin RNA, chemopreventive and chemotherapeutic chemicals, it is rare that one sees any reference to these studies by the mainstream investigators in these fields. Based on the assumption that the evolutionarily conserved connexin genes found in metazoans are needed for normal development and the maintenance of health and T. Dobzhansky's statement "Nothing in biology makes sense except in the light of evolution," a short review of the roles of endogenous and exogenous modulators of GJIC will be made in the context of the multistage, multimechanism process of carcinogenesis, the stem cell theory of carcinogenesis, the discovery and characterization of normal adult stem "cancer stem" cells and the observation that two distinct classes of GJIC-deficient cancer cells are known. The implications of these observations to a "systems biological" view of the role of gap junctions and the nutritional prevention and treatment of several chronic diseases and cancer will be discussed.

Keywords Connexin · Tumor promotion · Stem cell theory of cancer · Cancer stem cell · Systems biology · Epigenetic toxicology

J. E. Trosko (✉)
Center for Integrative Toxicology, National Food Safety Toxicology Center, Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, MI 48824, USA
e-mail: James.Trosko@hc.msu.edu

Introduction

There comes a time in every scientific discipline that (1) the current paradigm is no longer capable of adequately explaining new observations or (2) the old paradigm, via its past success, opens up new insights that allow one to modify the paradigm to integrate into new disciplines. This short commentary is based on the assumption that the latter option has occurred in the field of gap junction biology. From outside this field, it would be hard to imagine why a cellular structure, the gap junction, representing a biological function of metazoans, namely gap junctional intercellular communication (GJIC) of ions and small-molecular weight molecules, should be assigned a distinguishable role in evolution over that of any other cellular structure, such as a tight junction, nucleus, mitochondrion, etc. Clearly, all are required for the existence and function of a cell. Therefore, why would the gap junction be attributed a unique role in the evolution of the higher-order functions of a metazoan?

Recall the famous remark of Theodosius Dobzhansky (1975): "Nothing in biology makes sense except in the light of evolution." When the first multicellular metazoan appeared, this aggregated collection of cells was more than a collection of many cells; it was a new type of organism, different from the single-cell organisms from which it evolved. New phenotypes "emerged" that allowed this

new social society of cells to acquire a new adaptive survival strategy. The phenotype of cell division or growth control had to be associated with the acquisition of a group of cells to survive as a group. Had not that phenotype occurred, cells would have grown in an unlimited fashion, dependent only on temperature and nutrients, in a manner similar to a tumor. Next, this society of self-growth-controlled cells acquired the phenotype of cell specialization or differentiation. By assigning specific cellular functions within the group – secretory cells, neuronal cells, muscle cells, visual sensory cells, etc. – the society now had additional attributes to survive changes in the environment. The phenotype of self-annihilation or programmed cell death gave this group of cells the flexibility to remove cells within itself to add new functions during its development that were more adaptable for that stage, as well as remove to cells that inhibited the survival of the group because of acquired damage. The acquisition of the phenotype of "mortality" of the metazoan organism, after it survived long enough to reproduce and have its offspring survive, might seem a bit strange. That is because a single cell's status, being essentially "immortal" and able to proliferate indefinitely to maintain the survival of the species, would seem to be an admirable phenotype to maintain. The acquisition of the ability to terminally differentiate, i.e., to produce a red blood cell to carry oxygen to cells within tissues that had given up that ability in order to do other specialized functions, such as electronic stimulation of functions, had to be balanced with other new phenotypes, such as allowing the organism to survive long enough to reproduce and take care of offspring. This "mortality" phenotype was the phenotype of the whole organism, while the maintenance of "immortality" was assigned a few cells within the metazoan, namely, the adult stem cells and germ line stem cells. The former gave the whole organ the ability to expand the cell population of a tissue during growth and to replace damaged or dead cells within the tissue. The latter allowed the species genomic DNA to be passed on to future generations, ensuring the ability of the species to survive.

At the time in evolution when this transition took place, a family of new highly evolutionarily conserved genes appeared in metazoans that did not exist in the single-cell organisms, namely, the connexin genes coded for the membrane-associated proteins, connexins, which were organized into hexameric hemichannels and united with the hemichannel of neighboring cells to form the gap junction (Evans & Martin, 2002). This coupling of cells within a tissue ensured synchronized metabolic equilibration and/or electronic unification of cells. In effect, many cells of a metazoan do not exist as single cells but as a syncytium. Only those cells that needed mobility or freedom of function do not express their connexin genes or downregulate their gap junction function. For example, maintenance of an adult stem cell, in order to remain "stem" or to be primitive in the state of differentiation in the midst of their progenitor and differentiated daughter cells, seems to require nonexpression of the connexin genes or a nonfunctional GJIC (Trosko, 2000). On the other hand, the progenitor cells, which have the ability to proliferate a finite number of times before senescence or apoptosis, seem to have their ability to proliferate regulated by "contact inhibition," which appears to involve GJIC.

Clearly, this speculative interpretation of the potential role of the gap junction in evolution is not based on highly rigorous detailed mechanistic experiments of all disciplines (genetics, molecular biology, biochemistry, cell biology, physiology, etc.) related to the gap junction. However, there is a time in this science of gap junctions when newer information starts to create new views of the role of gap junctions in the evolutionary and developmental role of higher-order attributes of a metazoan. In effect, these observations assist in giving meaning to the reemergent science of "systems biology" (Cornish-Bowden, 2006). In other words, new phenotypes can "emerge" by the organization of different levels of structure/function. For example, when two types of differentiated cells (e.g., epithelial and stromal cells) emerge so that they exist side by side, they influence each other mutually to perform different functions from those they could have performed by themselves alone (Barcellos-Hoff, 2001). A few, widely dispersed neurons in brain tissue that secrete small amounts of hormones could now influence sexual development when they have their secretion synchronized by gap junctions, to make a biological impact when the individual secreted levels would be insufficient for bringing about any sexual development.

Systems biology is not simply the summation of all the reductionistic understanding of molecular mechanisms within a cell of a multicelled organism but the study of the complex integration of higher-level structures/functions that occurs due to the delicate timing and interactions within cells, within tissues and within and between organs that allow unique phenotypes to emerge that would not do so simply by adding together the component parts. Without the appearance of the connexin genes, their specific expression and selective ability to allow certain ions and small molecules to affect neighboring cells, as well as allow signals within a cell to trigger specific gene expression patterns for adaptation to a changing environment, a cell would not have the ability to divide, differentiate, apoptose or, if already differentiated, adaptively respond. The connexin genes are not simply just another set of genes in the genome of multicelled organisms. They are the genes that allow the whole organ to survive as a "system."

However, the hypothesis that the major role that gap junctions play in the evolution of the higher-order functions

of a metazoan and their primary role in systems biology is based on what appears to be an accumulation (or weight of the evidence) of experimental results linking the absence of GJIC to a wide range of developmental and disease states. The first is the speculation that the major difference between a normal cell and a cancer cell is the ability to perform GJIC. This early hypothesis was generated by Loewenstein & Kanno (1966). When one views the early emergence of phenotypes of metazoans – e.g., growth control, differentiation, apoptosis, mortality – it seems incredible that a cancer cell is characterized by (1) loss of contact inhibition or growth control, (2) inability to terminally differentiate, (3) dysfunctional apoptosis and "acquisition" of immortality. It is almost as though the emergence of a cancer cell within the finely evolutionarily honed cells of a healthy metazoan went through a process of de-evolution. In addition, was it a "coincidence" or was it "causal" that these cancer cells lacked functional GJIC? One of the general characterizations of cancer cells is that they lack GJIC either because they never expressed, transcriptionally, any connexin genes (e.g., HeLa or MCF-7 cells) (King et al., 2000; Momiyama et al., 2003) or because their expressed connexin genes were rendered nonfunctional by activated oncogenes or mutations (Trosko & Ruch, 1998).

In summary, the juxtaposition of evolution; stem cells; gap junction genes/function; higher-order functions of growth control, differentiation and apoptosis; and cancer, albeit based on the liberal splicing of selected observations and logic, does not constitute what most would agree to be rigorous scientific evidence. However, science makes its advances by the generation of testable hypotheses, which are born to challenge existing paradigms. The existing paradigm that is being challenged is that "carcinogens are mutagens" (Ames et al., 1973).

Hallmarks of Cancer: What Is the Role of Gap Junctions?

In a rather important fashion, the article "The Hallmarks of Cancer" (Hanahan & Weinberg, 2000) helped to focus the attention of the cancer research field. On careful analysis of the six hallmarks, an interesting idea jumps out: Three of the hallmarks, namely, immortality, invasive property and ability to induce angiogenesis, seem not to be unique properties of only cancer cells. All normal stem cells are naturally "immortal" until they are induced to terminally differentiate, and they are able to invade tissue and to induce angiogenesis during normal development. This begins to suggest that stem cells and cancer cells may share several common phenotypes.

However, taking another view of the multistage, multimechanism view of carcinogenesis, more evidence emerged that seems to be consistent with the paradigm of "carcinogen as mutagen." Those who strongly adhere to this paradigm feel that understanding mutations and mutagenesis is sufficient to explain the cancer process. After all, cancer cells contain mutations in oncogenes or tumor-suppressor genes. Inherited germ line mutations could predispose individuals to cancers; ultraviolet light-induced DNA lesions, which are not repaired in xeroderma pigmentosum skin cells, lead to mutations in *p53* genes of skin tumors (Brash et al., 1991). Chemicals associated with the induction of experimental tumors in rodents have been shown with in vitro "genotoxicity" assays to be capable of inducing cells having the phenotype consistent with having arisen because of presumptive mutations. However, with the exception of the chemical "carcinogen" being a mutagen, all other linkages of mutations and cancer are scientifically solid. The idea that chemicals which are associated experimentally in rodents or epidemiologically with cancers after exposure or with the mutations found in the cells of these tumors has been challenged (Trosko & Upham, 2005; Thilly, 2004). In brief, there are two possible interpretations of the origin of mutations in the tumor cells found in animals or humans exposed to the chemical in question. One is that the chemical-induced genomic DNA lesions, which, if repaired erroneously or not repaired, could be substrates for induced mutations and, if induced in proto-oncogenes or tumor-suppressor genes, could lead to cancer (the current paradigm). Alternately, if these chemicals actually selected preexisting mutations found in a cell that had a spontaneously induced mutation (i.e., error in DNA replication in a stem cell), then this "initiated" stem cell would be prevented from terminally differentiating or from dividing asymmetrically. This, then, is a new radical challenge to the idea that chemical carcinogens, teratogens, reproductive toxicants and neurontoxicants are toxicants because they damage genomic DNA and lead to mutations. Rather, it gives rise to the concept that chemical toxicants act epigenetically; i.e., they alter gene expression, in either stem cells, progenitor cells or terminally differentiated cells, by transcriptional, translational or posttranslational modifications (Trosko et al., 1998). In a stem cell these chemicals could induce abnormal proliferation, differentiation or apoptosis. In progenitor cells they might alter proliferation, apoptosis or senescence. In terminally differentiated cells, these chemicals could alter gene expression or apoptosis and possibly dedifferentiation.

That is best described as the juxaposition of the multistage, multimechanism concept of carcinogenesis meets gap junctions and stem cells. One of the earliest observations that a class of chemicals, which were classified as carcinogen promoters but not carcinogen initiators, was not able to damage genomic DNA or to cause mutations. To

understand this statement, the concepts of "initiation" and "promotion," both of which are operational concepts, were generated from whole-animal experimental cancer studies (Boutwell et al., 1982; Pitot & Dragon, 1991). An initiator is an agent that can induce an event in a normal cell, such that it now can exist long enough to accumulate additional irreversible genetic/epigenetic changes to become an invasive, metastatic cell. Promotion, on the other hand, operationally, is the process that will bring about the clonal expansion of that single initiated cell. Experimentally, promotion can be brought about by wounding, surgery, chronic inflammatory processes or processes leading to compensatory hyperplasia (Trosko & Tai, 2006). In addition, noncytotoxic stimulation of mitogenesis or blockage of apoptosis (Trosko et al., 1995) could lead to the dual processes of bringing about the expansion of the initiated cells (cell growth plus inhibition of cell death).

The operational terms "initiation" and "promotion" do not directly imply the underlying mechanisms. One hypothesis is that initiation occurs in an adult stem cell that prevents it from dividing asymmetrically but will allow it to divide symmetrically. Mechanistically, while mutations could explain this "irreversible" change, so could, in principle, a stable epigenetic change. In addition, mechanisms of promotion, leading to mitogenesis and blockage of apoptosis, need to be explained.

Promoting chemicals seem to be species-specific, tissue-specific and cell type-specific. An initiated organ needs to be exposed at a threshold level of the promoting agent or condition for a regular and extended period of time in the absence of an "antitumor promoter." A 7% relative decline in breast cancer incidence between 2002 and 2003 in the United States (Ravdin et al., 2006) has been interpreted as the result of millions of women having stopped taking postmenopausal hormone replacement therapy. It is not surprising that the results are what they were and that the explanation is correct. Estrogen is not a "carcinogen"; it is a tumor promoter. The minute the estrogen exposure in these women was stopped, the initiated breast cells (probably initiated breast cancer stem cells, which are estrogen receptor-positive) stopped proliferating.

Here, the role of gap junctions has been offered as a testable hypothesis. When it was shown that phorbol esters, a powerful rodent skin tumor promoter but not an initiator, blocked gap junction function in a reversible, noncytotoxic fashion after regular, chronic exposure at or above threshold dose levels (all properties of operational tumor promoters [Goodman, 2001; Trosko, 2001]), it was subsequently shown that many different classes of chemicals, working via different biochemical mechanisms to inhibit gap junction function, could also inhibit gap junctions (Trosko & Chang, 1989). This was not universally accepted as a mechanism of tumor promotion, as seen by the statement of Emmanuel Farber (2000): "There is a developing speculation and assumption that agents or circumstances are promoters or are promoting if they lead to decrease in cell-cell communication by interference with gap junction expression. This is a serious misconception that should be abandoned!"

Moreover, when one examines recent reviews of leading oncology researchers, especially those representing the field of molecular oncology, one never sees references to the potential role of gap junctions or the original hypothesis of Loewenstein & Kanno (1966). This seems to be an important observation related to the diffusion, or lack, of information within and between disciplines. One possible explanation is the success of molecular oncology in drawing the attention (and funding) away from basic cell biology of carcinogenesis and whole-animal carcinogenesis studies to studies on the single cancer cell and changes in the genome of these cancer cells. The introduction of sophisticated technologies, such as microarray analyses of cancers, has caused some fundamental biological understanding of complex cell interactions within both normal and cancer tissues, as well as the relatively newer insights of cancer stem cells (more to be said later on this matter), to be ignored. However, the insight by the late Van R. Potter (1945) is relevant to this dichotomy between the biology of cancer and molecular oncology, as seen with his statement:

> It was suggested earlier in this discussion that during the critical period, the cancer cells are susceptible to the influences of the host and are restrained by normal cells. The basis for this is the fact that the normal sequence to an injury is growth which reaches a certain level and then stops when the injury has been repaired. This growth must stop by some self-regulatory process which is possessed by normal cells but not possessed by tumor cells. The suppression of tumor growth by normal cells during the critical period undoubtedly occurs through the operation of a mechanism by which normal cells suppress their own growth when this is desirable.

There are two types of normal cells with regard to expressed or nonexpressed connexin genes and functional gap junctions. It appears that the fertilized egg and early blastocysts do not express their connexin genes or have functional gap junctions (Lo, 1996). In addition, many of the tested adult stem cells do not have functional GJIC (Trosko, 2000). These undifferentiated cells, while in their niches, do not normally proliferate. Therefore, growth control must occur via some non-gap junction fashion. Either or both extracellular substrate suppressive signals and secreted negative growth-suppressor molecules probably restrict proliferation of stem cells. The normal

progenitor cells are the so-called transit cells of a tissue, which, by their finite proliferative potential or the "Hayflict limit" (Hayflict, 1965), generate the bulk of the cells for tissue growth and replacement. Growth control or "contact inhibition" (Eagle, 1965) in these cells is most likely mediated by gap junctions. Reversible inhibition of these gap junctions by cytokines, hormones, growth factors and exogenous chemicals brings about the inhibition of contact inhibition and allows cells to proliferate.

In addition, there seem to be two kinds of cancer cells that are unable to perform GJIC and are not contact-inhibited. The first type, represented by the HeLa and MCF-7 cancer cell lines, do not perform contact inhibition or have functional GJIC because they have their normal connexin genes transcriptionally suppressed (King et al., 2000; Momiyama et al., 2003). The second type of cancer cell, which also has no functional GJIC, is unable to perform GJIC because their connexin proteins are rendered nonfunctional either by some activated oncogene or by some mutation in the connexin gene or some connexin regulatory gene (Trosko & Ruch, 2002).

These two types of cancer cells must be seriously considered as very different in terms of any strategy to prevent or treat them, assuming that restoration of normal gap junctions is the primary goal of the cancer chemopreventive or other therapeutic strategy (Trosko, 2003b).

In addition, these two types of normal and cancer cells, with nonexpressed connexin genes (normal stem cells, cancer stem cells) or with expressed connexins, are mitogenically stimulated ("promoted") by either secreted growth factors that trigger mitogenic signalling or endogenous or endogenous factors that trigger signaling that causes the expressed connexin proteins not to be trafficked, assembled or functional (i.e., their GJIC has been inhibited).

In both cases and in both classes of cells, gap junctions represent the ultimate downstream, fundamental biological function needed for proper growth control and differentiation.

Normal Stem Cells as "Targets" for Cancer Stem Cells and the Role of the Tumor Microenvironment in Tumor Cell Heterogeneity

Two major hypotheses of the origin of cancer were proposed decades ago, namely, the stem cell theory (Markert, 1968; Pierce, 1974; Potter, 1978) and the theory of dedifferentiation (Wicha, Liu & Dontu, 2006). While the jury is still out on these hypotheses, in the case of leukemia the evidence does seem to support more easily the stem cell theory (Al Hajj et al., 2003). On the other hand, the recent observation that within solid tumors there exist "cancer stem cells," cancer cells that have the potential of self-generating more cancer cells, as opposed to the other tumor cells which cannot self-sustain the tumor. These might be referred to as cancer "non-stem cells" or "partially differentiated" cancer stem cells, as the late Dr. V. R. Potter (1978) might have called them ("Oncogeny as partially-blocked ontogeny").

The recent demonstration of these cancer stem cells in many tumor types (breast, leukemia, brain [Wicha et al., 2006; Al Hajj et al., 2003; Singh et al., 2003; Kondo et al., 2004; Till & McCulloch, 1961]) and the demonstration that the cancer stem cell could sustain further growth of the tumor (Ponti et al., 2005) lead to the question, "Where did the original cancer stem cell come from?" Since the cells of the tumor, while being both genotypically and phenotypically heterogeneous, are clonally derived from a single cell (Fialkow, 1979), either that single "initiated" cell must have been a normal, immortal stem cell that was blocked from "mortalizing" or terminally differentiating and became the cancer stem cell or the normal cell was a "mortal" progenitor or terminally differentiated cell that was dedifferentiated by the initiation process to restore its immortality and then became the cancer stem cell.

Our laboratory, having isolated a number of normal adult human stem cells, utilized an observation that the *Oct-4* gene, a presumptive embryonic stem cell marker, that is not expressed in normal differentiated tissues (Tai et al., 2005), decided to test if these normal adult stem cells expressed the *Oct-4* gene. Not only did they express the *Oct-4* gene but the gene was suppressed when these stem cells were induced to differentiate. Yet, the *Oct-4* gene remained expressed (not reexpressed) in normal stem cells that were transfected with the SV40 large T or human papilloma *E6/E7* genes. They continued to be expressed in the neoplastically transformed derived cells or in tumor cell lines derived from the respective tissues. Moreover, when 83 canine tumors were examined, 100% of them from 21 different tumor sites expressed the *Oct-4* gene, but the frequency of expression between tumors was very variable (Webster et al., 2005). This suggests that the physiology of the dogs affected whether the cancer stem cells of the tumor divided symmetrically or asymmetrically because the tumor microenvironment was different among the animals.

Given the known influence of oxygen tension within the tumor and the known effect of oxygen tension on normal stem cell behavior within their niches (Csete, 2005), it might not be surprising that cancer stem cells might proliferate either symmetrically to produce a tumor with more *Oct-4*-expressing cancer stem cells or asymmetrically to produce some partially differentiated cells that might express connexins and are promoted by agents that block gap junctions. In addition, it seems that several tumor cells, having been separated by fluorescence-activated cell sorting using fluorescent toxicants, partition into two groups, one that fluoresces and another, a "side population," that

does not contain the fluorescent toxicant. These side population tumor cells seem to display stem-like properties (Asakura & Rudnicki, 2002). They seem to be resistant to the fluorescent toxicant because they express the multidrug-resistant gene *ABCG-2* (our unpublished data).

The recent demonstration that an endocrine-disrupting chemical, bisphenol-A, given to a pregnant rat, could increase the risk of prostate tumors in the male offspring might be viewed as the chemical increasing the male embryo/fetus prostate adult stem cell pool, thereby increasing the risk that one of these stem cells could be initiated/promoted later (Ho et al., 2006) In addition, if these pregnant rats were treated with both bisphenol-A and genistein, the male offspring did not have an increased risk for prostate cancer. Since genistein has been shown to induce differentiation in adult human breast stem cells (Hsieh & Chang, 1999), the explanation might simply be that the prostate stem cell pool was reduced, thereby reducing the risk for prostate cancer later in life. If this explanation is correct, modifiers of adult stem cell pools in different tissues/organs during pregnancy could influence disease states later in life.

If these ideas and observations are validated, the implications for both prevention and treatment of cancers (and other diseases) are enormous. First, as far as tumor promoters of initiated cells are concerned, if the initiated cell is a normal adult stem cell with no expressed connexin genes, then the promoter must be some agent that stimulates cell proliferation and blocks apoptosis by a secreted factor that stimulates mitogenic signaling in these cells or blocks the mitogenic suppression of some secreted factor. Antipromoters or chemopreventive agents of this class of promoters will have to be those that either induce differentiation of the stem cells by inducing connexin expression or that interfere with the secreted growth promoter or secreted growth suppressor.

Chemotherapeutic agents against this class of cancer stem cells would have to cause transcriptional activation of the repressed connexin genes and transcription repression of the *Oct-4* gene. The examples of HeLa (King et al., 2000) and MCF-7 (Momiyama et al., 2003), being treated with agents that cause transcriptional activation, might be an illustration of this approach. The recent demonstration that epigenetically induced transcriptional modulators, such as suberoylanilide hydroxamic acid (Ogawa et al., 2005), also affected connexin gene expression supports this approach.

Gap Junction as the Biological "Rosetta Stone" in Normal Development and Diseases

The gap junction is only one of the critical and vital structures/functions of a metazoan. However, it possesses a property that no other structure of a metazoan cell has: i.e., it helps create and integrate extracellular phenotypes and functions that the individual cell does not posses. It is truly the structure/function that allows new phenotypes, such as growth control and multiple types of gene patterns, to be expressed in cells containing the same genome and that allows groups of contiguous, but not gap junction-coupled, cells to differentiate independently of each other, permitting different phenotypes/functions to emerge when normal cells aggregate. Without gap junctions, the higher-order phenotypes and functions existing during different stages of embryonic/fetal/neonatal, adolescent, adult and geriatric development could not exist. The recent identification of chemicals and genetic factors that influence gap junction function and can cause a wide range of abnormal development and functional processes in many diseases illustrates that this structure/function is as vital to normal development and function as any gene. In fact, alteration of the many other non-gap junction genes that can influence either survival or disease state probably affects GJIC indirectly. The connection of these new concepts, namely, the role of the quality and quantity of adult stem cells (Trosko, 2003a) and of the expression and function of gap junctions, must be integrated into any "systems" approach to understanding the higher-order function of genomic information. After all, the genomic information is but a "blueprint." It is the delicate and systematic differential expression of that genetic information that leads to normal development and function. This was beautifully stated by C. Markert (1984):

> Cells interact and communicate during embryonic development and through inductive stimuli mutually direct the divergent courses of their differentiation. Very little cell differentiation is truly autonomous in vertebrate organisms. The myriad cell phenotypes present in mammals, for example, must reflect a corresponding complexity in the timing, nature, and amount of inductive interactions. Whatever the nature of inductive stimuli may be, they emerge as a consequence of specific sequential interactions of cells during embryonic development.
>
> The first embryonic cells, blastomers, of mice and other mammals are all totipotent. During cleavage and early morphogenesis these cells come to occupy different positions in the three-dimensional embryo. Some cells are on the outside, some inside. The different environments of these cells cause the cells to express different patterns of metabolism in accordance with their own developing programs of gene function. These patterns of metabolism create new chemical environments for nearby cells and these changed environments induce yet new programs of

gene function in responding cells. Thus a progressive series of reciprocal interactions is established between the cellular environment and the genome of each cell. These interactions drive the cell along a specific path of differentiation until a stable equilibrium is reached in the adult. Thereafter little change occurs in the specialized cells and they become remarkably refractory to changes in the environment. They seem stably locked into the terminal patterns of gene function characteristic of adult cells. The genome seems no longer responsible to the signals that were effective earlier in development.

Of course, changes can occur in adult cells that lead to renewed cell proliferation and altered differentiation as seen in neoplasms, both benign and malignant, but such changes are very rare indeed when one considers the number of cells potentially available for neoplastic transformation. Possibly, mutations in regulatory DNA of dividing adult cells can occasionally lead to new and highly effective programs of gene function that we recognize as neoplastic or malignant. However, most genetic changes in adult cells can probably lead to cell death since random changes in patterns of gene activity are not likely to be beneficial.

Acknowledgments This short personal review was written while the author (CORE B director) was supported by grants from the National Institute of Environmental Health Sciences: 2P42 ES004911-17. The content of this publication is solely the responsibility of the author and does not necessarily represent the official views of the NIEHS.

References

Al Hajj, et al. (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988

Ames BN, et al. (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285

Asakura A, Rudnicki AR (2002) Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. Exp Hematol 30:1339–1345

Barcellos-Hoff MH (2001) It takes a tissue to make a tumor: epigenetics, cancer and the microenvironment. J Mammary Gland Biol Neoplasia 6:213–221

Boutwell RK, et al. (1982) Mouse skin: a useful model system for studying the mechanism of chemical carcinogenesis. In: Carcinogenesis, vol 7. Raven Press, New York, 1–12

Brash DE, et al. (1991) A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinomas. Proc Natl Acad Sci USA 88:101

Cornish-Bowden A (2006) Putting the systems back into systems biology. Perspect Biol Med 49:475–489

Csete M (2005) Oxygen in the cultivation of stem cells. Ann N Y Acad Sci 1049:1–8

Dobzhansky T (1975) Nothing in biology makes sense except in the light of evolution. Am Biol Teachers 35:125–129

Eagle H (1965) Growth regulatory effects of cellular interaction. Isr J Med Sci 1:1220–1228

Evans WH, Martin PEM (2002) Gap junction: structure and function. Mol Membr Biol 19:121–136

Farber E (2000) Risk assessment for possible carcinogens: a critical look. Drug Metab Rev 32:143–151

Fialkow PJ (1979) Clonal origin of human tumors. Annu Rev Med 30:1345–1376

Goodman JA (2001) Operational reversibility is a key aspect of carcinogenesis. Toxicol Sci 64:147–148

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70

Hayflict L (1965) The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 37:614–636

Ho SM, et al. (2006) Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66:5624–5632

Hsieh CY, Chang CC (1999) Stem cell differentiation and reduction as a potential mechanism for chemoprevention of breast cancer. Chin Pharm J 51:15–30

King TJ, et al. (2000) Correlation between growth control, neoplastic potential and endogenous connexin43 expression in HeLa cell lines: implications for tumor progression. Carcinogenesis 21:311–315

Kondo T, et al. (2004) Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 101:781–786

Lo CW (1996) The role of gap junction membrane channels in development. J Bioenerg Biomembr 28:379–385

Loewenstein W R, Kanno Y (1966) Intercellular communication and the control of tissue growth: lack of communication between cancer cells. Nature 209:1248–1249

Markert C (1968) Neoplasia: a disease of differentiation. Cancer Res 28:1908–1914

Markert CL (1984) Genetic control of cell interactions in chimeras. Dev Genet 4:267–279

Momiyama M, et al. (2003) Connexin26-mediated gap junctional communication reverses the malignant phenotype of MCF-7 breast cancer cells. Cancer Sci 94:501–507

Ogawa T, et al. (2005) Suberoylanilide hydroxamic acid enhances gap junctional intercellular communication via acetylation of histone containing connexin43 gene locus. Cancer Res 65:9771–9778

Pierce GP (1974) Neoplasms, differentiation and mutations. Am J Pathol 77:103–118

Pitot H, Dragon YP (1991) Facts and theories concerning the mechanisms of carcinogenesis. FASEB J 5:2280–2286

Ponti D, et al. (2005) Isolation and in vitro propagation of tumorigenic breast cancer cells with stem cell/progenitor cell properties. Cancer Res 65:5506–5511

Potter VR (1945) The role of nutrition in cancer prevention. Science 101:105–109

Potter VR (1978) Phenotypic diversity in experimental hepatomas: the concept of partially blocked ontogeny. Br J Cancer 38:1–23

Ravdin PM, Cronin KA, Howlander N, Chlebowski RT, Berry DA (2006) A sharp decrease in breast cancer incidence in the United States in 2003. Abstract presented at the 29th San Antonio Breast Cancer Symposium, San Antonio, TX, December 14-17, 2006

Singh SK, et al. (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828

Tai MH, et al. (2005) Oct4 expression in adult stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis 26:495–502

Thilly WG (2004) Have environmental mutagens caused oncomutations in people? Nat Genet 34:255–259

Till JE, McCulloch EA (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow. Radiat Res 14:213–222

Trosko JE (2000) Gap junctions and the regulation of cellular functions of stem cells during development and differentiation. Methods 20:245–264

Trosko JE (2001) Is the concept of "tumor promotion" a useful paradigm? Mol Carcinog 30:131–137

Trosko JE (2003a) Human stem cells as targets for the aging and diseases of aging processes. Med Hypotheses 60:439–447

Trosko JE (2003b) The role of stem cells and gap junctional communication in carcinogenesis. J Biochem Mol Biol 36:43–48

Trosko JE, Chang CC (1989) Nongenotoxic mechanisms in carcinogenesis: role of inhibited intercellular communication. In: Hart R, Hoerger FD (eds) Banbury Report 31: New directions in the qualitative and quantitative aspects of carcinogen risk assessment. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 139–170

Trosko JE, Ruch RJ (1998) Cell-cell communication in carcinogenesis. Front Biosci 3:208–236

Trosko JE, Ruch RJ (2002) Gap junctions as therapeutic targets. Curr Drug Targets 3:465–482

Trosko JE, Tai MH (2006) Adult stem cell theory of the multi-stage, multi-mechanism theory of carcinogenesis: role of inflammation on the promotion of initiated stem cells. In: Dittmar T (ed) Infections and inflammation: impacts on oncogenesis. Karger, Amsterdam, pp 45–65

Trosko JE, Upham BL (2005) The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. Mutagenesis 20:81–92

Trosko JE, et al. (1995) Intercellular communication: a paradigm for the interpretation of the initiation/promotion/progression model of carcinogenesis. In: Arcos JC (ed) Chemical induction of cancer: modulation and combination effects. Birkhauser, Boston, pp 205–225

Trosko JE, et al. (1998) Epigenetic toxicant-induced changes in intracellular signaling leading to altered gap junctional intercellular communication. Toxicol Lett 102–103:71–78

Webster JD, et al. (2005) Oct-4 expression in canine neoplasms: a potential role for embryonic genes in cancer progression. Vet Pathol (in press)

Wicha MS, Liu S, Dontu G (2006) Cancer stem cells: an old idea – a paradigm shift. Cancer Res 66:1883–1890