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Endogenous Voltage Potentials and the Microenvironment: Bioelectric Signals that Reveal, Induce and Normalize Cancer

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Abstract

Cancer may be a disease of geometry: a misregulation of the field of information that orchestrates individual cells' activities towards normal anatomy. Recent work identified molecular mechanisms underlying a novel system of developmental control: bioelectric gradients. Endogenous spatio-temporal differences in resting potential of non-neural cells provide instructive cues for cell regulation and complex patterning during embryogenesis and regeneration. It is now appreciated that these cues are an important layer of the dysregulation of cell: cell interactions that leads to cancer. Abnormal depolarization of resting potential (V_{mem}) is a convenient marker for neoplasia and activates a metastatic phenotype in genetically-normal cells *in vivo*. Moreover, oncogene expression depolarizes cells that form tumor-like structures, but is unable to form tumors if this depolarization is artificially prevented by misexpression of hyperpolarizing ion channels. V_{mem} triggers metastatic behaviors at considerable distance, mediated by transcriptional and epigenetic effects of electrically-modulated flows of serotonin and butyrate. While *in vivo* data on voltages in carcinogenesis comes mainly from the amphibian model, unbiased genetic screens and network profiling in rodents and human tissues reveal several ion channel proteins as bona fide oncogene and promising targets for cancer drug development. However, we propose that a focus on specific channel genes is just the tip of the iceberg. Bioelectric state is determined by post-translational gating of ion channels, not only from genetically-specified complements of ion translocators. A better model is a statistical dynamics view of spatial V_{mem} gradients. Cancer may not originate at the single cell level, since gap junctional coupling results in multi-cellular physiological networks with multiple stable attractors in bioelectrical state space. New medical applications await a detailed understanding of the mechanisms by which organ target morphology stored in real-time patterns of ion flows is perceived or mis-perceived by cells. Mastery of somatic voltage gradients will lead to cancer normalization or rebooting strategies, such as those that occur in regenerating and embryonic organs, resulting in transformative advances in basic biology and oncology.

Keywords

Bioelectricity; Resting potential; Voltage gradient; Normalization; Reprogramming; Microenvironment

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Introduction

> "Cancer is no more a disease of cells than a traffic jam is a disease of cars. A lifetime of study of the internal-combustion engine would not help anyone understand our traffic problems--
>
> D. W. Smithers"

Ideas in cancer biology comprise two complementary paradigms. The mainstream view is that cancer cells are irreversibly damaged: they have accumulated genetic or epigenetic damage and are fundamentally a different kind of cell. On this view (the somatic mutation model), cancer cells have acquired cell-autonomous properties that underlie unlimited proliferation and metastasis [1,2]. Contrasting with this view is the idea that cells exhibit neoplastic behavior and form tumors due to a change of interactions with their environment. This view of cancer is focused on context-non-cell-autonomous signaling that activates cellular misbehavior in the host [3–8]. The latter class of models ranges from simple growth suppressive molecules (morphostats) secreted by healthy tissue [9,10] and averaging effects of cell neighbors that stabilize stochastic gene expression [11] to suppression of tumorigenesis by tissue-level organization [12,13] to global models of whole-body morphogenetic information fields [14–19].

While the mutation-centered paradigm has dominated work in this field for decades, increased attention is now focused on cancer as a progressive loss of the organization capacity of the environment over the heterogeneous behavior of isolated cells [18–24]. Interestingly, "no cancer exhibits any trait which cannot be found in some normal tissue as the expression of normal genomic activity; no cancer grows faster than an embryo nor is any cancer cell more invasive than a macrophage nor are cancer cell lines more immortal than are germ lines. The only distinction is that, in the cancer, the expression or lack of expression of many traits may be inappropriate for the tissue in which the cancer occurs" [25]. This is a view of cancer as fundamentally a developmental disorder of cell regulation.

Large-scale shape, or the correct geometric arrangement of organs and tissues in an organism, is a key concept in biological growth and development. To achieve optimal health, organisms strive to maintain shape at all levels, from the single cell to the whole organism. Cancer can be seen as an error of geometry, because tumor cells grow, migrate, and function without regard for the orderly structure within which they occur [26]. This is seen most acutely in teratomas - embryonic tumors that display extensive differentiation of a number of tissues combined with a complete absence of orderly organization of the whole. The idea that cancer is a developmental disease is an old one [9,10,26–29]. Needham and Waddington speculated that cancers represented an escape from the control of the morphogenetic field [30–32]. On this view, tumors form when cells stop obeying the normal patterning cues of the body: "cancer as part of an inexorable process in which the organism falls behind in its ceaseless effort to maintain order" [28].

Understanding cancer as a reversible physiological state of a multi-cellular dynamical system (as opposed to damage within single cancer stem cells) has significant medical implications because it suggests specific prevention and detection strategies focused on

modulating the physiological interrelationships among many cells instead of looking for DNA markers in single cancer stem cells. A mechanistic dissection of these pathways may give rise to strategies that reboot [23] or normalize cancer, in contrast to current approaches that all seek to kill tumors and thus risk a compensatory proliferation response by rogue cells that still remain [33]. Thus, biologists are beginning to explore the idea that cancer is not a genetic disease of specific loci but rather a kind of attractor in a multi-dimensional transcriptional space describing cell states [17]: "The topology of the attractor is the 'invisible hand' driving the system functions into coherent behavioral states: they are self-organizing structures and can capture the gene expression profiles associated with cell fates" [34]. Huang et al. also point out an interesting paradox: while many studies seek to "determine which gene is mutated to explain an incremental malignant trait, no one doubts that normal cells as distinct as a mature neuron vs. a blood or epithelial stem cell share the exact same genome! No mutations are invoked to explain the remarkable phenotypes during cell lineages in development" [34], and indeed aneuploidy is routinely present in normal brain, testes, and liver, but does not usually result in cancer (reviewed in 8).

A complete picture of cancer no doubt involves both an understanding of DNA damage and cell signaling dynamics, although considerable controversy exists as to the most appropriate level of organization at which to search for the origin and cure of cancer (ranging from genes, to stem cells, to tissues, to entire body plan organizing fields). Here we focus on bioelectrical information-exchanging processes occurring within and among cell groups in the suppression and progression of cancer, followed by an in-depth discussion of the importance of context and cell: cell signaling to the cancer problem in general. Our hypothesis is that while ion channels are increasingly revealed as important oncogenes, a focus on specific channel genes is just the tip of the iceberg because bioelectric cell states result from post-translational gating of ion channels and pumps, not only from genetically-specified complements of ion translocators. A more fruitful model of cancer and its reprogramming may be a statistical dynamics view of spatial gradients of resting potential as a systems-level property of multi-cellular physiological networks of cells linked by gap junctions.

Bioelectricity as an instructive component of microenvironment

Voltage gradients in non-neural cells control cell behavior—It has long been known that bioelectrical signals, or spatio-temporally patterned ion flows in non-excitable cells, are important determinants of cell behavior [35,36]. Steady-state endogenous ion currents, resting potentials (voltage gradients), and electric fields are produced by the activity of ion channel and pump proteins across cell membranes and their slow dynamics are distinct from the rapid action potentials of nerve and muscle (Figure 1). While related biophysical phenomena include transepithelial electric fields [37,38], ultraweak photon emission [39,40], and coherent AC electromagnetic fields [41,42], here we focus on distributions of V_{mem} or membrane potential [43]. The many interesting studies of applied field effects are discussed in several excellent recent reviews [44–47].

Bioelectric properties of cells and the electrical states of cells in the microenvironment are known to control several key behaviors of relevance to cancer [38,48–54]. For example,

electric fields generated by ion pumping across epithelia serve as migration cues for cellular galvanotaxis [55–59] - an important guidance modality for cell movement within the host. Cell shape changes, such as increased arborization, are also driven by endogenous electric fields and changes in V_{mem} [60–64]. Together, migration and shape properties are key elements of successful metastasis.

Moreover, resting potential established by ion channel and pump proteins is important for determination of differentiation state and proliferation; generally, a depolarized state is indicative of plastic, undifferentiated cells (e.g., stem cells), while differentiation is caused by increase of negative V_{mem}. Functional control of cell state by changes in V_{mem} has been observed in many kinds of stem and progenitor cells [65–75], including adult human mesenchymal stem cell [71,76] which can be kept stem-like despite the presence of chemical differentiation factors by forced depolarization, and in induced pluripotentent stem cells [77]. Even mature CNS neurons can be made to re-enter mitosis by sustained depolarization [78,79] revealing the power of transmembrane potential to regulate proliferative potential in adult somatic cells.

Importantly, the molecular mechanisms by which cell-autonomous [80] and non-autonomous [49,81] bioelectric events control downstream processes are now beginning to be fleshed out. Electric fields are transduced to cell migration machinery via Ca^{2+}-dependent mechanisms. A cell with a negative membrane potential, when exposed to an electric field, becomes more hyperpolarized near the anode, allowing the passive inflow of Ca^{2+} through voltage-gated calcium channels. An increase in Ca^{2+} in the anodal side of the membrane results in increased polymerization/depolymerization of actin, contraction of actomyosin, and decreased adhesion; collectively, the anodal side contracts and is propelled towards the cathode [82]. Inositol-phospholipid signaling, PI(3)Kγ, and cdc42/rho have been especially implicated in setting the directionality of cytoskeleton-mediated migration polarity in several cell types [83,84].

Changes in V_{mem} of cells, such as cancer-associated depolarization, can trigger transcriptional changes by 1) regulating the movement of morphogens such as serotonin, calcium, and inositol triphosphate through gap junctions [85–91], 2) controlling the import/export of small signaling molecules such as serotonin and butyrate across membrane exchangers [60,91–94], and 3) modulating the activity level of phosphatases such as PTEN [95–98]. Together, these transduction mechanisms convert an essentially biophysical state change into secondary messenger events that impact on transcriptional and epigenetic regulation of loci such as NODAL which are important for the cancer phenotype [94,99]. While bioelectric cues feed into the same molecular-genetic pathways that are known to regulate normal and neoplastic cell behaviors, they form a pathway that functions alongside biochemical gradients, but with significantly different spatial dynamics, to coordinate normal tissue morphology.

Spatio-temporal gradients of V_{mem} are instructive patterning cues—Disruption of the electrical gradients, or the mechanisms by which they are perceived by cells, are one way that complex anatomical order is subverted during carcinogenesis. Recent development of state-of-the-art tools for the detection and experimental manipulation of biophysical

signals in multicellular patterning contexts [80,100–102] has revealed how distributions of voltage gradients mediate positional information, organ identity of large cell groups, and initiation signals for complex developmental modules such as tail or limb regeneration. Using a combination of fluorescent voltage-reporter dyes to characterize spatial V_{mem} distributions and functional studies using targeted misexpression of a panel of well-characterized ion transporters to specifically modify those gradients *in vivo*, instructive signaling roles of transmembrane voltage gradients have been identified in embryogenesis and regeneration, adding to the list of such roles identified in earlier work using functional physiology [103,104].

During early frog development, the redistribution of maternal potassium channels and proton pumps in early blastomeres results in a V_{mem} difference across gap junction-coupled cells [89,90,105–108]. The resulting voltage gradient redistributes pre-nervous serotonin to the right side, which then interacts with a cytoplasmic receptor that binds histone deacetylase [1] and shuts off Nodal expression on the right side of the embryo [99,106,109]. Later, during craniofacial patterning, the position of the eyes [110] and other elements of the face [111] is determined by a regionalization of naïve ectoderm into distinct domains of hyperpolarized cells. These voltage gradients regulate the expression of genes like Frizzled, and artificially altering this pattern by misexpression of specific ion channels and pumps is sufficient to perturb normal craniofacial anatomy and to reprogram tissues far away from the head to form properly-patterned eyes [110]. Importantly, in such cases, as in the cancer phenotypes discussed below, it is really the V_{mem} that is the necessary and sufficient factor for inducing specific shape change – it does not matter which ion translocator protein is involved, or what ion species is used: a given voltage change, no matter how it is produced, activates specific downstream events.

During regeneration of flatworms, the patterning activity of adult stem cells (neoblasts) is regulated by gap junctional connectivity and a set of proton and potassium flows [112–114]. By regulation of apoptotic remodeling and downstream activity of genes such as Wnt11 [115], the physiological gradient determines the anatomy of the organs built after injury. In vertebrates, where electric fields were long ago implicated in limb regeneration [116–123], recent experiments showed that driving proton and sodium fluxes can initiate complete tail [124,125] or limb [126] regeneration in a range of non-regenerative conditions. The mechanisms involve guidance of innervation into the stump, activation of blastema genes such as MSX1, Notch, Delta, BMP2, and BMP4, and induction of cell proliferation in the wound mesenchyme.

Indeed, a number of recent molecular studies using unbiased approaches have identified a range of ion channels, gap junctions, and ion pumps in: morphogenesis of the trachea [127], development of skin pigmentation pattern [128,129], regeneration of the zebrafish fin [130], development of mammalian face [131–139], growth of the cerebellum [140–143], and formation of the skeletal [144], cardiac [145,146], and urogenital [147,148] systems. Thus in addition to experiments directly studying bioelectricity in amphibian, avian, and planarian systems, data from genetic models such as Drosophila also identifies channels such as Kir2.1 as important regulators of Dpp signaling and wing patterning [137].

With respect to wound healing, inhibition of which is known to be a tumor promoting agent [149–151], elegant molecular genetics experiments have now revealed some of the elements underlying endogenous electric field-mediated cell migrations. Epithelial wound closure involves Integrin Beta-4 (ITGB4), Cyclic AMP, betaphosphatidylinositol-3-OH kinase-γ (PI(3)Kγ) and phosphatase and tensin homolog (PTEN) [49,81,83,84,152]. Having seen that endogenous electric fields and V_{mem} gradients play an instructive role in normal patterning, what is the evidence that dysregulation of bioelectrical communication can underlie the cancer phenotype?

Bioelectric gradients in cancer at the cell level

Ion channels are oncogenes and important drug targets—The view that cancer is a developmental disorder predicts that molecular mechanisms known to be important mediators of the morphogenetic field would be involved in tumorigenesis. Indeed, there is mounting evidence (Figure 2) that the bioelectric cues that establish normal pattern can go awry and result in cancerous growth [51,153,154]. The function of ion channels is involved in the self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis [52]. Ion channels, pumps, and gap junctions are now recognized as oncogenes [51], predictive markers [52], and an important set of targets for new cancer drugs that's seek to modulate cell behavior by tweaking electrical controls of proliferation or metastatic behavior [155]. Importantly however, oncochannel misregulation occurs not only through mutations in channel genes but also by changes in the rich network of events that implement post-translational gating of wild-type ion channels.

Tumor cells differ from untransformed cells in terms of the type of ion channels and pumps they express and in the resulting membrane potential of the cells [156–165]. Some channel levels are thus used as markers, such as the K2P channel TREK-1 and the sodium channel NaV in prostate cancer [166,167], and the TRPM1 channel in melanoma [168,169]. Tumor cells' membrane voltage is often determined by a different transporter than that of normal cells and it has been suggested that this gives the cells a selective advantage [160]. For example, hepatocellular carcinoma up-regulates the V-ATPase, which is then localized to the plasma membrane [170].

The function of ion translocators, (Table 1) such as voltage-gated K^+ channels [171,172] and Cl^- channels [173], controls the proliferation rate of a number of cells that often form tumors [174–185] or leukemia [186]. ERG is particularly involved in cell growth signals [160,187–191], and is implicated in transformation of prostate epithelium [192]. Also implicated are 2-pore channels such as KCNK9 [193,194], and voltage-gated sodium channels, being definitive oncogenes – necessary and sufficient for a transformed phenotype [195]. Transfection of the EAG K^+ channel confers a transformed phenotype in mammalian cells [196], and hEAG1 channel expression is regulated by p53 (via miR-34 and E2F1) [197]. In human breast cancer cells, K^+ current controls progression through the cell cycle [198]; activation of an ATP-sensitive potassium channel is required for breast cancer cells to undergo the G1/G0-S transition [199]. Metastatic potential correlates with voltage-gated inward sodium current and it has been suggested that some sodium channels may be

oncofetal genes, encoding signals that are active during the rapid and autonomous growth of tumors and embryos [167,200–203].

Migration of cells including B-16 melanoma is dependent on K^+ channels [204]. The voltage-gated sodium channels (VGSCs) potentiate breast cancer metastasis [203], and indeed the involvement of NaV in the galvanotaxis that allows prostate and breast cancer cells to move across vessel lumens [82,195,205–211] is one of the leading stories on ion channels in cancer. Highly up-regulated activities of NaV confers on cancer cells directional motility and invasive characteristics via Ca^{2+} and pH-sensitive cytoskeletal remodeling processes which facilitate metastasis [195,203]. Certain channelopathies result in syndromes associated with cancer such as the lung cancer seen in Lambert-Eaton syndrome [212], and the tumors present in Beckwith-Wiedemann syndrome, which is caused by abnormal imprinting of a voltage-gated potassium channel [213–215]. As will be discussed below, V_{mem} induces cancer-like cell states; while a complete picture of the process remains to be worked out in mammalian cells, a few entry-points have been identified. For example, KID-1, a kinase induced by depolarization [216,217], is a member of the Pim family of proto-oncogenes [218].

Voltage control functions together with the more commonly-studied signals such as growth factors and adhesion molecules. For example, adhesion to specific substrate molecules (mediated by integrin) causes a 20 mV hyperpolarization of resting potential in murine neuroblastoma cells; the hyperpolarization is due to Kir channels and works through a G protein; this hyperpolarization is gone after 1 hour and is necessary for neurite outgrowth [219,220]. Proton pump blockers such as concanamycin and bafilomycin are known anti-tumor agents [221–228] and repression of pancreatic tumor cells occurs after selective blockade of IK-type channels [229]. The involvement of neurotransmitters in cancer [230,231] could also be explained by a voltage-dependent mechanism. For example, GABA is a tumor suppressor [232–234] and GABAA and nAChR are ligand-gated ion channels often expressed in tumors [51].

The involvement of ion channels in transformation, growth control, and metastasis has led to efforts to develop potassium, chloride, and sodium channel and pump modulators as clinical agents for ovarian [235], breast [236], and prostate [237] cancer [155,238]. Unbiased drug screens for inhibitors of cancer stem cells have identified salinomycin (a potassium ionophore) [239] and tetraethylammonium (TEA, a potassium channel blocker) as anticancer drugs that target tumor initiating cells. For example, TEA was found to suppress colony formation in endometrial cancer cells while its withdrawal resulted in a significant enhancement of tumorigenesis [240].

Which ion channels should be targeted by therapeutic drugs? In an important sense, focusing on the channel gene or protein may be missing the bigger picture. In the current literature, ion translocators are usually treated as single genes or proteins responsible for a specific cell behavior (metastasis, hyperproliferation, etc.) – a cell-level view that neglects the fact that numerous channels and pumps contribute to the V_{mem} gradients that mediate large-scale patterning cues [54,242]. The true impact of bioelectricity in cancer will only occur when we understand and target the storage of patterning information in physiological networks

that is misprocessed in cancer [243,244]; such networks are dynamical systems with complex feedback between the post-translational gating of many different channel and pump proteins.

Resting potential: a statistical dynamics view

"For those who believe in the simplification and rationalization of the cancer process, the actual course of research on the molecular basis of cancer has been largely disappointing. Rather than revealing a small number of genetic and biochemical determinants operating within cancer cells, molecular analyses of human cancers have revealed a bewilderingly complex array of such factors." [245]. It is now appreciated that the essence of cancer may not be in specific driver genes but in the dynamics of cells traversing state spaces and shifting between different attractors [34,246]. While these state spaces are commonly thought of in terms of transcription (gene-regulatory networks), the data on bioelectricity in cancer suggests that another important concept may be the physiological state space.

It has long been realized that cancer differs from normal cells by the relatively depolarized state of its cells [247–250]. As far back as the 1930's, Burr was able to detect tumors based on voltmeter readings [251,252]. What these classical data (and the molecular data summarized below) had in common is a focus on bioelectrical state of the cell rather than its genetics: a given resting potential level is contributed to by all of the ion channels and pumps in the cell. Thus, while V_{mem} relies on gene products, it is a complex function of all of them and cannot even in principle be reduced to genetics or transcriptional profiles because all of these translocator proteins are gated at the post-translational level. The dependence of voltage upon the activities of a myriad channels which are regulated by each other and additional physiological events (e.g., phosphorylation), and the ability of voltage change to induce specific outcomes (regardless of which ion channels are used to alter the V_{mem} of this cell), suggest a powerful paradigm borrowed from physics: statistical mechanics.

We propose that the right concept to describe the role of V_{mem} in cellular control is akin to "pressure" in physics. Pressure is a systems property – it is created by the contribution of individual molecules' motions, but tracking any individual molecule in an attempt to understand or manipulate what the system will do would be missing the point entirely. There is no "driver particle" in a gas under pressure any more than there is anything special about a particle that happens to be at the "center of gravity" of a complex object. Pressure is a concept that exists at a higher level of organization than individual molecules, but is causal in the sense that appropriate measurement and control of pressure as such is sufficient to efficiently predict and rationally alter the behavior of systems. We propose that a statistical mechanics view of V_{mem} is the right level to understand its involvement in cancer. Focusing on the details of specific channel genes obscures the "necessary and sufficient causal state" for inducing or suppressing cancer (e.g., depolarized V_{mem}, see below). Specific channels may dominate the V_{mem} in specific cell types, and in those cell types serve as convenient and simple genes to be targeted. However, the general situation is that one can often use any well-characterized channel or pump to make the necessary (and functionally sufficient) changes in V_{mem}. True, databases like Gene Expression Omnibus and Oncomine are

revealing many associations between ion channels and cancer, and specific network analyses do implicate ion channels [253] in processes such as invasiveness. We argue that this is only the tip of the iceberg – an immense under-estimate of the true importance of voltage in oncology, because most of the physiological changes are occurring at the post-translational gating level, and thus are utterly invisible to the mRNA or protein profiling that is so extensively used today.

This was seen for example in the regeneration and left-right asymmetry field, where any number of appropriate gain-of-function channel and pump constructs could be used to induce specific anatomical outcomes such as randomization of the asymmetric organs' laterality or the regrowth of a tail [80,100,126]. Focusing on individual channels as "genes for regeneration" is missing the point that the necessary and sufficient factor is often a voltage state, such as the narrow range of V_{mem} that induces eye formation in any region of the Xenopus larva [110]. With increasingly detailed profiling data, the picture is going to only become more complicated unless we define the distinct physiological states that are responsible for inducing specific cell behaviors such as transformation or metastasis and formulate models at that level of biological organization. This in turn will enable us to rationally design reagents (e.g., select specific gain-of-function channels or pharmacological cocktails) to control V_{mem} appropriately *in vivo*.

But, if distinct V_{mem} levels are transduced by various second-messenger mechanisms into transcriptional and epigenetic responses, why not simply focus on those downstream endpoints directly? This is the situation with every complex regulatory network – any node event (transcriptional or biophysical) has an upstream cause and a downstream effect. The trick is to find "key nodes" – components of the functional network that are convenient to manipulate because they offer optimal control over complex downstream events. This is seen for example in the regeneration field, where a single hour's treatment of a non-regenerative tail blastema is sufficient to induce the entire 8-day regeneration program of this complex appendage (containing spinal cord) [125]. Bioelectric states appear to be powerful master regulators that trigger complex downstream cascades (self-limiting and self-organizing patterning modules) without the need to micromanage the process. Importantly, recent data reveal that V_{mem} is a similarly potent control node in the genetic and biophysical networks that underlie cancer.

Bioelectrical regulation of cancer *in vivo*

V_{mem} signature detects cancer—A variety of bioelectric properties have been used as detection modalities for tumors; these capitalize on cancer cells' distinct electrical impedance [254–268] or ion content [269,270]. Zeta potential is also associated with cancer; for example asbestos fibers and sheets of positively-charged materials (but not powders of the same material) induce tumors, probably by acting as a capacitor for bioelectric potential, the positive side corresponding to the electron sink existing at a wound [271]. In this section, we focus on depolarized V_{mem}, which has been suggested to correspond to the cancer state [247–250].

One way to probe the physiology of the effects of canonical mammalian oncogenes (Gli1, Xrel3 and KRASG12D) and a mutant tumor suppressors (p53Trp248) *in vivo* is to

misexpress them in Xenopus and zebrafish embryos [272–275], which induces tumor like structures (ITLS, Figure 3A,A′). ITLS's thus form as a result of genetic interference with signaling pathways altered in several cancer types including basal cell carcinoma, lung cancer, leukemia, melanoma, and rhabdomysarcoma [276–279]. Examination of injected animals using fluorescence reporters of V_{mem} [280] revealed unique depolarization of tumors (and increased sodium content) compared to healthy surrounding tissues (Figure 3B) [93,281]. Moreover, depolarization foci are present in oncogene-expressing, preneoplastic cells that are yet to undergo transformation or show any morphological phenotype. Such depolarized foci, while present in only 19–30% of oncogene-injected embryos (depending on oncogene used), predict tumor formation with 50–56% success rate (15–21% false negatives). For comparison, prostate specific antigen (PSA) level in the serum, when used as a biomarker for prostate cancer, has ~29% predictive value [282,283]. An added advantage of V_{mem} as a biomarker is that it is associated with tumors of diverse molecular origin, suggesting a general role for V_{mem} change as an early indicator of tumorigenesis.

The next major areas of opportunity for bioelectric detection of cancer are four-fold. First, a more specific physiological signature needs to be developed (to distinguish tumor cells from adult stem cells – another depolarized population) and appropriate voltage-sensitive dye technology implemented as a diagnostic tool to visualize areas of pre-cancer on patients as well as observe tumor margins during surgery. In addition to visualization, a better characterization of bioelectric state could be used to guide drug delivery vehicles such as nanoparticles [284,285]. Second, this strategy needs to be validated in a mammalian model system, and in a range of well-characterized human tumor cell lines. Third, it is critical to begin to tackle the long-range aspects of biological disturbance introduced by cancer. While body-wide morphogenetic fields and the role that V_{mem} distributions play in these are only beginning to be understood [14,15], it is imperative to establish molecular models in which to investigate the fact that transplanted or chemically-induced tumors can be detected by aberrant voltmeter readings taken at locations far away from the tumor [251,252,286–290]. Lastly, modern work on bioelectricity in non-excitable cells has not yet addressed the information encoded in time-dependent changes in V_{mem}. For example, fibroblasts expressing Ras-oncogene respond to the drug bradykinin with V_{mem} oscillations, while control cells exhibit a single transient hyperpolarization. In human carcinoma cells, fluctuations of membrane potential are triggered by EGF and persist for long periods of time after EGF application [291]. The mechanisms and significance of the temporal V_{mem} changes for cancer initiation and progression remain to be discovered.

Depolarization of specific cells induces metastatic phenotype at a distance

Given that a depolarized V_{mem} is an indicator of tumorigenic potential, is it merely a side-effect of cancer, or is it functionally instructive? This question was addressed for the first time *in vivo* in a frog model [60], by the selective depolarization of a sparse set of cells expressing the glycine-gated chloride channel (Figure 4). Using a pharmacological strategy designed to depolarize this subpopulation, a remarkable phenotype was observed: hyperpigmentation of the animals due to over-proliferation, increased migration, and drastic arborization of melanocytes (pigment cells). By transiently depolarizing cells in the body, a different cell type underwent a metastatic-like conversion, turning on expression of genes

such as Sox10 and SLUG 61. The melanocytes acquired a dendritic morphology, upregulated mitotic activity, and invaded blood vessels and soft tissues like the neural tube lumen and brain (Figure 5). In addition to melanocytes, disorganization and ectopic growth of blood vessels was also observed [281], but otherwise the tadpoles were remarkably normal in terms of overall growth and development. Importantly, the same exact effect was induced by any method of depolarization, including by the movement of chloride, sodium, potassium, or hydrogen ions – truly an effect initiated by V_{mem} depolarization, not any specific gene product or chemical ion species. This metastatic-like conversion occurred by a bioelectrical signal alone, without any oncogene, DNA damage, or cancer-causing chemical being applied. Also of note here is that, like in some blood cancers, there was no primary tumor site but a direct metastatic behavior of a normal embryonic stem cell (neural crest) derivative. Thus, while this is an example of non-genotoxic cell-cell communication effect, it differs from the epithelium:stroma interaction at a primary tumor site described by others [292].

Most interesting was the non-cell-autonomous nature of the effect: the cells that acquired a melanoma-like phenotype were not the cells whose resting potential was changed (the GlyR-bearing cells were thus called “instructor cells”) [60,61]. Indeed, only a small number of instructor cells had to be depolarized in order to induce the hyperpigmentation phenotype (which is all-or-none within any individual animal). How was the communication between these two cell types mediated? A suppression screen testing the several known methods of transducing voltage change into transcriptional cascades revealed (Figure 6) that the serotonin transporter SERT, which powers uptake or efflux of serotonin depending on resting potential, was involved (much like in the bioelectric regulation of left-right patterning); blockade of SERT could rescue the hyper pigmentation effect, and direct application of serotonin could trigger a similar phenotype.

A number of key questions remain. First, although it is clear that serotonin signaling is involved in the imposition of a metastatic phenotype by depolarization, serotonin is too small to be fluorescently tagged without drastically altering its transport properties. Thus, the movement of serotonin across long distances has not been imaged directly in this model system. Second, it remains to be understood how hyperpigmentation occurs in an all-or-none manner: treatments that inhibit specific serotonin receptors for example partially rescue the effect, but they do not inhibit metastatic phenotype in some melanocytes, but instead completely rescue only some animals in a test cohort. The current model of this effect [281] relies on a model of amplification and antagonistic function among three different subtypes of serotonin receptors, leading to stochastic effects in the downstream activation of cAMP signaling. However this model remains to be tested in detail. Finally, how does this pathway relate to mammalian cells? It is known that ivermectin, a specific opener of the glycine-gated chloride channel, can regulate the growth of neuroblastoma [293] and leukemia cells [294], although neither of these studies looked at the V_{mem} changes that would have been induced by ivermectin in mammalian tissue culture medium. The findings in the Xenopus model make a number of predictions for human medicine that could be tested. First, the GlyR channel opener Ivermectin, which was used to induce the melanoma-like conversion, could have increased the rate of melanoma and other cancer in human patients. While this drug is no longer widely prescribed as an antiparasitic agent, this class of molecules was

used in human medicine [295] and is known to cause cancer in the parasites that it (usually) kills by muscle depolarization and paralysis [296]. Conversely, we predict that patients taking Prozac (the SERT blocker fluoxetine) may have lower incidence of melanoma. These predictions await epidemiological testing.

Hyperpolarization inhibits oncogene-induced tumorigenesis

he above data show that depolarization of V_{mem} can, itself, activate a metastatic-like phenotype. What role could V_{mem} play in carcinogenesis induced by genetic perturbation? Well-characterized channels such as inward rectifying potassium channel (Kir 4.1) and constitutively open glycine-gated chloride channel mutant (GlyRF99A) can be used to generate strong hyperpolarizing currents [297,298]. When GlyRF99A is co-injected with the Xrel3 oncogene in Xenopus larvae [93], it significantly suppresses the incidence of ITLS formation compared to oncogene alone (Figure 7). Florescent tags on the oncogene protein revealed that hyperpolarization could prevent the formation of tumor-like structures despite very robust expression of oncogene in cells. The use of several different hyperpolarizing channels based on Cl^- and K^+ confirmed that suppression of neoplastic transformation was due to V_{mem} hyperpolarization per se, as opposed to ion-specific or scaffolding functions of the ion channel proteins. Consistent with this, data in rats showed that the ion channel modulator drug ivermectin can likewise modulate the effectiveness of carcinogenic compounds [299].

How do changes in V_{mem} transduce into suppression of oncogene-mediated tumorigenesis? A pharmacological suppression screen of several candidate mechanisms, followed by molecular-genetic loss-of-function validation [93], implicated the sodium-coupled monocarboxylate transporter (SLC5A8). SLC5A8 has previously been identified as a tumor-suppressor whose transport of butyrate or other short chain fatty acids (HDAC inhibitors) is essential in maintaining a healthy colon and/or control colon cancer invasion [300–306]. A model of the bioelectrical regulation of oncogene activity is shown in Figure 8: oncogene expression causes the observed depolarization, which limits the intake of Na+ through SLC5A8, also limiting butyrate intake (co-transport, Figure 8A). Lack of HDAC regulation due to reduced butyrate presence leads to hyperpolarization and tumor progression (Figure 8B). However, forced hyperpolarization within oncogene-expressing cells facilitates the uptake of the positive Na^+. This powers the import of butyrate through SLC5A8, resulting in continuous suppression of HDAC and thus reduced proliferation and ITLS suppression (Figure 8C). The specific genetic targets of hyperpolarization in this context remain to be thoroughly explored, however p21 is a likely mediator. Hyperacetylation of histones – as a result of butyrate-induced HDAC inhibition – has been shown to up-regulate p21 at both at the mRNA and protein level [307,308]. p21 inhibits cyclins/cdk's activities, thus inhibiting downstream substrate phosphorylation and causing cell cycle arrest (reduced proliferation) at the G1/S transition and subsequent suppression of ITLSs (Figure 8D). It should be noted however that many other (physiological, non-genetic) events can induce a similar depolarization as that initiated by oncogene function, thus inducing the rest of the downstream steps in this tumorigenesis pathway without necessitating oncogenic mutation.

Cancer: a disease of geometry?

Given the data implicating physiological cell properties and bioelectric cell:cell communication in cancer and its normalization, it is important to place this body of work in the context of developmental signaling. Bioelectric gradients are one component of morphogenetic cues mediating positional information, tissue specification, and intercellular coordination [14,43].

Morphogenetic Field as tumor suppressor: importance of community

The sum total of the instructive patterning signals that impinge upon cells in the organism (Figure 9A) is referred to as the Morphogenetic Field [14,18,309,310]. This instructive information is mediated by a range of dynamically-varying spatio-temporal gradients of secreted biochemical factors, extracellular matrix properties, stresses/strains/stiffness values, and electric properties. Disturbances of the normal interactions between cells and the signals that normally orchestrate individual cell activities into maintenance of host tissues and organs can manifest as cancer. The hypothesis that cancer is fundamentally a phenomenon at the level of multicellular organization makes a number of unique predictions confirmed by experimental data (that are not predicted by the somatic mutation model). For example, one way to perturb field structure is to introduce ectopic organizer nodes. Indeed, implantation of early embryos (which organize their own field of signals) under the kidney capsule of an adult makes transplantable malignant teratomas despite a lack of any infective, chemical, or radiation initiator to cause genetic damage [8], while normal adult Xenopus kidney implanted in the non-amputated forelimbs of recently-etamorphosed larvae will make lymphosarcomas as well as accessory limb structures [311]. Implantation of mouse embryos into adults causes teratocarcinomas [312], possibly due to an interference between the host and implanted embryo’s morphogenetic field signals.

Cancerous failure of morphostasis can occur because a morphogenetic field is missing, altered, or not successfully perceived (all three of which can occur due to genetic or physiological state change). Cells in dispersed monolayer culture are several orders of magnitude more sensitive to chemical carcinogenesis than are organized tissues within an intact organism [313], and placing normal primary mammalian cells in culture results in the appearance of cells with malignant potential [313–316]. Chick embryos infected with the v-Src virus exhibit no malignant phenotype, but the same cells in culture undergo massive transformation [317]. A number of recent papers stress the suppressive nature of signals from neighboring tissues [4,9,10,29]. Consistently, re-establishing appropriate interactions of human cancer cells with the microenvironment and normal neighbors underlies the observed reversion of malignant phenotype in a number of cell lines [318–320].

Consistent with the need for cell: cell interactions in suppressing cancer are data showing that tumorigenesis is promoted when cells are isolated from their neighbors (and thus from the morphogenetic guidance they would otherwise receive) by physical barriers. Implanting into connective tissue of the rat rectangles of inert plastic, metal foil, or glass cover slips induces sarcomas when the material is >1cm^2. If the material is perforated, the incidence is reduced, and the effect does not occur with powders of the same material (which actually

increases surface area, ruling out chemical induction or genetic damage mechanisms) [321–324].

More recent data has focused attention on interruption of cell:cell communication via ions and other small molecules through gap junctions (GJs) – aqueous channels made of connexin proteins that allow physiological signaling directly between the cytoplasmic interiors of docking adjacent cells [325–330]. For example, Connexin32-deficient mice have a 25-fold increased incidence of spontaneous liver tumors [331]. Gap junctional isolation is known to be a tumor-promoting agent [330,332–337], although there are counter-examples [338]. Active GJ communication allows cells to make sophisticated decisions comparing relative levels of specific compounds between themselves and their neighbors [339] and thus can underlie the transmission of physiological patterning signals [89,90,114,340–347].

Another mechanism of coordination across large cell fields that was recently implicated in cancer is the planar cell polarity (PCP) pathway – a set of protein components designed to coordinate orientation and function of cells over long distances [348]. PCP has now been shown to function as a non-canonical tumor suppressor [349,350]. While the direct causal relationship between loss of PCP and tumor initiation in humans is not yet proven, it is clear that loss of polarity can be an initiating event in tumor formation in Drosophila [351]. Consistent with conserved mechanisms underlying coordination and maintenance of long-range order in cancer and normal development, PCP is also involved in dynamic morphostasis: grafts of embryonic skin (after the planar polarity of hair becomes evident), when implanted into adults, realign their hair polarity to match that of the hosts [352] – this dynamic readjustment to local conditions is a factor that distinguishes cancerous tissue from its normal counterpart (see below). PCP allows cells to align axes orthogonal to their apical-basal polarity with each other, and with major anatomical axes of the organism, linking large-scale order with regulation of single-cell behavior.

Another mechanism used to coordinate cell activity away from cancer is communication via the nervous system. Tumors are readily induced by denervation in salivary organ and alimentary canal in cockroach [353,354] and in mammalian skin [355]. Similarly, tumors are chemically induced more easily in denervated rabbit ears as compared with contralateral controls bearing normal innervation [355]; the same has been observed in sarcomas implanted into normal or denervated frog limbs [356]. These remarkable results are predicted by models in which nervous system components transmit long-range morphogenetic field cues [357–360], but have been unfortunately neglected in the modern literature focused on DNA. Much works remains to characterize the role of the nervous system in providing information and cancer-suppressive cues to existing adult tissue, and to dissect which signals are broadcast via the long-range communication systems provided by planar cell polarity, nerves, and gap-junctional networks.

Positional information and cancer

Models of suppression of cancer by long-range morphogenetic cues, as opposed to simpler models of growth-inhibitory signals from any normal neighbors, predict that tumorigenesis would be modulated by global position within the host, as are events operating during embryonic development and regeneration. Microarray analysis reveals quite different

profiles of human glioma cell lines grown in leg vs. brain [361]. Moreover, tumors grow on posterior regions of Triturus less readily than they do on anterior regions [362], and numerous such differences are observed in human tumors as well [363–368]. These studies suggest a link between large-scale axial patterning in adult organisms and the potential for failed perception of morphogenetic cues by cells.

A most impressive example of the importance of location and environment in cancer growth is provided by patients with peritoneo-venous shunts, who have a steady infusion of peritoneal fluid that carries billions of desquamated cancer cells into the systemic circulation, for months or years [7]. Known metastases in some organs before insertion of the shunt exhibited additional deposits in the same organs but not anywhere else, despite millions of viable cancer cells being distributed to every organ. This work (which ruled out immune clearance of cancer cells) revealed that the disseminating cancer cells were capable of establishing secondary tumor colonies in some anatomical sites in a given patient but could not do so in other organs, raising the question of whether some body regions have more active morphostasis pathways.

Even more interestingly, surgical disruption of normal topographical tissue relationships tends to induce cancer, which suggests a feedback model where the morphogenetic field can be altered by scrambled anatomy, or perhaps difficulty in cells' reading instructions at the borders of fields that are not supposed to be geometrically adjacent. For example, despite lack of DNA damage or cytotoxic chemical stressor, transplantation of rat testis to the spleen induces formation of interstitial cell tumors [369], while normal rat ovary tissue put into normal rat spleen results in malignant neoplasm [370]. Cancer thus is not only a disruption of normal patterning within the tumor but also reveals an interplay between its activity and the context of the large-scale spatial organization of the host.

Normalization of cancer by developmental and regenerative patterning

The morphogenetic field ought to be the most active and accessible during embryogenesis. It is thus not surprising that despite considerable malignancy and aneuploidy, tumor cells introduced into wild-type embryos become integrated as normal tissue [371–381]. Human metastatic melanoma cells injected into zebrafish embryos acquire a non-neoplastic phenotype, but form tumors when injected into zebrafish after organogenesis [382,383]. Likewise, implanted sarcoma progressed in 80% of adult rats but only in 6.4% of rat embryos. Similar data have been recently shown for chick and other kinds of embryos that are able to tame aggressive cancer cells when these are implanted [383–386]. Cancer normalization can occur cell-autonomously [387], or induced by communication from other cells, such as the mammary stroma [371,388–393]. Indeed the embryonic field present in the blastocyst can normalize several types of cancer cells including those isolated from embryonic carcinoma, leukemia and neuroblastoma [381], although the limits of this normalization process (with respect to large-scale chromosome aberrations found in some tumors) remain to be probed fully. Thus, active patterning signals can normalize cancer (over-ride genetic defects and reboot cell behavior programs); this is a finding that is not predicted by the cell-level view of cancer as embryos have high levels of many growth

factors that could be expected to potentiate tumor growth (and do, in experimental contexts such as cell culture which is devoid of large-scale patterning structure).

Tumors have also been described as wounds that do not heal – areas of disruption and cell growth without an appropriate patterning program that reaches a terminal goal state [149,394]. This analogy is supported by profiling data showing the molecular similarity of repair vs. carcinoma in renal tissue [149]. Successful tumors have developed the capacity to preempt and subvert the wound-healing response of the host [395]. What about wounds that not only heal but successfully rebuild a missing structure? Some animals, such as salamanders, can regenerate entire limbs, eyes, hearts, and jaws. Even mammals regenerate some organs (e.g., liver in humans, and antlers – meters of innervation, bone, and skin – in deer).

It has been long known that regeneration and cancer are closely related [396–401]. Highly-regenerative organisms are resistant to carcinogenesis and indeed activating regenerative response can normalize existing tumors [396–399,401–405], although this does not always occur [406]. The inverse relationship between regeneration and cancer susceptibility [407,408] is more compatible with the importance of morphogenetic field guidance than with cancer risk associated with the presence of highly-active, undifferentiated cells [18]. Mammalian liver regeneration can overcome cancer - early nodules initiated by carcinogens are remodeled to normal-appearing liver [409,410], hepatocarcinoma cells can be normalized by injection into wild-type liver [411,412], and over 95% of nascent tumor sites remodel into normal tissue by the highly-regenerative liver [413–415]. In zebrafish brain regeneration, a remarkable degree of aneuploidy does not lead to cancer -an active patterning program trumps chromosomal damage [416], and amphibian limb regeneration can likewise normalize tumors [31,32,400]. Thus, tumors may be wounds that do not pattern.

Modern molecular model systems are now available for the study of these still poorly-understood mechanisms: regeneration of the zebrafish tail prevented tumor formation from BRAFV600E mutation + p53 knockout [417], offering the opportunity to use the numerous available zebrafish reporter lines and functional morpholino strategies to investigate the relationship between regeneration and tumorigenesis. Remarkably, such influence is not necessarily local. Induction of anterior regeneration in planaria turns posterior infiltrating tumors into differentiated accessory organs such as the pharynx [362], which suggests the presence of regulatory long-range signals that are initiated by large-scale regeneration. It is likely that the normalization of tumors by active remodeling represents one of the most profound and exciting areas for future work in understanding morphogenetic fields and their interpretation by growing tissue.

Interestingly, the interplay between proper patterning and cancer suppression is retained throughout life; for example, if the endocrine gland is removed in Dixippus, regenerative capacity is lost, and spontaneous tumors begin to appear [418–421]. Work in highly-regenerative model species such as amphibia and some invertebrates is likely to be the fastest route to understanding this fascinating phenomenon of tumor and cancer cell reprogramming but it is important to note that these signaling pathways are likely to be of

relevance to human patients. For example, childhood neuroblastoma has a high rate of spontaneous regression [422,423], and a number of other cancers often regress spontaneously [7]. The efforts of regenerative medicine to improve regeneration prospects in man may thus have a significant side benefit of impacting cancer treatment.

Explanations at above the single-cell level—Is cancer fundamentally a cell-level property or a multicellular phenomenon that, like the wetness of water, which is not applicable to individual H_2O molecules, applies only to collections of cells and characterizes the interactions between them? The current paradigm focuses on cell-level activity (proliferation, differentiation, migration), but tissue- or organ-level systems properties might be the right basal concepts with which to formulate models and intervention modalities [424–427].

The difference between these approaches is not mere philosophy – it has testable implications that allow data to distinguish between the two classes of models. For example, a focus on cell cycle checkpoints and TGF-β molecules (a view at the cell level) leads to the prediction that cancer and regenerative potential should go together: animals with ready access to plastic, highly proliferative cells should be prone to neoplasia, and long-lived humans would be forever barred from powerful regenerative pathways because of the evolutionary pressure to suppress cancer over decades. Conversely, a morphogenetic field model (cancer as a failure to transmit or receive anatomical cues) suggests that regeneration and cancer should be inversely related, as robust patterning pathways necessary for regenerating complex organs from new cell growth would also keep cells within a coherent morphological plan and away from tumorigenesis during normal lifespan.

In fact, the most highly regenerative animals tend to have the lowest incidence of cancer [396,399–401]. Moreover, if a tumor is induced on the limb of a salamander and the limb is amputated through the tumor (Figure 9B), the remaining cancer tissue becomes part of the newly regenerating limb [396–401]! This readily illustrates the profound relationship between cancer and regeneration and the importance of studying systems-level concepts (the mechanistic details of “exerting strong patterning control at the level of a whole appendage”) for what is often thought of as a cellular- or gene-level process. It also suggests a highly optimistic view of the potential for regenerative pattern control in human cancer medicine.

As in regenerative medicine, the answer to this question impacts treatment strategies: do we micromanage individual gene products, or attempt to initiate complex patterning cascades? Up-regulating embryonic genes in adults results in cancer [428–430], while, as discussed above, inducing embryonic genetic programs leads to cancer normalization [18]. Moreover, drugs that target upstream functions in signaling networks have less general toxicity than those that interact with targets further downstream [431]; this is consistent with the view that the right level of intervention to optimize effectiveness and compatibility with overall health (lack of toxicity) is by activating large-scale physiological modules that have been evolved to implement mutually compatible (healthful) downstream events, instead of specifically impacting individual downstream players which may induce unwanted interference with other functions in the organism.

A closely related question to the scale and level of organization of cancer state is the spatial distance over which the disturbance acts [432–434]. In mammalian breast cancer [292] and frog melanoma-like transformation [60,61], clear roles for non-local (long-range) influence over carcinogenesis have been found and can now be dissected. This is clinically relevant, as seen in field effects in many different kinds of cancer in which surrogate sites are not necessarily adjacent to the main tumor [435,436]. The characteristic size scale of the "cancer field" is still not mechanistically understood and is relevant not only for understanding the basic biology of signaling but for designing detection and treatment modalities intending to impact specific tumor sites. Although most work on cell:cell interactions today deals with chemical gradients as a signaling modality, bioelectricity is an ideal medium for long-range coordination and information exchange among cells during pattern maintenance and repair.

Future Prospects/Speculations

Tumor boundaries and selves

The fundamental fact of cancer is that cells cease to work towards the anatomical needs of a host organism and narrow their dynamic goal-seeking behavior to the level of single cells – an increase in "selfish" behavior away from the normal cooperativity of multicellular life. Cancer could result from a failure of the host to impose or transmit necessary patterning information within a particular region; it is also possible that tumor cells are those that stopped attending to the morphogenetic field cues [27,349,398]. Anticipating recent discoveries of the importance of gap-junction cell:cell communication for planarian regenerative patterning [112,114], in 1965 Seilern-Aspang described planarian experiments in which a carcinogen led to formation of many head teratomas with irregular nerves and un-oriented eyes concluding that "the cell-isolating action of the carcinogen prevents formation of a single morphogenetic field and leads to the establishment of several separated fields of reduced dimensions" [362]. Thus, tumors could also represent establishment of a local "subfield" – a fragmentation of the host's morphogenetic field such that integration with the host body plan is lost. Unlike normal somatic tissues, which remodel when transplanted into foreign locations [437–439], the histopathological structure of metastasis reflects the tissue of origin, not of their destination [8], confirming an inability to respond to neighboring signals such as positional information and remodeling cues.

Interestingly, cancer is not only a loss of patterning, but also a coherent, goal-seeking subsystem: tumors are not just aggregates of replicating neoplastic cells but complex living entities composed of numerous cell types that work together to acquire nutrients, survive, and evade the efforts of an environment that is trying to kill them [5,440]. One way to model such changes in dynamics is as a reduced scope of "self" – the view that a tumor is, in some practical sense, an independent organism [441] with its own (primitive) morphogenetic field. In a tumor, the boundary of self has been reduced from the whole body to that of a much smaller structure, making the rest of the body just part of the pseudo-organism's outside environment. Such a view is suggested by a number of findings. First, histological analysis indicates that tumors can indeed be regarded as complex tissues with a distinct internal organization [314,442]. Tumors reproduce themselves via metastasis, and execute many adaptive strategies (such as up-regulating multi-drug resistance proteins in the face of

chemotherapy) to preserve their homeostasis and existence – just as organisms within an ecological niche do [443–445]. Much like organisms maintaining morphostasis, tumors maintain their identity during massive cell turnover during selection for founder cells resistant to chemotherapy drugs [446]. Recent work describes the highly malignant brain tumor as an "opportunistic, self-organizing, and adaptive complex dynamic biosystem" [447]; proper characterization of the essential principles predictive of the properties of tumor invasion makes uses of concepts such as least resistance, most permission, and highest attraction – these are systems-level, goal-directed elements that are very compatible with the conceptual modeling techniques suggested for understanding embryogenesis and regeneration of whole organisms.

The defection of cells from the goal states of the body to those of a much smaller entity (a tumor, or perhaps individual cells) implements a contraction of the functional boundaries of the self-organizing system [448,449]. Tumors of course pursue goals quite at odds with those of their host. "Glioma cells are ill-equipped to participate in ion and amino acid homeostasis, those important altruistic tasks performed by their nonmalignant counterparts. Instead, gliomas are more concerned about their relentless growth and invasive migration" [450]. Interestingly, cooperation occurs among the tumor cells that can be analyzed via the same mathematical tools that explain cooperation and competition among somatic cells and members of societal groups [339,451,452]. While tumors typically lose heterologous gap-junctional communication to surrounding stroma, they often maintain good gap junctional connections among their own cells. Interestingly, gap-junctional connections have been proposed as a mechanism by which cells can recognize "self" [453,454].

The questions of size control and field boundaries are central to developmental biology as well as cancer. During planarian regeneration, a regenerating head will inhibit the formation of heads elsewhere, but parts of the regenerating head do not inhibit the rest of that same head from forming. A specific voltage range causes tissues to reorganize into eyes [110], but these eyes are of normal (limited) size, and at the same time contain numerous distinct tissues that clearly result from a process more complex than simple control of cell fate and differentiation by a resting potential value. To really understand the fascinating ability of active morphogenetic fields during regeneration to normalize or prevent tumors will require new, molecularly-tractable models of tumor normalization. Axolotls are a powerful system in which this could readily be dissected [455,456]. Future work must uncover the mechanisms that establish size and scope of morphogenetic fields, to understand how boundaries are established and altered during pattern formation. Cybernetic models of goal-seeking behavior among dynamical systems such as embryos and tumors are needed to understand the kinds of signals that can be manipulated for desired outcomes in regenerative biomedicine and oncology contexts [457–459].

Genetics and physiology

One of the most important lessons to come from the recent work on bioelectrical controls of morphology is the fact that significant patterning information can be generated and maintained at the level of physiology and de-coupled from changes in transcription and translation (Figure 10). While biophysical events are certainly transduced into genetic

cascades, the laws of physics and the post-translational gating of channel and pump proteins guarantee that cells expressing precisely the same complement of proteins can be in very different bioelectrical states. Of course this is akin to familiar phenomena such as action potentials propagating down an axon and calcium fertilization waves moving over an oocyte, neither of which requires changes in mRNA or protein levels for the dynamics of bioelectrical cell state to evolve. On longer time scales, networks of voltage-gated ion channels and voltage-regulated gap junctional current paths can form cell fields with rich feedback dynamics in the distribution of voltage levels that are not at all captured by analysis of protein content. Protein profile does not determine physiological state: not only can cells with the same protein be in different physiological states, cells in very similar physiological states could reach them by making use of very different ion channels. The ability of many different channels to be combined towards the same physiological end-goal results in a huge degree of compensation and redundancy [460]; this is a benefit for ensuring physiological robustness of bioelectrical control systems, but also means that the popular single-channel knockout experiments will rarely reveal phenotypes indicative of the patterning roles of transmembrane voltage potentials.

As seen in the several examples discussed in detail above, changes in bioelectric state can alter patterning and induce/suppress tumorigenesis without DNA damage, and by the modulation of any number of channels/pumps at the post-translational level. Cracking the bioelectrical code to allow prevention and normalization of cancer will require fleshing out the interaction between information stored in truly epigenetic (in the original sense of the word) physiological networks, transcriptional responses, and the goal-seeking control algorithms of single cells and multicellular host organisms. This in turn will require new technique development - establishment of model systems in which voltage can be controlled directly in any cell/tissue of interest; one exciting candidate is the extension of optogenetics to non-neural, non-excitable tissues [461,462], and conceptual apparatus for modeling information processing and autonomous dynamical system properties in silico that can be applied to the initiation and reprogramming of cancer *in vivo*.

Speculations: cancer as a failure of morphogenetic field memory

Taken together, recent and classical data suggest that morphogenesis and morphostasis are core concepts unifying three major areas of study – development, regeneration, and cancer. Understanding the ability of systems to self-assemble complex anatomy, to repair damage, and maintain shape against aging and cancer is paramount to progress in all three fields, and will drive radical advances in biomedicine [463]. It is imperative that we identify and quantitatively model the information-processing and computational activities of patterning systems to gain control of molecular mechanisms by which morphogenetic information orchestrates low-level (cell) behaviors towards the patterning needs of the host. Cancer biology may be an ideal context in which to consider predictive, quantitative models of top-down causation and control as an alternative to the current paradigm focused on molecular pathways and emergence [464–468]. What criteria (degree of predictive control in functional experiments? parsimony of model? conformance with reductionism?) are to be used to decide among top-down and bottom-up models? This has important implications beyond philosophy and basic developmental biology. Our choice of strategies for

regenerative and cancer medicine depends crucially on finding the easiest path towards gaining rational control over complex biological shapes and understanding the still mysterious link between the equally rapid growth of cancer vs. regenerative repair.

Bioelectric events have properties that make them ideal components for implementing the morphogenetic field, and indeed recent data has shown that their manipulation is a good entry-point into a molecular-level understanding of these mechanisms [80,469]. Bioelectricity is central to development, regeneration, and cancer. But the transformative impact of integrating biophysics into our genetic paradigm will be the ability to move beyond V_{mem} in single cells and understand the dynamics of multicellular bioelectric networks and how they store and specify shape. A key next step is the construction of specific dynamical systems models of patterning information stored in real-time physiological networks. Multidimensional spaces of many different bioelectric measurements will require concerted physiomics profiling efforts; such data may turn out to contain attractors that map to anatomical states, and may implement the “dynamically preformed morph” envisioned by Gurwitsch [470].

The data of modern bioelectricity reveal the instructive control of shape (and its derangement during cancer) by endogenous voltage gradients and ion currents. Dynamic control of morphogenesis requires processing large amounts of information about tissue and organ structure, as well as a mechanism for ceasing growth when particular target morphology has been reached (a step that is likely to be completely short-circuited in tumors). Fortunately, we have two solid precedents for storing information in dynamic patterns of ion flow. The first is storage of bits in a magnetic core memory – the information is literally encoded in the direction of ion flows and their resulting magnetic fields. Much as the ion flows among electrically active cells are invisible to techniques focused on the material structure of cells and the mRNA/proteins expressed in them, the information content of electronic storage media is invisible to a description of the material components of a computer memory system - energy flow patterns can store distinct bits among identical bi-stable units, whether they are implemented in cells [471–473] or transistor flip-flop circuits.

Even more simply, the conservation of basic molecular elements in the central nervous system and in non-neural embryonic cells reminds us that cognitive science has a mature and well-developed history of investigating spatial maps encoded in the dynamics of electrically-active cells - navigational memory in the brain [474,475]. The neurobehavioral community is quite comfortable with the storage of memory in neural networks, and techniques and results in this field should be combined with modern understanding of pattern formation and disorders. After all, both study information – spatial information processed in reorganization of geometry (morphogenesis), and temporal information remembered as patterns from the environment (learning and memory); the parallels in information-processing algorithms of neural networks and developmental dynamics was pointed out by Grossberg decades ago [476], but not seriously investigated yet. Not surprisingly, ion translocators are involved in learning and memory storage [477–479], placing these molecules at an important focal point at the intersection of morphogenesis and cognition. Likewise, heart cells have been modeled as a neural-like network to explain

memory effects relevant to remodeling [471,480]. While most somatic cells process voltage change signals much more slowly than do rapidly spiking neurons, it is tempting to speculate that the analogy may indicate a real, mechanistic relationship. Such computational tissues would be ideal media in which to store and manipulate the information used by morphogenetic fields. Given that many cell types are communicating electrically via membrane potential and highly-tunable electric synapses, gap junctions [88,481–483], there may not be any fundamental difference between the information-processing functions of neural networks and similar electrical dynamics in non-neural cells. Thus, cancer could be a regenerative response that cannot remember what target morphology is to be recognized as the “end of growth signal”, and a reprogramming solution could be sought in repairing the ability of cells to access the electrically-mediated memory of appropriate tissue organization and the cell behaviors that are needed to achieve that state.

If these highly speculative parallels hold, many novel, mechanistically-tractable questions are suggested with respect to how normal tissue architecture is “remembered” in cell networks and what processes of memory failure may result in neoplasm. For example, the glutamate receptor, metabotropic [1], also known as GRM1, is an ion channel and an oncogene; notably, the knockout mice exhibited problems with long-term potentiation (memory) [484–488]. Likewise, signaling via the neurotransmitter serotonin has been implicated in cancer [60,281,489–491]. The use of cognitive modulator compounds in cancer assays is being pursued in our lab, to test predictions of a hypothesis linking bioelectric mediation of morphogenetic cues, stored memory of correct organ/tissue morphology, and its disruption in cancer.

Conclusion and Summary

Much classical and recent data reveal that cancer is not simply a result of damaged genomes but rather involves a disruption of normal developmental mechanisms and cell:cell communication across large distances. It is now known that endogenous bioelectrical gradients underlie an important layer of such cell coordination, and it is thus not surprising that ion channels are increasingly revealed as not only markers of the transformed state but also bona fide oncogenes and thus important drug targets. Work in the amphibian model demonstrated that depolarization of resting potential by voltage-sensitive fluorescent dyes is a promising modality for detecting cancer *in vivo*. Moreover, depolarization of key cell groups in the body is sufficient to activate metastatic behavior in other cell types through a serotonergic signaling mechanism. Finally, oncogene-induced tumor structure formation requires depolarization, and artificial hyperpolarization is able to suppress this effect, despite high levels of oncogene protein, through a butyrate and histone deacetylate mechanism. Thus, the tumor microenvironment is not only chemical but also bioelectrical [492]; indeed, it may function over considerable distances. Transformative applications in cancer medicine and beyond await our molecular dissection of the bioelectrical and other mechanisms by which active morphogenesis and nervous system activity suppresses tumorigenesis, and regenerative and embryonic environments actively reprogram tumors.

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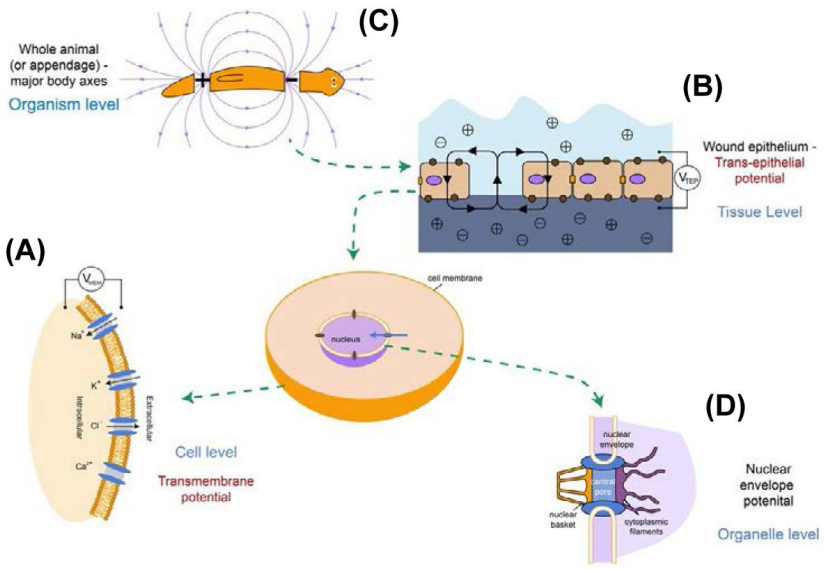

Figure 1. Bioelectric cues *in vivo*

(A) Resting potential gradients in all cells result from the imbalance of ion movement across cell membranes. The resulting V_{mem} is a function of the internal and external concentrations of the major ion species, as well as the conductivity to each ion (open/closed states of specific ion channel proteins).

(B) At the level of tissues, trans-epithelial potentials (TEPs) result from ion flows across sheets of cells connected by tight junctions [493–494]. Breaks in epithelia provide electric fields that serve as migratory cues for galvanotaxis of cells involved in wound healing and metastasis.

(C) At a yet greater level of biological organization, spatial gradients of V_{mem} and TEP correlate with whole body organs or primary anatomical axes[113,495,496], providing patterning cues such as positional information that guide growth and form.

(D) Still mysterious with respect to their function in patterning are the gradients across intracellular membranes, such as the nuclear envelope potential [497–498]. The role of such gradients for active targeting and distribution of intracellular components or conformation/transcriptional status of components of the DNA in the nucleus remain to be analyzed.

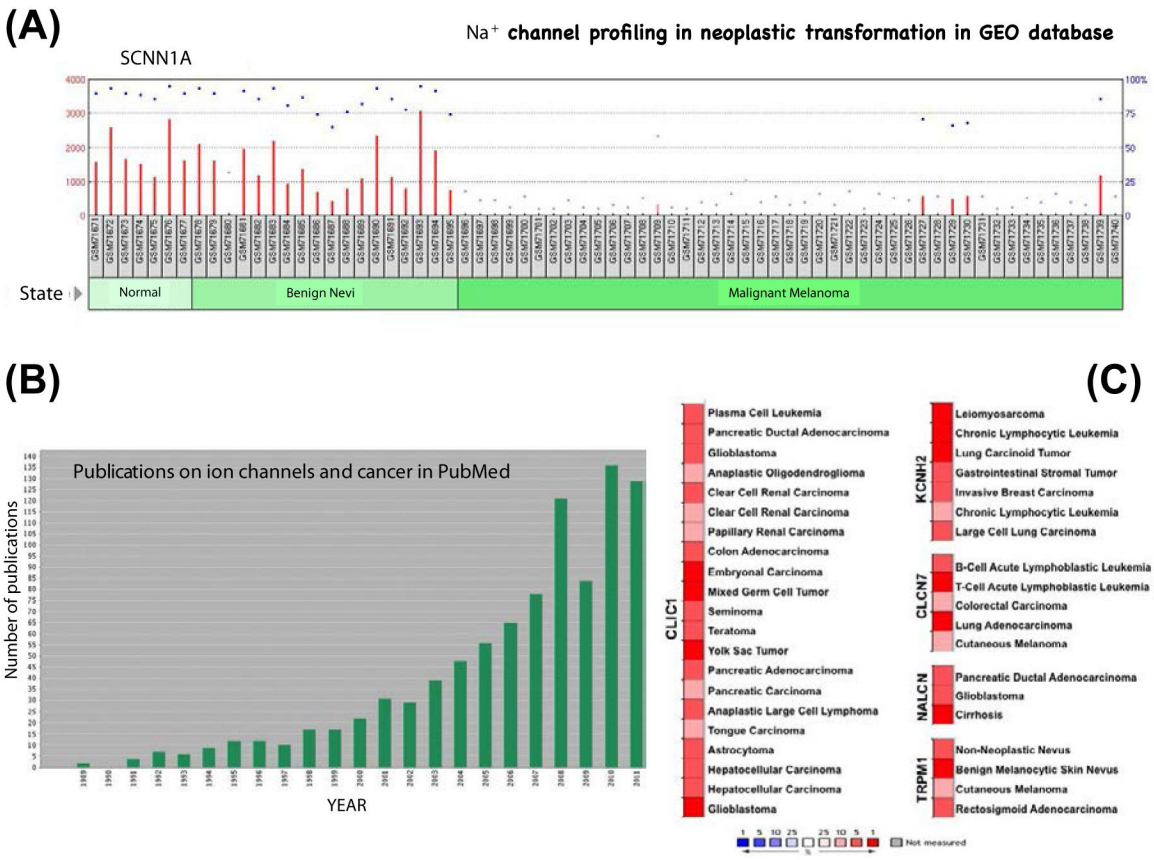

Figure 2. Molecular genetics implicates ion translocators in cancer

(A) The Gene Expression Omnibus (GEO) database consists of high-throughput functional genomic data on collections of biologically and statistically comparable samples. A profile generated from the cutaneous malignant melanoma dataset readily reveals changes of transcription of many ion channel genes during neoplastic progression; here is shown the down-regulation of a specific sodium channel at the transition from benign nevi to malignant melanoma.

(B) The amount of published data on ion channels is growing more voluminous every year. Importantly, the current level of interest in oncochannels as genetic and pharmacological targets is a significant underestimate of their true importance, since current studies take place almost exclusively at the levels of mRNA or protein profiling. Since channels do most of their regulation post-translationally (being opened or closed by a range of local and non-cell-autonomous signals), analyses such as profiling, microarray, deep sequencing, knockout screens, etc. inevitably miss all of the regulation that takes place at the level of physiology.

(C) Oncomine is a cancer profiling database consisting of genes, pathways, and networks deregulated in more than 50,000 cancer gene expression profiles. Analysis of genes with highly altered expression levels (fold change compared to relevant normal tissue in the top 10 percentile) in multiple cancer types implicates several chloride, potassium, and sodium channels as oncogenes.

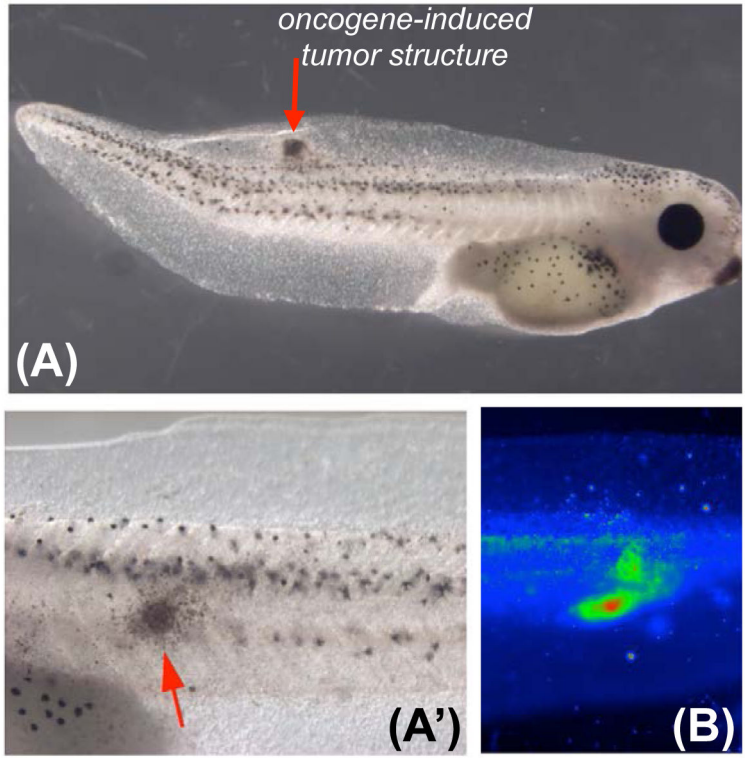

Figure 3. Transmembrane potential as a diagnostic modality for tumor detection

(A, closeup in A′) Tumors (red arrow) can be induced *in vivo* in tractable model systems such as *Xenopus* larvae using targeted misexpression of mammalian oncogenes such as dnP53, Rel3, Gli1, RAS, etc.).

(B) Using voltage-sensitive fluorescent dyes, areas of depolarization (green, red) are detected non-invasively [93,281]. While a tightly-defined physiological signature remains to be developed (likely necessitating concomitant use of several different physiological dyes, such as those reporting voltage, sodium content, and pH), the scanning of bioelectric properties with light-emitting dyes *in vivo* is a promising modality for early detection of pre-cancerous tissue and tumor margins during surgery.

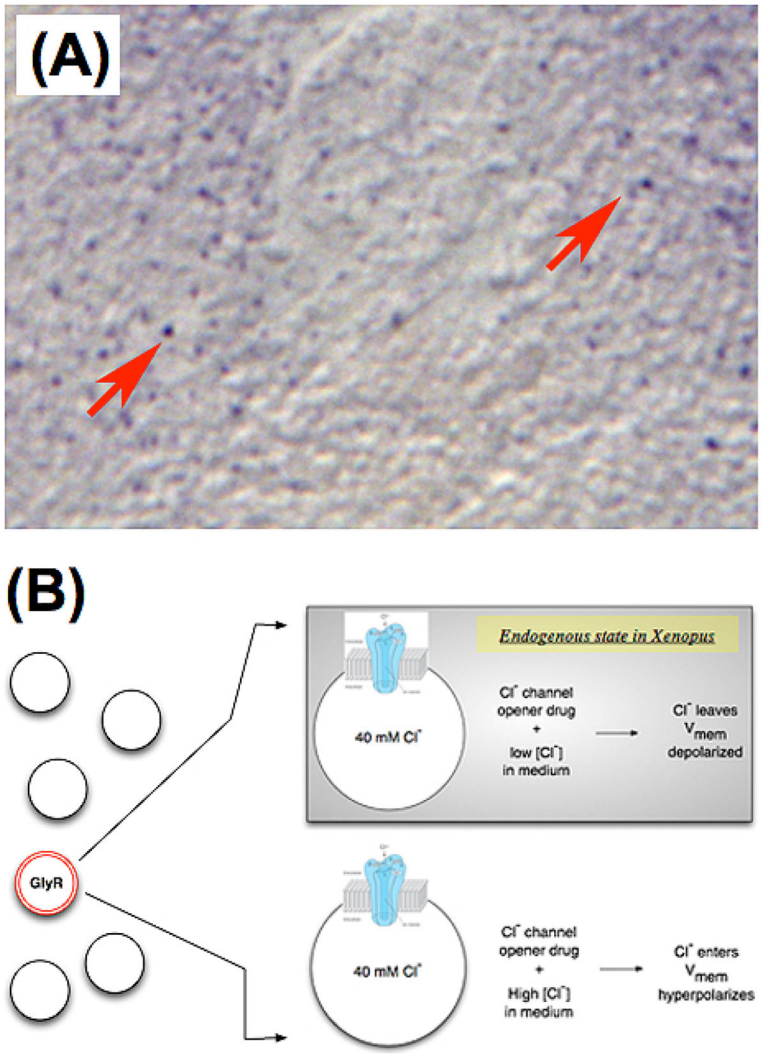

Figure 4. Experimental control of V_{mem} in a defined cell subpopulation

(A) In *Xenopus*, a sparse but ubiquitous population of cells expresses the Glycine-gated chloride channel (GlyR or GlyCl) [60]. Here a section of a frog larva has been subjected to immunohistochemistry revealing these cells as purple dots (red arrows).

(B) A strategy for selective depolarization of these cells *in vivo* uses a specific channel opener, ivermectin, to render these cells permeable to chloride. Then the level of chloride in surrounding medium is varied, to induce depolarization (efflux of negative Cl^- ions) or hyperpolarization (influx of negative chloride ions) at will. This technique allows the experimenter to study the effects of V_{mem} change in a specific cell population within a living organism.

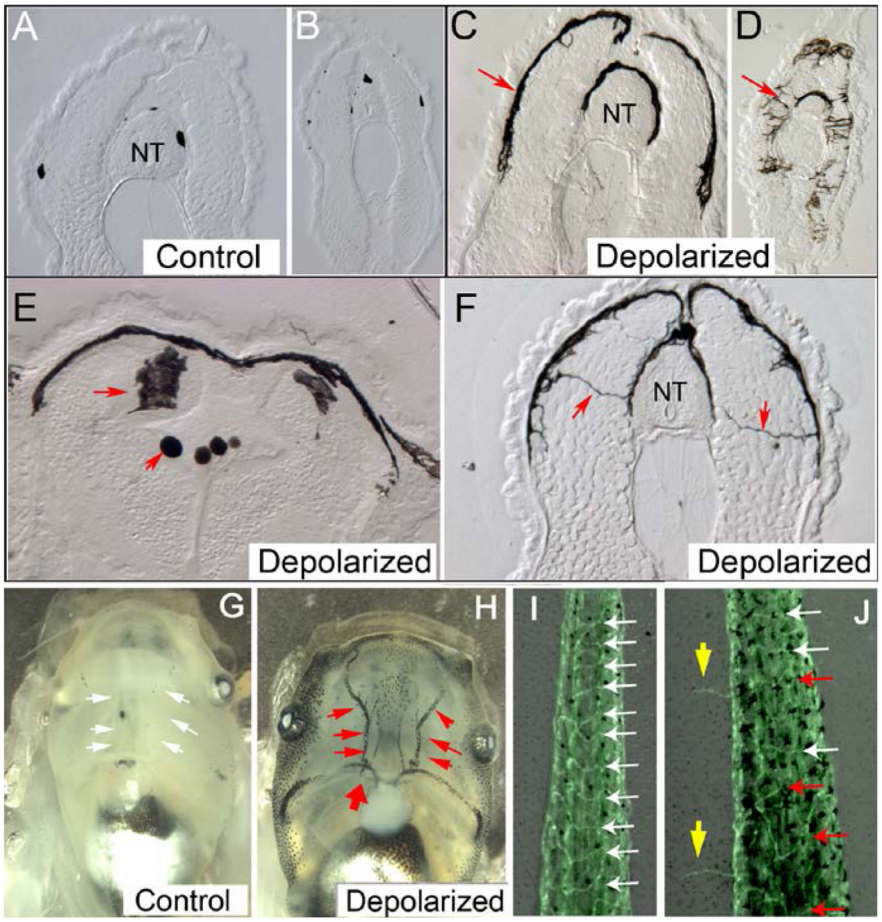

Figure 5. Instructor cell depolarization induces metastatic phenotype

When the GlyR-expressing cells are depolarized, a remarkable phenotype is observed among melanocytes – pigment cell derivatives of the neural crest [60–61]. Panels A–F shows cross sections of tadpoles.

(A, B) in control sections across the anterior and posterior trunk, small numbers of normal round melanocytes are observed (nt = neural tube). In contrast (C,D), animals in which the instructor cells have been depolarized show high numbers of highly arborized melanocytes. In fact these melanocytes not only become much more dendritic and overproliferate, but also thoroughly invade soft body tissues such as the neural tube and its lumen (E, red arrows) and form long nerve-like projections across the entire somatic mesoderm (F). These cells preferentially target the blood vessels (H, red arrows, compares to G).

Not only melanocytes are affected: blood vessels (visualized in a transgenic animal in which all flk1-positive cells express GFP) also lose their normal patterned organization and grow ectopically (blue arrows, compare J to I) [281]. When depolarized using any method (whether relying on chloride or another ion), the instructor cells activate a metastatic-like program of behavior in several target cell types, without the involvement of genetic damage or the presence of canonical oncogenes.

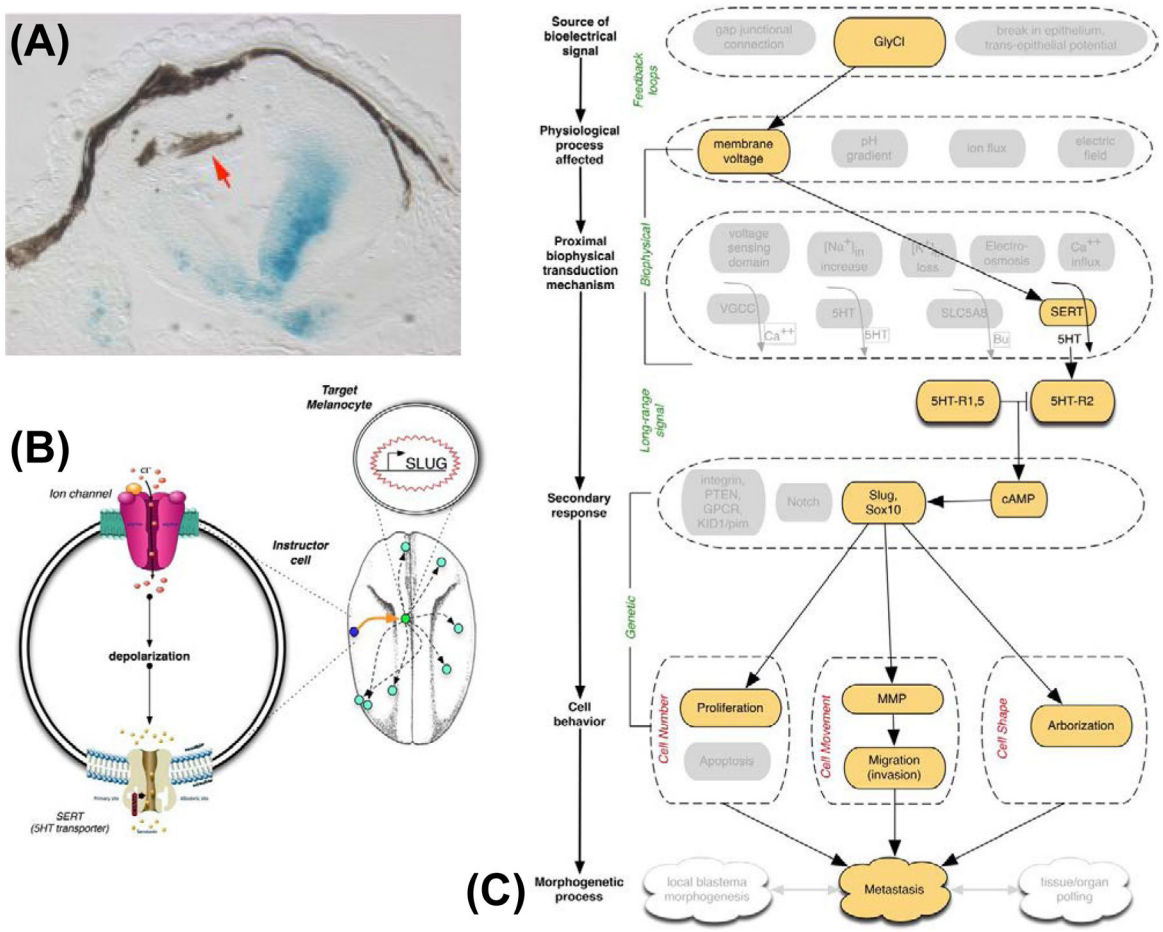

Figure 6. Instructor cells manipulate cell behavior using serotonin

(A) The cells that overproliferate, change shape, and migrate inappropriately (brown melanocytes, red arrow) are not the same cells that are depolarized (blue lineage label, showing the location of a depolarizing channel's misexpression in this section of a tadpole) [60,61]. Indeed, the effect takes place at considerable distance and recent studies showed that a very few depolarized cells at one end of a *Xenopus* larva is sufficient to induce the metastatic behavior of melanocytes at the far end of the animal [281].

(B) The non-cell-autonomous transformation of melanocyte behavior is mediated by a non-neural function of the neurotransmitter serotonin, and can be completely rescued by fluoxetine – the blocker of SERT (the 5HT transporter). A current model of these events is that an instructor cell, when depolarized (causing SERT to run backwards instead of performing serotonin reuptake), begins to secrete serotonin. Serotonin itself can induce the same hyperpigmented phenotype, turning on genes like Slug and Sox10.

(C) The induction of metastatic behavior by voltage change forms a paradigm case for understanding bioelectric events in cancer. In this case, all of the key points are known: the endogenous channel regulating V_{mem}, the physiological parameter that is necessary and sufficient for activating the effect (depolarization, no matter which ion species is used), the transduction mechanism that converts biophysical property into movement of a small molecule chemical signal (SERT), the receptor machinery (5HT-R1,2,5 and cAMP) and key

transcriptional downstream responses, and the cell behaviors regulated by these downstream events.

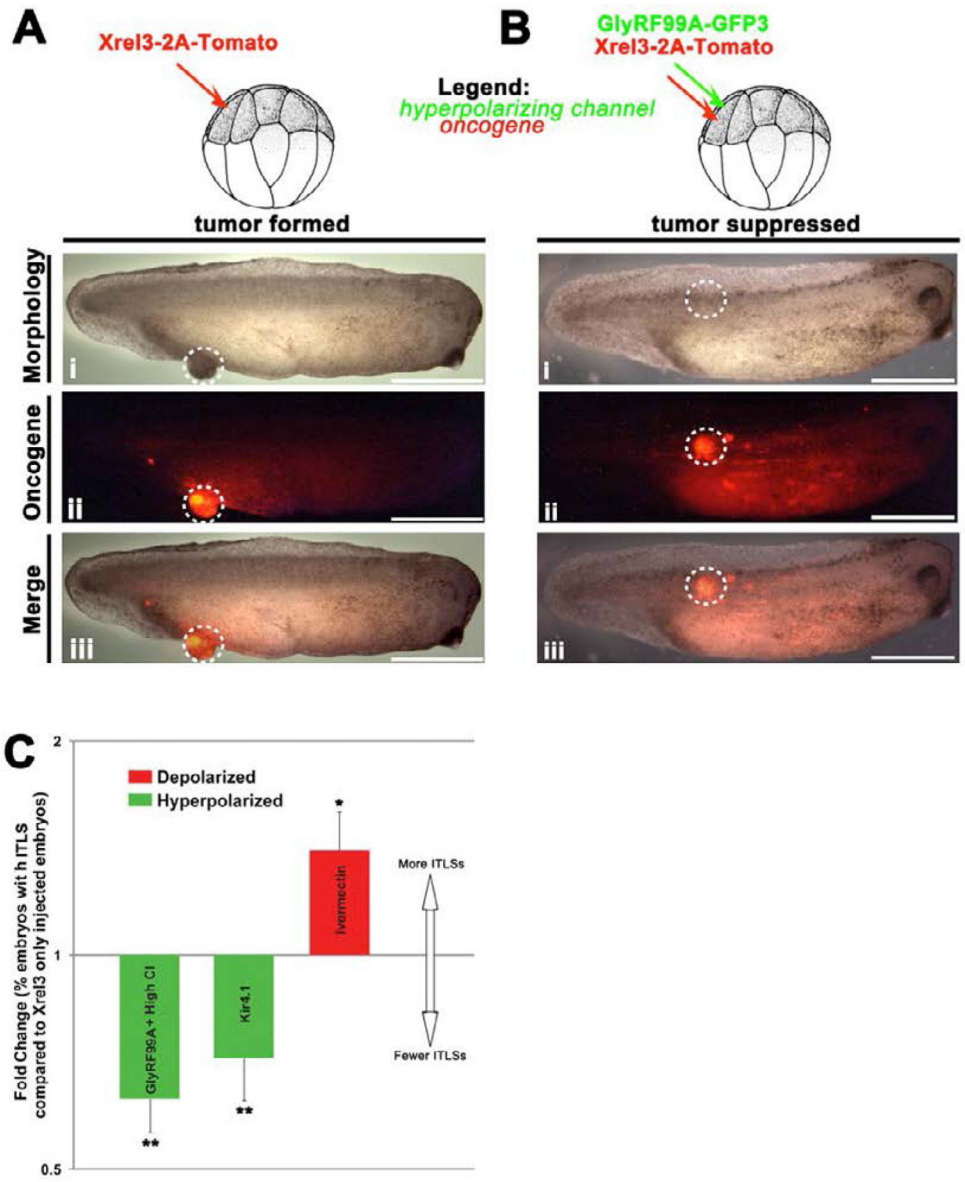

Figure 7. V_{mem} as a regulator of oncogene-mediated tumorigenesis

(A) When an oncogene (Xrel3) fused with a red fluorescent tag (tdTomato) is injected into a frog embryo, the larvae develop fluorescently-labeled tumors. Remarkably, when a hyperpolarizing channel mRNA is co-injected with the oncogene (B), the incidence of tumors is significantly reduced (C). The ability of a hyperpolarized state to suppress tumorigenesis despite the strong presence of oncogene protein (dashed circle in panel B-ii) reveals the functional importance of the depolarized state acquired by prospective tumor cells, and shows that at least in some contexts, cancer can be suppressed by physiological signals despite the presence of a genetic component normally sufficient to induce a tumor [93].

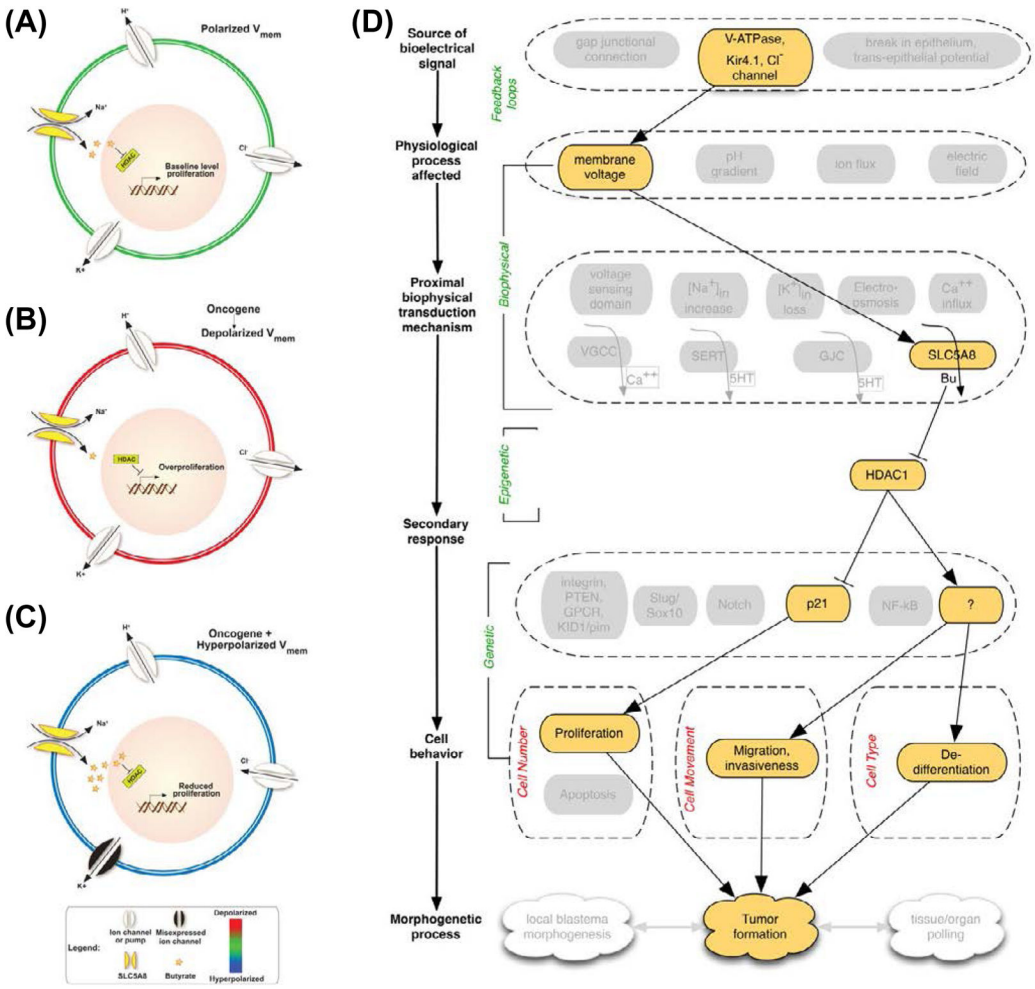

Figure 8. SLC5A8, Butyrate, and HDAC mediate tumor suppression by hyperpolarization

The current model of how V_{mem} regulates ability of cells to form tumors is as follows [93]. (A) In unperturbed embryos, polarized V_{mem} is generated and maintained by several ion channels and pumps present in the plasma membrane; this condition allows moderate amounts of butyrate to influx through SLC5A8 and inhibits histone deacetylases (HDACs). This epigenetically regulates transcription machinery thereby maintaining baseline level proliferation and differentiation compatible with normal somatic morphostasis.

(B) Expression of oncogenes, or other physiological events (e.g., non-genetically induced depolarization, as described in [60–61]), results in the inability of SLC5A8 to import butyrate. Higher HDAC activity then leads to overproliferation and other neoplastic changes leading to appearance of tumor structures.

(C) The effect can be blocked by forced hyperpolarization via molecular and/or pharmacological targeting of H^+, K^+, or Cl^- ion translocators. Forced hyperpolarization of the overall transmembrane potential efficiently powers the uptake of Na^+ through SLC5A8. This energetically favorable intake of Na^+ drives the inward flux of butyrate through SLC5A8. High levels of butyrate continually block HDAC, which leads to hyperacetylation of important genes resulting in cell cycle arrest and suppression of tumor formation.

(D) The bioelectric pathway is similar to that of the depolarization-induced metastasis (Figure 7C), except that the transduction mechanism involves SLC5A8 and butyrate (instead of SERT and serotonin), and initially regulates a chromatin modification enzyme (HDAC) upstream of transcriptional changes that lead to tumor formation.

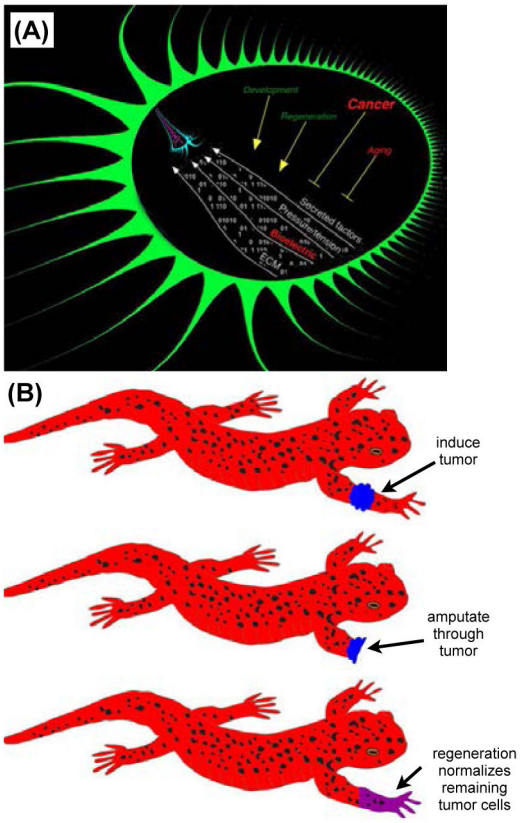

Figure 9. Cancer as a disease of geometry

(A) Within any organism, substructures are provided with a field of instructive information that continually orchestrates individual cell activities into large-scale anatomical target morphologies [14,15]. These signals are mediated by gradients of chemical factors, pressures/tensions, extracellular matrix components, and bioelectrical events. This morphogenetic field operates during embryogenesis and regenerative repair, as well as maintains the organism for decades against aging, and disorders of cell: field interaction manifest as cancer.

(B) When half of a tumor is removed during a limb amputation in regenerative organisms such as salamanders, remaining tumor tissue is normalized and participates in the formation of a healthy limb [397,398,400]. The ability of embryonic and regenerative contexts to reprogram cancer cells towards correct anatomy reinforces the idea that cancer is a disease of organization and cell:cell communication, and suggests normalization strategies as alternatives to biomedical paradigm of mandatory killing of permanently damaged (malignant) cells.

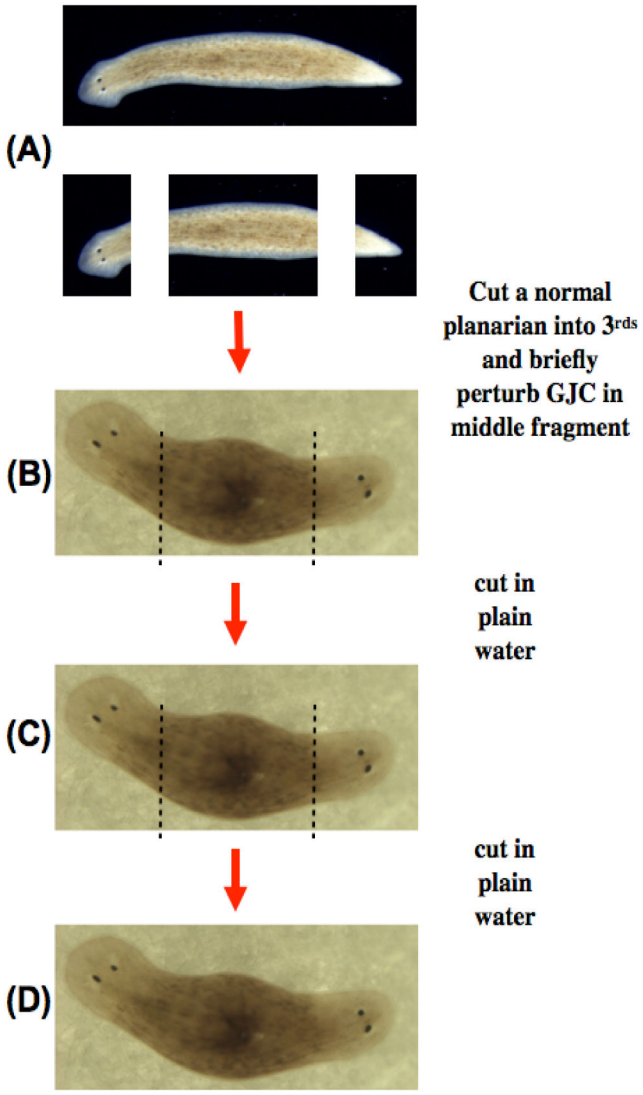

Figure 10. Bioelectric control of growth and form overrides genetic information

The interplay of physiological and genetic information represents a key area for future research in the cancer field, as well as developmental and regenerative biology. Recent work on the bioelectric control of stem cell-mediated regeneration in planaria illustrates one example in which information stored in bioelectrical states dominates the genetically-encoded tissue pattern [113,115].

(A) An intact worm is cut into 3 pieces, and the middle fragment is subjected to transient pharmacological gap junctional inhibition [112,114]. This isolation of the wound cells from long-range signals throughout the fragment result in a 2-headed worm (B). Remarkably, when these worms are cut again (C) and again (D), without any further manipulation, the 2-headed phenotype persists (indefinitely). The ability of a brief perturbation of physiological signaling to permanently change the target morphology (the shape to which the animal regenerates upon damage) *despite* an undamaged genome hints at the power of bioelectrically-encoded signals to regulate cell behavior toward specific tissue outcomes. Note that this target morphology is specified non-locally and cannot be explained by a

simple epigenetic modification of wound tissue, because the tail cells that would have been epigenetically reprogrammed into head identity are *removed* on each round of cutting, and it is the normally-differentiated central trunk cells that must know to generate an ectopic head at each wound site. Such distributed storage of target morphology in the real-time bioelectrical network present throughout the organism may explain the classical data on detection of tumors by electrical readings taken far away from the actual site of the cancer, and serves to focus attention on events outside of the immediate *micro*environment of a neoplastic lesion.

Table 1

Ion translocators implicated in cancer.

Ion channel/pump Protein	Species	Reference
NaV1.5 sodium channel	Human	253,499
EAG-1 potassium channel	Human	196
KCNK9 potassium channel	Mouse	194
Ductin (proton V-ATPase subunit)	Mouse	495
SLC5A8 sodium/butyrate transporter	Human	496
KCNE2 potassium channel	Mouse	497
KCNQ1 potassium channel	Human	214,215
SCN5A sodium channel	Human	498
Metabotropic glutamate receptor	Mouse, Human	236,485,499