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Cell hydration as the primary factor in carcinogenesis: A unifying concept

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Summary The paper discusses the unifying concept that cell hydration is the primary factor in the mechanism of carcinogenesis. The concept includes the following hypotheses: (1) Increased cell hydration causes cancer not only by promoting cell division and oncogene expression, but also by inactivating genes inducing cell differentiation, and by preventing apoptosis. Conversely, factors that reduce cell hydration prevent cancer by inhibiting cell division and oncogene expression, while activating genes inducing cell differentiation, and by promoting apoptosis. The *unique* ability of cell hydration to have these opposite effects on cell behavior and gene expression can account for its postulated role as the primary factor in both the promotion and prevention of cancer. (2) A progressive increase in cell hydration, induced by successive mutations and/or epigenetic changes, is the basic mechanism of multi-step carcinogenesis, the degree of malignancy increasing with the degree of cell hydration. (3) The increased hydration of cancer cells accelerates their respiration rate, thereby enhancing their ability to compete for nutrients with their normal counterparts. This effect may play a major role in promoting tumor growth and in the postulated mechanism of multi-step carcinogenesis. (4) Increased cell hydration is also proposed as an alternative or additional explanation of the carcinogenetic effect of inflammatory agents and of hormones. A survey of the literature provides evidence consistent with these hypotheses, but suggestions are included for further investigations to test their validity and their implications. From a clinical perspective, the abnormally high water content of cancer cells permits the use of microwave technology for tumor detection and treatment. Also of considerable therapeutic significance is the increased sensitivity if cancer cells to desiccation, postulated to result from genetic changes induced by increased hydration. This may well be the achilles heel of cancer, and recent investigations indicate that it may be exploited very effectively in the treatment of the disease. In conclusion, I suggest that the need for studies on the molecular biology of cancer to be supplemented by more information on environmental effects on gene expression and on the biochemical and physiological factors that mediate genetic effects at the cellular level. This approach might also be used to assess the validity of the postulated role of cell hydration as a factor of particular significance.

Introduction

In view of the general recognition of water as the most important requirement for all forms of life, it is difficult to account for the relatively little attention it has received in biological research

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(see comments by Szent-Gyorgyi [1]). This apparent neglect may be attributed, at least partly, to the greater interest of biologists in more complex organic compounds and, especially in recent years, to an increasing focus on studies at the molecular level. However, the need for more attention to be given to the influence of water was provided by evidence that small changes in cell water content, mediated by hormones or by the osmotic effect of ions or organic osmolytes, can play an important role in the regulation of cell metabolism and gene expression (reviewed in [2]), and in the pathology of various diseases [3].

There is also considerable evidence that cell water content may be a major factor in the mechanism of carcinogenesis. A characteristic feature of cancer cells is that their water content is similar to that of embryonic tissue, but consistently higher than that of normal cells of similar origin [4]. Evidence that this difference may be of carcinogenetic significance was provided by numerous reports from early investigations of a positive correlation between increased tissue water content and carcinogenesis (reviewed in [5]). However, these investigations provided no explanation of this relationship, or of the nature of the mechanism(s) involved. The unifying concept discussed in this paper addresses both of these questions. Suggestions are also included for further investigations that would enable the validity of the concept to be more critically assessed.

Hypotheses

The concept discussed includes the following related hypotheses: (1) Increased cell hydration causes cancer not only by promoting cell division and oncogene expression, but also by inactivating genes inducing cell differentiation, and by preventing apoptosis. Conversely, factors that decrease cell hydration prevent cancer by inhibiting cell division and oncogene expression, while activating genes inducing cell differentiation, and by promoting apoptosis. The *unique* ability of cell hydration to have these opposite effects on cell behavior and gene expression can account for its postulated role as the primary factor in both the promotion and prevention of cancer. (2) On this hypothesis, a progressive increase in cell hydration, induced by successive mutations or epigenetic changes, is the basic mechanism of the well-established multi-step nature of carcinogenesis [6], the degree of malignancy increasing with the degree of cell hydration. (3) The increased hydration of cancer cells and their mitochondria is postulated to accelerate their respiration rate and other metabolic reactions, thereby enhancing their ability to compete for nutrients with normal cells. This effect may play a major role in the promotion of tumor growth and in the postulated mechanism of multistep carcinogenesis. (4) Increased cell hydration is also postulated to provide an alternative or additional explanation of the promotion of cancer by inflammatory agents and by hormones.

When this concept is viewed from an evolutionary perspective, carcinogenesis may be regarded as a reversal of the changes that occurred during the transition of primitive multicellular organisms from an aqueous to a terrestrial environment. Adaptations to this transition are postulated to have involved a reduction of cell proliferation and the evolution of genes promoting increased cell differentiation, especially of a kind that would provide the cells with greater protection from desiccation by accumulating organic osmolytes [7]. Inactivation of such genes by the increased cell hydration postulated to promote carcinogenesis may account for the greater sensitivity of cancer cells to increased extracellular molarity as compared with their normal counterparts [8]. I suggest that this may be the achilles heel of cancer, and experimental evidence indicates that it may be exploited very effectively in the treatment of the disease [9].

When viewed from an ontogenetic perspective, cancer may be regarded as a progressive reversal to an embryonic condition, the increasing degree of cell hydration associated with carcinogenesis causing activation of primitive genes that promote cell division, while inactivating genes inducing cell differentiation, and which were activated epigenetically during embryo development. The frequent occurrence of fetal proteins in tumors [10], and the similarity in the high water content of tumors and embryos, are consistent with this hypothesis.

Evidence consistent with the proposed hypotheses

Cell behavior

The hypothesis that increased cell hydration contributes to cancer by promoting cell division and oncogene expression, while inhibiting cell differentiation and apoptosis, finds considerable support in the literature. Numerous studies, involving a wide range of cells, have shown that cell proliferation is correlated with an increase in cell volume [11]. In fibroblasts, this increase in cell volume is

associated with the transition from the G1 to the S phase of the cell cycle [12], suggesting that factors affecting water uptake may limit the rate of cell division. This may account, at least partly, for the promotion of cell proliferation by the ras oncogene [13], whose expression increases cell volume by approximately 30% [14]. This effect may also contribute to cancer by promoting the hypotonic induction of the gene encoding for ornithine decarboxylase [15], the rate limiting enzyme in the synthesis of polyamines [16], which are essential for cell proliferation and a critical requirement for cell transformation [17]. Also consistent with the present hypothesis is the report that hypotonic induction of cell swelling causes rapid tyrosine kinase activation, which is essential for cell swelling-induced ERK1/2 activation, and for induction of c-*fos* expression in cardiac myocytes [18]. A similar treatment induces an increase in c-*jun* mRNA levels in rat hematoma cells [19].

Since cell division and cell differentiation are alternative and mutually exclusive pathways in the cell cycle, the reduction of differentiation in malignant tissue is usually attributed to the associated increase in cell proliferation. The present hypothesis suggests a mechanistic explanation of this inverse relationship by proposing that increased cell hydration not only promotes cell division but also inactivates genes that induce differentiation. This hypothesis is consistent with evidence that the activated ras oncogene can inhibit myogenic differentiation by a direct inhibition of gene expression [20], and by a mechanism that is not dependent on continued cell proliferation [21]. Further investigations are required to test the hypothesis that the increase in cell hydration caused by ras gene expression may have contributed to these effects, and that a similar mechanism may account for the inhibition of cell differentiation by other oncogenes [22]. The additional hypothesis that increased cell hydration prevents apoptosis is consistent with evidence that insulin-like growth factor (IGF1), the major mediator of insulin, and which induces cell swelling [23], is a potent inhibitor of apoptosis [24].

The evidence that genes inducing differentiation are directly inhibited by increased cell hydration suggests that such genes would be activated by a reduction in cell hydration. Results consistent with this assumption were provided by a study in which cells of a human cell cancer line (HT29) were treated with a non-toxic, non-absorbed polymer, polyethylene glycol (PEG, MW 8000) [25]. This substance increases the osmotic pressure of aqueous solutions in a dose dependent manner [26], and may therefore be expected to reduce the degree of cell hydration. After a 3-week delay, this treatment induced the characteristic appearance of cells undergoing differentiation, referred to as ''flat foci''. When subcultured, these cells produced two kinds of colon cells, namely enterocytic and mucus-secreting cells, which were stable in long term culture in standard solution. However, further studies are needed to determine the degree of PEG-induced reduction of cell hydration, and its relation to the induction of differentiation.

Of more practical significance is evidence that PEG not only induces cell differentiation but may also be a very effective treatment for colon cancer. Corpet et al. [9] reported that when rats were provided with 5% PEG in their drinking water and injected with a carcinogen (azoxymethane) they developed 10-times fewer colon tumors than the controls, while a 16d treatment with PEG resulted in a 5-fold reduction in carcinogen-induced aberrant crypt foci, a putative early stage in colon cancer. A later in vitro investigation [27] showed that PEG induced a marked and dosage-dependent inhibition of cell division. This response was correlated with the osmotic pressure of the PEG solution, and was similar to that produced by an equiosmolar solution of sorbitol. It was therefore postulated that the PEG-induced reduction of colon cancer was mediated by an osmotic effect. On the present hypothesis, this osmotic effect may be attributed, at least partly, to a reduction in the degree of hydration of the colonic epithelial cells. The same investigation also showed that similar PEG treatments of two non-cancerous cell lines had relatively little effect. It was suggested that this could be due to a greater sensitivity of transformed cells to extracellular hyperosmolarity, a difference that could be attributed on the present hypothesis to the transformation-induced inactivation of genes promoting osmolyte accumulation [8]. In a similar investigation [28], the use of PEG at a higher range of concentration induced apoptosis rather than arresting cell division, thus suggesting that a greater reduction of cell hydration may be required to induce apoptosis than to arrest cell division. This explanation, which should be tested experimentally, is consistent with numerous studies showing that apoptosis is typically associated with cell shrinkage [11].

Multi-step carcinogenesis

The hypothesis that an increase in cell hydration is a major factor in the mechanism of multi-step carcinogenesis can not claim any direct experimental support. There are, however, some data consistent with this hypothesis. A morphometric study of oral cancer, using computer assisted quantitative image

analysis, showed that histopathological identification of different stages in neoplastic progression in the basal layer of the oral epithelium were correlated with increases in both nuclear and cellular dimensions [29]. These changes were considered to be of diagnostic value for lesions with a high risk of malignant transformation. A similar relationship between increasing cell size and neoplastic progression was reported from nickel-induced cancer of the nasal mucosa [30]. There is, however, a need to investigate the relationship between increases in cell volume (i.e., cell hydration) and neoplastic progression in other forms of cancer.

Respiration

The hypothesis that the increased hydration of cancer cells enhances their respiration rate is consistent with numerous studies showing that hormonal induction of increased cell volume or swelling of the mitochondria stimulates respiration and other aspects of metabolic activity (reviewed in [31,32]). Support for the present hypothesis is also provided by Halpern et al. [33], who reported that, in mice, the average microviscosity of tumor cells, which is inversely related to their water content, was approximately 38% lower than that of normal tissue. The authors suggested that this effect might be expected, from theoretical considerations, to cause a general increase in chemical reaction rates (see references cited), thereby generating an overall acceleration of metabolic activity of the tumor cells, which might provide them with a growth advantage over their normal counterparts. In discussing the composition of both plant and animal cells, Steward [34] remarked that ''the higher their water content the greater is their metabolic activity as measured by respiration''. Although supported by experimental evidence (see [34], Fig. 3-1), this conclusion has received very little attention. It is, however, consistent with my suggestion [35] that since growth and metabolic activity must be closely integrated, evolution would be expected to favor a mechanism by which both are controlled by a common factor, and that water would seem to be the only substance that could adequately perform this dual function. Also, as previously suggested, this relationship may play an essential role in the postulated mechanism of multi-step carcinogenesis.

Inflammation

Chronic inflammation is a major cause of various forms of cancer [36], but there is a lack of agreement about the mechanism involved. Numerous studies (reviewed in [37]) are consistent with the hypothesis that oxy radicals, generated by inflammation, contribute to cancer by causing DNA damage and consequent mutagenesis. However, the validity of this hypothesis has been questioned, mainly because of a lack of conclusive evidence of the occurrence of mutations resulting from oxidative damage in tumors or cultivated cells, and also because of the failure of several extensive trials to demonstrate any protective effect of anti-oxidants [38]. An alternative explanation, based on the present hypothesis, attributes inflammation-induced carcinogenesis, at least partly, to the associated increase in water content (edema) of the inflamed tissue. It is postulated that the latter effect, especially if prolonged, and gradually increased by the continued action of inflammatory agents, will not only promote persistent hyperplasia, but may also induce oncogene expression and the associated inhibition of cell differentiation and apoptosis. The increase in cell hydration, and the associated hyperplastic response, may also be enhanced by phagocytosis of dead or injured cells and/or by the absorption of their exudates, which may function osmotically to promote increased water uptake by uninjured cells. It should be noted that this could provide a mechanistic explanation of the hyperplastic response to injury, often attributed teleologically to the need for replacement of dead cells. Support for the postulated role of edema as a major factor in carcinogenesis is provided by experiments in which quantitative measurements of edema in the mouse ear, induced by application of 12-*O*-tetradecanoylphorbol-13-acetate (PTA), showed that all the substances tested that suppressed TPA-induced edema were also potent inhibitors of tumor promotion [39]. In addition, the TPA-induced expression of c-*fos*, c-*jun* and c-*myc* proto-oncogenes may contribute to its carcinogenetic effect [40]. On the present hypothesis, these genetic effects may be attributed to the TPA-induced edema and the associated increase in cell hydration.

A second factor that may contribute to inflammation-induced carcinogenesis is the serum released by the vascular leakage commonly associated with inflammation [41]. Evidence of the effectiveness of serum in promoting cell division was provided by in vitro investigations with an established line of mouse fibroblast cells (3T3), which showed that quiescent cells in a confluent monolayer could be reactivated by increasing the serum concentration from 10% to 30%, a treatment that caused the cells to pile up on top

of each other, reaching a density approximately 10 times that of the confluent monolayer [42].

Although the serum constituent(s) responsible for this response were not determined, one might speculate that insulin, which induces cell swelling, and was reported to replace the serum factor(s) needed for in vitro multiplication of chicken and duck cells [43], may promote the G1 phase of cell division, where water may be limiting factor [12], while DNA synthesis may be enhanced by an increased supply of polyamines, which may be a limiting factor in cell transformation [17].

Although changes in cell hydration are known to influence gene expression, the mechanism is still obscure [44]. It would seem, however, that such genetic effects are more likely to be epigenetic rather than mutational. Thus, the postulated role of cell hydration in inflammation-induced carcinogenesis is consistent with the suggestion that, in view of the lack of conclusive evidence of the importance of mutagenic effects, the role of epigenetic effects should receive more attention [37]. This change in focus was also advocated for other studies on the genetic control of carcinogenesis [45].

Hormones

Endogenous hormones are a major cause of human cancer [46]. One explanation of this relationship is that hormone-induced cell proliferation contributes to cancer not only by promoting tumor growth but also, less directly, by increasing the occurrence of various carcinogenetic mutations resulting from genetic errors during cell division [47]. However, the role of the latter effect as a major factor in cacinogenesis has been questioned owing to the relative infrequency of cancer in organs which undergo continuous cell proliferation as compared with those in which cell division is of less frequent occurrence [48]. An alternative or additional explanation based on the present hypothesis, is similar to that proposed above to account for the effect of persistent inflammation, i.e., that the primary mechanism by which hormones promote cancer is by increasing cell hydration, thus promoting cell proliferation and oncogene expression, while reducing cell differentiation and preventing apoptosis. This explanation is consistent with evidence that the major carcinogenetic hormones, i.e., insulin, estrogen and testosterone, all induce cell swelling (see references cited in [44], Table 2). The mechanism promoting cell swelling by insulin, and the associated metabolic effects, are described and reviewed in [23]. An elevation of insulin-like growth factors in blood serum is associated with several kinds of cancer, its carcinogenetic potency being attributed to a combination of mitogenic and antiapoptotic effects [49]. In addition, hormones may also contribute to cancer by promoting oncogene expression, insulin inducing ras expression [50], while estrogen induces expression of c-*myc*, c-*fos* and c-*jun* proto-oncogenes (see [51], Table 1). On the present hypothesis, these effects can be directly attributed to hormone-induced increases in cell hydration.

Interestingly, certain plant hormones, e.g., auxin, cytokinins and gibberellins, share the ability of animal hormones to promote water uptake, a common effect that was postulated to account for their similar effects on plant development [35]. Of particular relevance to the present hypothesis are in vitro studies on plant cancer (reviewed in [52]), showing that an exogenous supply of hormones was required for the attainment of '' habituation'', a stage at which the tissues have become hormone independent, and have also acquired an increased degree of cell hydration. This is considered to be an early stage of carcinogenesis. Subsequent neoplastic development, characterized by increased cell proliferation and decreased differentiation, is associated with observational evidence of a progressive increase in cell hydration, an observation consistent with the present suggestion that such an increase plays a major role in multi-step carcinogenesis.

Testing the hypotheses and their implications

Methodology

Testing the proposed hypotheses and their implications would require a critical quantitative study of the relationship between cell hydration and those changes in cell behavior, physiology and gene expression associated with the promotion or prevention of cancer. In early investigations of this relationship [5], cell hydration was usually measured by expressing tissue water content on a fresh or dry weight basis. Recent advances in technology now enable cell hydration to be determined less directly by measuring small changes in cell volume using the Coulter Counter/channelyzer system [53], of which a new, improved model is also available [54]. Korchev et al. [54] reviewed other methods of measuring cell volume, including the recently developed technique of scanning ion conductance microscopy, which permits rapid, high

resolution of volume changes, while retaining normal cell function. It is particularly suitable for studying cell expansion in growing monolayers, as might be required in the present investigation.

Cell behavior

As indicated previously, the promotion of cell division by factors that increase cell volume has been well documented, and no testing of this effect is required. The additional hypothesis that increased cell hydration can directly inactivate genes that induce cell differentiation could be tested using cultured cells grown at a low serum level to induce differentiation [21], and determining whether expression of selected, differentiation-inducing genes could be prevented by reducing the osmolarity of the medium, or by increasing the concentration of insulin to promote water uptake. The latter treatments could also be used to test the hypothesis that increased cell hydration inhibits apoptosis.

As noted previously, treatment of cultured cells with PEG can either arrest cell division, promote cell differentiation, or induce apoptosis. An investigation could be conducted to determine if these different responses are correlated with differences in the degree of PEG-induced reduction of cell hydration. Such an investigation could include a comparison of normal and cancerous cells to provide further evidence of the greater sensitivity of cancer cells to desiccation as compared with their normal counterparts.

An experiment could also be conducted to test a related hypothesis that the proliferative behavior of stem cells is dependent on their high water content, which may be maintained, at least partly, by the environment of the ''stem cell niche'', and that an environmentally-induced decrease in cell hydration may be the primary factor that *initiates* the differentiation of the stem cell progeny. This hypothesis is consistent with the greater sensitivity of adult stem cells to γ radiation-induced apoptosis as compared with their differentiating progeny [55], and which could be attributed to their greater degree of cell hydration [56]. However, direct measurements of the relative degree of hydration of the stem cells and their progeny would be required to test this hypothesis.

Cell physiology

The hypothesis that increased cell hydration enhances respiration, and also that this factor plays a major role in neoplastic progression, could both be tested in the same investigation by measuring respiration and cell hydration at successive stages of tumor development. Such measurements could be made on tissue samples from the different, well-defined and readily accessible stages of colorectal tumorigenesis, a system found to be very suitable for studying genetic changes associated with multi-step carcinogenesis [57].

Genetic factors

The unifying nature of the proposed concept suggests a new approach to the study of cancer at the molecular level. The concept implies that many, if not all of the genetic effects on cancer are mediated, directly or indirectly, by effects on cell hydration. While speculation on the many genetically-controlled mechanisms in which effects on cell hydration might be involved is beyond the scope of this paper, one example which would seem to merit further investigation is the approximately 30% increase in cell volume caused by expression of the ras oncogene. As suggested earlier, this effect might contribute to cancer not only by promoting cell proliferation but also, less directly, by enhancing the synthesis of polyamines, which are essential for cell transformation. This hypothesis might be tested by treating the ras-expressing cells with a combination of dimethylamiloride and furosemide, which enables the ras-induced increase in cell volume to be eliminated by increased extracellular molarity [14]. The following are some other questions related to the ras-induced increase in cell volume that might be addressed: (1) Is the enhanced transformation induced by the experimental amplification of the ras oncogene [58] correlated with an increase in cell hydration? It was postulated that the ras-amplified cells may produce an increased amount of transforming ''growth factors'', which may function in an autocrine manner to promote transformation. The present hypothesis suggests that one of these growth factors, or perhaps the only one, may be water. This suggestion is supported by the observation that ras amplification is positively correlated with an increase in ornithine decarboxylase, which is promoted in vitro by hypotonicity [59]. (2) Injection of cells with the protein encoded by the normal cellular ras gene induced transformation, but only when injected at a considerably higher concentration than that required for transformation by the protein of the ras oncogene [60]. It would be of interest to determine if the degree of transformation induced by the increased concentration of the normal ras protein was correlated with an

increase in cell volume. (3) Do other point mutations that induce ras oncogenicity also increase cell volume, and if so, is the degree of cell volume increase correlated with the difference in transforming potential shown by these various mutations [61]? (4) Does the increase in cell volume induced by the ras gene play an essential role in its ability to inactivate genes inducing differentiation, as previously suggested? Such a mechanism would be consistent with the present hypothesis, and might be tested by suppressing the ras-induced increase in cell volume as described above. This postulated mechanism implies that the various other oncogenes that suppress differentiation also do so by increasing cell hydration. This suggestion raises the further question as to whether the carcinogenetic effect of other oncogenes is mediated, either directly or indirectly, by their ability to increase cell volume (i.e., cell hydration). A survey of the literature showed that the c-myc oncogene causes a 1.6-fold increase in cell volume [62], while expression of the Akt oncogene induces a 2-fold increase in cell size, and a 80—90% increase in cell volume [63]. These data suggest that oncogenes may function in an autocrine manner [64], thus, maintaining the increased cell hydration postulated to be the primary factor in carcinogenesis. Consideration might also be given to testing the hypothesis that the reported collaboration between ras and myc oncogenes required to induce transformation of primary (i.e., non-immortalized) embryo fibroblasts [65] may depend on the ability of both genes to increase cell volume, this combined effect raising the degree of cell hydration to the level required for transformation. The difference in the site of action of these genes, i.e., in the cytoplasm and nucleus, respectively, might perhaps be a significant factor in their collaborative behavior.

There is increasing evidence in recent years that tumor suppressor genes (e.g., p53, RB) are functionally equivalent to genes that induce differentiation or apoptosis. Experiments could be conducted to test the hypothesis that expression of these genes and their phenotypic effects are induced by factors that reduce the degree of cell hydration. As noted above, in vitro experiments using PEG, which is assumed to reduce cell hydration, induced either differentiation [25] or apoptosis [28]. Although these experiments differed in both the PEG concentration used and the duration of treatment, their comparison suggests that the reported induction of cell differentiation by a relatively low level of p53 protein, and of apoptosis by a higher level [66], may be caused by differences in the reduction of cell hydration, and that apoptosis, which is typically associated with cell shrinkage, may be induced by a greater reduction of cell hydration than that required to induce cell differentiation. However, in testing this hypothesis, consideration should be given to the probable interaction between the tumor suppressor genes and other factors that may also affect the degree of cell hydration.

Clinical aspects

A detailed discussion of the clinical applications of this concept would not be appropriate until its validity has been critically assessed. It may be noted, however, that the abnormally high water content of cancer cells is a major factor in the use of microwave technology for the detection [67] and treatment [68] of cancer. A second characteristic feature of the cancer cell of potential therapeutic significance is its increased sensitivity to desiccation. As previously suggested, this may be cancer's achilles heel, and may account, at least partly, for the remarkable effectiveness of PEG in the treatment of colon cancer. A desiccating effect may also contribute to the ablation of tumors by ethanol injections, a promising new treatment whose therapeutic potential has yet to be fully assessed [69]. Further investigations could also include comparative studies of the effectiveness of other desiccating agents, and the development of novel or improved application techniques.

Concluding comments

Since the structure of DNA was elucidated more than 50 years ago, cancer research has been increasingly restricted to studies at the molecular level. While this approach has provided a wealth of knowledge of the genes that play a role in both the promotion and prevention of cancer, and of their complex interactions, in my opinion the contribution of this knowledge to a more complete understanding of the mechanism of carcinogenesis has been limited by the relatively little attention given to environmental effects on gene expression, and to the biochemical and physiological factors that mediate genetic effects at the cellular level. I suggest that an increased focus on these questions is urgently required, and also that such investigations should include a critical assessment of the postulated role of cell hydration as a factor of particular significance.

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