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Carcinogenesis and the plasma membrane

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Summary Presented is a two-stage hypothesis of carcinogenesis based on: (1) plasma membrane defects that produce abnormal electron and proton efflux; and (2) electrical uncoupling of cells through loss of intercellular communication. These changes can be induced by a wide variety of stimuli including chemical carcinogens, oncoviruses, inherited and/or acquired genetic defects, and epigenetic abnormalities. The resulting loss of electron/proton homeostasis leads to decreased transmembrane potential, electrical microenvironment alterations, decreased extracellular pH, and increased intracellular pH. This produces a positive feedback loop to enhance and sustain the proton/electron efflux and loss of intercellular communication. Low transmembrane potential is functionally related to rapid cell cycling, changes in membrane structure, and malignancy. Intracellular alkalinization affects a variety of pH-sensitive systems including glycolysis, DNA synthesis, DNA transcription and DNA repair, and promotes genetic instability, accounting for the accumulation of genetic defects seen in malignancy. The abnormal microenvironment results in the selective survival and proliferation of malignant cells at the expense of contiguous normal cell populations.

INTRODUCTION

Despite advances in molecular genetics and identification of various oncogenes and tumor-suppressor genes, no consistent pattern of genetic or epigenetic defects have been found in all tumors (1,2). Indeed, genotypic and phenotypic instability is a fundamental observation in cancer (3).

Notwithstanding this cellular heterogeneity, malignancies uniformly possess common traits. They are morphologically and functionally more primitive than the tissue of origin, exhibit increased growth rate, invade normal tissue, metastasize, and preferentially derive energy from glycolysis. These common traits must represent critical, interrelated parameters necessary for the development of cancer. For example, neoplastic invasion may be linked to excess tumor acid production at the advancing tumor edge (4,5). These new insights suggest reconsideration of the role of the cell–environment interaction in developing and maintaining the malignant phenotype and focus attention on the critical structure in this interaction – the cell membrane.

In general, the role of non-DNA cellular elements in carcinogenesis has received little attention. However, in hybrid studies, non-nuclear cellular elements can propagate malignant phenotype in the absence of transmitted nuclear genetic material. Indeed, it has been shown that fusion of cytoplasts from malignant cells with karyoplasts of normal cells resulted in a 97% incidence of tumors, while the opposite combination (normal cytoplasts and malignant karyoplasts) yielded 0% tumors (*6*). Thus, Prehn has proposed that genetic changes in malignancy are secondary phenomena caused by rapid proliferation and loss of self-correcting mechanisms rather than the primary events in carcinogenesis (*7*).

In this paper, we propose a model of carcinogenesis based on abnormalities of the plasma membrane, loss of electron and proton homeostasis, and reversion to a glycolytic state. This establishes a self-sustaining microenvironment that is favorable for tumor cell proliferation

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and invasion. DNA abnormalities are considered contributory or secondary phenomena.

We demonstrate that this membrane-based model of carcinogenesis is simple, complete, and consistent with existing data. The hypothesis yields predictions which are testable both in vitro and in vivo.

ELECTRON HOMEOSTASIS

A role for the plasma membrane in carcinogenesis has been proposed previously. In 1971, Cone postulated a functional relationship between transmembrane potential and mitotic activity in general, including both normal proliferative activity (e.g. growth, wound healing) and malignancy (8). Specifically, he proposed that cells with a high transmembrane potential (E_m) demonstrated virtual absence of mitotic activity, while cells with a low E_m showed greatly increased proliferation. His observations included: (1) high E_m among cell types that exhibit low mitotic activity (e.g. muscle, neurons); (2) markedly decreased E_m among mitotically active cells, especially in transformed and malignant cells; (3) adaptation of cells in culture to a low E_m that persists or progresses with proliferation and transformation of those cells; and (4) morphologic membrane changes during adaptation to culture and diminishing E_m that result in dissociation of tissue into individual cells similar to the decreased cell adhesion seen in malignancy. He proposed that low E_m resulted in steric alterations of the membrane, affecting the activity of membrane-bound proteins, subsequently affecting DNA and protein synthesis and altering metabolic pathways. Of particular note, he discussed the possibility that similar structural alterations could occur in the mitochondrial membrane, affecting oxidative phosphorylation.

Beech later proposed another theory (*9*) based on one or more of three mechanisms acting at the plasma membrane: (1) oxidation of molecules at the extracellular surface; (2) chronic, persistent electron impingement at the extracellular surface; (3) relocation of basic molecules to the cytoplasmic surface causing attraction of acidic molecules to the extracellular surface via electrostatic forces. The result of these mechanisms is increased electronegativity of the extracellular surface. Since the cytoplasmic surface is negative with respect to the extracellular side, this results in a decrease in E_m (the absolute transmembrane potential), consistent with Cone's theory and supported by other data (10–14). Incorporated into this theory were a variety of carcinogenic stimuli including chronic injury, asbestos, viruses, ionizing radiation, and chemical carcinogens, all of which resulted in an increase in extracellular surface electronegativity (or decrease in E_m). He proposed that these changes were carcinogenic via conformational changes in the cell membrane (15), enhanced lateral diffusion of membrane molecules (16–18), and alteration of membrane-cytoskeleton attachments. Ultimately, these changes lead to molecular movements of polyamines, other basic molecules and dissociation of histones from DNA and then to mitosis. Interestingly, it was proposed that transmission of the malignant phenotype was not through passage of altered membrane components or DNA abnormalities, but from the persistent presence of the carcinogenic stimulus acting upon daughter cells.

However, the mechanism by which these changes become persistent and heritable and their precise role in malignant transformation are unclear. Our hypothesis proposes electron and proton flow across the cell membrane as the critical factor in this development of the transformed phenotype.

Intracellular electron flow exists as the basis for the electrochemical proton gradient in oxidative phosphorylation. Recently, a transplasma membrane electron transport system has been demonstrated. Though the purpose and structure of this system has yet to be determined, transmembrane electron flow likely contributes to E_m via alterations of surface electrostatic charge, which has been proposed as a parameter in mathematical models of E_m origin and magnitude (19). The result of electron efflux is increased electronegativity of the extracellular surface and an absolute decrease in E_m.

Transplasma membrane electron transport is coupled to the Na^+/H^+ antiport and electron efflux is associated with proton efflux, decreasing external pH, and increased intracellular pH (20,21). Several studies show involvement of membrane electron transport in regulation of growth (22–24). Mitogens stimulate the electron transport system in the range of concentration that causes proliferation in culture and associated increased proton efflux (25). The Ha-ras oncoprotein stimulates electron transport in Ha-ras transformed cells with increased proton release (26). Conversely, inhibition of electron transport by a membrane impermeable chelator is associated with inhibition of DNA synthesis in transformed cells (27).

The nature of cellular electron movement outside of transport systems is poorly understood, though several theories have been proposed, including semiconduction along proteins and macromolecules (28,29) and quantum-mechanical electron tunnelling (30). Passage of electrons through actin (a major cytoskeleton component) produces ionic currents. Thus, cytoskeletal structures can behave as electrical 'wires' and are capable of functioning as nonlinear inhomogeneous transmission lines (31).

A variety of intercellular junctions exist that could participate in intercellular electron flow, including anchoring junctions (actin filaments, intermediate filaments) and communicating junctions (gap junctions).

Gap junctions possess much lower electrical resistance than nonjunctional plasma membrane (32).

HYPOTHESIS: CARCINOGENESIS AND THE PLASMA MEMBRANE

Given clear evidence for an intracellular electron transport chain for energy production, transplasma membrane electron flow, electrically conducting cytoskeletal elements, and electrical coupling of cells within organs, we propose that cells must maintain electron homeostasis. The resulting electron steady state and associated H^+ transport strongly affect the transmembrane potential and determine the functional and mitotic activity of the cell. In a given cell, steady-state electron homeostasis is based upon: (1) the supply of electrons from energy sources and inflow via intercellular (e.g. gap junctions, adherens junctions) and extracellular matrix contact points; and (2) electron outflow via these same contact points and transplasma membrane transport or efflux.

We postulate the primary defect in carcinogenesis to be an architectural, organizational, and/or compositional change in the plasma membrane that results in loss of electron homeostasis, with a net depletion from the intracellular steady-state. Such changes could occur in response to a variety of stimuli including chemical carcinogens, viruses, and chronic microenvironmental changes such as chronic cellular injury or local acidosis. Inherited or acquired genetic abnormalities and epigenetic defects could cause, predispose to, or augment plasma membrane aberrations by affecting integral, peripheral, or amphitropic membrane proteins.

Loss of electron homeostasis will lead to a malignant phenotype if two conditions are met: (1) persistent, heritable electron/proton efflux; and (2) intercellular electrical uncoupling. For analytic purposes, these steps may be understood in the context of the generally accepted 'initiation/promotion' tumor model, to establish clearly defined steps for testing and verification.

In our model, initiation is characterized by a persistent, heritable increased transplasma membrane efflux of electrons and protons. This may result from several mechanisms: conformational changes in membrane-bound proteins affecting transport systems and/or semiconduction; changes in membrane fluidity; and phase transitions (crystalline vs liquid crystalline) that alter the intrinsic electron 'conductivity' of the plasma membrane (33). The coupling of the Na^+/ H^+ antiport with electron transport results in simultaneous increase in proton efflux.

Transmission of such membrane abnormalities to daughter cells may be based on several mechanisms. Since each progeny receives one half of the already altered plasma membrane, donated architecture can affect newly integrated membrane components via intermolecular associations (33). Genetic (inherited and/or acquired) defects involving membrane-associated proteins would be transmitted by transcription. Transmissible epigenetic phenomena include altered phosphorylation and dephosphorylation cascades of both protein and lipid membrane components. Persistent microenvironment accumulation of chemical carcinogens, persistent local pH changes, and persistent negative extracellular surface charge could maintain membrane architectures promoting electron/proton efflux. Such mechanisms may be self-sustaining or self-promoting when resulting electrical and pH changes contribute to or promote stabilization of induced membrane abnormalities. Despite the persistently altered electron and proton flow, these initiated cells are not tumorigenic due to intact feedback loops that include other membrane pumps and intercellular contact points such as gap junctions which will maintain electron homeostasis and intracellular pH.

In our model, tumor promotion corresponds to loss of negative feedback mechanisms. A cell with increased electron efflux could maintain electron homeostasis and remain untransformed through electrical coupling with adjacent normal cells via gap junctions. Disruption of the negative feedback loop provided by intercellular communication combined with persistent electron/proton loss would result in progressive intracellular electron and proton depletion.

Increased electron efflux results in accumulation of negative charge on the extracellular surface, causing an absolute decrease in E_m and stimulation of the Na^+/H^+ antiport. Because it is a 1:1 exchange, this is an electrically neutral pump, but the ratio of protons released as compared to electrons is high. This causes a decrease in extracellular pH and increase in cytoplasmic pH in malignant cells (34,35). Since H^+ ions pass freely through gap junctions, such intracellular pH changes could not be sustained without functional gap junction loss.

The resulting intracellular electron and proton deficit could interfere with oxidative phosphorylation by altering the cumulative redox state of respiratory chain electron carriers. A more oxidized state resulting from net intracellular electron deficit could result in lower electron transfer rates and diminished energy production per unit time and lead to increasing glycolytic metabolism to meet cellular energy requirements. The associated intracellular proton deficiency could compromise the electrochemical proton gradient of oxidative phosphorylation via a reduction of total proton-motive force generated by mitochondrial inner membrane potential and altered local pH gradients (36). Additionally, several key rate-limiting enzymes in aerobic glycolysis are known to be pH sensitive (37), suggesting that intracellular and extracellular pH changes may be regulatory factors in the transition from aerobic to anaerobic glycolysis.

The reversion to a sustained glycolytic state and stimulated Na^+/H^+ antiport causes continuous and persistent acidification of the immediate extracellular space, functioning as a positive feedback loop. Low extracellular pH further decreases gap junctional intercellular communication (38), contributing to additional electrical uncoupling.

Diminished transmembrane potential and increased intracellular pH are linked with malignant transformation. Transmembrane potential increases when normal cells come in contact with each other, suggesting possible involvement in contact inhibition (8). Tumor cells do not undergo this change, presumably because of absent or incomplete electrical coupling (39). Aberrant gap junctional intercellular communication is seen in most tumor cells; transformed phenotypes are suppressed when transformed cells are placed in physical contact with intact gap junctions of normal cells (40).

Increased intracellular pH is associated with increased glycolysis, DNA synthesis, transcription, and microtubule assembly (41). Insertion of a gene for the yeast plasma membrane H^+-ATPase into fibroblasts results in continuous proton efflux, intracellular alkalinization, and malignant transformation (42).

The direct correlation of specific genes (e.g. retinoblastoma) and the statistical association of some genetic defects with malignancy clearly indicate a significant genetic role in cancer. Since DNA only codes for protein synthesis (as opposed to lipids), our hypothesis predicts that genetic defects predisposing to malignancy must result in protein-induced changes at the membrane level, causing architectural changes that lead to electron/proton efflux and loss of electron and pH homeostasis.

To our knowledge, very little is known about the nature of such protein interactions. However, the DCC gene has been identified as a tumor-suppressor gene in colorectal cancer, which is frequently lost during carcinogenesis (43). Sequencing studies of the DCC protein suggest that it is a cell adhesion protein, thus acting at the level of the membrane, and potentially affecting membrane function and/or intercellular communication.

Alternatively, the retinoblastoma gene can regulate the transcription and expression of other genes, including growth-inhibitory (TGFB1) and regulatory cell-cycle (44). It is postulated that such proposed oncogenes could alter the expression of genes responsible for integral, peripheral, and amphitropic proteins, affecting membrane architecture and function.

For example, in vitro studies show gap junction intercellular communication decreased by tumor promoters (phorbol esters) and the *ras* oncogene via a mechanism of phosphorylation and diminished expression and modification of connexins, the protein components of the junction (45). In this manner, both chemical carcinogens and genetic abnormalities can alter the expression of another gene and affect electron homeostasis via electrical uncoupling. Oncogenes have also been shown to alter the activity of NADH oxidase, which can transfer electrons across the membrane, thereby affecting electron efflux (46).

Loss of electron homeostasis and pH changes can alter epigenetic and self-correcting mechanisms, accounting for the genetic instability and accumulation of genetic defects seen in malignancy. For example, lowering extracellular pH of irradiated cells causes inhibition of DNA repair (47). It is postulated that such effects are based upon inhibition of pH-dependent DNA repair enzymes and the induction of DNA misrepair. This may serve as a positive feedback loop if these resultant protein abnormalities affect the membrane and lead to further increased electron/proton efflux. Genetic defects may also accumulate due to rapid cell cycling causing decreased time spent in G_o, with most of the cell's energy and time spent in replication and not DNA repair. The end result is the evolution of morphologically heterogeneous cell populations competing in an abnormal microenvironment. Based on competitive growth advantages, a dominant single cell line would emerge as a malignancy of 'single cell origin'.

Mathematical modeling and experimental data show that an acidic microenvironment results in the selective survival and proliferation of malignant cells and the death of contiguous normal cell populations (48). The proposed pH changes related to stimulation of transplasma membrane electron transport and proton efflux support such a theory and provide a basis for persistent microenvironmental conditions conducive to tumor cell survival at the expense of normal cell populations.

PREDICTIONS AND THERAPEUTIC IMPLICATIONS

There are testable predictions to determine the validity of our hypothesis, based upon the individual conceptual components of the model. The following testable predictions are made:

1. The transmembrane electron transport system contributes significantly to the low transmembrane potential seen in cells lacking normal homeostatic mechanisms. Thus, stimulation of the electron transport system in uncoupled cells will result in a decrease in absolute E_m, while stimulation of the electron transport system in cells with intact intercellular communication will demonstrate significantly less or no change in E_m due to intact feedback loops.
2. Under similar microenvironmental conditions,

electron and proton efflux in transformed or malignant cells will be greater than that seen in their derivative cells (e.g. hepatocellular carcinoma cell vs hepatocyte)

3. Preventive strategies that promote electrical coupling of cells will modulate development of the malignant phenotype. For example, carotenoids have been presumed to have anticancer action based on statistical analysis of some cancer rates. In vivo studies show carotenoids inhibit neoplastic transformation in the post-initiation phase of carcinogenesis via modification of gene expression and upregulation of gap junctions (49).
4. Blockage of the Na^+/H^+ antiport (e.g. drugs, gene therapy) will reduce the development, viability and invasiveness of transformed cells (4). Studies have already shown that amiloride, a Na^+/H^+ antiport blocking agent, can inhibit the development of induced colon tumors in rats (50). Other strategies to alkalinize the extracellular microenvironment will have a similar effect.
5. Agents that uncouple the electron transport system from the Na^+/H^+ antiport will have antitumor activity.
6. Interruption or blockage of the electron transport system in malignant cells will reduce tumor viability and kill tumor cells. Some established chemotherapeutic agents may be acting at the level of the plasma membrane. Adriamycin inhibits the transplasma membrane electron transport system and, when conjugated with diferric transferrin (blocking transfer to the nucleus), has been shown to act specifically at the plasma membrane. The adriamycin conjugate is ten-fold more inhibitory than an equivalent concentration of adriamycin alone (51).

SUMMARY

The critical components of the hypothesis of membrane-based carcinogenesis are as follows:

1. A wide variety of agents (e.g. carcinogens, oncoviruses, inherited and/or acquired genetic defects, epigenetic abnormalities) act at the level of the plasma membrane to cause architectural, organizational or compositional alterations that result in a heritable, persistent increased efflux of electrons and protons across the membrane.
2. Such 'initiated' cells are not transformed in the presence of intact feedback loops which include intercellular communication (metabolic and electrical).
3. 'Promotion' involves the loss of these feedback mechanisms via intercellular uncoupling by either primary agents (e.g. genetic mutations, epigenetic abnormalities, oncoviruses, carcinogens) or microenvironmental phenomena (e.g. decreased extracellular pH, chronic inflammation, wounding).
4. These initiation and promotion steps lead to a progressive intracellular electron/proton deficiency, decrease in extracellular pH and increase in intracellular pH.
5. Increased intracellular pH is associated with glycolysis, altered gene expression, and altered gene repair, which promotes genetic instability and the accumulation of genetic defects seen in malignancy. Some of these genetic defects may serve to further destabilize the membrane or interfere with intercellular communication, acting as a positive feedback loop.
6. Low extracellular pH results in the selective survival of malignant cells at the expense of contiguous normal cells and also serves as a positive feedback loop by its negative effect on intercellular communication.
7. These changes result in morphologically heterogenous cell populations. Based on competitive growth advantages in an abnormal microenvironment, a single cell line emerges to develop into a malignant tumor.

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