

## Review Article

# A Step Beyond pH: Electrochemical Network Deregulation in Cancer, Mechanisms, Consequences, and Therapeutic Implications: A Narrative Review

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Cancer has long been viewed through the lenses of genetic mutations, epigenetic reprogramming, and dysregulated kinase cascades. Yet, an underappreciated dimension of oncogenesis lies in the subversion of cellular electrochemical networks, intricate systems of ion channels, pumps, exchangers, transporters, and gap junctions that maintain membrane potentials, intracellular pH, and bioelectric gradients essential for cellular identity and tissue architecture. These networks, evolutionarily conserved from single-celled organisms to complex metazoans, dictate fundamental processes like proliferation, differentiation, migration, and programmed cell death. In cancer, their deregulation transforms physiological bioelectric signals into pathological drivers, enabling tumor initiation, progression, survival, and resistance to programmed death.

At the molecular level, this dysregulation manifests through diverse mechanisms. Voltage-gated potassium channels (e.g. KV10.1/Eag1 and KV11.1/hERG), frequently over-expressed in solid tumors, hyperpolarize the plasma membrane, stabilizing resting potentials that suppress apoptosis and enhance survival under stress. Concurrently, calcium-permeable channels like TRP family members unleash oscillatory  $\text{Ca}^{2+}$  waves that activate pro-invasive pathways, including calpain-mediated cytoskeletal remodeling and NF- $\kappa$ B-driven inflammation. Sodium-hydrogen exchangers (e.g. NHE1) acidify the extracellular tumor milieu, fostering matrix degradation and immune suppression, and increase intracellular pH favoring proliferation and survival, while  $\text{Na}^+/\text{K}^+$ -ATPase dysfunction disrupts redox homeostasis, amplifying reactive oxygen species (ROS) that fuel genomic instability. Gap

junctional intercellular communication mediated by connexins such as Cx43, often collapses in early carcinogenesis, isolating neoplastic cells from suppressive cues and promoting autonomous growth. The consequences ripple across scales. Intracellularly, electrochemical shifts reprogram metabolism toward glycolysis (Warburg effect or aerobic glycolysis), sustaining hypoxia-adapted proliferation. At the tissue level, bioelectric gradients guide collective cell behaviors: depolarized tumor fronts orchestrate angiogenesis via VEGF up-regulation, while synchronized depolarization waves propel epithelial-to-mesenchymal transitions (EMT). Systemically, these networks influence metastatic niches, where cancer-associated fibroblasts and immune cells co-opt shared ion fluxes to erect protective barriers. Clinically, such deregulation correlates with poor prognosis, hERG expression portends aggressive gliomas, and TRPC1 variants heighten breast cancer recurrence, underscoring its biomarker potential.

Therapeutically, this electrochemical vulnerability offers novel paradigms beyond conventional targeted therapies. Small-molecules like ivermectin (Kir3 activator) and carvedilol (hERG modulator) have demonstrated anti-proliferative synergy with chemotherapeutics in xenografts. Optogenetic tools and bioelectric scaffolds are reemerging from developmental biology, enabling precise reprogramming of tumor potentials in vivo. Moreover, nanoparticle-delivered ionophores and proton pump inhibitors exploit pH gradients for selective cytotoxicity. By integrating electrochemical profiling into precision oncology, via patch-clamp electrophysiology or voltage-sensitive dyes, we can unmask "electric fingerprints" of tumors, guiding combination regimens that restore homeostasis and thwart adaptation.

This review discusses cutting-edge insights into the mechanisms of electrochemical network deregulation, their multifaceted consequences on cancer hallmarks, and translational implications for innovative therapies. In an era of immunotherapy plateau and resistance crises, recalibrating cancer's bioelectric code may unlock lasting cures.

This manuscript is not based on experiments performed by the author but on putting together already available information and interpreting these tidbits of knowledge in a major frame of systems biology.

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## Introduction

The investigation into the pH of tumors likely began with Otto Warburg's seminal research in the 1920s, which identified the acidic nature of tumors. At that time it was not possible to distinguish between intracellular and extracellular pH [1]. Furthermore, it was not known that intracellular and extracellular pH had different values [2][3]. At the point, low oxygen and acidic microenvironments were mainly seen as consequences of abnormal metabolism and not as primary pathogenetic signals. Membrane exchangers and electrolyte channels were also unknown fundamental regulatory mechanisms. Knowledge of the compartmentalized nature of pH in cell biology started to develop in the 1970s.

A brief history of scientific progress in this area begins in 1975 with Leopoldo Anghileri [4] who found that inducing metabolic acidosis with ammonium chloride decreased the growth of many experimental tumors. The mechanism involved was not clarified, however, the author wrote that "*the observed tumor inhibition appears to be related to a systemic impairment of the anabolic mechanisms*". Soon after, in 1980, Harguindey and Gillis initially proposed that proton alterations in the cancer environment were related to carcinogenesis and that active interference with these alterations could prevent/modify carcinogenesis [5].

It took some time, until the 1980s–2000s, for microelectrode and imaging studies to show that the extracellular pH (pHe) of solid tumors is consistently acidic ( $\approx 6.5$ – $6.9$ ), whereas normal tissue pHe is  $\approx 7.2$ – $7.4$ . [6]

The studies by Albers et al. [7] in 1981, were the first to show that there was an inversion of the pH gradient in malignant tumors. They wrote: "*pH<sub>i</sub> exceeded pH<sub>e</sub> if either P<sub>CO2</sub> and/or the concentration of lactic acid were raised above a critical level*". However, the authors expressed doubts about their own results. This publication did bring three new concepts to light:

- the pH gradient inversion in cancer;
- that lactic acid was not the only cause of acidity, with CO<sub>2</sub> being equally important;
- that pH<sub>i</sub> and pHe are different. Table 1.

| Table 1: pH of       | NORMAL CELL                                                                       | CANCER CELL                                                                         |
|----------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| EXTRACELLULAR MATRIX | 7.3 -7.4                                                                          | 6.5-6.8                                                                             |
| CYTOPLASM            | 7.1-7.2                                                                           | 7.4-7.8                                                                             |
| pH GRADIENT          |  |  |

Table 1.

A follow up was that in 1987, Rotin et al. [8] showed that manipulation of intracellular acidity by “pushing” it towards acidosis had a cytotoxic effect. In 2000, Reshkin et al. demonstrated that cytoplasmic alkalization is a primary event in cellular transformation, a process inhibited by amiloride, an NHE1 inhibitor. Furthermore, amiloride used at this very early stage of transformation was able to block malignization [9].

This was the starting point.

A so-called “pH-centric cancer model” has been developing over the last 45 years. The fundamentals of this model consists of cancer cells inverting the normal pH gradient; they become intracellularly alkaline and extracellularly acidic, and this inversion drives proliferation, invasion, metastasis, and therapy resistance rather than being a passive consequence. This inversion is created by metabolic rewiring (Warburg effect) plus overactive proton-export systems such as  $\text{Na}^+/\text{H}^+$  exchanger isoform 1 (NHE1), monocarboxylate transporters (MCTs), V-ATPase, sodium-bicarbonate cotransporter1 (NBC1) and carbonic anhydrases. We have coined the term pHtome for the set of participants in the pH gradient reversal process. The pHtome is frequently over-expressed and/or over-active in most cancers, and especially in advanced tumors.

It was the deepening into the pH conundrum that led us to consider that pH is only one aspect of a multi-prong phenomenon, such as the electrochemical network (ECN). For the sake of clarity we shall study the ECN through different sections. However, this network is a highly intertwined, interdependent, and dynamic process of apparently diverse cell phenomena such as metabolism, membrane electrolyte channels, transporters and exchangers, membrane enzymes, intracellular buffers, and antioxidant

systems. While there is no malignization this ECN works in a certain manner but when cancer enters the scene, ECN works in a different way we can call ECN deregulation.

To fully understand what is discussed here, let's suppose that the ECN deregulation is the script of a play, where there are actors (electrolyte channels, metabolism, buffers, and others) that “do things”. As a consequence of what they do, there are immediate effects, that are usually measurable, and mediate effects that drive cancer hallmarks. What we shall try to achieve in this paper is the knowledge of the script. With this objective in mind, we shall discuss first about the actors, what they do, and the immediate effects of their actions on the stage. Fig1.

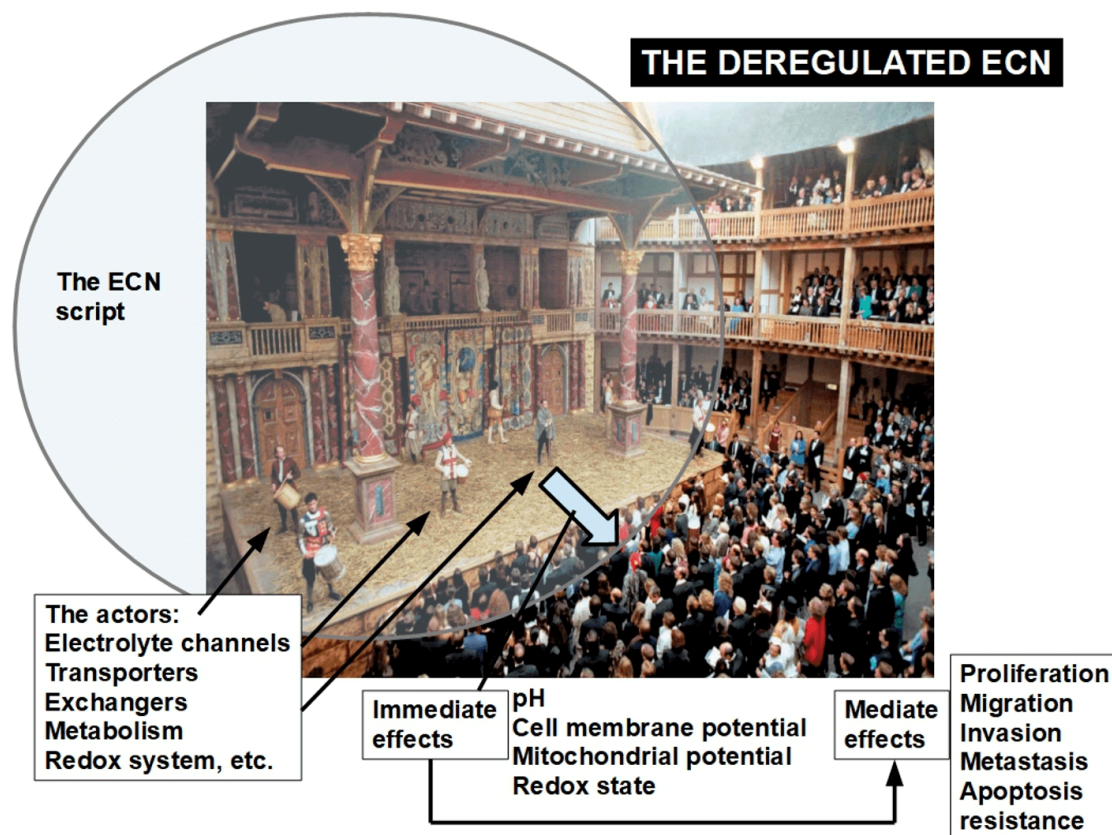


**Figure 1.** A Shakespearian representation of the ECN. The script (ECN) determines what the actors will do and how they will interact among them. The public receives the immediate effects of the play. When they leave the theater they develop the mediate effects, in this case the hallmarks of cancer.

Bioelectricity in cells primarily refers to the resting membrane potential, an electrical voltage difference across the plasma membrane (typically  $-40$  to  $-90$  mV, inside negative relative to outside). This is foundational for nerve impulses, muscle contraction, cell signaling, development, and even wound

healing. However, this bioelectricity is also related to mitochondrial membrane potential, the intracellular pH, and the redox homeostasis. Unfortunately, most of the texts on this issue have a limited view to cell membrane potential, without pondering how the network in reality works.

Bioelectricity is established and maintained by two main processes:

1. **Active transport** (energy-consuming pumps create concentration gradients).
2. **Passive diffusion** through ion channels (selective permeability turns gradients into voltage).

The primary “battery charger” is the sodium-potassium ATPase channel. This battery uses ATP to pump 3 Na<sup>+</sup> ions out of the cell and 2 K<sup>+</sup> ions in. This results in high [K<sup>+</sup>] inside (~140 mM) vs. outside (~4 mM), and high [Na<sup>+</sup>] outside (~145 mM) vs. inside (~12 mM). Its main role is building concentration gradients. Compartmentalization determined by the bilayer lipid membrane is the key player that allows building different concentration gradients.

Other important players in this process are the potassium leak channels. The membrane is much more permeable to K<sup>+</sup> than Na<sup>+</sup> at rest (due to open K<sup>+</sup> leak channels). So that K<sup>+</sup> diffuses down its concentration gradient out of the cell. As positive K<sup>+</sup> leaves, the cell interior becomes negatively charged (due to leftover anions like proteins, Cl<sup>-</sup>, and organic phosphates that can't easily cross the membrane). This negative charge creates an electrical gradient that pulls K<sup>+</sup> back in. Equilibrium is reached when the chemical driving force outward equals the electrical force inward.

In summary:

1. Pump creates steep gradients (high K<sup>+</sup> in, high Na<sup>+</sup> out).
2. K<sup>+</sup> leaks out leading to negative inside.
3. Negative interior attracts K<sup>+</sup> back and repels Na<sup>+</sup>.
4. Steady state: small ongoing pump activity counters the slow leaks.

Importantly, in non-excitabile cells membrane potential influences proliferation, differentiation, migration, and tissue patterning (e.g., during embryonic development or regeneration).

After all these preliminary concepts discussed, we shall study the ECN, approaching it through its immediate effects, rather than the actors. This will be divided into four sections that will be integrated at the end.

- **Section 1:** the pH gradient inversion.
- **Section 2:** The redox balance

- **Section 3:** The cell membrane potential
- **Section 4:** The mitochondrial membrane potential

## Section 1. Mechanistic Summary of the pH-Centric Cancer Model

We decided to initiate this journey with the altered pH in cancer cells, because this was the starting point that led us to investigate the rest of the system in which this cancer hallmark is embedded, that is the ECN.

The following is a concise outline of the mechanisms of the pH-centric cancer model.

### 1.1. The Foundational Observation: pH Gradient Inversion

| Normal cells:                 | Cancer cells:                   |
|-------------------------------|---------------------------------|
| pHi ≈ 7.2 (slightly acidic)   | pHi becomes alkaline (~7.4-7.6) |
| pHe ≈ 7.4 (slightly alkaline) | pHe becomes acidic (~6.5-6.9)   |

**This inversion is now considered a hallmark of malignancy.**

Importantly, the creation of an increased alkaline cytoplasm precedes any metabolic shift, because it is a very early event of transformation <sup>[9]</sup>. It takes place even before proliferation starts.

### 1.2. Upstream Drivers of pH Inversion

#### 1.2.1. Metabolic Rewiring (Warburg Effect)

Cancer cells shift to a predominantly aerobic glycolysis, producing large amounts of lactate + protons. This does not mean that the cancer cell fully abandons oxidative phosphorylation, because both metabolic pathways coexist as a tunable metabolic continuum rather than being mutually exclusive pathways. This has been clearly shown by Sidney Weinhouse in the 1950s <sup>[10][11][12][13][14][15]</sup>.

- Proton accumulation would be lethal if retained.
- Therefore, cancer cells must export protons aggressively.

- Increased intracellular pH selectively activates glycolysis and the pentose phosphate pathway through key metabolic enzymes such as HK1, PKM2, G6PDH <sup>[16]</sup>. The result is increased nucleotide levels, NADPH/NADP<sup>+</sup> ratio, and cell proliferation.
- Increased production of CO<sub>2</sub> participates in pH acidosis through diffusion and hydration at the cell membrane via the activity of membrane carbonic anhydrases IX and XII. Figure 2.

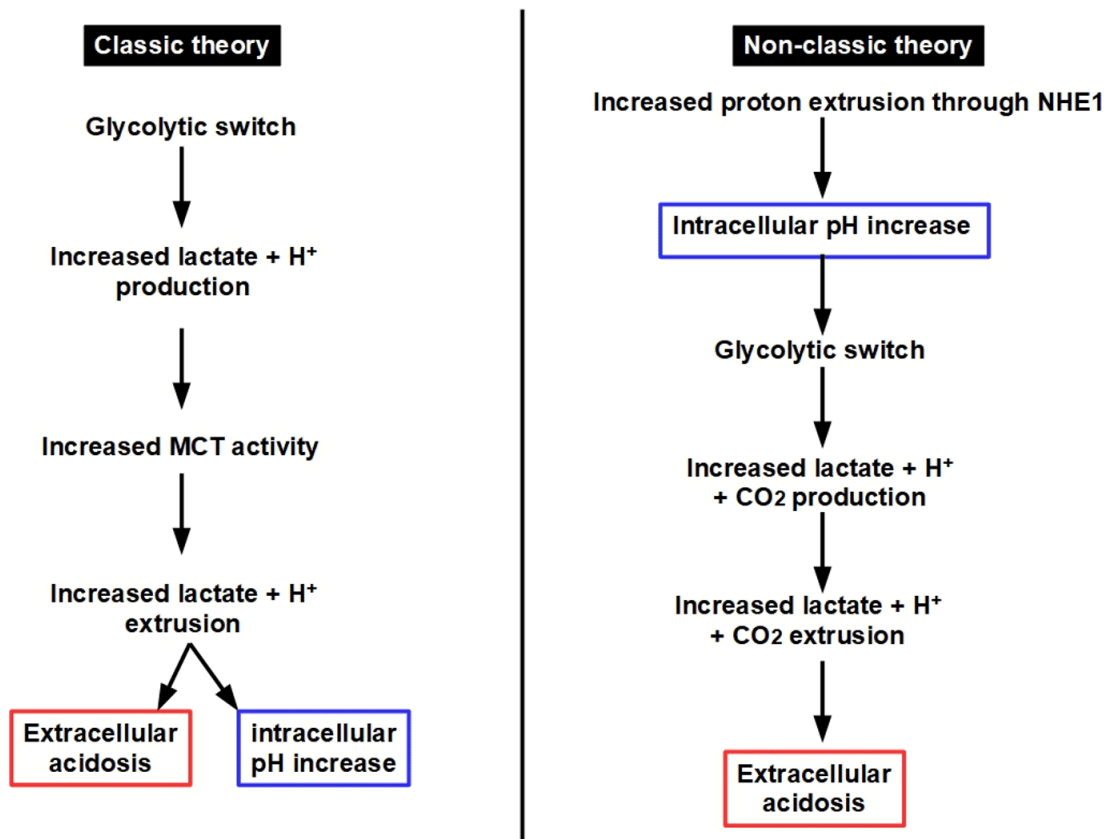


**Figure 2.** Almost all the publications consider intracellular pH modifications in cancer as a consequence of the Warburg effect. On the other hand, we believe that intracellular alkalization, that precedes the Warburg effect, is the cause of the predominantly glycolytic metabolism. That is, the Warburg effect is not the cause of intracellular increased pH, but rather the consequence. Extruding lactate plus its cognate proton does not explain the reason for increased intracellular pH. The conceptual difference may be important regarding eventual treatments. Man et al. <sup>[16]</sup> have shown that “Simply increasing the lactate/proton symporter monocarboxylate transporter 4 (MCT4) or the sodium–proton antiporter NHE1 was sufficient to increase intracellular pH and give normal hematopoietic cells a significant competitive growth advantage in vivo.” Further they say: “Intracellular alkalization is a primitive mechanism by which proton partitioning can directly reprogram carbon metabolism for cell growth”. Other references that support this figure are <sup>[17]</sup>.

Furthermore, MCT's lactate and proton transport does not consumes energy, and therefore, it is gradient dependent. This means that the concentration of lactate + proton should be higher inside than outside the cell. The meaning of this is that the Warburg effect does not alkalize the cytoplasm, on the contrary it acidifies it. The classic theory shown in Figure 2 is invalid according to this elementary reasoning. Another important issue is that monocarboxylate transporters (MCTs) have a finite, saturable transport capacity characterized by classic Michaelis–Menten kinetics ( $K_m$  and  $V_{max}$ ). They are not infinite proton + lactate conduits; they saturate at high substrate concentrations and their maximal flux depends on isoform, pH gradient, and CD147 chaperoning <sup>[18]</sup>. When the amount of lactate and proton concentration in the extracellular milieu achieves a certain threshold level, MCTs simply stop working and lead to increasing intracellular acidity <sup>[19]</sup>. This further confirms that the Warburg effect is not the cause of increased intracellular pH. In conclusion, the Warburg effect contributes to extracellular acidity but not to intracellular alkalinity.

### 1.2.2. Hypoxia and Perfusion Defects <sup>[20]</sup>

- Poor vascular clearance leads to proton trapping in the extracellular matrix.
- Hypoxia (through the HIF transcription factors) further enhances glycolysis, expression of membrane-bound carbonic anhydrases, and acid production.

## 1.3. The Proton-Export Machinery (Core Mechanistic Module of the pHtome)

Cancer cells over-express multiple and sometimes overlapping proton-extrusion systems:

### 1.3.1. NHE1 ( $Na^+/H^+$ Exchanger 1) <sup>[21][22][23]</sup>

- Primary driver of intracellular alkalization.
- Over-expressed at invadopodia, enabling invasion.
- Blocking NHE1 can prevent malignant transformation <sup>[9]</sup>.

### 1.3.2. MCT1 / MCT4 (Monocarboxylate Transporters) <sup>[24][25]</sup>

- Export lactate +  $H^+$  from glycolysis.
- Create an acidic peritumoral zone.
- However, according to our criteria, they are not involved in increased intracellular pH.

### 1.3.3. V-ATPase Proton Pumps [26][27].

- Pump H<sup>+</sup> into extracellular space and intracellular vesicles.
- Promote matrix degradation and invasion.
- Promote metastasis [28].
- Promote anoikis resistance [29].

### 1.3.4. Membrane Carbonic Anhydrases (CAIX, CAXII)

- Reversibly convert CO<sub>2</sub> into HCO<sub>3</sub><sup>-</sup> + H<sup>+</sup>
- Support both intracellular alkalinity and extracellular acidity [30][31][32]. The mechanism for intracellular alkalinity is different from that of extracellular acidity. This last one is the result of carbonic acid production that dissociates into bicarbonate and proton. On the other hand, intracellular alkalinity is the consequence of the tandem work between CAs and NBC1 that reintroduces bicarbonate into the cell.

### 1.3.5. Voltage gated sodium channels (VGSCs)

- Although not part of the pHtome, because VGSCs do not export protons, they can induce NHE1 activity (the main cause of intracellular alkalinity) by lowering the threshold for its action [33][34].

## 1.4. Downstream Consequences of pH Inversion

### 1.4.1. Intracellular Alkalinity (pHi ↑)

- Stimulates **DNA synthesis, cyclin activation, and proliferation.**
- Inhibits apoptosis (acidification is required for caspase activation [35]). Acidification is a *permissive signal* for caspase 1, 4, and 11. Acidic pH increases the catalytic activity of caspase 3, 7, 8, and 9, while caspase 2, 6, and 14 are pH-independent.

|                                                                                                                                                                                                                                                               |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>If pHi drops (intracellular acidification):</b></p> <p><b>Caspase-1,-4,-5,-11</b> activate strongly</p> <p><b>Caspase-3,-7,-8,-9</b> activation is moderately enhanced</p> <p>Inflammasome assembly increases</p> <p>Apoptosome formation increases</p> |
| <p><b>If pHi remains alkaline (NHE1 active):</b></p> <p><b>Caspase-1/4/5/11</b> are suppressed</p> <p><b>Caspase-3/7/8/9</b> are inefficient</p> <p>Cell avoids apoptosis and pyroptosis</p> <p>Cancer cell survival is favored</p>                           |

#### 1.4.2. Extracellular Acidity (pHe ↓)

- Activates proteases (cathepsins, MMPs).
- Promotes ECM degradation, migration, invasion, and metastasis <sup>[36][37]</sup>.
- Selects for acid-resistant, aggressive clones. Acid-adapted cancer cells are tumor cells that have evolved mechanisms to survive and thrive in the acidic microenvironment typical of solid tumors <sup>[38]</sup>. The cells that adapt to extracellular acidosis show several distinct characteristics and survival mechanisms such as:

**1.4.2.1. Metabolic Rewiring:** Acid-adapted cells shift toward oxidative metabolism, increasing mitochondrial activity and lipid utilization while reducing glycolysis <sup>[39]</sup>. This is regulated by pathways, such as PPAR $\alpha$  signaling, which enhance fatty acid oxidation and mitochondrial function <sup>[40]</sup>.

**1.4.2.2. Aggressiveness and Invasiveness:** Extracellular acidosis selects for more invasive phenotypes, enabling cancer cells to invade surrounding tissues. This is linked to extracellular matrix remodeling and epithelial-mesenchymal transition (EMT) <sup>[41][42][43]</sup>. To this we must add that it has been suggested that extracellular acidosis can have a “vicinity effect” inducing malignization of normal cells<sup>[44]</sup>. There is no experimental confirmation of this issue.

**1.4.2.3. Stem Cell Properties:** Acidic conditions promote cancer stem cell-like traits, enhancing tumor aggressiveness and metastatic potential [45].

**1.4.2.4. Survival Mechanisms:** there are many mechanisms involved, some of them with overlapping functions. Membrane channels, ion exchangers, transporters, and enzymes are in the first line of the survival mechanism employed by AACCs (acid adapted cancer cells) [46].

**Sodium-hydrogen exchanger 1 (NHE1) and 3 (NHE3)** are key figures among ion exchangers safeguarding pH homeostasis. NHE1 is an antiporter spanning through the cell membrane that exports protons ( $H^+$ ) while importing  $Na^+$  [47][48][49]. NHE1 thus alkalinizes the cytoplasm while it acidifies the extracellular matrix playing a role in invasion, migration and metastasis [50][51].

**1.4.2.5. Chronic acidosis induces autophagy and cytoplasmic vacuolization**, helping cells cope with stress [52]. Mikl et al. [53] found that in cancer cells extracellular acidity inhibited mTOR signaling with two consequences: lysosome activation and AE2 degradation. AE2 is the acid-loading anion exchanger 2 (SLC4A2) that imports protons into the cytoplasm. Its lysosomal degradation prevents the acidic matrix from acidifying the cytoplasm

**1.4.2.6. Lipid Uptake:** Acid-adapted cells rapidly accumulate lipids via transporter-independent mechanisms, supporting their metabolic needs [54][55].

These adaptations make acid-adapted cancer cells more resilient and aggressive, presenting challenges for treatment strategies targeting the tumor microenvironment. They also contribute to creating an immunosuppressive environment. Acid-adapted cancer cells create an immunosuppressive environment by suppressing T-cell and NK-cell function, recruiting immunosuppressive myeloid cells, and enhancing Treg activity [56][57][58].

### **1.4.3. Therapy Resistance**

- An acidic microenvironment reduces uptake of weakly basic chemotherapy compounds.
- An alkaline cytosol protects against apoptosis-inducing drugs by activating AMPK-mTORC2-AKT signaling [59].

**In summary:** Cancer cells survive and progress by exporting protons to maintain an alkaline cytosol and acidic microenvironment; this pH gradient inversion fuels proliferation, invasion, metastasis, and resistance. This makes proton-handling systems actionable therapeutic targets [60][61][62][63]. Figure 3.

Recent evidence suggests that while the pH-centric model is broadly applicable to most cancers and likely relevant at specific stages of cancer progression, it is still incomplete. This framework does not account for several critical factors that interact with pH, resulting in a distinct deregulated electrochemical network that promotes various cancer hallmarks. These players are the cancer cell redox state, the cell membrane potential, and the mitochondrial membrane potential. In this paper we discuss the strong interrelation and inter-dependence among the four main players of the electrochemical network. Importantly, we show the place that the peculiar pH of cancer cells and their environment occupies in the electrochemical network.

### A summary of how the proton export machinery increases $pH_i$ and decreases $pH_e$

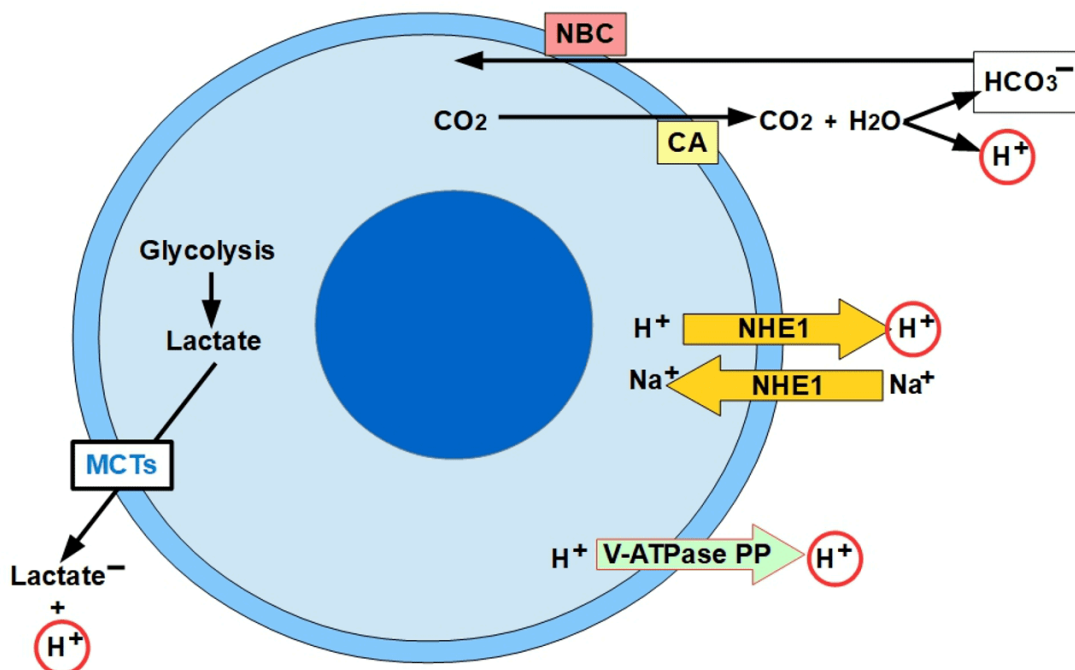


**Figure 3.** Key components involved in the functioning of proton export systems. CA: carbonic anhydrases IX and XII; NHE1: Sodium, hydrogen exchanger 1; MCTs: Monocarboxylate transporters; V-ATPase PP: NBC: Sodium bicarbonate cotransporter; Vacuolar ATPase proton pump.

The objective of this paper is to analyze the relationship between the pH-centric model and the peculiarities of the cancer cell redox system integrating into a wider pH-redox model that includes both

mitochondrial and cell membranes. In cancer cells, pH gradient inversion, redox changes, and altered mitochondrial and plasma membrane potentials are closely linked as elements of a deregulated electrochemical network.

This paper analyzes the relationship of the four manifestations (immediate effects) of the deregulated electrochemical network integrating them into a major frame of systems biology. These four manifestations are:

- A. The inverted pH gradient that has been summarized above;
- B. The peculiar redox state of the cancer cell;
- C. The cell membrane hypopolarization;
- D. The mitochondrial membrane hyperpolarization

These manifestations are summarized in Figure 4. The interrelations among the four are powerful drivers of tumor progression and therefore a possible integral targeting will be suggested as a corollary.

## CAUSES

Somatic mutations in channel-encoding genes  
Copy number variation and gene amplification  
Splice variant switching  
Oncogenic signaling and transcriptional reprogramming  
Microenvironment driven deregulation  
Post-translational modifications  
Epigenetic modifications  
EMT and cancer stem cell-associated channel remodeling  
Chemotherapy-associated deregulation

## DEREGULATION OF ION CHANNELS



## ELECTROCHEMICAL EFFECT

Cell membrane hypopolarization

## CAUSES

Increased activity/increased expression of proton extrusion exchangers, transporters, enzymes, and cotransporters.  
  
Increased proton production in the form of lactate, carbonic acid (hydrated CO<sub>2</sub>) and phosphate from ATP

## DEREGULATION OF NHE1, MCT1, MCT4, CAIX, CAXII, NBC1



## ELECTROCHEMICAL EFFECT

Inverted pH gradient

## CAUSES

Increased ROS production  
Increased anti-oxidants productions  
ENOX2 replaces ENOX1 splice variant

## Eh DEREGULATION



## ELECTROCHEMICAL EFFECT

Altered Eh: mild oxidative stress

## CAUSES

Increased electron-transport chain (ETC) activity → more proton pumping.  
Altered mitochondrial ion transporters.  
Reverse proton-pumping mode of ATP synthase  
Reversed or altered orientation of the adenine nucleotide translocator (ANT).  
Mitochondrial Ca<sup>2+</sup> uptake stimulates TCA cycle and NADH  
Mitochondrial structural remodeling and biogenesis.  
Hypoxia and mechanical stress can further increase  $\Delta\Psi_m$  by altering mitochondrial signaling and metabolic pathways

## MITOCHONDRIAL DEREGULATION



## ELECTROCHEMICAL EFFECT

Mitochondrial membrane hyperpolarization

Cell membrane hypopolarization  
pH gradient inversion  
Eh deregulation  
Mitochondrial membrane hyperpolarization

## TUMOR BEHAVIOR

Proliferation, migration, invasion, metastasis, resistance to apoptosis



**Figure 4.** The four manifestations of the electrochemical network deregulation of the cancer cell.

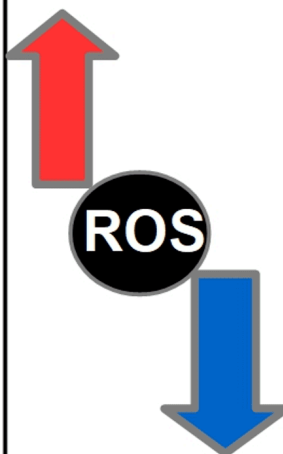
## Section 2. The redox state of a cell

The redox state of a cell (often quantified as the reduction potential ( $E_h$ ) of major couples like  $\text{NAD}^+/\text{NADH}$  or  $\text{GSSG}/2\text{GSH}$ ) represents the potential of oxidation vs. reduction potential.  $E_h$  effectively measures electron activity showing the drive to move from a reducing agent to an oxidizing agent. Redox state and intracellular (cytosolic) pH are bidirectionally coupled through thermodynamic principles, proton-coupled electron transfer (PCET) reactions, and physiological feedback mechanisms. Changes in one, directly influence the other. This interdependence is critical for metabolism, stress responses, signaling, and processes such as the cell cycle. Dynamic changes in redox status and pH are interdependent processes, which is sometimes overlooked in cellular studies [64]. So that our first step beyond pH will be understanding how pH and  $E_h$  are related.

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, and reactive nitrogen species (RNS) such as peroxynitrate, play a dual role in cancer [65][66][67][68][69]. At low to moderate levels, they act as signaling molecules that favor cancer progression by promoting multiple hallmarks of cancer [70]. Cancer cells often maintain elevated but tightly controlled ROS levels through increased production (e.g. via mitochondrial metabolism, and NADPH oxidases) and enhanced antioxidant defenses, allowing pro-tumorigenic effects without triggering cell death [71]. The mechanisms of these pro-tumorigenic effects are described below. Figures 4 and 5.

## Cancer Cells Maintain Elevated but Controlled ROS Levels by:

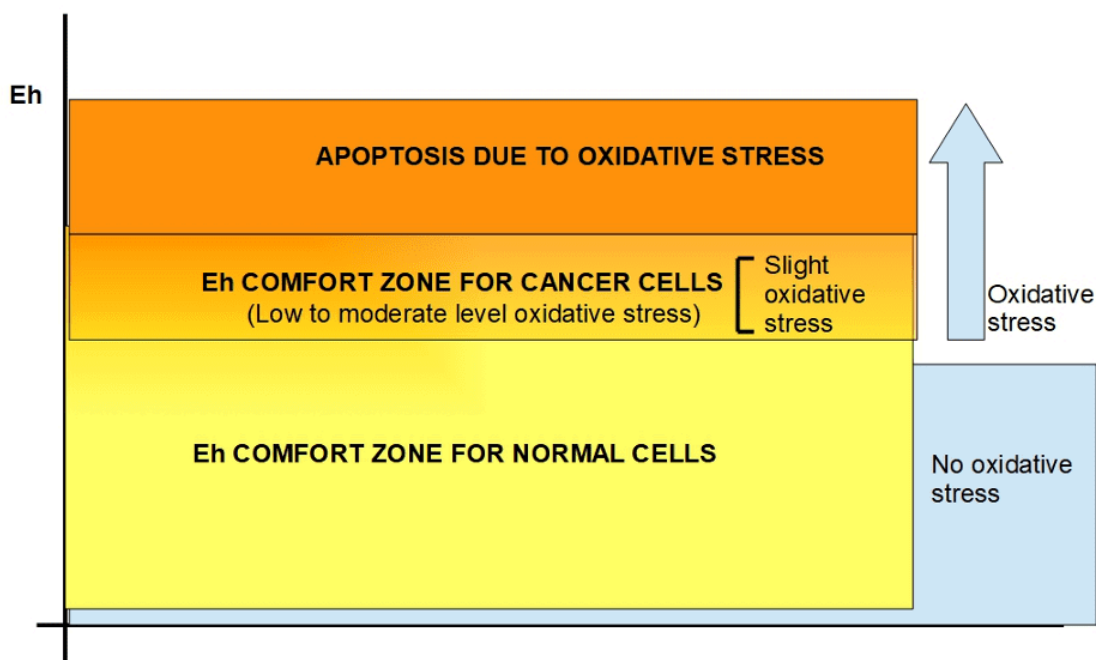
1. Increasing ROS production  
 - Mitochondrial metabolism (ETC complexes I–III) is a major ROS source.  
 Cancer cells often have mutations or metabolic rewiring that increase mitochondrial ROS (mROS).  
 - NADPH oxidases (NOX family) generate superoxide at the plasma membrane and are frequently up-regulated in tumors.  
 This contributes to redox-sensitive signaling pathways that promote growth and invasion.



2. Enhancing antioxidant defenses to prevent ROS from reaching lethal levels, cancer cells upregulate:  
 - Glutathione (GSH) system  
 - Thioredoxin (Trx) system  
 - Superoxide dismutases (SOD1/2)  
 - NADPH-generating pathways (PPP, malic enzyme, IDH1/2, folate cycle).  
 These systems maintain redox homeostasis and buffer ROS-driven signaling.  
 NRF2 in cancer cells acts as a master regulator of oxidative-stress defenses by permanent activation through - KEAP1 loss-of-function mutations or - NFE2L2 gain-of-function mutations.

## Cancer Cells Adapt to Low to Moderate Levels of Chronic Oxidative Stress

**Figure 5.** Increased but controlled ROS levels in cancer cells. Based on references [\[72\]\[73\]\[74\]\[75\]\[76\]\[77\]\[78\]\[79\]\[80\]\[81\]\[82\]\[83\]](#).



#### How ROS shifts Eh (redox potential)

##### 1. ROS oxidize redox-active thiols → Eh becomes more positive

ROS such as  $\text{H}_2\text{O}_2$  and superoxide oxidize cysteine thiolates ( $\text{Cys-S}^-$ ) to sulfenic acids or disulfides. This shifts the glutathione and thioredoxin redox couples toward their oxidized forms, which raises (makes more positive) the redox potential Eh.

- ROS oxidize cysteine residues as part of redox signaling.
- These oxidative modifications shift redox couples toward oxidized states.

##### 2. ROS increase the oxidized/ reduced ratio of major redox couples

ROS increase:

- GSSG/GSH ratio (more oxidized glutathione)
- $\text{NAD}^+/\text{NADH}$  ratio
- Oxidized thioredoxin / reduced thioredoxin

All of these changes increase Eh, because Eh is defined by the Nernst equation, where a higher oxidized/reduced ratio yields a more positive redox potential.

##### 3. ROS shift redox microdomains toward oxidation

ROS generation is compartmentalized — mitochondria, peroxisomes, and other organelles have distinct redox environments. When ROS rise in a compartment, the local Eh becomes more positive, reflecting a more oxidizing environment.

**Figure 6.** The comfort zone for cancer cells is a moderately oxidative stress intracellular milieu <sup>[84]</sup>. Cancer cells maintain a “redox comfort zone” in which the intracellular redox potential (Eh) is kept *mildly reducing*, enough to prevent ROS-induced death, but not so reducing that proliferation-linked ROS signaling is lost. This zone is maintained through antioxidant up-regulation and metabolic rewiring <sup>[85][86][87]</sup>.

## *2.1. Pro-tumoral effects of a moderate oxidative cytoplasm*

### *2.1.1. Induction of Genomic Instability and Mutations*

Moderate ROS cause oxidative damage to DNA (e.g. 8-oxoguanine formation, strand breaks, base modifications). This leads to mutations, genomic instability, and activation of oncogenes (e.g. KRAS) while inactivating tumor suppressors (e.g. p53). These genetic alterations drive malignant transformation and tumor initiation/progression <sup>[88][89]</sup>.

### *2.1.2. Activation of Proliferative and Survival Signaling Pathways*

ROS function as second messengers that oxidize key proteins (e.g. inhibiting phosphatases like PTEN or PTP1B) <sup>[90][91][92]</sup>. This hyper-activates pathways such as:

- PI3K/AKT/mTOR
- MAPK/ERK
- JNK/p38
- NF- $\kappa$ B
- HIF-1 $\alpha$

These cascades promote cell cycle progression, inhibit apoptosis, enhance survival, and support metabolic reprogramming (e.g. Warburg effect). ROS also activate transcription factors like HIF-1 $\alpha$  (under hypoxia and in some normoxic conditions), further boosting proliferation and angiogenesis <sup>[93][94][95][96]</sup>.

### *2.1.3. Promotion of Angiogenesis*

ROS stabilize HIF-1 $\alpha$  and up-regulate vascular endothelial growth factor (VEGF) <sup>[97][98][99]</sup>. They also stimulate endothelial cell migration and new vessel formation in the tumor microenvironment, supplying nutrients and oxygen for tumor growth and enabling metastatic spread.

### *2.1.4. Facilitation of Invasion and Metastasis*

ROS drive epithelial-mesenchymal transition (EMT) by altering cytoskeletal dynamics and up-regulating matrix metalloproteinases (MMPs), which degrade the extracellular matrix. They also enhance cell migration/invasion through pathways like NF- $\kappa$ B and Rho GTPases, while modulating the tumor microenvironment to support dissemination <sup>[100]</sup>.

### *2.1.5. Modulation of the Tumor Microenvironment and Inflammation*

ROS promote chronic inflammation by activating NF- $\kappa$ B and recruiting/polarizing immune cells (e.g. macrophages toward pro-tumor M2 phenotype). They suppress anti-tumor immunity (e.g. inhibiting T cells and NK cells) while fostering a supportive niche for tumor growth, immune evasion, and therapy resistance <sup>[101][102]</sup>.

### *2.1.6. Contribution to Therapy Resistance*

Moderate ROS levels help cancer cells adapt to stress, up-regulate antioxidants (e.g. via Nrf2), and activate survival pathways, leading to resistance against chemotherapy, radiotherapy, and targeted treatments <sup>[103][104][105]</sup>.

**ROS duality:** While these mechanisms highlight how ROS favor progression at controlled levels, excessively high ROS (beyond cancer cells' adaptive capacity) can induce oxidative damage leading to apoptosis, ferroptosis, or senescence, forming the basis for some ROS-inducing anticancer therapies. Cancer cells exploit moderate redox imbalance to their advantage, making ROS homeostasis a key vulnerability <sup>[106][107]</sup>.

In summary, moderate ROS drive cancer progression primarily through oxidative damage-induced mutagenesis, hyper-activation of oncogenic signaling, angiogenesis, EMT/metastasis, and microenvironmental remodeling. This context-dependent role explains why strategies targeting ROS (e.g. antioxidants in prevention vs. pro-oxidants in therapy) must consider tumor stage and concentration.

## *2.2. Links between pH and redox potential (integrating the electrochemical network)*

### **2.2.1. Thermodynamic link: pH directly modulates redox potential (via the Nernst equation)**

Many biological redox reactions are proton-coupled (PCET), meaning electrons and protons are transferred together. The Nernst equation for such half-reactions explicitly includes a pH term (Figure 7).

## Nernst equation

The Nernst equation quantifies how the electrical potential of a redox reaction changes when concentrations change.

$$E_h = E^0 - \frac{m * 0.0591}{n} * \text{pH} + \frac{RT}{nF} \ln \left( \frac{[\text{Ox}]}{[\text{Red}]} \right)$$

↑  
Q

Where:

- $E_h$  = cell potential under actual conditions
- $E^0$  = standard cell potential
- $R$  = gas constant
- $T$  = temperature (K)
- $n$  = number of electrons transferred
- $m$  = number of protons
- $F$  = Faraday constant
- $Q$  = reaction quotient (products/reactants)
- $0.0591 = RT/F \log$  base 10 under 298 K temperature.

pH appears in the Nernst equation only if the redox reaction consumes or produces protons. Examples where pH matters:

- $\text{O}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O}$
- $\text{MnO}_4^- + 8\text{H}^+ + 5\text{e}^- \rightarrow \text{Mn}^{2+} + 4\text{H}_2\text{O}$
- **$\text{NAD}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADH}$**

Examples where pH does not appear:

- $\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$
- $\text{Cu}^{2+} + 2\text{e}^- \rightarrow \text{Cu(s)}$

**Figure 7.** The Nernst equation. pH appears whenever the redox reaction involves  $\text{H}^+$  ions. pH and  $E_h$  are formally integrated through the *Nernst equation*, and this relationship is well-established in electrochemistry. The Nernst equation explicitly links redox potential ( $E_h$ ) to proton activity (pH), meaning that any redox couple involving  $\text{H}^+$  will show a predictable  $E_h$ -pH dependence. The relationship between  $e_h$  (electrode potential) and pH (measure of acidity) is described by the Nernst equation, where a higher pH (indicating lower  $\text{H}^+$  concentration) generally results in a lower  $E_h$ . As pH increases, the tendency for reduction decreases, altering the redox behavior of species in a solution <sup>[108][109]</sup>.

For the  $\text{NAD}^+/\text{NADH}$  couple ( $\text{NAD}^+ + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{NADH} + \text{H}^+$ ), acidification (lower pH) shifts to  $E_h$  positive (more oxidizing) by mass action, favoring oxidation of protonated forms. <sup>[110]</sup>

Experimentally, cytoplasmic acidification (via external pH jump or butyrate loading) in intact *Nitella* (alga) cells, causes a positive shift in measured redox potential (more oxidized state), independent of membrane voltage changes. The mechanism is enhanced oxidation of protonated redox couples driven by mass action. This pH dependence means that in experimental conditions, redox potential values must be corrected for local pH (typically ~60 mV shift per pH unit) for accurate interpretation <sup>[111]</sup>. Figure 8.

## Core mathematical integration of pH ↔ Eh when protons participate

### Effects of the Nernst equation

According to the Nernst equation

Increasing pH → Eh becomes more negative (more reducing)

Decreasing pH → Eh becomes more positive (more oxidizing)

Cancer cells maintain

High GSH/GSSG ratio (via NRF2, glutathione synthesis)

Alkaline cytosolic pH (pHi 7.3- 7.6) via NHE1

Both push towards a more negative Eh, creating a mildly reducing environment that protects against ROS-induced death.

Cancer cells maintain

High NADP/NADP<sup>+</sup> via PPP, ME1, IDH1/2

High NADH via glycolysis and mitochondrial rewiring

Alkaline cytosolic pH (pHi 7.3- 7.6) via NHE1

Together, these shift towards a more negative Eh, supporting reductive biosynthesis and ROS buffering.

**Figure 8.** NERNST equation for GSH/GSSG and NADH/NAD<sup>+</sup> redox couples explains tolerance to oxidative stress thanks to the cooperative relationship with intracellular pH.

### 2.2.2. Redox state influence

Conversely, shifts toward a more oxidizing environment (higher Eh) often acidify the cytosol:

**Oxidative stress examples:** Treatment with H<sub>2</sub>O<sub>2</sub> (an oxidant) simultaneously raises cytosolic redox potential and lowers pH (by ~1 unit in yeast). Adding GSSG causes an initial alkalization (H<sup>+</sup> consumption during GSH/GSSG re-equilibration) followed by acidification <sup>[112]</sup>.

**GSH depletion:** Loss of reduced glutathione (more oxidizing state) impairs the Na<sup>+</sup>/H<sup>+</sup> antiporter (a key pH regulator), leading to cytosolic acidification. This occurs in viral infection models and chemically induced GSH loss <sup>[113]</sup>.

**Metabolic links:** Mitochondrial respiration generates NADH + H<sup>+</sup>, CO<sub>2</sub> (forming carbonic acid), and proton leaks, linking redox (electron flow) to net proton production in the cytosol. Uncoupled respiration can increase cytosolic protons <sup>[114]</sup>.

### 2.2.3. Integrated cellular observations

**Simultaneous measurements:** Genetically encoded biosensors (e.g. pHaROS in yeast) and SERS nanosensors in mammalian cells confirm real time cross-talk. Redox and pH oscillate together during the cell cycle (e.g. nuclear oxidation peaks coincide with pH shifts), and perturbations in one affect the other.

**In the cell cycle:** pHi and redox couples oscillate in a reproducible, phase-locked pattern across G1/S/G2/M. pH and redox oscillations are mechanistically coupled, with pH acting as a coordinator of mitochondrial and cytosolic redox cycles. Dual-sensor imaging confirms that redox shifts and pH shifts occur together in real time during cell-cycle progression.

**Compartmental and stress specificity:** Cytosolic pH is normally ~7.0-7.4; redox is tightly buffered (typically ranging from -240 to -300 mV in healthy cells). Oxidative stress or mutants disrupting redox enzymes (e.g. glutathione peroxidase) alter both parameters.

In summary, the relationship is not one-way but reciprocal and homeostatically regulated: pH sets the thermodynamic “landscape” for redox reactions, while redox poise modulates proton-handling enzymes, metabolic acid production, and signaling. Disruptions (e.g. oxidative stress) propagate changes in both, influencing cell fate. This coupling is fundamental in prokaryotes and eukaryotes alike and is increasingly studied with dual-function sensors.

**Proton-coupled electron transfer (PCET)** is a fundamental chemical process in which an electron ( $e^-$ ) and a proton ( $H^+$ ) are transferred together—either in a single concerted step or through tightly linked sequential steps—between donor and acceptor sites. This coupling is essential in biology, chemistry, and energy conversion because it allows efficient redox reactions while managing charge, avoiding high-energy intermediates, and linking electron flow to proton gradients.

Pure electron transfer often creates charged species (e.g. radicals or ions) that are energetically costly in aqueous or protein environments. By coupling the electron to a proton, the net reaction can be more neutral or balanced, lowering the activation barrier. In cells, PCET connects **redox state** (electron transfer) directly to **pH** (proton movements), as seen in the  $NAD^+/NADH$  couple or glutathione redox buffering. This explains why intracellular acidification can shift redox potentials and vice versa <sup>[115][116][117]</sup>.

### 2.3. Concerted vs. Stepwise Mechanisms

PCET encompasses several pathways:

1. **Concerted PCET (also called CPET, EPT, or CEPT):** Electron and proton transfer occur **simultaneously** in one elementary step, without a stable intermediary molecule <sup>[118]</sup>. There is a single transition state. This is often favored thermodynamically when stepwise paths would involve unstable species (e.g. high-energy radicals or charged intermediates). The electron and proton may go to the same acceptor (e.g. in some hydrogen-bonded systems) or different ones (bidirectional or multisite PCET).

2. **Stepwise (sequential) mechanisms:**

- **ET-PT:** Electron transfer first, then proton transfer.
- **PT-ET:** Proton transfer first, then electron transfer.

These involve populated intermediates and are more likely when one step is strongly favored energetically.

The concerted pathway is frequently observed in biology because it avoids high-energy charged intermediates and can be tuned by the protein environment (hydrogen-bond networks, pKa values, distances).

#### 2.4. *Distinction from Hydrogen Atom Transfer (HAT)*

- **HAT:** Both the electron and proton originate from the **same bond** (typically a C–H or O–H) and transfer together to the same acceptor site, resembling neutral H(hydrogen atom) movement. It is often adiabatic (electronic wave function changes smoothly) <sup>[119]</sup>.
- **PCET (non-HAT):** The electron and proton come from **different orbitals** and often go to **different atomic orbitals** or sites (e.g. electron to a metal center or  $\pi$ -system, proton to a separate base or solvent). In many cases, this is electronically nonadiabatic <sup>[120]</sup>.

Many reactions classically called "HAT" are actually concerted PCET when analyzed in detail. The distinction matters for kinetics, isotope effects, and distance dependence.

#### 2.5. *The overall picture*

The cancer cell's pH gradient and redox state are not two separate entities, they are two faces of the same electrochemical adaptation. Some publications say that the Warburg effect is their shared metabolic source <sup>[121][122]</sup>. We do not agree on this point. The Warburg effect acidifies extracellular pH but does not increase intracellular pH, as explained above. We believe that the link is probably NHE1 activity which is the main engine for cytoplasmic alkalization.

NHE1 and ROS regulate each other bidirectionally: ROS activate NHE1, and NHE1 activity amplifies ROS production. This creates a feed-forward loop linking pH regulation, Na<sup>+</sup> homeostasis, and oxidative stress. ROS can modulate NHE1 expression and activity, while NHE1-driven intracellular alkalinization and tumor microenvironment acidosis feed back onto cellular redox balance. This bidirectional crosstalk positions the ROS–NHE1 axis as a key integrator of pH homeostasis, metabolic reprogramming, and signal transduction in cancer cells [\[123\]\[124\]\[125\]\[126\]\[127\]\[128\]\[129\]\[130\]\[131\]](#).

The ROS–NHE1 relationship includes the following concepts:

- NHE1 is not only a pH regulator but also a polymodal sensor node that can be modulated by redox-sensitive kinases and phosphatases [\[132\]](#).
- Paradoxically, prolonged or intense oxidative stress can repress NHE1 expression. In non-apoptotic contexts, hydrogen peroxide exposure has been shown to downregulate NHE1 promoter activity and protein levels via an iron-dependent, caspase-3/-6–mediated pathway. This suggests that NHE1 is dynamically tuned by ROS concentration and duration: moderate ROS promote NHE1 upregulation and pro-tumorigenic phenotypes, whereas severe oxidative stress may suppress NHE1, potentially contributing to cell-death programs or metabolic paralysis [\[133\]](#).
- An alkaline cytoplasm (such as generated by NHE1 activity) can indirectly potentiate ROS signaling by modulating the activity of NADPH oxidases (NOX), antioxidant enzymes (e.g., catalase, peroxiredoxins), and mitochondrial respiration, thereby shaping the overall redox landscape [\[134\]\[135\]](#).
- The low pHe, generated by NHE1's proton export activity, promotes ECM degradation, immune evasion, and stromal activation, all of which can further stimulate ROS production in both tumor and stromal cells. Thus, NHE1-driven acidification can indirectly sustain a chronic oxidative microenvironment that supports tumor progression and resistance.

The proton–electron coupling in every half-reaction is the mathematical bridge between them [\[136\]\[137\]](#). The result is a self-sustaining electrochemical identity: alkaline and reducing inside the cell, acid and oxidizing outside [\[138\]](#) it is thermodynamically stable, proliferative, apoptosis-resistant, immune-evasive, and invasive. All are direct consequences of the Nernst equation applied to a cell that has rewritten its own electrochemical constitution [\[139\]\[140\]\[141\]](#).

Cell replication is a good example for observing how pH and redox state evolve as a team throughout the process.

## 2.6. How does intracellular pH vary through cell replication?

- Starts relatively stable in early G1.
- Drops at the G1/S transition.
- Rises in mid-S phase.
- Drops again in late S.
- Peaks (alkalinization) at G2/M.
- Sharp acidification during mitosis, followed by recovery in daughter cells.

These ~0.1-0.3 unit oscillations have been well-documented in recent single-cell studies and correlate with cell-cycle checkpoints <sup>[142][143][144][145][146][147]</sup>.

## 2.7. Eh also varies throughout cell replication

Eh (right y-axis, GSH/GSSG redox couple; more negative = more reducing environment):

Typically more reducing (~ -230 to -255 mV) during proliferative phases (G1/S and mid-S).

Gradually becomes less reducing / more oxidizing toward G2/M (~ -200 mV).

Oxidation peaks often align with ROS increases in late S/G2/M.

Returns to more reducing state post-mitosis.

This “redox cycle” helps regulate progression from quiescence (G0) through proliferation and is widely reported in the literature Zhen et al. <sup>[148]</sup> showed that intracellular redox reactions occur in temporally distinct patterns and that proliferating cells require phase-specific redox states to support biosynthesis and antioxidant defense. Sies et al. <sup>[149]</sup> defined the dynamic maintenance of redox homeostasis, showing that redox potentials shift with metabolic and signaling demands, including those of the cell cycle. There is also evidence that ROS levels and the redox state fluctuate across G1, S, G2 and M, influencing cyclones, CDKs, checkpoints, and proliferation <sup>[150]</sup>. In a recent review Kiyama T. <sup>[151]</sup> discusses how intracellular redox potential is regulated by electron carriers and proton availability, reinforcing the concept that Eh is a dynamic physiological parameter.

In summary:

- **Eh is not static**—it oscillates with metabolic demands during the cell cycle.
- **G1 → S transition requires a more reducing environment**, supporting nucleotide synthesis and antioxidant buffering.

- **G2/M is associated with transient ROS increases**, which act as signaling molecules for mitotic entry.
- **Redox-sensitive proteins** (e.g. CDKs, phosphatases, transcription factors) respond to these Eh shifts.
- **Electron carriers** ( $\text{NAD}^+/\text{NADH}$ ,  $\text{NADP}^+/\text{NADPH}$ ,  $\text{GSH}/\text{GSSG}$ ) define the redox landscape that gates cell-cycle progression.

### Section 3. Cell Membrane potential

The **membrane potential** (also called resting membrane potential or  $V_{\text{mem}}$ ) is the voltage difference across a cell's plasma membrane, typically negative inside relative to outside (e.g.  $-10$  mV to  $-90$  mV depending on cell type). It arises primarily from unequal ion distributions (especially  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ) and selective ion channel permeabilities, maintained by pumps like the  $\text{Na}^+/\text{K}^+$ -ATPase. The  $\text{Na}^+/\text{K}^+$ -ATPase is not  $V_{\text{mem}}$ 's master regulator as some books say. The master regulator is the selective ion permeability, especially through  $\text{K}^+$  leak channels. The  $\text{Na}^+/\text{K}^+$  ATPase pump actively pumps three sodium ions out and two potassium ions in per hydrolyzed ATP, generating both a chemical gradient and an electrogenic current. However, its contribution to  $V_{\text{mem}}$  is only about 5 to 10 mV. Leak channels for  $\text{K}^+$ , particularly the two-pore-domain channels (K2P family), allow potassium to flow down its concentration gradient, further hyperpolarizing the membrane <sup>[152]</sup>.  $\text{K}^+$  channelsa such as the K2P leak channels are the true master regulators of  $V_{\text{mem}}$ .  $V_{\text{mem}}$  is approximately equal to  $E_{\text{K}}$  <sup>[153][154]</sup>.

In summary, the  $\text{Na}^+/\text{K}^+$ -ATPase builds the gradients, but  *$\text{K}^+$  leak channels* set the voltage, making selective permeability the true master regulator of  $V_{\text{mem}}$ .

The membrane potential is not static during the cell cycle ( $\text{G0/G1} \rightarrow \text{S} \rightarrow \text{G2} \rightarrow \text{M}$ ). It undergoes dynamic changes that correlate with, and in many cases help regulate, progression through phases. These shifts involve differential expression and activity of ion channels (particularly  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$  channels). Figure 9.

**Na<sup>+</sup>/K<sup>+</sup>-ATPase (ATP-driven)**

- Maintains Na<sup>+</sup> OUT / K<sup>+</sup> IN
- Creates steep ion gradients
- Electrogenic: +1 charge out
- Enables V<sub>mem</sub> but does NOT set its value

**Ion Gradients (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, H<sup>+</sup>)**

- Chemical driving forces for diffusion
- Provide the "potential energy" that channels convert into voltage

**SELECTIVE PERMEABILITY (MASTER REGULATOR OF V<sub>mem</sub>)**

Dominant: K<sup>+</sup> leak channels (K2P, Kir)

- Set resting V<sub>mem</sub> ≈ E<sub>K</sub>
- Hyperpolarization during quiescence/differentiation

Modulators: Na<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, H<sup>+</sup> channels

- Depolarization during proliferation (G1/S, G2/M)
- Dynamic oscillations during the cell cycle

Figure 9. V<sub>mem</sub> regulators

### 3.1. General Pattern of Membrane Potential Changes

- **Quiescent/non-proliferating cells** (often in G0 or early G1) tend to have a hyperpolarized (more negative) membrane potential. This state is associated with reduced proliferation.
- **Proliferating cells** (including many cancer cells) tend to have a relatively depolarized (less negative or more positive) membrane potential overall, which supports cell cycle advancement [\[155\]](#).
- **Cancer stem cells** are depolarized in relation to non-cancer stem cells [\[156\]](#).

Specific phase-dependent changes (observed in various mammalian cell types, such as CHO (chinese hamster ovarian cancer) cells, astrocytes, lymphocytes, and others):

- **G0/G1 phase:** Cells often start relatively depolarized in early G1. Hyperpolarization (becoming more negative) frequently occurs during the G1 progression or at the G1/S transition. This is linked to up-regulation of inwardly rectifying K<sup>+</sup> channels (e.g. Kir) or other K<sup>+</sup> conductances, driving the potential closer to the K<sup>+</sup> equilibrium potential (around -90 mV). Hyperpolarization in this window can facilitate Ca<sup>2+</sup> signaling or other events needed for DNA synthesis. In some contexts, if too extreme, sustained hyperpolarization acts as a brake, blocking G1-to-S transition.
- **S phase (DNA synthesis):** The membrane potential is often maintained in a relatively **hyperpolarized** or elevated (more negative) state compared to early G1. This supports ongoing DNA replication. Arrest

in the S phase can lead to depolarization in some models due to down-regulation of certain  $K^+$  currents and up-regulation of outwardly rectifying currents.

- **G2 phase:** The hyperpolarized state is generally maintained from S into G2.
- **M phase (mitosis):** The membrane potential **depolarizes** (becomes less negative) as cells enter mitosis. This depolarization has been causally linked to promoting mitotic entry or progression (e.g. via activation of pathways like ERK signaling). Artificial depolarization can enhance mitosis in some systems, while extreme hyperpolarization can induce mitotic arrest. The potential declines (depolarizes) from the elevated levels seen in S/G2.

These oscillations are not universal across all cell types but represent a common theme, especially in proliferating somatic or embryonic cells. Early studies (e.g. on Chinese hamster ovary cells) showed increased (hyperpolarized) potential maintained through S and G2, with depolarization upon mitotic entry <sup>[157]</sup>.

### 3.2. Mechanisms Linking Membrane Potential to Cell Cycle

Membrane potential influences the cell cycle through:

- **Ion fluxes and signaling:** Hyperpolarization increases the driving force for  $Ca^{2+}$  influx (via voltage-gated or store-operated channels), which is critical for progression (e.g. G1/S). Depolarization can activate voltage-gated  $Na^+$  or  $Ca^{2+}$  channels to trigger downstream pathways <sup>[158][159]</sup>. In summary:
  1. Hyperpolarization is required for G1/S transition.
  2. Depolarization is required for entry into mitosis (M phase).
  3. Ion channels ( $K^+$ ,  $Na^+$ ,  $Cl^-$ ) generate phase-specific  $V_{mem}$  oscillations <sup>[160][161]</sup>.
    - $K^+$  channels** produce hyperpolarization during G1/S
    - $Na^+$  channels** depolarize transitions
    - $Cl^-$  channels** modify volume, and charge that tune  $V_{mem}$ .
  4. Blocking channels arrests the cell cycle <sup>[162][163][164][165][166]</sup>.

**Threshold hypothesis:** A "boundary"  $V_{mem}$  level may act as a checkpoint for DNA synthesis; crossing it (often via depolarization from a hyperpolarized state or vice versa) permits progression.

### 3.3. $K^+$ channels as key regulators

$K^+$  channels are conventionally classified into four major families based on topology and gating mechanisms:

**Voltage-gated (Kv)**

**Calcium-activated (KCa: BK/large, IK/intermediate, SK/small)**

**Inward-rectifying (Kir, including KATP)**

**Two-pore domain (K2P)**

Voltage-gated (e.g. Kv11.1/hERG), inwardly rectifying (Kir), and  $Ca^{2+}$ -activated  $K^+$  channels modulate  $V_{mem}$ . Their expression/activity cycles with the cell cycle phases. Blocking  $K^+$  channels often arrests cells in G1. <sup>[167][168][169][170][171]</sup>,  $K^+$  channels are the dominant controllers of  $V_{mem}$  because:

The plasma membrane is most permeable to  $K^+$  at rest;

The Nernst potential for  $K^+$  ( $E_K$ ) is the main determinant of resting  $V_{mem}$ ;

Opening  $K^+$  channels leads to hyperpolarization (Table 2).

Closing/blocking  $K^+$  channels leads to depolarization

And  $V_{mem}$  changes regulate:

G1/S transition;

$Ca^{2+}$  influx (via voltage-sensitive  $Ca^{2+}$  channels);

Cyclin expression;

Proliferation and differentiation;

Oncogenic signaling (e.g. Eag1/Kv10.1 in cancer).

| Channel                                      | Phase                 | Effect on Vmem                 | Mechanistic Role                                          | Reference                                     |
|----------------------------------------------|-----------------------|--------------------------------|-----------------------------------------------------------|-----------------------------------------------|
| Kv1.3                                        | G1 to S               | Hyperpolarization              | Permits Ca <sup>2+</sup> influx and cyclin activation     | Urrego et al. 2014 <a href="#">[168]</a>      |
| Kv10.1 (Eag1)                                | G1 to S               | Hyperpolarization              | Required for S-phase entry and oncogenic proliferation    | Pardo & Stuhmer 2014 <a href="#">[169]</a>    |
| Kir2.1                                       | G1                    | Strong hyperpolarization       | Sets resting Vmem and stabilizes G1                       | Huang et al. 2015 <a href="#">[167]</a>       |
| Kv11.1 (hERG)                                | S to G2               | Mild depolarization            | Coordinates Ca <sup>2+</sup> signaling and metabolic flux | Urrego et al. 2014 <a href="#">[168]</a>      |
| BK (KCa1.1)                                  | G2 to M               | Depolarization bursts          | Supports mitotic entry and spindle formation              | Sundelacruz et al. 2009 <a href="#">[172]</a> |
| KATP channels                                | G1/S metabolic gating | Hyperpolarization              | Links ATP/ADP ratio to Vmem and proliferation             | Huang et al. 2015 <a href="#">[167]</a>       |
| Two-pore K <sup>+</sup> channels (TASK/TREK) | All phases            | Background K <sup>+</sup> leak | Maintains baseline Vmem and tune responsiveness           | Pardo & Stühmer 2014 <a href="#">[169]</a>    |

**Table 2.** Different cell cycle-linked K<sup>+</sup> channels and their role in Vmem and mitosis

| Channel        | Gene   | Cancer-Relevant Functions                             | Mechanisms / Notes                                                          | Evidence                                                                              |
|----------------|--------|-------------------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Kv1.3          | KCNA3  | Proliferation, apoptosis resistance, immune evasion   | Regulates membrane potential; mitochondrial Kv1.3 modulates apoptosis       | Peer-reviewed studies on Kv1.3 in melanoma, glioma, CLL <a href="#">[173]</a>         |
| Kv1.5          | KCNA5  | Metabolic reprogramming, proliferation control        | Links membrane potential to metabolic state; down-regulated in some cancers | Evidence in breast, lung cancer, and lymphoma <a href="#">[174]</a>                   |
| Kv7.1 (KCNQ1)  | KCNQ1  | Tumor suppressor in GI cancers; oncogenic in others   | Controls epithelial polarity, Wnt/ $\beta$ -catenin modulation              | Strong evidence in colorectal, and gastric cancer <a href="#">[175]</a>               |
| Kv10.1 (Eag1)  | KCNH1  | Strong oncogene; promotes proliferation, angiogenesis | Over-expressed in many tumors; regulates cell cycle entry                   | Extensive evidence across solid tumors <a href="#">[176]</a>                          |
| Kv11.1 (hERG1) | KCNH2  | Proliferation, migration, angiogenesis                | Forms complexes with integrins; modulates PI3K/AKT                          | Strong evidence in colorectal, pancreatic cancers, and leukemia <a href="#">[177]</a> |
| KCa1.1 (BK)    | KCNMA1 | Migration, invasion, metastasis                       | Ca <sup>2+</sup> -activated; regulates cytoskeletal dynamics                | Evidence in breast, prostate cancers, and glioma                                      |
| KCa3.1         | KCNN4  | Proliferation, migration, TME modulation              | Controls Ca <sup>2+</sup> influx; supports T-cell and cancer cell motility  | Evidence in glioma, pancreatic, immune cells                                          |
| Kir2.1         | KCNJ2  | EMT, metastasis                                       | Hyperpolarization-driven signaling; regulates $\beta$ -catenin              | Evidence in gastric, breast cancer                                                    |
| Kir3.1         | KCNJ3  | Hormone-driven cancers                                | GPCR-linked signaling; promotes proliferation                               | Evidence in breast, ovarian cancer                                                    |
| Kir4.1         | KCNJ10 | Glioma biology                                        | Regulates K <sup>+</sup> buffering; altered in glioma                       | Evidence in astrocytoma, glioblastoma                                                 |

| Channel            | Gene        | Cancer-Relevant Functions         | Mechanisms / Notes                                  | Evidence                                   |
|--------------------|-------------|-----------------------------------|-----------------------------------------------------|--------------------------------------------|
| K2P (TASK1/3)      | KCNK3/9     | Hypoxia adaptation, proliferation | Regulates resting potential; TASK-3 often amplified | Evidence in melanoma, breast, lung cancers |
| K2P (TREK1)        | KCNK2       | Migration, stress adaptation      | Mechanosensitive; modulates cytoskeleton            | Evidence in glioma, prostate cancer        |
| K2P (TWIK-related) | KCNK family | TME adaptation                    | pH-sensitive channels supporting survival           | Evidence across solid tumors               |

**Table 3.** K<sup>+</sup> channels relevant to cancer. Some are also cell cycle-linked K<sup>+</sup> channels

*Note: shaded channels are relevant for cancer and for the cell cycle and are shown in both Tables.*

Potassium channel mutations in cancer are less common than potassium channel dysregulation/overexpression, but several channels do harbor cancer-associated variants, and KCNQ1, KCNJ5, and KCNJ2 are among the best-described examples. Potassium channel genes are subject to somatic mutation. KCNQ1, encoding the voltage-gated K<sup>+</sup> channel Kv7.1, which is frequently mutated or deleted in colorectal cancer, where its loss disrupts normal epithelial polarity and promotes dedifferentiation. KCNH2 (hERG) mutations have been identified in leukemias, glioblastoma, and other solid tumors, and are associated with alterations in apoptotic sensitivity, since hERG current is required for the normal decrease in apoptotic volume [178]. Gain-of-function mutations in KCNK9, encoding the two-pore domain channel TASK3, have been identified in breast and lung cancers, where they confer resistance to serum starvation-induced apoptosis by hyperpolarizing the mitochondrial membrane potential [179][180][181].

Calcium channels are also affected. CACNA1C and CACNA1D, encoding L-type voltage-gated calcium channel subunits, are mutated in prostate cancer and endometrial cancer respectively. Mutations in TRPV4, a member of the transient receptor potential vanilloid family, have been identified in squamous cell carcinomas and mesotheliomas. In glioblastoma multiforme, mutations and amplifications of TRPM7 — a channel-kinase that regulates Mg<sup>2+</sup> homeostasis and actomyosin contractility — are associated with invasive phenotypes.

Copy number variations profoundly influence ion channel expression in cancer. Amplification of chromosomal regions harboring channel genes can dramatically increase transcript and protein levels, resulting in gain-of-function phenotypes even in the absence of activating point mutations.

For example, loss-of-function mutations in KCNQ1 enhance Wnt/ $\beta$ -catenin signalling disrupting epithelial homeostasis in cancer [\[182\]](#). KCNH2 was found to harbor point mutations, copy number mutations and DNA methylation in many tumors.

The chromosomal region 11q13, which is amplified in 15-20% of breast and head-and-neck cancers, contains KCNK4 and several calcium signaling genes. Amplification of chromosome 1q32, observed in multiple myelomas and hepatocellular carcinomas, encompasses KCNN3, encoding the small-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channel SK3.

Conversely, hemizygous or homozygous deletions at channel gene loci result in loss-of-function alterations. The loss of KCNQ1 at 11p15.5, KCNRG at 13q14, and CLCA2 at 1p31 are recurrent events in colorectal, chronic lymphocytic leukemia, and epithelial cancers, respectively. Such deletions typically reduce channel expression to levels insufficient to maintain normal electrophysiological homeostasis, thereby promoting the proliferation and survival of cancer cells [\[183\]](#), [\[184\]](#), [\[185\]](#).

**Other effects:** Changes in  $V_{\text{mem}}$  can influence cell volume, pH, YAP/TAZ signaling (Hippo pathway), mitochondrial function, and gene expression tied to proliferation [\[186\]](#), [\[187\]](#), [\[188\]](#). Metabolic/ionic states feed directly into Hippo signaling [\[189\]](#).

Depolarization generally promotes proliferation/mitosis, while strong hyperpolarization can inhibit it or maintain quiescence. Cancer cells often exploit depolarized states for unchecked growth [\[190\]](#), [\[191\]](#), [\[192\]](#).

Figure 10.

### Cancer cells show a characteristic bioelectric signature:

- ▶ Membrane depolarization (resting potentials ranging from -10 to -40 mV compared to the -70 to -90 mV found in normal cells).
- ▶ This is the result of alterations in the expression and function of ion channels such as:
  - ▶ Down-regulation of  $K^+$  channels (Kv1.3, Kv1.5, and Kv10.1),
  - ▶ Up-regulation of voltage-gated  $Na^+$  channels (Nav1.5, Nav1.7)
  - ▶ Up-regulation of  $Ca^{2+}$  channels (Cav3.1, Cav3.2).

This bioelectric signature plays an important role in cancer cell physiopathology because:

Depolarized  $V_{mem}$  affects  $Ca^{2+}$  dynamics by altering the electrochemical gradient for  $Ca^{2+}$  influx:  $\longrightarrow$  disrupted intracellular signaling impacts proliferation and migration.

$V_{mem}$  influences voltage-sensitive enzymes and transporters modulating  $Na^+$ -dependent nutrient transporters  $\longrightarrow$  affect cellular metabolism.

Disruption of intercellular communication through gap junctions.

Figure 10. It is based on references [\[193\]](#)[\[194\]](#)[\[195\]](#)[\[196\]](#)[\[197\]](#)[\[198\]](#)

Mitochondrial membrane potential ( $\Delta\Psi_m$ ) also plays a role: its decrease can delay G1-to-S transition as a stress signal. Mitochondrial membrane potential ( $\Delta\Psi_m$ ) is a central regulator of cell proliferation because it controls ATP production, ROS signaling, and metabolic flux, each of which feeds directly into cell-cycle progression [\[199\]](#)[\[200\]](#)[\[201\]](#).

### 3.4. Experimental Evidence and Implications

- Artificial manipulation (e.g. via ionophores, channel blockers, or altering extracellular ions) shows causal links: forcing hyperpolarization can block DNA synthesis; depolarization can drive mitotic activity [\[202\]](#)[\[203\]](#).
- These bioelectric changes are part of broader "bioelectric signaling" that complements biochemical checkpoints (cyclins, CDKs, etc.) [\[204\]](#).
- Therapeutic potential: Targeting ion channels or  $V_{mem}$  (e.g. with drugs modulating  $K^+$  channels) is being explored for cancer (to arrest proliferation).

Note that exact mV values and directions can vary by cell type, species, and context (e.g. embryonic vs. adult somatic cells). Immature or stem-like cells often have more depolarized potentials that become

hyperpolarized upon differentiation.

- **Hyperpolarization** (more negative) increases the electrochemical driving force for  $\text{Ca}^{2+}$  entry, helping trigger signals for S-phase entry [205].
- **Depolarization** in mitosis can activate voltage-sensitive pathways (e.g. involving ion channels like Kv or  $\text{Ca}^{2+}$  channels) that facilitate chromosome segregation and cell division.
- Cancer cells often maintain a persistently depolarized state, which correlates with uncontrolled proliferation.

### 3.5. *Eh and Vmem maintain an indirect relationship*

Vmem and Eh are not the same quantity and are not directly interchangeable:

- Vmem is an electrical potential driven by ion charge separation across the impermeable lipid bilayer (maintained by pumps like  $\text{Na}^+/\text{K}^+$ -ATPase and channels).
- Eh is a chemical potential describing electron transfer in redox reactions (e.g. via ROS, antioxidants, or metabolic couples).

They operate on overlapping but distinct levels (both in mV) and influence each other indirectly through bidirectional feedback in cellular physiology, especially during the cell cycle, proliferation, differentiation, and stress responses.

#### 3.5.1. *Key Links and Correlations*

##### 1. Cell Cycle and Proliferation Context:

Proliferating cells (including many cancer cells) tend to have depolarized Vmem (less negative, e.g. -10 to -40 mV) and a more reducing Eh (more negative, e.g. around -240 mV for GSH/GSSG) [206][207][208].

Quiescent/differentiated cells often show hyperpolarized Vmem (more negative) and a relatively more oxidizing Eh [209][210][211].

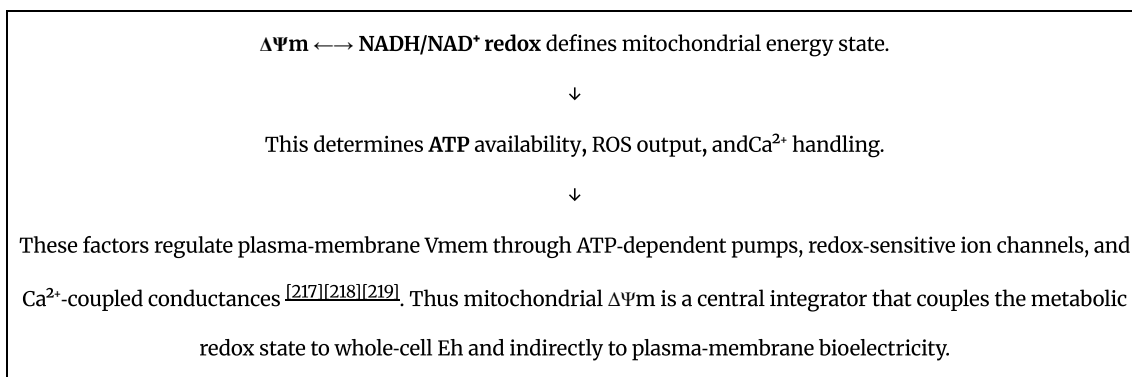
Periodic redox cycles (oscillations in Eh) parallel bioelectric changes and help drive cell-cycle progression ( $\text{G0} \rightarrow \text{G1/S} \rightarrow \text{S/G2} \rightarrow \text{M}$ ). ROS (reactive oxygen species) act as second messengers that can modulate ion channels, thereby altering Vmem [212]. Conversely, changes in Vmem can influence ROS production or mitochondrial function, which shifts Eh.

##### 2. Mechanistic Interactions:

**NADPH oxidases (NOX)** at the plasma membrane: These enzymes transfer electrons from intracellular NADPH to extracellular O<sub>2</sub>, generating ROS (which affect Eh) while also being electrogenic, the electron flow itself can depolarize V<sub>mem</sub> (make it less negative).

**ROS as signals:** Moderate ROS levels (shifting Eh) can open or close ion channels (e.g. K<sup>+</sup> or Ca<sup>2+</sup> channels), leading to V<sub>mem</sub> hyperpolarization or depolarization. This feeds back into Ca<sup>2+</sup> signaling, which is voltage-sensitive and critical for G1/S transition. ROS regulate ion channels primarily by oxidizing redox-sensitive cysteines, altering channel gating, trafficking, and signaling, thereby reshaping membrane potential, Ca<sup>2+</sup> dynamics, and downstream oncogenic pathways [\[213\]\[214\]\[215\]\[216\]](#).

**Mitochondrial coupling:** Mitochondrial membrane potential ( $\Delta\Psi_m$ ) links energy metabolism (redox state via NADH/NAD<sup>+</sup>) to overall cellular Eh. Disruptions here can indirectly affect plasma V<sub>mem</sub> through ATP levels or ion homeostasis. The mechanisms consist of:



**Bioelectric-redox feedback loops:** Early bioelectric events (V<sub>mem</sub> changes) can modulate redox-modulated pathways (e.g. via NADPH oxidase), and vice versa. In regeneration models, inhibiting ROS production alters both electric currents and V<sub>mem</sub> dynamics. NADPH oxidases (NOX enzymes) require NADPH as the electron donor for ROS production [\[220\]](#).

### 3. Functional Outcomes:

A reducing Eh (more negative) often supports proliferation because it supplies the reducing equivalents needed for DNA synthesis and anabolic metabolism, aligning with the depolarized V<sub>mem</sub> window that facilitates certain Ca<sup>2+</sup> or growth signals.

An oxidizing shift in Eh can promote differentiation, apoptosis, or quiescence, sometimes coinciding with V<sub>mem</sub> hyperpolarization [\[221\]](#).

In cancer or stem cells, a persistently reducing Eh and depolarized Vmem create a pro-proliferative state.

### 3.6. Summary of Typical Patterns

- **Proliferative state:** Depolarized Vmem + reducing Eh (more negative redox potential) → favors growth and cell-cycle progression.
- **Quiescent/differentiated state:** Hyperpolarized Vmem + relatively oxidizing Eh → supports stability and specialized function.
- These are correlated but not strictly causal in one direction; they form integrated **bioelectric-redox networks** that complement cyclin/CDK biochemical oscillations.
- **Reducing Eh (more negative)** → increases NADPH/NADH availability → supports DNA synthesis and anabolic metabolism.
- **Depolarized Vmem** → increases  $\text{Ca}^{2+}$  influx → activates growth pathways.
- Both states are characteristic of **proliferative cells**, including cancer cells. <sup>[222]</sup>

Exact values vary by cell type, compartment (cytosol vs. extracellular vs. organelles), and conditions. Measurements of Eh often use fluorescent sensors or couples like GSH/GSSG, while Vmem uses voltage-sensitive dyes or patch-clamp. In the context of the cell cycle graphs we discussed earlier, the hyperpolarization around G1/S and depolarization in M phase often occur alongside redox shifts that fine-tune ROS signaling for checkpoint passage. Figure 10.

Figure 11 shows the interdependent chronology of changes in pH, Eh, and Vmem during the cell cycle.

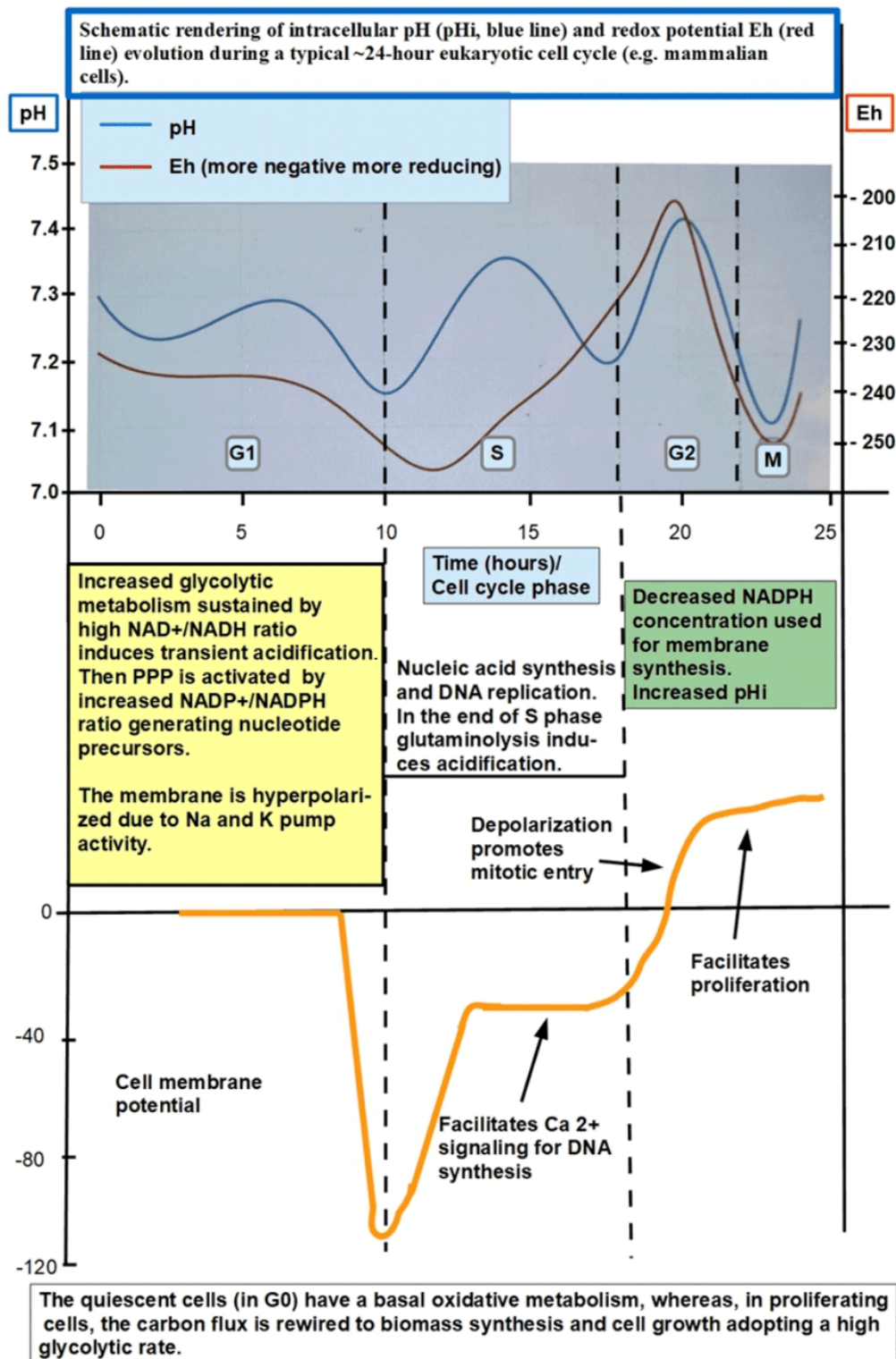


**Figure 11.** It shows how the three factors: pH, Eh and Vmem work together in a coordinated and interdependent manner to drive cell proliferation. The x-axis shows time (hours) with major phases labeled:

G1 (0-10 h); S (10-18 h); G2 (18-22 h); M (mitosis, 22-24 h).

pHi (left y-axis, typical cytoplasmic range ~7.1-7.4 in normal cells; often ~0.2-0.4 units higher in proliferating/cancer cells):

#### Important notes

Values are approximate and cell-type dependent (e.g. yeast vacuolar pH or Tetrahymena show slightly different patterns). Normal resting pHi is ~7.2; cancer cells are often constitutively more alkaline.

Eh is a composite measurement (commonly derived from glutathione ratios); absolute values vary with measurement method.

The diagram illustrates coupled oscillatory dynamics observed across studies, no single “canonical” combined plot exists, so this is a summary for visualization.

### 3.7. Characteristic States in Cancer Cells

- **Vmem:** Strongly depolarized (less negative, typically -5 to -40 mV, often around -10 mV) compared to normal differentiated cells (-60 to -90 mV or more negative). This arises from altered ion channel/transporter expression and activity (e.g. reduced K<sup>+</sup> conductance, increased Na<sup>+</sup> or Cl<sup>-</sup> fluxes).
- **Eh (redox potential):** Shifted toward a more reducing environment (more negative Eh for key couples like GSH/GSSG or NADP<sup>+</sup>/NADPH). Cancer cells often maintain elevated reductive power (higher NADP<sup>+</sup>/NADPH and sometimes NAD<sup>+</sup>/NADH ratios), supporting biosynthesis, ROS detoxification, and redox signaling despite increased ROS production from mitochondria or NADPH oxidases <sup>[223]</sup>.
- **pHi:** Constitutively alkaline (higher, typically 7.12-7.65 vs. ~7.0-7.2 in normal cells). This reversal of the normal pH gradient (alkaline inside, acidic outside) is driven by up-regulated proton extruders such as NHE1 (Na<sup>+</sup>/H<sup>+</sup> exchanger), V-ATPase, MCTs (monocarboxylate transporters), and carbonic anhydrases.

Mitcheson et al. <sup>[224]</sup> provided the foundational electrochemical basis of Vmem, including its dependence on ionic gradients and proton-coupled processes.

Together, these sources form a rigorous, cross-disciplinary reference set for the biochemical–bioelectrical integration of redox potential (Eh), membrane voltage (Vmem), and intracellular pH (pHi).

These changes are not isolated; they create positive feedback loops that lock cancer cells into a pro-proliferative, stress-resistant state.

### 3.8. Interconnections and Feedback Loops

#### 1. Depolarized Vmem and Alkaline pHi:

Depolarization reduces the electrochemical driving force for  $H^+$  influx (or equivalently, aids net acid extrusion) because the driving force equals the difference between membrane potential ( $V_m$ ) and the ion's equilibrium potential ( $V_{eq}$ ). When  $V_m$  becomes less negative, the electrical component favoring inward proton movement decreases. Even though cancer cells face a constant passive  $H^+$  load (due to high membrane permeability and metabolism), the less negative  $V_{mem}$  helps maintain elevated pHi against the acidic extracellular environment (pHe often ~6.5-7.0) [225].

Conversely, alkaline pHi influences ion channel activity (especially  $K^+$  channels). In many systems, alkalization tends to hyperpolarize, but in cancer cells the dominant effect is sustained depolarization via pH-sensitive modulation of channels/transporters. Acidification (if pHi drops) causes stronger depolarization, while the alkaline baseline supports volume regulation, migration, and proliferation.

pHi alkalinization activates glycolytic enzymes and inhibits mitochondrial oxidative phosphorylation, reinforcing the Warburg effect and further metabolic acid production—which the cell must extrude, perpetuating the cycle [226].

#### 2. Redox (Eh) Integration with Vmem and pHi:

A reducing Eh supports NADPH-dependent antioxidant systems and biosynthetic pathways (lipids, nucleotides) needed for rapid proliferation. NADPH oxidases (NOX) at the plasma membrane are electrogenic: electron transfer from cytosolic NADPH to extracellular  $O_2$  generates extracellular ROS (affecting Eh and pHe) while contributing to membrane depolarization.

The redox state modulates ion channels and transporters (e.g. ROS or redox-sensitive cysteines alter  $K^+$ ,  $Ca^{2+}$ , or proton exchanger activity), linking Eh shifts directly to  $V_{mem}$  and pHi control [227][228].

Alkaline pHi and reducing Eh together favor glycolytic flux and reduced mitochondrial membrane potential ( $\Delta\Psi_m$  often lower in cancer), which decreases ATP from OXPHOS but increases reliance on glycolysis—producing more lactate/ $H^+$  that must be exported, further acidifying pHe while keeping pHi high.

Mitochondrial redox ( $NADH/NAD^+$ ) and plasma  $V_{mem}$  are coupled via ATP levels and ion homeostasis; disrupted mitochondrial function in cancer feeds back to support depolarized  $V_{mem}$  and alkaline pHi.

**In summary:**

**Redox → Vmem:** ROS and NADPH oxidase activity modulate membrane potential by altering ion channel activity and transepithelial currents (Ferreira et al., 2016).

**Vmem → Redox:** Bioelectrical states influence mitochondrial ROS production and redox-sensitive signaling pathways (Schofield et al., 2020).

**pH ↔ Eh:** Proton concentration directly shifts redox potentials through Nernstian relationships (Kiyama, 2025 <sup>[151]</sup>, Aamer et al., 2022).

**pH ↔ Vmem:** Proton-coupled transporters and pH-sensitive channels alter membrane voltage (Mitcheson & Stanfield, 2019 <sup>[224]</sup>).

## Section 4. The mitochondrial membrane potential ( $\Delta\Psi_m$ ) and its multiple relations

$\Delta\Psi_m$  and  $\Delta\Psi_p$  are electrically independent but functionally coupled through ion homeostasis, metabolism, and signaling. The plasma membrane potential ( $\Delta\Psi_p$ ) sets the ionic environment that mitochondria operate in, while mitochondria generate  $\Delta\Psi_m$  using the electron transport chain. Changes in one can influence the other through  $\text{Ca}^{2+}$  flux, ATP availability, and  $\text{Na}^+/\text{H}^+$  gradients. This shows the relationship between pH, Eh,  $\Delta\Psi_m$  and  $\Delta\Psi_p$ .

### 4.1. What Each Potential Represents

**A. Plasma Membrane Potential ( $\Delta\Psi_p$ ):** Altered Vmem (depolarized state) is now recognized as a component of cancer cell identity, influencing communication, tissue organization, and cell polarity <sup>[229]</sup>. It is:

- Generated mainly by selective ion channels and secondarily by  $\text{Na}^+/\text{K}^+$ -ATPase pump.
- Typical value:  $-40$  to  $-90$  mV (inside negative).
- Governs:
  - Excitability
  - Nutrient uptake
  - $\text{Ca}^{2+}$  entry

Cell volume

Cell polarity

**B. Mitochondrial Membrane Potential ( $\Delta\psi_m$ ):**  $\Delta\psi_m$  is consistently elevated in many cancers, and this hyperpolarized state supports proliferation, invasion, and metastasis. Recent sources describe  $\Delta\psi_m$  as both a biophysical hallmark and a functional driver of malignant progression [\[230\]\[231\]\[232\]](#). It is:

- Generated by proton pumping of complexes I, III, IV of the electron transfer chain (ETC).
- Its Typical value is:  $-150$  to  $-200$  mV (matrix negative).

A high mitochondrial membrane potential:

- drives ATP synthesis needed for G1/S and G2/M transitions;
- $\Delta\psi_m + \Delta pH$  together form the proton-motive force;
- this electrochemical gradient is harnessed by ATP synthase to generate ATP [\[233\]\[234\]\[235\]](#);
- supports ROS-mediated signaling that activates proliferative pathways;
- enhances mitochondrial  $Ca^{2+}$  uptake, boosting TCA-cycle flux [\[236\]](#);
- stabilizes mitochondrial structure and biosynthetic output;
- promotes survival under hypoxia and mechanical stress in the tumor microenvironment.

Together, these functions make  $\Delta\psi_m$  a powerful integrator of metabolic and signaling cues that support cancer growth.  $\Delta\psi_m$  links directly to cell-cycle control because it governs ATP availability, mitochondrial  $Ca^{2+}$  uptake, and redox signaling, each of which feeds into G1/S and G2/M checkpoints. The same electrochemical variables that shape  $\Delta\psi_m$  also integrate with  $V_{mem}$ ,  $pH_i$ , and  $E_h$ , forming a single bioenergetic-bioelectric axis that regulates proliferation.

Decreased  $\Delta\psi_m$  delays G1/S, and experimentally increasing  $\Delta\psi_m$  restores normal timing. This checkpoint effect arises because  $\Delta\psi_m$  controls mitochondrial ATP synthesis, TCA-cycle flux, and  $Ca^{2+}$ -dependent activation of biosynthetic enzymes required for DNA replication [\[237\]](#).

## 4.2. How They Are Mechanistically Connected

### 4.2.1. $Ca^{2+}$ Coupling (the strongest link)

- Plasma membrane depolarization increases  $Ca^{2+}$  influx because voltage-gated  $Ca^{2+}$  channels (VGCCs) open in response to depolarization [\[238\]\[239\]\[240\]](#) triggering a wide range of cellular responses [\[241\]\[242\]](#)

[243][244].

- Mitochondria take up  $\text{Ca}^{2+}$  via MCU (mitochondrial calcium uniporter), which hyperpolarizes  $\Delta\Psi_m$  by stimulating the TCA (tricarboxylic acids) cycle and ETC activity [245][246][247][248][249][250][251][252].

Together, these references support the consensus that:

MCU is the main  $\text{Ca}^{2+}$  entry channel in the inner mitochondrial membrane.

$\text{Ca}^{2+}$  entry through MCU activates TCA-cycle dehydrogenases.

This increases NADH/FADH<sub>2</sub> supply to the ETC.

ETC acceleration hyperpolarizes  $\Delta\Psi_m$  and increases ATP synthesis.

|                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>In summary:</b> $\text{MCU} \rightarrow \text{TCA} \rightarrow \text{ETC} \rightarrow \Delta\Psi_m \rightarrow \text{cell-cycle progression}$ . |
|----------------------------------------------------------------------------------------------------------------------------------------------------|

This is also supported by recent super-resolution work showing  $\text{Ca}^{2+}$ -driven cristae hyperpolarization [253]. Thus:  $\Delta\Psi_p \rightarrow \text{Ca}^{2+} \text{ influx} \rightarrow \Delta\Psi_m \text{ increases}$ .

#### 4.2.2. ATP/ADP Coupling

- $\Delta\Psi_p$  is maintained (but not generated) by  $\text{Na}^+/\text{K}^+$ -ATPase, which consumes 30–50% of cellular ATP [254][255]. This is why  $\Delta\Psi_p$  maintenance is considered one of the largest ATP sinks in the cell
- ATP is produced by mitochondria using  $\Delta\Psi_m$  [256][257][258].
- If  $\Delta\Psi_m$  drops (e.g. ETC inhibition), ATP falls  $\rightarrow \text{Na}^+/\text{K}^+$  pump weakens  $\rightarrow \Delta\Psi_p$  depolarizes.

**Thus:  $\Delta\Psi_m \rightarrow \text{ATP supply} \rightarrow \Delta\Psi_p \text{ stability}$ .**

#### 4.2.3. $\text{Na}^+/\text{H}^+$ Coupling (newer insight)

In this regard, Erich Gnaiger [225] wrote: “The proton motive force (pmF) is central to oxidative phosphorylation (OXPHOS), coupling oxygen consumption (OX) in cell respiration to phosphorylation of ADP to ATP (PHOS). Defined as an electrochemical potential difference, the pmF consists of two components: the electric part  $\text{pmF}_{el}$ , linked to the transmembrane potential difference ( $\Delta\Psi$ ), and the diffusive part  $\text{pmF}_{d}$ , related to the pH difference ( $\Delta\text{pH}$ ) across the mitochondrial inner membrane. Although  $\text{pmF}_{el}$  is dominant in animal mitochondria,  $\text{pmF}_{d}$ , often overlooked, contributes significantly under physiological conditions”.

A 2024 Cell paper [259] showed that  $\Delta\Psi_m$  is controlled not only by  $\text{H}^+$  but also by a mitochondrial  $\text{Na}^+$  gradient via a  $\text{Na}^+/\text{H}^+$  exchanger in complex I.

This means that:

- $\Delta\Psi_m$  is not solely determined by  $H^+$ ; a mitochondrial  $Na^+$  gradient contributes ~50% of  $\Delta\Psi_m$  in coupled-respiring mammalian mitochondria.
- The P-module of Complex I acts as a mitochondrial  $Na^+$ -specific  $Na^+/H^+$  exchanger (mNHE).
- This exchanger uses the  $H^+$  gradient to build a  $Na^+$  gradient, which in turn controls half of  $\Delta\Psi_m$ .
- Mutations affecting this  $Na^+/H^+$  exchange function (without impairing canonical Complex I enzymatic activity) alter  $\Delta\Psi_m$  and mitochondrial  $Ca^{2+}$  handling,

**Thus:  $\Delta\Psi_p$  (via  $Na^+$  channels/pumps)  $\rightarrow$  cytosolic  $Na^+$   $\rightarrow$   $\Delta\Psi_m$ .**

This is the first demonstration that  $\Delta\Psi_m$  is a dual-ion electrochemical potential, integrating both

$H^+$  pumping by the ETC, and  $Na^+$  gradient formation via a built-in  $Na^+/H^+$  exchanger within Complex I. This fundamentally revises the classical chemiosmotic model by showing that Complex I is bifunctional: an NADH dehydrogenase and a  $Na^+/H^+$  exchanger shaping  $\Delta\Psi_m$ .

#### 4.2.4. pH Coupling

Plasma membrane transporters (NHE1, MCTs) regulate cytosolic pH.  $\Delta\Psi_m$  depends on matrix pH and  $\Delta pH$  across the IMM (inner mitochondrial membrane), because  $\Delta\Psi_m$  and  $\Delta pH$  together form the proton-motive force ( $\Delta p$ ) that drives ATP synthesis. Importantly,  $\Delta\Psi_m$  and  $\Delta pH$  contribute independently to the proton-motive force ( $\Delta p$ ) that drives ATP synthesis [260]. Acidic cytosol reduced  $\Delta\Psi_m$ ; alkaline cytosol led to increased  $\Delta\Psi_m$  [261][262]. The mechanism behind these events is the following:

**Acidic cytosol reduces  $\Delta\Psi_m$  because:**

Acidic cytosol increased  $H^+$  influx into mitochondria via  $H^+$ -coupled transporters

Matrix pH drops thus  $\Delta pH_m$  (differential pH in the mitochondria) decreases

To maintain  $\Delta\mu H^+$  (proton force),  $\Delta\Psi_m$  partially collapses

The net effect is reduced  $\Delta\Psi_m$

**Alkaline cytosol increases  $\Delta\Psi_m$**

Alkaline cytosol reduced  $H^+$  entry into mitochondria

Matrix pH rises and so does the  $\Delta pH_m$  increases

ETC increases proton pumping to rebalance  $\Delta\mu H^+$

The net effect is increased  $\Delta\Psi_m$

This is fully consistent with the chemiosmotic theory mentioned above.

### 4.3. Functional Consequences of the Coupling

**When the plasma membrane depolarizes:**

- $\text{Ca}^{2+}$  floods in and mitochondria take it up increasing  $\Delta\Psi_m$ , which in turn rises ATP production.
- But excessive  $\text{Ca}^{2+}$  can collapse  $\Delta\Psi_m$  (permeability transition). This is well-documented and mechanistically linked to mPTP opening, ROS amplification, and failure of oxidative phosphorylation [263][264][265]. This causes dissipation of  $\Delta\Psi_m$  and release of apoptotic factors.
- Overload also increases ROS, further destabilizing  $\Delta\Psi_m$ .

**When  $\Delta\Psi_m$  collapses:**

- ATP production stops, the  $\text{Na}^+/\text{K}^+$  pump fails and  $\Delta\Psi_p$  depolarizes.
- Cell becomes electrically silent which leads to apoptosis or necrosis.

**When cytosolic  $\text{Na}^+$  rises:**

- Mitochondrial  $\text{Na}^+/\text{H}^+$  exchanger activity changes,  $\Delta\Psi_m$  shifts. The strongest primary evidence is that the  $\text{Na}^+$  gradient across the inner mitochondrial membrane directly contributes to  $\Delta\Psi_m$ , and mNHE (the mitochondrial  $\text{Na}^+/\text{H}^+$  exchanger) controls this gradient [266].

### 4.4. Conceptual Summary

$\Delta\Psi_p$  and  $\Delta\Psi_m$  are not electrically continuous, but they are metabolically and ionically coupled.

Changes in  $\text{Ca}^{2+}$ , ATP/ADP ratio,  $\text{Na}^+$  gradients, and pH allow the plasma membrane potential to influence mitochondrial function and vice versa [267].

**In summary: The plasma membrane potential sets the ionic and energetic environment of the cytosol, while the mitochondrial membrane potential generates ATP and buffers  $\text{Ca}^{2+}$ ; the two potentials are linked through  $\text{Ca}^{2+}$  flux,  $\text{Na}^+$  gradients, ATP demand, and pH, forming a tightly integrated bioelectric network.**

The electrochemical network deregulation and the interaction between its components support the cancer process through various mechanisms:

Warburg metabolism increases glycolysis/lactate/ $\text{H}^+$  production, that when extruded collaborates in the generation of an acidic pHe. Alkaline pH<sub>i</sub> is maintained by proton extruders such as NHE1 whose activity is facilitated by depolarized  $V_{\text{mem}}$ .

Depolarized  $V_{mem}$  alters  $Ca^{2+}$  and other ion signaling (increased driving force or dysregulated influx) that promotes proliferation, migration, and survival pathways while reducing sensitivity to apoptotic signals (see Figure 6).

Reducing Eh enables ROS signaling at moderate levels (pro-proliferative) while preventing the oxidative threshold that triggers cell death. Aoyama & Nakaki explain <sup>[268]</sup> that glutathione (GSH) maintains intracellular redox homeostasis, allowing cells to tolerate and use ROS for signaling while preventing oxidative damage (see Figure 3)

Supporting NADPH for reductive biosynthesis and glutathione recycling; modulates pH regulators and channels.

All these elements converge on cell cycle progression: alkaline  $pH_i$  and depolarized  $V_{mem}$  favor G1/S and G2/M transitions; reducing Eh supports DNA synthesis and repair.

The network also promotes:

- Epithelial-to-mesenchymal transition (EMT), by modulating membrane potential, ion fluxes, and downstream transcriptional programs. Electrochemical networks, including membrane potential, ion fluxes ( $Ca^{2+}$ ,  $Na^+$ ,  $H^+$ ), and ROS-redox signaling, promote EMT by activating TGF- $\beta$ /EGF pathways, destabilizing epithelial polarity, and enabling migration/invasion <sup>[269][270][271][272][273]</sup>.
- Invasion (via pH-dependent proteases and adhesion remodeling), and immune evasion (acidic  $pH_e$  suppresses immune cells) <sup>[274][275]</sup>.

In normal proliferating cells, these parameters show dynamic oscillations coordinated with the cell cycle (e.g. transient  $pH_i$  shifts,  $V_{mem}$  hyperpolarization at G1/S). In cancer, they become constitutively dysregulated and less oscillatory, creating a stable "proliferative lock" with reduced mitochondrial activity, lower average ATP in some contexts, and heightened dependence on glycolysis.

## Section 5. Unified Frame: $pH$ , Eh, $\Delta\Psi_p$ , and $\Delta\Psi_m$ in One Thermodynamic System

The cell is a multi-compartment electrochemical machine where  $pH$ , Eh,  $\Delta\Psi_p$ , and  $\Delta\Psi_m$  are linked because protons and electrons are thermodynamically conjugate variables <sup>[276][277]</sup>. Where protons go, electrons follow, and vice versa <sup>[278][279]</sup>. The integrated analysis of the four players led to building the following conceptual frame:

### 5.1. $pH \leftrightarrow Eh$ (Nernst coupling)

Higher pH (lower  $[H^+]$ ) → Eh becomes more negative → stronger reductant

Lower pH -----→ Eh becomes more positive → weaker reductant

This is why cytosolic alkalinization strengthens NADH as a reductant and shifts metabolic flux. The mechanistic explanation is that higher pH shifts the  $NAD^+/NADH$  redox couple toward the reduced (NADH) state, increasing reductive pressure and driving metabolic flux toward pathways that consume NADH [280]. Going into more detail: at higher pH, protons are less available, shifting the equilibrium toward NADH, making it a stronger reductant. This increases the  $NADH/NAD^+$  ratio, which is the biochemical definition of reductive stress. NADH accumulation, affecting glucose, lipid, amino-acid, and nucleotide metabolism [281], including:

- increased flux through reductive biosynthetic pathways [282];
- inhibition of  $NAD^+$ -requiring dehydrogenases;
- diversion of carbon away from oxidative metabolism.

Another way of expressing the same concepts:

Higher cytosolic pH → stronger NADH reductive power (via Nernst shift).

Higher  $NADH/NAD^+$  ratio → reductive stress.

Reductive stress → metabolic reprogramming across glycolysis, lipid synthesis, amino-acid metabolism, and nucleotide synthesis.

### 5.2. $pH (\Delta pH) \leftrightarrow \Delta \Psi m$ (proton-motive force)

The proton-motive force (pmF) is the stored energy created when the electron transport chain pumps protons across the inner mitochondrial membrane, generating both a voltage ( $\Delta \Psi$ ) and a pH gradient ( $\Delta pH$ ). ATP synthase uses this combined electrochemical force to make ATP.

The electron transport chain (ETC) pumps protons from the matrix across the inner mitochondrial membrane (IMM) into the intermembrane space. Because the IMM is impermeable to protons, this

pumping creates two linked gradients:

- **An electrical gradient ( $\Delta\Psi$ )** — the matrix becomes negatively charged relative to the intermembrane space.
- **A chemical gradient ( $\Delta\text{pH}$ )** — the matrix becomes more alkaline than the intermembrane space.

Together, these form the **proton-motive force (pmF)**, the energy stored in the electrochemical separation of protons. This is explicitly described in the chemiosmotic hypothesis: the proton gradient stores potential energy that ATP synthase captures to convert ADP + Pi into ATP [283]

Matrix alkalization *increases the proton-motive force (pmF)* because  $\Delta\text{pH}$  is a formal component of  $\Delta p$ , and a more alkaline matrix increases the  $\Delta\text{pH}$  term [284].

|   |                                                                                                                     |
|---|---------------------------------------------------------------------------------------------------------------------|
| • | <b>Matrix alkalinity increases <math>\Delta\text{pH}</math></b>                                                     |
| • | <b>Cytosolic acidification decreases <math>\Delta\text{pH}</math></b>                                               |
| • | <b>Cytosolic alkalization increases <math>\Delta\Psi\text{m}</math> indirectly by reducing proton back-pressure</b> |

Recent work shows that  $\Delta\Psi\text{m}$  is also partly controlled by a **mitochondrial  $\text{Na}^+$  gradient** via a  $\text{Na}^+/\text{H}^+$  exchanger in complex I, meaning  $\Delta\Psi\text{m}$  is not purely protonic.

### 5.3. $\text{pH} \leftrightarrow \Delta\Psi\text{p}$ (plasma membrane potential)

The plasma membrane potential ( $\Delta\Psi\text{p}$ ):

- is generated mainly by  $\text{K}^+$  channels (K2P) and also by other ion channels. The  $\text{Na}^+/\text{K}^+$ -ATPase pump is a minor collaborator;
- is stabilized by  $\text{H}^+$  fluxes and proton-coupled transporters;
- is sensitive to extracellular pH because proton gradients influence antiporters and proton motive force generation in bacteria and eukaryotes.

Extracellular acidification, such as seen in cancer, depolarizes the plasma membrane by altering proton-dependent ion transport and lipid topology which, in turn, affects membrane potential ( $V_{\text{mem}}$ ) [285][286][287].

Thus:

- Extracellular acidification → depolarizes  $\Delta\Psi_p$
- Extracellular alkalization → hyperpolarizes  $\Delta\Psi_p$

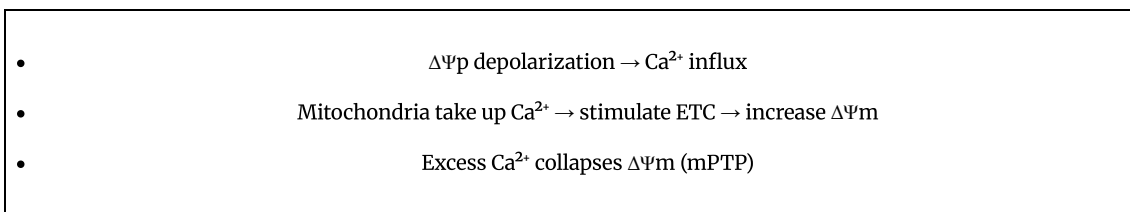
#### 5.4. $\Delta\Psi_p \leftrightarrow \Delta\Psi_m$ (ion-metabolic coupling)

These two potentials are electrically separate but functionally coupled through ATP supply, ion-transport energetic,  $\text{Ca}^{2+}$  signaling, and redox/ROS feedback.

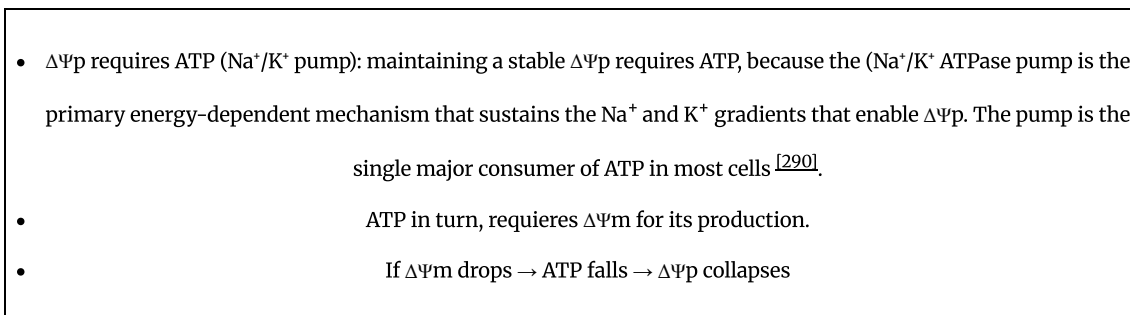
##### 5.4.1. $\text{Ca}^{2+}$ flux

Depolarization of the plasma membrane opens voltage-gated  $\text{Ca}^{2+}$  channels, increasing cytosolic  $\text{Ca}^{2+}$ . Mitochondria rapidly take up  $\text{Ca}^{2+}$ , and Gottschalk et al. [253] showed that mitochondrial  $\text{Ca}^{2+}$  elevation hyperpolarizes crista membranes by stimulating TCA cycle flux and OXPHOS.

$\Delta\Psi_m$  is influenced not only by protons but also by other ions transported across the IMM such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ . Changes in  $\Delta\Psi_p$  that alter cytosolic  $\text{Na}^+$ ,  $\text{H}^+$ , or  $\text{Ca}^{2+}$  directly modify the ionic environment feeding mitochondrial exchangers that introduce  $\Delta\Psi_m$  shifts [288][289].



##### 5.4.2. ATP demand



### 5.4.3. $\text{Na}^+$ gradients

- $\Delta\Psi_p$  determines cytosolic  $\text{Na}^+$  (This statement is an oversimplification; in reality, cytosolic  $[\text{Na}^+]$  is determined by a balance of  $\text{Na}^+$  influx (via channels, cotransporters, exchangers) and  $\text{Na}^+$  efflux (mainly  $\text{Na}^+/\text{K}^+$ -ATPase), modulated by the plasma-membrane potential ( $\Delta\Psi_p$ ) [291].
- Cytosolic  $\text{Na}^+$  influences mitochondrial  $\text{Na}^+/\text{H}^+$  exchange (elevated cytosolic  $\text{Na}^+$  does not directly “drive” the mitochondrial  $\text{Na}^+/\text{H}^+$  exchanger (mNHE) in the same way it drives the mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCLX), but it indirectly influences mitochondrial  $\text{Na}^+$  and  $\text{H}^+$  handling by altering the  $\text{Na}^+$  gradient and pH regulation across the inner membrane) [292].
- This directly modulates  $\Delta\Psi_m$

### 5.4.4. ROS-based coupling of $\Delta\Psi_p$ and $\Delta\Psi_m$

ROS modulate  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$  channels at the plasma membrane by oxidizing lipids and channel cysteines, altering their gating and thereby shifting  $\Delta\Psi_p$  [293][294][295].

High  $\Delta\Psi_m \rightarrow \uparrow \text{ROS} \rightarrow \text{oxidation of PM channels} \rightarrow \Delta\Psi_p \text{ depolarization or hyperpolarization depending on channel type.}$

### 5.5. $E_h \leftrightarrow \Delta\Psi_m$ (electron transport chain)

The ETC converts redox potential differences ( $E_h$ ) into electrical potential ( $\Delta\Psi_m$ ) across the inner mitochondrial membrane. As electrons flow from NADH or  $\text{FADH}_2$  to  $\text{O}_2$  through Complexes I  $\rightarrow$  III  $\rightarrow$  IV, each step involves a drop in redox potential ( $E_h$ ).

This redox energy is equivalent to a Gibbs free-energy decrease, which can be converted into electrical work [296][297][298].

- NADH/ $\text{NAD}^+$  and Q/ $\text{QH}_2$  redox couples donate electrons
- Electron flow pumps protons  $\rightarrow$  generates  $\Delta\Psi_m$
- $\Delta\Psi_m$  then drives ATP synthesis

Thus:

- More negative Eh (stronger reductants) → higher  $\Delta\Psi_m$
- Oxidized redox pools → lower  $\Delta\Psi_m$

Another way of looking at the same events consists of:

- Electrons flow from low Eh (NADH) to high Eh ( $O_2$ ).
- This redox drop (-1.1 V) provides free energy.
- Complexes I, III, IV convert this energy into vectorial proton pumping.
- Proton pumping creates charge separation across the IMM.
- Charge separation = electrical potential ( $\Delta\Psi_m$ ).
- Therefore: the ETC is a redox-to-electrical energy converter.

### 5.6. $Eh \leftrightarrow \Delta\Psi_p$ (redox-electrical coupling)

Redox state influences  $\Delta\Psi_p$  indirectly by modulating ROS production, redox-sensitive ion channels, and redox-regulated signaling at the plasma membrane. The key point is that redox changes do not directly alter  $\Delta\Psi_p$  the way ion flux does, but they reshape the activity of the proteins that set  $\Delta\Psi_p$  [299][300][301][302].

- NADH availability controls ATP production
- ATP powers  $Na^+/K^+$ -ATPase
- Pump activity determines  $\Delta\Psi_p$
- ROS oxidize cysteine residues on ion channels
- This oxidation changes channels gating, altering ion flux and thus shifting  $\Delta\Psi_p$ .

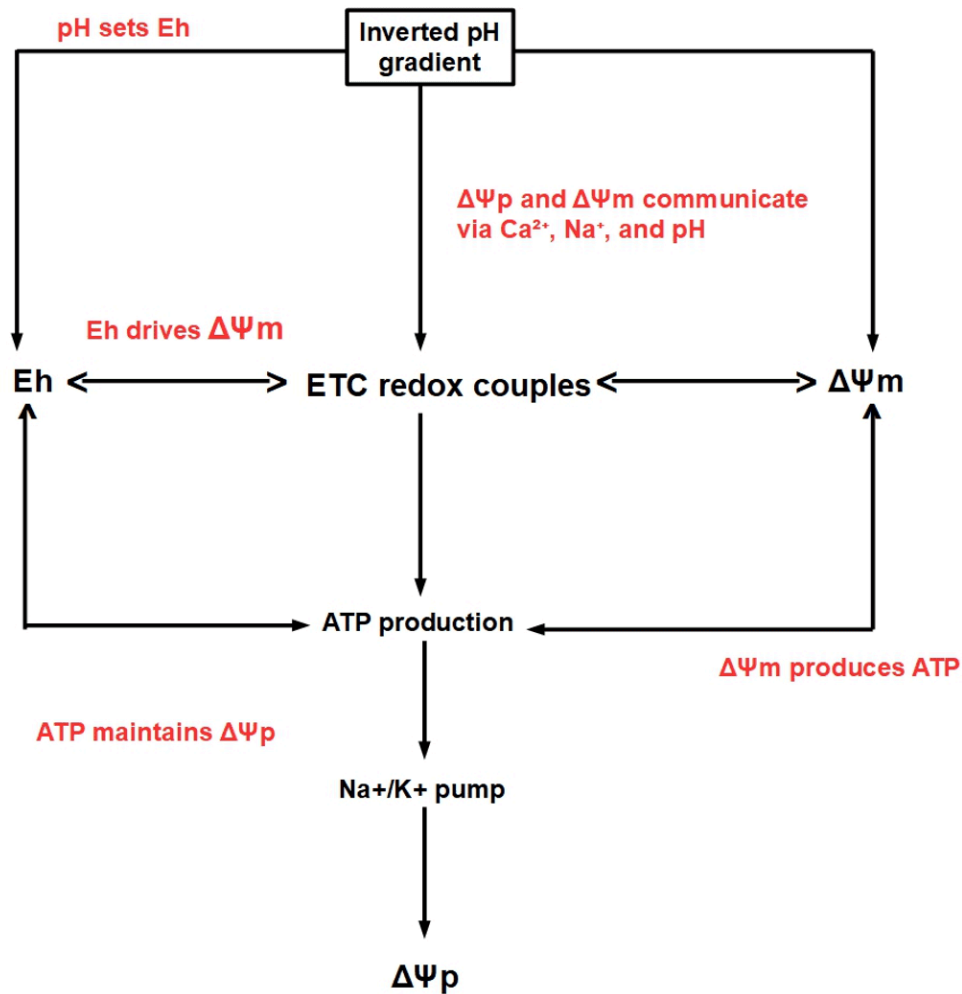
Thus:

- Reduced cytosol (high NADH) → strong  $\Delta\Psi_p$
- Oxidized cytosol → weak  $\Delta\Psi_p$

Redox state influences  $\Delta\Psi_p$  through metabolic coupling. Redox state is tightly linked to metabolic reprogramming and ROS homeostasis <sup>[303]</sup>.

Figures 12 and 13 summarize these relational facts.

## Integrating pH, Eh, $\Delta\psi_m$ , and $\Delta\psi_p$ in a single bioenergetic frame



pH sets redox potential (Eh), Eh drives mitochondrial  $\Delta\psi_m$  through the ETC,  $\Delta\psi_m$  generates ATP that sustains  $\Delta\psi_p$ , and  $\Delta\psi_p$  feeds back to  $\Delta\psi_m$  through  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , and pH — forming a single integrated bioenergetic network.

Figure 12. Integrating the electrochemical network in a bioenergetic framework

## Intracellular electrochemical relationship network

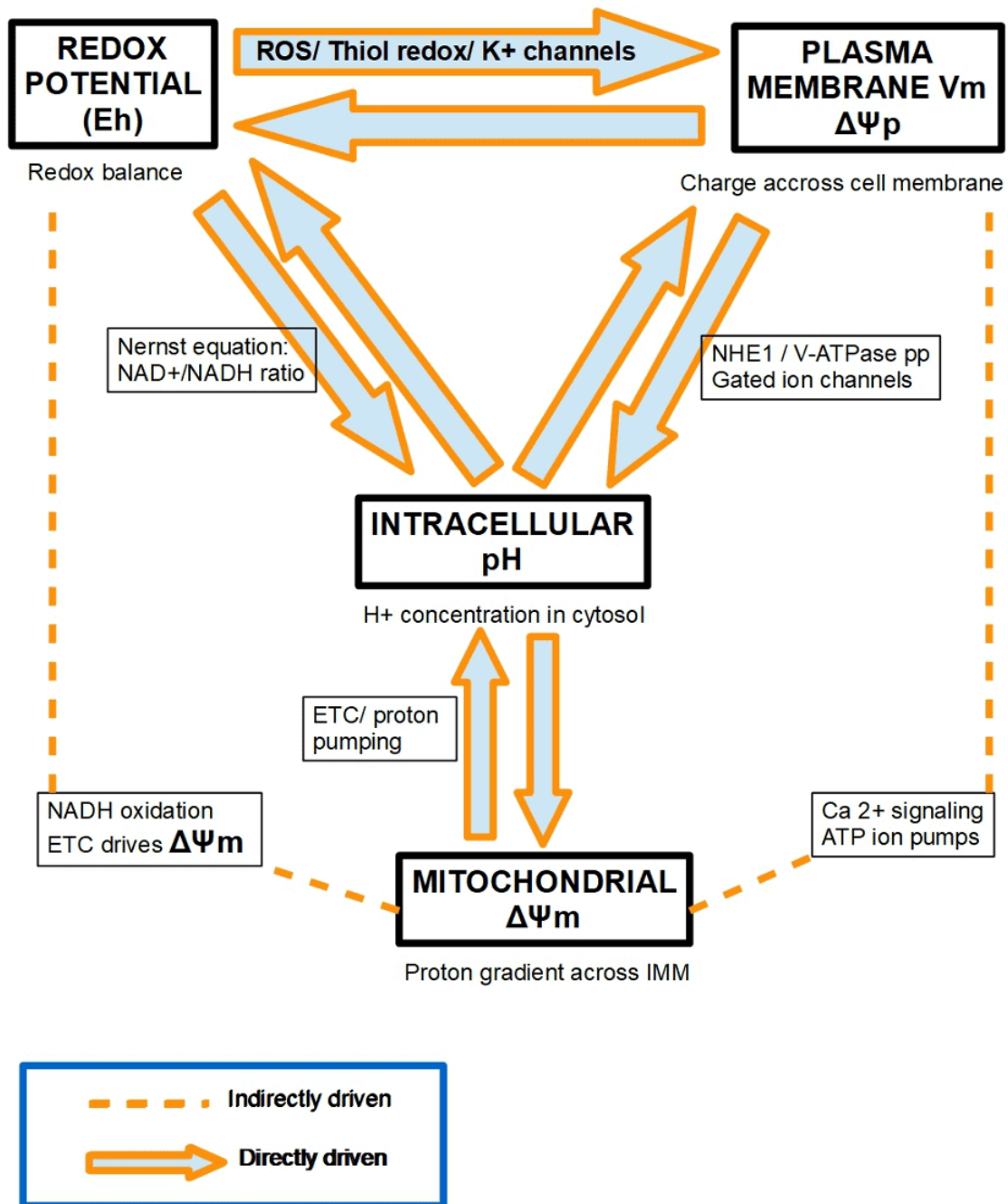


Figure 13. Another view of the same integration of the intracellular electrochemical network.

### In summary

**Proliferative state:** Depolarized  $V_{mem}$  + reducing  $E_h$  (more negative redox potential) → favors growth and cell-cycle progression.

**Quiescent/differentiated state:** Hyperpolarized  $V_{mem}$  + relatively oxidizing  $E_h$  → supports stability and specialized function.

These are correlated but not strictly causal in one direction; they form integrated **bioelectric-redox networks** that complement cyclin/CDK biochemical oscillations.

**Intracellular pH ↔  $E_h$  (redox potential)** These two are deeply coupled via the Nernst equation. Every unit change in pH shifts  $E_h$  by ~59 mV. Essentially, the  $NAD^+/NADH$  and  $GSH/GSSG$  ratios link them: more reducing conditions (lower  $E_h$ ) tend to alkalinize the cytosol, and vice versa. Metabolic states (aerobic vs. anaerobic) shift both simultaneously.

**Intracellular pH ↔ Plasma membrane  $V_m$**   $H^+$  is itself a charged ion, so pH and membrane potential are electrically coupled. The  $Na^+/H^+$  exchanger (NHE) and  $H^+$ -ATPases use or generate membrane voltage to move protons. Conversely, cytosolic acidification can directly open or close voltage-sensitive and pH-sensitive ion channels, altering  $V_m$ .

**Intracellular pH ↔ Mitochondrial  $\Delta\Psi_m$**  The inner mitochondrial membrane maintains a large proton-motive force ( $pmf = \Delta\Psi_m + \Delta pH$ ). When the cytosolic pH drops (acidosis), proton backflow into the mitochondrial matrix and can collapse  $\Delta\Psi_m$ . Conversely, ETC-driven proton pumping alkalinizes the mitochondrial matrix and directly contributes to cytosolic acid load. The mitochondrial permeability transition pore (mPTP) is exquisitely sensitive to pH. The mPTP opening is strongly inhibited by acidic pH and sensitized by alkaline pH <sup>[304][305][306]</sup>.

**$E_h$  ↔ Mitochondrial  $\Delta\Psi_m$**  Linked indirectly through the electron transport chain: NADH oxidation at Complex I feeds electrons into the ETC, which drives proton pumping and therefore generates  $\Delta\Psi_m$ . A collapse of  $\Delta\Psi_m$  leads to reverse electron transport and ROS production, shifting  $E_h$  toward oxidized states.

**$V_m$  ↔ Mitochondrial  $\Delta\Psi_m$**  Connected primarily through  $Ca^{2+}$  signaling and ATP supply. Depolarization of the plasma membrane (e.g. action potentials) triggers  $Ca^{2+}$  entry, which is taken up by mitochondria and stimulates ETC, transiently boosting  $\Delta\Psi_m$ . ATP produced by  $\Delta\Psi_m$ -driven ATP synthase then fuels the  $Na^+/K^+$ -ATPase, restoring  $V_m$ .

**$E_h$  ↔ Plasma membrane  $V_m$**  ROS generated by a shifted  $E_h$  can oxidize cysteine residues on  $K^+$  and  $Ca^{2+}$  channels, opening or closing them and directly altering  $V_m$ . Some voltage-gated channels (e.g. Kv) carry thiol-sensing domains, making this a bidirectional redox-electrical loop.

## Section 6. Voltage-Gated Ion Channel Dysregulation in the electrochemical network [307]

The oncogenic remodeling of voltage-gated channel expression is so consistent that it has prompted the term "channelome reprogramming." Key observations include:

**Sodium channels (NaVs):** NaV1.5 (SCN5A) is over-expressed in strongly metastatic breast cancer cells and in other tumors. NaV1.5 differs from other voltage-gated sodium channels (VGSCs) in its structure, regulation, tissue distribution, gating behavior, and disease associations. NaV1.5 functional and structural differences make it more sensitive to anti-arrhythmic drugs (e.g. flecainide) [308][309]. Unlike other VGSCs, NaV1.5 (especially the neonatal splice variant) is strongly involved in cancer invasion and metastasis, particularly in breast and prostate cancers.

NaV activity promotes invadopodium formation, extracellular matrix degradation via cysteine cathepsin activation, and epithelial-mesenchymal transition (EMT). The mechanism involves channel-independent signaling through the NaV1.5 C-terminal tail, which interacts with the Nedd4-2 ubiquitin ligase and multiple scaffolding proteins. NaV1.7 (SCN9A) promotes prostate cancer invasiveness through a similar mechanism involving pH dysregulation [310][311][312][313]. Increased NaV1.5 expression has been related to increased migration and metastasis [314][315][316][317]. Furthermore, NaV1.5 regulates EMT transcription factors. However, NaV1.5 also seems to play a different role in the electrochemical network: it acts not as an action-potential generator, but as a driver of metastatic bioelectric remodeling, specifically by enhancing Na<sup>+</sup> influx, indirectly stimulating H<sup>+</sup> efflux (through NHE1 because NaV1.5 does not conduct protons[318]), acidifying the pericellular space, and activating EMT and invasion programs. NaV1.5 does not act as an action-potential generator in cancer. Instead, it is a bioelectric amplifier that rewires Na<sup>+</sup>/H<sup>+</sup> coupling.

**Potassium channels:** The hERG channel (KCNH2) is aberrantly expressed in colorectal, gastric, and hematologic cancers, contributing to cell cycle progression through G1/S. EAG1 (KCNH1) promotes tumor cell proliferation by interacting with focal adhesion kinase (FAK) and regulating integrin signaling [319][320][321]. The small-conductance calcium-activated K<sup>+</sup> channel SK3 mediates breast and colon cancer cell migration by colocalizing with TRPC1 channels to form a calcium-entry/potassium-efflux module in lipid rafts [322].

**Calcium channels:** Dysregulation of  $\text{Ca}^{2+}$  homeostasis is ubiquitous in cancer. TRPV1, TRPM7, TRPM8, and ORAI1 (the pore subunit of SOCE) are all involved in cancer-specific  $\text{Ca}^{2+}$  signaling. Aberrant  $\text{Ca}^{2+}$  influx activates calcineurin/NFAT pathways, promotes VEGF production and angiogenesis, and desensitizes the mitochondrial permeability transition pore to apoptotic stimuli [\[323\]](#)[\[324\]](#)[\[325\]](#).

**Chloride channels:** TMEM16A (ANO1) is amplified in head and neck squamous cell carcinoma (chromosome 11q13 amplicon) and activates EGFR signaling independently of ion conduction. CLC-3 chloride channels govern regulatory volume decrease (RVD) and are required for the cell shrinkage that facilitates apoptosis. Their loss contributes to apoptotic resistance in glioblastoma [\[326\]](#).

## Section 7. Therapeutic and Pathophysiological Implications

This integrated tetrad (sections 1, 2, 3, 4) explains cancer's robustness: targeting one element (e.g. forcing  $V_{\text{mem}}$  hyperpolarization via  $\text{K}^+$  channel openers, acidifying  $\text{pH}_i$  via NHE1/V-ATPase inhibitors, or oxidizing Eh with pro-oxidants) can disrupt the others, leading to proliferation arrest, apoptosis, or sensitization to chemotherapy.

Simultaneously targeting intracellular pH ( $\text{pH}_i$ ), plasma membrane potential ( $V_m$ ), redox potential (Eh), and mitochondrial membrane potential ( $\Delta\Psi_m$ ) in cancer cells leverages their tight electrochemical interdependence. Cancer cells characteristically show alkaline  $\text{pH}_i$  (~7.1-7.6 vs. ~7.0-7.2 normal), depolarized  $V_m$  (less negative resting potential), altered Eh (often with elevated ROS or reductive stress supporting survival), and hyperpolarized  $\Delta\Psi_m$  (more negative, ~-180 to -220 mV vs. ~-140 to -160 mV normal). These drive proliferation, invasion, and therapy resistance via the electrochemical network. For example:

- alkaline  $\text{pH}_i$  maximizes proliferation in models; lowering it inhibits growth [\[327\]](#)[\[328\]](#);
- depolarized  $V_{\text{mem}}$  correlates with aggressiveness [\[329\]](#); normalizing it can restore differentiation cues or gap-junction communication;
- the reducing Eh and alkaline  $\text{pH}_i$  enhance uptake or effects of certain weak-acid drugs (e.g. ascorbate) [\[330\]](#).

Cancer cells' reliance on this network makes them selectively vulnerable compared to normal cells with hyperpolarized  $V_{\text{mem}}$ , near-neutral  $\text{pH}_i$ , and balanced redox.

Disrupting one often cascades to others (e.g.  $\Delta\Psi_m$  collapse increases mitochondrial ROS → Eh shift and  $\text{pH}_i$  acidification;  $\text{pH}_i$  drop impairs ion pumps →  $V_m$  change). Most strategies are preclinical/experimental (ionophores, uncouplers, mito-targeted conjugates, or multi-responsive nanocarriers). They exploit

cancer-specific hallmarks for selectivity (e.g. hyperpolarized  $\Delta\Psi_m$  drives uptake of lipophilic cations). No single approved therapy does this clinically and combinations or advanced delivery systems are under study.

Many authors, including us, have been targeting the inverted pH gradient with the hope of inhibiting growth and inducing apoptosis mainly through intracellular acidification or extracellular alkalization [331][332][333][334][335][336][337][338][339]. Although some minor successes have been achieved, manipulating the inverted pH gradient seems insufficient for this purpose. The facts exposed above led us to believe that targeting cancer cell physiopathology requires an integral attack on its electrochemical network. We also believe that there are drugs that can contribute to this endeavor.

### *7.1. Therapeutic implications*

We, and others, have proposed blocking proton export as a mechanism to attack cancer hallmarks. This type of targeting has shown some encouraging results in vitro and in vivo. However, all attempts by inhibiting the proton extrusion mechanisms have failed to yield significant and long term results. Regarding patients, stable disease was achieved in a few cases, but there was no evidence of complete or partial response according to RECIST criteria [340].

It is evident that blocking proton extrusion as the sole targeting mechanism is insufficient for achieving significant results. Considering the Nernst equation, it seems that targeting intracellular pH needs to be complemented by increasing intracellular oxidative stress and also acting on  $\Delta\Psi_p$  and  $\Delta\Psi_m$ .

The  $\Delta\Psi_m$  - $V_{mem}$ - $E_h$ - $pH_i$  tetrad in cancer cells creates a self-reinforcing physiopathologic network that supports proliferation, metabolic reprogramming (Warburg effect), invasion, metastasis, apoptosis resistance, and therapy evasion. Therapeutic targeting aims to disrupt this network by normalizing one component (or even more) which often cascades to destabilize the others due to tight interconnections (e.g. depolarized  $V_{mem}$  facilitates acid extrusion that maintains alkaline  $pH_i$ ; reducing  $E_h$  supports NADPH-dependent proton handling and ion channel modulation).

Therefore, we believe that the whole electrochemical tetrad needs to be targeted in an integral and coordinated manner. In this regard, there are many possibilities that we shall analyze below.

#### *7.1.1. Targeting Depolarized $V_{mem}$ ( $\Delta\Psi_p$ ) (Bioelectric Normalization)*

Cancer cells maintain a persistently depolarized plasma membrane potential (less negative, often -5 to -40 mV), which reduces the electrochemical driving force for  $H^+$  influx, helps maintain alkaline  $pH_i$ , and

modulates  $\text{Ca}^{2+}$ /ROS signaling for growth. Table 4

| Cell Type                      | Typical Vmem (mV) | Notes                                                            |
|--------------------------------|-------------------|------------------------------------------------------------------|
| Normal epithelial cells        | -60 to -75        | Highly polarized low proliferation                               |
| Stem cells / progenitors       | -40 to -55        | Intermediate polarization high plasticity                        |
| Cancer cells (solid tumors)    | -10 to -30        | Strongly depolarized correlates with proliferation and migration |
| Highly metastatic cancer cells | 0 to -20          | Extreme depolarization high NaV/CaV activity                     |

**Table 4.** Vmem in different cells

#### Strategies:

**K<sup>+</sup> channel modulation:** Open or up-regulate certain K<sup>+</sup> channels (e.g. Kv1.5) to increase K<sup>+</sup> efflux and drive hyperpolarization (more negative Vmem). **Dichloroacetate (DCA)** indirectly achieves this by reversing mitochondrial hyperpolarization and restoring Kv channel expression, selectively promoting apoptosis in cancer cells without harming normal cells. Dichloroacetate is a functional modulator of Kv channels, most clearly demonstrated for Kv2.1 in pulmonary artery smooth muscle cells. These effects occur independently of DCA's canonical metabolic action on pyruvate dehydrogenase, indicating a non-metabolic signaling mechanism linking DCA to Kv channel modulation [341][342]. According to Bonnet et al. a mitochondrial K<sup>+</sup> channel axis is abolished in cancer [343]. Its normalization not only suppresses growth but induces apoptosis. This landmark paper by Bonnet et al. shows that DCA activates Kv currents and up-regulates Kv1.5 expression (NFAT1-dependent) in cancer cells (but not normal cells). This occurs via decreased  $\Delta\Psi_m$ , increased mitochondrial  $\text{H}_2\text{O}_2$ , and a positive feedback loop involving reduced  $[\text{Ca}^{2+}]$  ions. DCA induces apoptosis selectively in cancer cells with high  $\Delta\Psi_m$  and low Kv1.5. siRNA against PDK2 mimics the effect. DCA's effects on Kv are linked to redox balance (oxidized state favoring Kv activity) and are observed in contexts like glioblastoma, endometrial cancer, and vascular remodeling [344]. DCA is not a classic direct Kv opener/blocker (like 4-aminopyridine or NS1643 analogs)

but exerts its Kv-modulating effects downstream of PDK inhibition and mitochondrial remodeling. For a review on potassium channels in cancer see [\[345\]](#).

**Inhibition of depolarizing channels:** Blocking voltage-gated or other channels (e.g. certain Na<sup>+</sup>, Ca<sup>2+</sup>, or Cl<sup>-</sup> channels) or EAG/HERG family members that contribute to the depolarized state in specific cancers can arrest proliferation and enhance sensitivity to chemotherapy.

**Ion channel blockers overall:** Many repurposed drugs (e.g. astemizole for EAG1 (Kv11.1)) show preclinical efficacy in reducing tumor growth and invasiveness. Cryo-EM work directly visualizes astemizole bound inside the hERG pore, confirming its role as a high-affinity channel blocker and stabilizing a non-conducting state [\[346\]\[347\]\[348\]\[349\]](#). Multiple studies demonstrated astemizole's effects on proliferation, apoptosis, tumor progression, and synergy with other anticancer drugs [\[350\]\[351\]](#). Some of astemizole's anti-cancer effects seem to be independent of EAG1 inhibition [\[352\]\[353\]\[354\]\[355\]](#). Importantly, astemizole synergizes with another K<sup>+</sup> channel acting drug such as calcitriol [\[356\]](#).

**Cascade effects:** Hyperpolarization can increase the driving force for H<sup>+</sup> influx (acidifying pHi) and alter redox-sensitive channel activity, shifting Eh toward oxidizing conditions that favor apoptosis.

### *7.1.2. Targeting Alkaline pHi (Acidification Strategies)*

Cancer cells exhibit constitutively alkaline intracellular pH (pHi ~7.1-7.7 vs. ~7.0-7.2 in normal cells), driven by up-regulated proton extruders. This alkalinity optimizes glycolysis, inhibits apoptosis, and supports migration.

#### **Key targets and agents:**

- **NHE1 (Na<sup>+</sup>/H<sup>+</sup> exchanger 1) inhibitors:** Amiloride, cariporide, or newer compounds acidify pHi, induce apoptosis, and sensitize cells to drugs like lapatinib or temozolomide. Combinations with mild systemic alkalization have shown promise in models like clear cell renal cell carcinoma by exploiting differential pH handling. The most relevant evidence comes from studies combining mild alkaline treatment with pH-regulating transport inhibition, particularly NHE1, which is strongly up-regulated in ccRCC [\[357\]](#).
- **Mild systemic alkalization** reduces extracellular proton concentration (↑pHe). Because cancer cells rely heavily on NHE1 and other proton exporters to maintain an alkaline pHi, raising pHe reduces the proton gradient they normally exploit [\[358\]](#).

- **V-ATPase inhibitors:** Bafilomycin A1, concanamycin A, archazolid, or clinical-stage agents (e.g. NiK12192, saliphenylhalamide). These block vacuolar proton pumping, disrupt lysosomal pH, inhibit autophagy, and reduce invasion/metastasis. Preclinical and early-phase trials explore them in lung, breast, and other cancers, and often in combination to overcome resistance.
- **Other proton regulators:** MCT (monocarboxylate transporter) or carbonic anhydrase inhibitors to impair acid extrusion.

**Cascade effects:** pHi acidification often causes membrane depolarization (via pH-sensitive K<sup>+</sup> channels), which can further stress redox balance by impairing NADPH production or increasing ROS <sup>[359][360]</sup>.

### 7.1.3. Targeting Reducing Eh (Redox Modulation)

Cancer cells maintain a more reducing intracellular environment (more negative Eh for couples like GSH/GSSG or NADPH/NADP<sup>+</sup>), supporting biosynthesis, ROS detoxification at moderate levels (pro-proliferative signaling), and resistance to oxidative stress.

#### Strategies:

**Pro-oxidant therapies:** High-dose ascorbate (vitamin C) exploits the triad, its uptake and oxidation are favored by depolarized V<sub>mem</sub> and alkaline pHi in cancer cells, leading to selective H<sub>2</sub>O<sub>2</sub> generation, glutathione depletion, and ferroptosis-like death while sparing normal cells (which have hyperpolarized V<sub>mem</sub> and balanced pHi) <sup>[361]</sup>. Although a clinical trial with high dose vitamin C failed to show benefits in advanced cancer <sup>[362]</sup>, we think that the cause of failure is attributable to the stand-alone approach. We insist that the electrochemical network targeting requires a multipronged scheme.

**Antioxidant system inhibitors:** Target glutathione (e.g. via xCT/SLC7A11 inhibitors), thioredoxin, or GPX4 to overwhelm reductive capacity and induce lethal oxidative stress. Nrf2 pathway modulators or agents like arsenic trioxide also tip the balance <sup>[363][364]</sup>.

**Redox-active compounds:** Manganese porphyrins <sup>[365]</sup> or other metal complexes that generate ROS or disrupt redox enzymes selectively in cancer. See also Figure 14 prooxidants.

**Cascade effects:** Oxidizing Eh shifts can inhibit pH regulators (many are redox-sensitive), alter ion channel gating, and promote V<sub>mem</sub> changes that favor apoptosis.

#### 7.1.4. Targeting $\Delta\Psi_m$ (Mitochondria-Targeted Lipophilic Cations (TPP<sup>+</sup> Conjugates) – Exploit Hyperpolarized $\Delta\Psi_m$ for Selective Multi-Parameter Disruption)

Triphenylphosphonium (TPP<sup>+</sup>) cations accumulate 100–500-fold in cancer mitochondria due to the Nernstian drive from hyperpolarized  $\Delta\Psi_m$  (and  $V_m$  plasma contribution). Once inside, conjugates release payloads or act directly as ionophores/uncouplers, hitting  $\Delta\Psi_m$ ,  $E_h$  (via mtROS),  $pH_i$  (proton/ion shuttling), and  $V_m$  (via ion homeostasis disruption).

##### Key examples and mechanisms:

- **TPP<sup>+</sup>-conjugated ionophores** (e.g. salinomycin or monensin derivatives): These polyether ionophores transport K<sup>+</sup>/H<sup>+</sup> across membranes. Mitochondrial accumulation dissipates  $\Delta\Psi_m$  (depolarization), perturbs proton gradients (acidifies mitochondrial matrix →  $pH_i$  effects), generates mtROS ( $E_h$  oxidation/shift), and alters cytosolic ion balance (indirect  $V_m$  depolarization). Enhanced cytotoxicity and selectivity vs. parent compounds; some promote mtROS explicitly [\[366\]\[367\]\[368\]\[369\]](#).
- **Mito-DCA** (TPP<sup>+</sup>-linked dichloroacetate scaffold): TPP drives mito uptake → esterase releases DCA, inhibiting PDK1. This shifts metabolism from glycolysis to oxidation, collapsing hyperpolarized  $\Delta\Psi_m$  (depolarization confirmed by JC-1/TMRM assays), reducing lactate (normalizes alkaline  $pH_i$  indirectly), lowering ATP, and increasing ROS in malignant cells ( $E_h$  perturbation). Far more potent than free DCA; selective for cancer mitochondria [\[370\]\[371\]](#).
- **TPP<sup>+</sup>-uncouplers** (e.g. TPP+C10, GA-TPP+C10): Mild uncoupling dissipates  $\Delta\Psi_m$ , oxidizes NAD(P)H ( $E_h$  shift), reduces ATP, and opens mPTP. Synergistic with other agents; effective in breast/colon models [\[372\]\[373\]\[374\]](#).

These provide built-in simultaneity via  $\Delta\Psi_m$ -driven targeting + direct ion/redox effects.

#### 7.1.5. Protonophores: Direct Proton Gradient and Electrochemical Disruption

Protonophores, mitochondrial uncouplers, and UCP inhibitors each modulate mitochondrial bioenergetics in distinct ways, sometimes pro-tumorigenic, sometimes anti-tumorigenic, depending on context.

Some of these protonophores (e.g. FCCP, CCCP, niclosamide) can shuttle H<sup>+</sup> across the inner mitochondrial membrane (IMM), collapsing the proton motive force and reducing ATP production. [\[375\]\[376\]](#).

They dissipate  $\Delta p$ , forcing cells to increase oxygen consumption and glycolysis. They can reduce ROS by lowering electron pressure on the ETC, or increase ROS if ETC flux becomes unstable. They often trigger

AMPK activation, autophagy, and cell death in metabolically fragile tumors.

**How they hit all parameters:**

- **Depolarize  $\Delta\Psi_m$**  (primary action).
- **Acidify pHi** (proton influx/leak modulation; counter alkaline pHi hallmark).
- **Shift Eh** (increased mtROS or NAD(P)H oxidation; deplete GSH).
- **Indirect Vm effects** (via ATP drop impairing Na<sup>+</sup>/K<sup>+</sup>-ATPase or ion exchangers).

**Examples:**

- Classic/protonophores (DNP, FCCP, CCCP, BAM15): Uncouple OXPHOS →  $\Delta\Psi_m$  dissipation, ROS burst (Eh), pHi drop. Cancer-selective toxicity in some models; BAM15 milder/systemic potential.
- **Bo-Mt-Ge** (Gardenia-derived UCP2 inhibitor): Blocks uncoupling protein 2 (over-expressed in CSCs, promotes H<sup>+</sup> leaks). Cuts H<sup>+</sup> influx → regulates/stabilizes electrochemical gradient ( $\Delta\Psi_m$  effect), depletes GSH (Eh oxidation), synergizes with apoptosis. Selective for CSCs via UCP2-stemness link; down-regulates resistance factors (P-gp, Bcl-2).

Combinations (e.g. uncoupler + NHE1 inhibitor like cariporide) amplify NHE1 blockade acidifies pHi directly → mito fragmentation/ROS ( $\Delta\Psi_m$ /Eh) while uncoupler adds proton leak.

### *7.1.6. Multi-Responsive Nanocarriers or Dual-Targeted Systems – Engineered Simultaneity*

Nanoparticles responsive to multiple cues (pH, redox,  $\Delta\Psi_m$ ) release payloads that reinforce effects across parameters.

- pH/redox/ $\Delta\Psi_m$ -sensitive designs accumulate in tumors (acidic pHe, high ROS, hyper- $\Delta\Psi_m$ ), then release ion channel modulators, protonophores, or redox catalysts inside cancer cells/mito.
- Dual mito/nucleus-targeting (e.g. mitaplatin conjugates): Alters  $\Delta\Psi_m$  (cytochrome c release) while hitting nuclear DNA; secondary pHi/Eh via apoptosis signaling.
- Examples include TPP<sup>+</sup>-dendrimers or probes that track/perturb pHi +  $\Delta\Psi_m$  + ROS simultaneously.

### *7.1.7. Bioelectric and Electrical Field Approaches – Non-Pharmacological Network-Wide Modulation*

External fields or electroceuticals can perturb the entire electrochemical network at once.

- **Tumor-treating fields (TTFields) or modulated electro-hyperthermia (mEHT):** Alter  $V_m$  (depolarize), ion fluxes (pHi via exchangers),  $Ca^{2+}$ /ROS (Eh), and  $\Delta\Psi_m$  indirectly. Cancer cells (with altered excitability) are more sensitive [377].
- **Periodic impulse electrostimulation (IES):** Induces MMP ( $\Delta\Psi_m$ ) oscillations with self-healing responses; cancer cells show exaggerated effects vs. normal. Can couple to pHi/redox via ion dynamics.
- **Direct current electrochemical therapy (EChT, from prior discussion):** Local pH extremes + ion/redox shifts +  $V_m$  disruption.

## 7.2. Challenges, Selectivity, and Outlook

- **Interconnectivity enables synergy:** e.g.  $V_m$  depolarization → reduced driving force for pHi regulators → acidification → mito  $Ca^{2+}$ /ROS overload →  $\Delta\Psi_m$  collapse/Eh shift.
- **Selectivity:** Cancer hyper- $\Delta\Psi_m$ , alkaline pHi, and high ROS create therapeutic windows; normal cells resist better.
- **Monitoring:** Use TMRM/JC-1 ( $\Delta\Psi_m$ ), pH-sensitive dyes, roGFP (redox/Eh), and voltage dyes for  $V_m$ .
- Most are preclinical (cell lines, xenografts); some TPP conjugates/uncouplers are in advanced testing. Clinical translation often via repurposing (DCA analogs, ionophores) or trials combining with standard care.

## 7.3. Integrated/Multi-Target Approaches and Synergies

Single-targeting often faces compensatory mechanisms, so combination strategies are most promising:

- **pHi +  $V_{mem}$ :** NHE1/V-ATPase inhibition +  $K^+$  channel modulation to simultaneously acidify cytosol and hyperpolarize membrane.
- **Redox + pHi:** Pro-oxidants (e.g. ascorbate) with proton pump inhibitors—alkaline pHi and depolarized  $V_{mem}$  enhance selective drug accumulation and ROS toxicity.
- **Triad-disrupting agents:** DCA (mitochondrial/redox/ $V_{mem}$ ) or repurposed ion channel drugs combined with pH/redox modulators.
- **Nanoparticle or smart delivery:** pH- and redox-responsive polymers or nanoparticles that release payloads in the acidic tumor microenvironment (low pHe) or upon intracellular redox shifts, improving specificity.
- **Adjuvant use:** These approaches sensitize tumors to chemotherapy, radiotherapy, or immunotherapy by disrupting the protective triad (e.g. normalizing pHi/ $V_{mem}$  restores gap junctions or immune

recognition; oxidizing Eh reduces antioxidant defenses).

## Targeting the intracellular electrochemical network

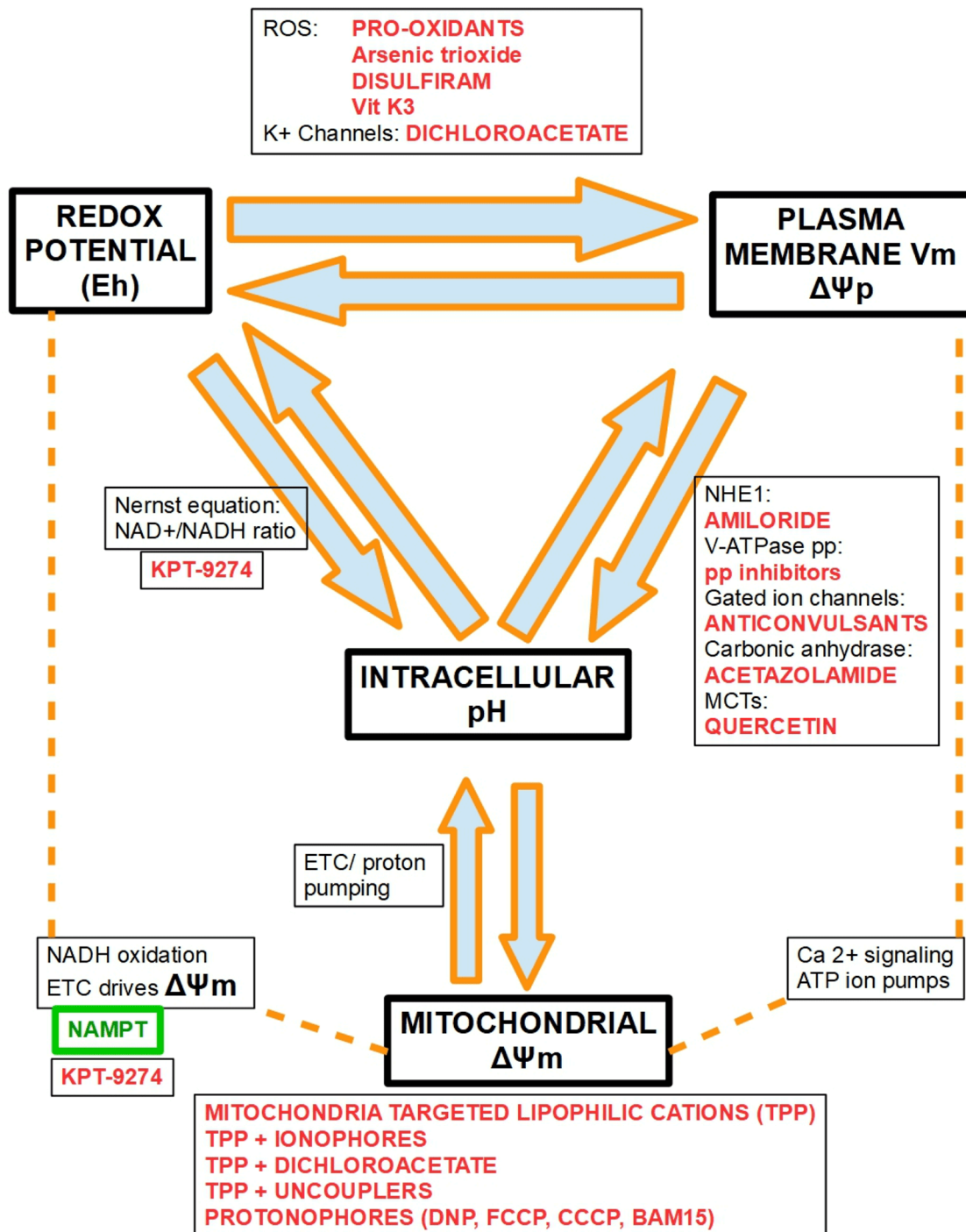


**Figure 14.** A drawing that shows the different levels and drugs that can target the pro-tumoral electrochemical network. Potential drugs are in red.

## PRO-OXIDANTS

### 1. Chemotherapeutic Drugs (Clinically Used Pro-oxidants) induce ROS as part of their anticancer mechanism:

Doxorubicin (and other anthracyclines like epirubicin), redox cycling, generates superoxide and hydroxyl radicals via Fenton chemistry.

Cisplatin (and other platinum drugs like carboplatin): mitochondrial ROS production and antioxidant depletion.

Arsenic trioxide: inhibits SOD and thioredoxin reductase (TrxR), leading to ROS accumulation; approved for acute promyelocytic leukemia.

Paclitaxel and other taxanes: can elevate ROS in certain contexts.

Etoposide and podophyllotoxins: contribute to oxidative DNA damage.

Bleomycin: iron-dependent ROS generation causing DNA strand breaks.

Imexon: depletes glutathione (GSH) pools.

Motexafin gadolinium: acts as an electron acceptor, increasing superoxide.

### 2. Natural Phytochemicals with Pro-oxidant Activity

Piperlongumine (from long pepper): highly selective; increases ROS and triggers cancer-specific apoptosis by inhibiting antioxidant systems.

Curcumin (from turmeric) — dual role; prooxidant at higher concentrations via ROS generation and mitochondrial disruption.

Epigallocatechin gallate (EGCG) (from green tea): can induce ROS and apoptosis in tumor cells.

Resveratrol (from grapes, red wine): context-dependent prooxidant effects.

Quercetin (flavonoid in onions, apples, etc.): increases ROS and depletes GSH in cancer cells.

Plumbagin (naphthoquinone from Plumbago): potent ROS inducer, inhibits NF-κB and other pathways.

Parthenolide (from feverfew): depletes GSH and elevates ROS.

Phenethyl isothiocyanate (PEITC) and sulforaphane (from cruciferous vegetables) — thiol-reactive, deplete GSH.

### 3. Other Notable Pro-oxidants or Redox-Active Agents

High-dose vitamin C (ascorbic acid): acts as a pro-drug generating extracellular hydrogen peroxide ( $H_2O_2$ ); selective for cancer cells in pharmacologic doses.

Disulfiram (used for alcoholism): redox-cycling and copper chelation leading to ROS.

Buthionine sulfoximine (BSO): inhibits γ-glutamylcysteine synthetase, depleting GSH (research tool).

Menadione (vitamin K3): classic redox cyler generating superoxide.

Redox-cycling quinones and certain metal complexes (e.g. involving copper or iron).

**Figure 15.** A list of possible clinically useful pro-oxidants that can be employed to increase oxidative stress as part of a scheme targeting the altered pro-tumoral electrochemical network.

## Section 8. The fifth pillar of the electrochemical network: the NAD<sup>+</sup>/NADH ratio

We have discussed the four pillars of the electrochemical network. However, there is a fifth pillar which we did not mention because rather than a pillar it is one of the network's main objectives: the NAD<sup>+</sup>/NADH ratio. The electrochemical network ( $\Delta\Psi_p$ ,  $\Delta\Psi_m$ , ion fluxes, pH, ROS) and the NAD<sup>+</sup>/NADH ratio are deeply interdependent. Each regulates and reshapes the other. The NAD<sup>+</sup>/NADH ratio sets the redox driving force for electron flow, which determines  $\Delta\Psi_m$ , ROS, and ATP production. In turn,  $\Delta\Psi_m$ ,  $\Delta\Psi_p$ , and ion fluxes regulate NADH oxidation, NAD<sup>+</sup> regeneration, and metabolic pathway selection. It is a tightly coupled bidirectional system. The next six boxes summarize the NAD<sup>+</sup>/NADH ratio relationship with the electrochemical network. They are based on references [\[378\]](#)[\[379\]](#)[\[380\]](#)[\[381\]](#)[\[382\]](#)[\[383\]](#)[\[384\]](#)[\[385\]](#)

### 8.1. NAD<sup>+</sup>/NADH ratio → controls $\Delta\Psi_m$

NADH donates electrons to Complex I.

Electron flow down the ETC pumps protons → generates  $\Delta\Psi_m$ .

The magnitude of  $\Delta\Psi_m$  depends on the rate of NADH oxidation.

High NADH/NAD<sup>+</sup> → ETC saturated →  $\Delta\Psi_m$  hyperpolarizes → ↑ ROS

High NAD<sup>+</sup>/NADH → ETC flows smoothly →  $\Delta\Psi_m$  stabilized → ↓ ROS

### 8.2. $\Delta\Psi_m$ → feeds back to regulate the NAD<sup>+</sup>/NADH ratio

If  $\Delta\Psi_m$  becomes too high, proton pumping slows (backpressure).

ETC flux drops → NADH oxidation slows → NADH accumulates → NAD<sup>+</sup>/NADH ratio ↓

If  $\Delta\Psi_m$  collapses (uncoupling), ETC accelerates → NADH is rapidly oxidized → NAD<sup>+</sup>/NADH rises.

Thus,  $\Delta\Psi_m$  sets the rate at which NADH can be reoxidized

### 8.3. $NAD^+/NADH$ ratio $\leftrightarrow$ controls cytosolic pH and $\Delta\Psi_p$

Lactate dehydrogenase (LDH) couples NADH oxidation to proton consumption/production.

NADH-driven glycolysis acidifies the cytosol  $\rightarrow$  activates NHE1  $\rightarrow$  increases  $Na^+$  influx  $\rightarrow$  depolarizes  $\Delta\Psi_p$ .

Conversely, high  $NAD^+/NADH$  shifts metabolism toward OXPHOS  $\rightarrow$  reduces glycolytic proton production  $\rightarrow$  stabilizes  $\Delta\Psi_p$ .

Thus, redox state shapes  $\Delta\Psi_p$  through metabolic proton production and  $Na^+/H^+$  exchange.

### 8.4. $\Delta\Psi_p$ and ion fluxes $\rightarrow$ regulate $NAD^+/NADH$ via metabolic pathways

Depolarized  $\Delta\Psi_p \rightarrow \uparrow Ca^{2+}$  influx  $\rightarrow \uparrow$  mitochondrial  $Ca^{2+} \rightarrow \uparrow$  TCA cycle dehydrogenases  $\rightarrow \uparrow$  NADH production.

$Na^+$  influx (e.g. via NaV1.5 in cancer)  $\rightarrow \uparrow$  NHE1 activity  $\rightarrow \uparrow$  glycolysis  $\rightarrow \uparrow$  cytosolic NADH.

$K^+$  channel activity modulates mitochondrial volume and ETC flux  $\rightarrow$  indirectly alters NADH oxidation.

Thus, the electrochemical network controls NADH generation and oxidation.

### 8.5. $NAD^+/NADH$ ratio $\leftrightarrow$ ROS $\leftrightarrow$ ion channels

High  $NADH/NAD^+ \rightarrow$  ETC over-reduction  $\rightarrow \uparrow$  ROS.

ROS oxidize redox-sensitive ion channels ( $K^+$ ,  $Ca^{2+}$ ,  $Cl^-$ )  $\rightarrow$  alter  $\Delta\Psi_p$ .

Ion-channel modulation changes  $Ca^{2+}$  and  $Na^+$  flux  $\rightarrow$  reshapes mitochondrial metabolism  $\rightarrow$  alters NADH oxidation.

Thus, ROS is the redox-electrical coupling intermediate

## 8.6. $\text{NAD}^+/\text{NADH}$ ratio $\leftrightarrow$ DNA repair $\leftrightarrow \Delta\Psi\text{m}$ and $\Delta\Psi\text{p}$

$\text{NAD}^+$  is consumed by PARP during DNA repair.

High DNA damage  $\rightarrow \text{NAD}^+$  depletion  $\rightarrow$  ETC slows  $\rightarrow \Delta\Psi\text{m}$  collapses.

$\Delta\Psi\text{m}$  collapse  $\rightarrow$  ATP depletion  $\rightarrow$  ion pumps fail  $\rightarrow \Delta\Psi\text{p}$  depolarizes.

Thus, genome stability feeds back into the electrochemical network via  $\text{NAD}^+$  availability.

The electrochemical network ( $\Delta\Psi\text{p}$ ,  $\Delta\Psi\text{m}$ , pH, ion fluxes) is both a driver and a consequence of  $\text{NAD}^+/\text{NADH}$  dynamics.

The  $\text{NAD}^+/\text{NADH}$  ratio controls cancer cell behavior by regulating energy metabolism, mitochondrial function, redox balance, DNA repair, and proliferation. The most recent evidence shows that cancer cells actively manipulate this ratio to support growth, resist stress, and maintain biosynthetic flux.

## 8.7. How the $\text{NAD}^+/\text{NADH}$ Ratio Controls Vital Factors in Cancer Cells

### 8.7.1. Controls Mitochondrial Metabolism & Proliferation

Cancer cells require a sufficiently **oxidized mitochondrial  $\text{NAD}^+/\text{NADH}$  ratio** to maintain oxidative metabolism and biomass production.

- **SLC25A51**, the mitochondrial  $\text{NAD}^+$  transporter, is up-regulated in aggressive cancers such as AML.
- Increasing mitochondrial  $\text{NAD}^+$  supports respiration, glutamine utilization, and proliferation.
- Lowering SLC25A51 collapses the oxidative  $\text{NAD}^+/\text{NADH}$  ratio inducing apoptosis and reduced tumor growth [\[386\]](#).

### 8.7.2. Regulates DNA Repair, Epigenetics, and Survival Pathways

$\text{NAD}^+$  is the required substrate for **PARPs** and **sirtuins**, which control:

- DNA repair
- Chromatin remodeling
- Stress resistance

- Cell survival

Cancer cells maintain high NAD<sup>+</sup> to support these pathways. When NAD<sup>+</sup> drops, DNA repair fails with cell death one of its consequences <sup>[387]</sup>.

### 8.7.3. Drives Glycolysis and the Warburg Effect

NAD<sup>+</sup> is an essential cofactor for glycolysis, the TCA cycle, fatty-acid oxidation, and OXPHOS. Disruption of NAD<sup>+</sup>/NADH balance alters these pathways and contributes to cancer's metabolic rewiring <sup>[388][389][390]</sup> <sup>[391]</sup>. The NAD<sup>+</sup>/NADH ratio determines glycolytic flux:

- GAPDH requires NAD<sup>+</sup>, so glycolysis depends on maintaining NAD<sup>+</sup> regeneration.
- Cancer cells regenerate NAD<sup>+</sup> through lactate production, maintaining high glycolytic rates.
- This supports rapid ATP production and biosynthesis <sup>[392]</sup>.

### 8.7.4. Supports Lipid Synthesis and Biomass Production

NAD<sup>+</sup> regeneration is essential for **de novo lipid synthesis**, especially when mitochondrial respiration is limited.

- Cancer cells rely on NAD<sup>+</sup> regeneration to maintain anabolic growth.
- Limiting NAD<sup>+</sup> regeneration restricts lipid synthesis thus impeding or reducing proliferation <sup>[393]</sup>.

### 8.7.5. Controls Redox Homeostasis & ROS Detoxification

A balanced NAD<sup>+</sup>/NADH ratio:

- Maintains redox equilibrium
- Prevents excessive ROS
- Supports antioxidant systems

Cancer cells fine-tune this ratio to avoid oxidative damage while maintaining metabolic flexibility <sup>[394]</sup>.

In **summary**: The NAD<sup>+</sup>/NADH ratio is a **master regulator** of cancer cell physiology. It controls:

- **Energy production** (glycolysis, OXPHOS)
- **Biosynthesis** (lipids, nucleotides, amino acids)
- **DNA repair & epigenetics** (PARPs, sirtuins)
- **Redox balance**

- **Proliferation & survival**

Cancer cells actively manipulate this ratio, via NAD<sup>+</sup> transport (SLC25A51), salvage synthesis (NAMPT), and metabolic rewiring—to sustain growth and resist therapy.

## **Section 9. The sixth pillar of the electrochemical network: ENOX2**

ENOX2 (Ecto-NOX Disulfide-Thiol Exchanger 2) is a cell-surface NADH oxidase found predominantly in cancer cells, where it supports growth and redox cycling. ENOX2 modifies the electrochemical network primarily by acting as a terminal oxidase at the outer plasma membrane that shuttles electrons from the cytosol to the extracellular space, thereby altering local redox potentials, membrane potential, and redox-coupled signaling pathways <sup>[395]</sup>.

It alternates between two enzymatic activities every ~22-24 minutes:

### **NADH or hydroquinone oxidation**

#### **Protein disulfide–thiol interchange**

Together, these activities support plasma-membrane electron transport, growth regulation, and ultradian timekeeping <sup>[396]</sup>. The main effects of ENOX2 are:

#### **Redox-driven changes in membrane potential**

ENOX2 serves as the terminal oxidase of plasma-membrane electron transport (PMET), accepting electrons from cytosolic NAD(P)H via an internal quinone reductase and hydroquinone intermediates, then reducing external acceptors such as oxygen or protein disulfides. This unidirectional outward electron flow generates a small but measurable outward current, which can slightly hyperpolarize or depolarize the membrane depending on the overall ion-channel balance, thereby shifting the local electrochemical gradient sensed by voltage- and redox-sensitive proteins <sup>[397]</sup>.

#### **Alteration of redox and pH microenvironments**

Electron transfer from ENOX2 to O<sub>2</sub> generates reactive oxygen species (ROS) at the cell surface, which can oxidize nearby membrane proteins and lipid-linked thiols, changing their conformation and activity (e.g. channels, transporters, receptors). Because ROS and thiol-disulfide reactions are coupled to protonation states of cysteine residues and nearby groups, ENOX2 activity can indirectly influence local pH and proton fluxes, especially when cooperating with other transporters or channels (e.g. Ca<sup>2+</sup>- or K<sup>+</sup>-channels whose gating is redox-sensitive).

## **Oscillatory activity and signaling networks**

ENOX2 exhibits rhythmic, ultradian oscillations in its two main activities (NADH/hydroquinone oxidation and protein disulfide-thiol interchange), which are linked to growth-related processes and cell-cycle-like cycling. These oscillations create time-dependent, periodic perturbations in the electrochemical network, modulating the activity of redox-sensitive pathways (e.g. RAS, NOTCH, integrin signaling, circadian-related outputs), and thereby coordinating growth and stress responses.

In short, ENOX2 shapes the electrochemical network by acting as a redox-active terminal oxidase at the plasma membrane, generating outward electron flow, localized ROS, and thiol-redox cycles that tune membrane potential, pH-sensitive targets, and downstream signaling cascades <sup>[398]</sup>.

### **6.1. ENOX2 in cancer**

As part of the electrochemical network, ENOX2 (tNOX) is also a player in cancer. It promotes cancer cell growth by acting as a tumor-associated, growth-related ecto-NADH oxidase that supports proliferation, redox homeostasis, and stem-like properties, while its inhibition blocks entry into the cell-cycle and reduces tumor growth.

#### **Pro-proliferative role at the cell surface**

ENOX2 is a cancer-specific ecto-oxidase located at the outer plasma membrane; it accepts electrons from cytosolic NADH and hydroquinones and transfers them to extracellular acceptors, coupling this to growth-related oxidations. Blocking ENOX2 activity (e.g. with isoflavones such as ME-143 or idronoxil) prevents cell enlargement and blocks the transition to the S-phase, indicating that ENOX2 is required for cells to initiate a new round of the cell cycle <sup>[399]</sup>.

#### **Link to proliferation, migration, and metastasis**

High ENOX2 expression correlates with increased proliferation, migration, and expression of mesenchymal markers in several cancers, and is associated with aggressive phenotypes (e.g. MDA-MB-231 breast, PANC-1 pancreatic, HSC-3 oral cancers) <sup>[400]</sup>. ENOX2 also interacts functionally with invasion-related pathways such as MMP2, and its down-regulation by drugs (e.g. mebendazole) reduces invasion- and metastasis-related markers.

#### **Enrichment in cancer stem-like cells and tumors**

ENOX2 is up-regulated in multiple tumor types versus adjacent normal tissue and is enriched in cancer stem cell-like populations. Its over-expression enhances sphere formation, stemness markers (SOX2,

Nanog, c-Myc, ABCG2), and tumor-initiating capacity in xenografts. Silencing or pharmacologically inhibiting ENOX2 reduces tumor growth, stemness, and in some contexts improves therapy response (e.g. by enhancing T-cell infiltration and chemotherapy efficacy in nasopharyngeal carcinoma).

In short, ENOX2 acts as a redox-driven, growth-permissive node at the cancer cell surface: it supports proliferation, invasive and stem-like behavior, and is increasingly viewed as both a biomarker and a therapeutic target in several malignancies <sup>[401]</sup>.

## 6.2. ENOX2 inhibitors

Several ENOX2 (tNOX) inhibitors are being explored or used experimentally in cancer treatment, mostly as investigational or repurposed agents rather than as standard-of-care drugs. The main ones include:

**Idronoxil (NV-X-014 / IDX):** Idronoxil is a first-in-class ENOX2 inhibitor developed by Noxopharm; it is a synthetic derivative of phenoxodiol that selectively targets cancer-associated ENOX2. It has been evaluated in clinical trials (often combined with chemotherapy or radiotherapy) for prostate, ovarian, and other cancers; recent work shows that idronoxil plus cisplatin enhances T-cell infiltration and tumor control in nasopharyngeal carcinoma <sup>[402]</sup>.

**Isoflavone-based ENOX2 blockers (phenoxodiol / ME-143):** ME-143 (NV-143), a synthetic isoflavone structurally related to phenoxodiol, is a well-studied ENOX2-targeting compound that inhibits the enzyme's hydroquinone-oxidase and NADH-oxidase activities. It induces G<sub>1</sub> arrest and apoptosis in cancer cells and has been investigated in ovarian, breast, prostate, and leukemic models, both as monotherapy and as a chemosensitizer <sup>[403][404]</sup>.

### Repurposed drugs and natural compounds

**Mebendazole:** Anthelmintic mebendazole has been identified as an ENOX2-inhibiting agent that reduces ENOX2-driven redox metabolism and suppresses tumor growth and metastasis-related signaling in several cancer types. Mebendazole inhibits ENOX2 in cancer cells primarily by down-regulating its expression and disrupting ENOX2-driven redox metabolism, rather than by acting as a classical, high-affinity enzyme inhibitor. Multi-cancer profiling shows that mebendazole treatment reduces ENOX2 mRNA and protein levels in several tumor lines (e.g. HEPG2 hepatocellular carcinoma, K562 leukemia), indicating transcriptional and/or post-transcriptional suppression of ENOX2 <sup>[405][406]</sup>.

This down-regulation is heterogeneous: some aggressive cancers (e.g. MDA-MB-231, PANC-1) exhibit decreased ENOX2, while others show modest or no change, suggesting cell-type-specific regulation rather

than universal occupancy of a single ENOX2 binding site.

ENOX2 supports cancer growth by maintaining plasma-membrane redox cycling and NADH-dependent electron transport, Mebendazole-induced reduction of ENOX2 expression perturbs this redox equilibrium, leading to reduced cancer-cell growth and enhanced apoptosis. By weakening ENOX2-driven metabolism, mebendazole also indirectly suppresses downstream pro-invasive pathways such as MMP2-mediated invasion and metastasis-related signaling, reinforcing its anti-tumor effect beyond simple microtubule inhibition.

**Capsaicin and green-tea catechins (e.g. EGCG):** These natural products show ENOX2-inhibitory activity in experimental models and can trigger apoptosis and anti-tumor effects, though they are not yet used clinically as specific ENOX2-targeting therapies <sup>[407][408]</sup>.

In practice, ENOX2-targeted therapy remains largely in the investigational or early-clinical space, with idronoxil and ME-143 being the most advanced pharmacologic ENOX2 inhibitors, while mebendazole and certain dietary compounds are being explored for repurposed or adjunctive roles.

## Section 10. Discussion

Cancer cells are not stable systems and they depend on controlled instability. They are high-flux, high turnover and high pace systems. Importantly, the pace of events and the magnitude of changes must stay within a survivable band. They must generate ATP fast. They must export protons fast. They must recycle NADH to NAD<sup>+</sup> fast. They must buffer ROS fast. They must maintain membrane potential within tight limits. Regarding the pace of events, if they are too slow lead to quiescence and if they accelerate too much they can end up in collapse. Therefore, cancer cells live in the middle of instability and they are skilled survivors in the middle of this instability.

This paper introduces the novel concept that cancer cell viability is governed not only by metabolic and growth pathways selection but also by the kinetic stability of electron and proton fluxes (the electrochemical network balance) a dimension of metabolic control that has been largely overlooked. By identifying ENOX2 as a redox pacemaker and demonstrating that its inhibition destabilizes the temporal coordination of NADH turnover, and mitochondrial compensation, we hint towards a new class of therapeutic vulnerability rooted in metabolic tempo rather than metabolic identity. The proposed ENOX2–NRF2 axis reframes redox homeostasis as a dynamic, pace-regulated system whose collapse can be therapeutically induced. This represents a conceptual departure from traditional approaches that target static metabolic dependencies, Instead, it offers a strategy that exploits the cancer cell's inability to

tolerate rapid deviations in redox and proton flux. By combining ultradian redox oscillations, mitochondrial overload, and NRF2-dependent buffering into a unified kinetic model, this project opens a new frontier in cancer metabolism: targeting the speed at which malignant cells must adapt to survive.

### *10.1. Why Cancer Cells Maintain a Negatively Charged Cytoplasm*

Cancer cells require a strongly negative intracellular environment because this electrical and proton-handling state is fundamental for:

- sustaining high metabolic flux;
- maintaining membrane potential;
- driving nutrient import;
- supporting rapid biosynthesis; and
- preventing acid-induced apoptosis.

A negatively charged cytoplasm is not a passive property, it is an actively maintained, energy-dependent state that enables the malignant phenotype to survive and flourish.

### *10.2. How Cancer Cells Maintain a Negatively Charged Cytoplasm*

Cancer cells use a coordinated set of mechanisms to keep the cytoplasm negative and the extracellular space acidic. These mechanisms operate continuously and at high speed.

#### *10.2.1. Proton Export Systems (the main drivers of negativity)*

Cancer cells aggressively pump protons out of the cytoplasm through:

- NHE1 ( $\text{Na}^+/\text{H}^+$  exchanger);
- V-ATPase proton pumps;
- MCT1/4 (lactate- $\text{H}^+$  cotransporters);
- CAIX/CAXII (carbonic anhydrases).

This proton export removes positive charge, alkalinizes the cytosol, acidifies the extracellular space, and maintains a net negative intracellular potential. This is essential for survival because intracellular acidification can trigger apoptosis.

#### *10.2.2. Electron Export and Redox Cycling (ENOX2's role)*

ENOX2 contributes to the negative cytoplasm by:

oxidizing extracellular NADH;  
enabling continuous outward electron flow;  
regenerating NAD<sup>+</sup> at the membrane;  
and preventing intracellular electron accumulation.

Electron export is electrically equivalent to removing the negative charge from the cytosol, which helps stabilize the membrane potential and maintain the cytoplasm's net negativity.

When ENOX2 is inhibited:

electrons accumulate;  
NADH rises;

Growth arrest can occur due to failure of the ENOX2-dependent protein disulfide–thiol interchange required for cell enlargement <sup>[409]</sup>.

### *10.3. Mitochondrial Membrane Potential ( $\Delta\Psi_m$ ) as a Charge Sink*

Cancer cells maintain hyperpolarized mitochondria:

$\Delta\Psi_m$  is often -180 to -220 mV (more negative than normal cells).

This acts as a sink for electrons and protons, helping stabilize cytosolic charge.

If mitochondria are forced to take too much load (as happens when ENOX2 is inhibited), they:

overproduce ROS;;  
depolarize;  
and lose their ability to buffer cytosolic charge.

This is where NRF2 becomes essential.

### *10.4. NRF2 as the Master regulator of redox homeostasis, metabolic flux, ROS detoxification, and electrophile defense <sup>[410][411][412][413][414]</sup>*

NRF2 maintains the negative cytoplasm indirectly by:

supporting glutathione and thioredoxin systems;  
maintaining NADPH pools;  
supporting mitochondrial integrity;  
regulating proton-handling enzymes;  
and preventing ROS-induced depolarization.

Without NRF2, the cell cannot maintain:

redox balance;

mitochondrial membrane potential;

or cytoplasmic negativity.

This paper has tried to build an explanation of the physiopathology of the electrochemical network in cancer and suggest an “electro-energetic trap” that can eventually have lethal effects on cancer cells. This electro-energetic trap is based on the core ideas that cancer cells maintain a negatively charged cytoplasm through high-paced proton export, electron disposal, and mitochondrial hyperpolarization. ENOX2 inhibition disrupts this charge-handling tempo, causing electrons and protons to accumulate faster than the cell can compensate. Without NRF2 to stabilize redox and proton flux, the loss of cytoplasmic negativity becomes a lethal energetic crisis for cancer cells.

In cancer, the four interrelated aspects of the electrochemical network, namely: pH, Eh,  $\Delta\Psi_m$ , and  $\Delta\Psi_p$  and the added actions of ENOX2, NAD<sup>+</sup>/NADH ratio, and the NRF2 system adopt a particular phenotype that favors proliferation, migration, EMT, invasion, metastasis, and resistance to apoptosis. These components of the electrochemical network are the four horsemen of cancer progression. The phenotype these four horsemen collaboratively and interdependently create is characterized by increased intracellular pH, decreased extracellular pH, increased intracellular oxidative profile, reduced cell membrane potential and increased mitochondrial potential.

However, if we try to simplify the activity of the four horsemen we can find that the common denominator of their activity is the governance of intracellular electron flow.

A defining mechanistic divergence between normal and malignant cells lies in the governance of intracellular electron flow. In non-transformed cells, electron production, mitochondrial oxidation, and membrane-associated electron export are tightly coupled, such that growth factor signaling is required to elevate metabolic flux and thereby sustain proliferation. Electron disposal capacity is rate-limiting, and growth is constrained by the ability of mitochondria and ENOX1-mediated ecto-oxidation to maintain redox homeostasis.

By contrast, cancer cells acquire the ability to uncouple electron control from exogenous growth factor input, enabling autonomous proliferation. This shift is driven by a coordinated rewiring of redox regulation, splicing control, and growth signaling. Oncogenic AKT–mTORC1 activity suppresses FOXO1, relieving repression of the splicing factor hnRNP F, which in turn enforces EXON-4 skipping in the ENOX2 transcript. The resulting cancer-specific ENOX2 isoform provides a high-capacity, oscillatory electron

export system at the plasma membrane, capable of oxidizing NADH and hydroquinones at a rate that matches the elevated electron load generated by glycolysis, glutaminolysis, and hyperactive mitochondria. ENOX2 thereby functions as a surface-level electron sink that stabilizes the NAD<sup>+</sup>/NADH ratio, supports sustained glycolytic throughput, and maintains ATP levels sufficient to suppress AMPK activation. This creates a self-reinforcing loop: ENOX2-mediated electron disposal sustains AKT–mTORC1 signaling, which maintains hnRNP F activity and thus preserves ENOX2 expression. In this state, electron pressure becomes a driver rather than a restraint for proliferation, allowing cancer cells to grow independently of external mitogenic cues. There is **no evidence** that “electron pressure” (as a physical or electrochemical force) is a *primary driver* of cancer initiation. However, cancer cells **do** exploit and reshape **electron** flow, redox pressure, and NAD(P)H/NAD(P)<sup>+</sup> disequilibria to support proliferation, resist apoptosis, and maintain biosynthetic flux. In that sense, *altered electron pressure is not a cause of cancer, but it becomes a requirement and amplifier of malignant behavior.*

By “electron pressure” we mean:

**High NADH/NAD<sup>+</sup> ratios** (reducing pressure)

**High NADPH/NADP<sup>+</sup> ratios** (anabolic and antioxidant pressure)

**Elevated mitochondrial membrane potential ( $\Delta\psi_m$ )**

**Increased electron flux through ETC complexes**

**Cytosolic redox poise shifted toward reduction**

**PMRS/ENOX-driven electron export maintaining surface charge**

These are all well-documented features of cancer metabolism. So while “electron pressure” is not a recognized term, the underlying idea, excess reducing equivalents and altered electron flow, is absolutely central to cancer cell physiology. This is fully consistent with: Warburg effect, mitochondrial hyperpolarization, PMRS/ENOX2 activity, redox-driven signaling autonomy, and NRF2-dependent redox stabilization.

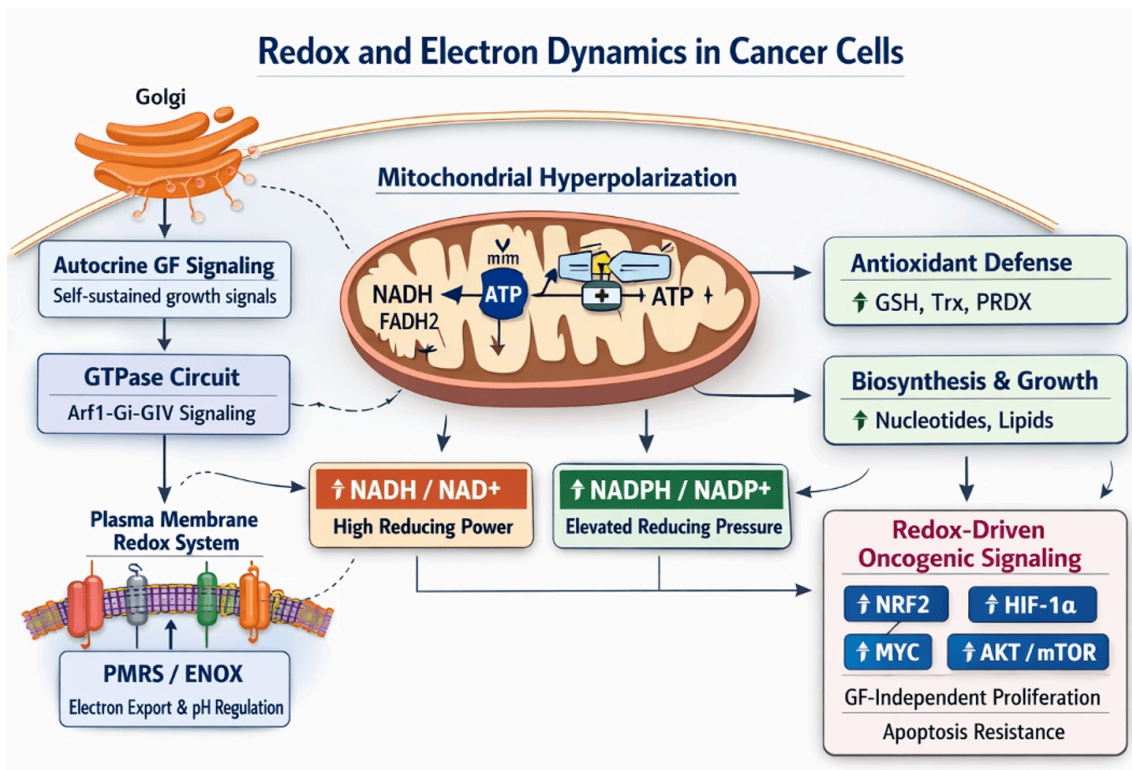


**Figure 16. Mechanistic Map of Electron and Redox Dynamics in Cancer Cells.** This drawing shows how malignant cells maintain proliferation by coupling metabolic and redox circuits that collectively generate and exploit *electron pressure*. At the center, the mitochondrion maintains **hyperpolarization** ( $\uparrow\Delta\Psi_m$ ) through excess electron transport chain (ETC) flux, driven by elevated NADH and FADH<sub>2</sub> input. This produces ATP while preserving a **high reducing power** ( $\uparrow\text{NADH}/\text{NAD}^+$ ) and **reducing pressure** ( $\uparrow\text{NADPH}/\text{NADP}^+$ ) that feed biosynthetic and antioxidant pathways. On the left, **autocrine growth factor signaling** and the **Arf1-Gi-G1V GTPase circuit** create self-sustained secretory loops, enabling growth signaling independent of exogenous stimuli. The **plasma membrane redox system (PMRS/ENOX)** exports electrons to the extracellular space, maintaining surface charge and pH inversion—an electrochemical adaptation that supports continuous proliferation. In the right panel, elevated NADPH fuels antioxidant defenses (glutathione, thioredoxin, peroxiredoxin systems) and biosynthetic programs (nucleotide and lipid synthesis). These redox-driven processes stabilize oncogenic signaling networks—NRF2, HIF-1α, MYC, AKT/mTOR, that reinforce growth factor independence and apoptosis resistance. Overall, the map depicts cancer cells as self-charging systems, where metabolic rewiring and redox control converge to maintain autonomous proliferation through persistent electron flux and redox homeostasis.

In normal cells, mitochondrial stress, NADH accumulation, or ROS elevation activate ceramide-PP2A signaling and AMPK, which collectively inhibit AKT, reactivate FOXO1, suppress hnRNP F, and restore

exon-4 inclusion, collapsing ENOX2 expression and reinstating the ENOX1-dominant, growth-restricted state. Thus, the capacity to maintain electron flow through ENOX2 despite high metabolic flux represents a fundamental biochemical innovation of malignant cells, enabling proliferation under conditions that would otherwise trigger growth arrest or apoptosis.

In this framework, cancer can be conceptualized as a state in which electron disposal capacity has been decoupled from growth factor dependency, allowing redox pressure itself to support the signaling architecture required for continued proliferation. This reframes tumorigenesis as a disorder of electron governance, in which the acquisition of ENOX2-driven electron export constitutes a pivotal enabling event. For this to happen uninterruptedly, the four horsemen need to first create the malignant phenotype described above that keeps the particular electron governance that favors cancer progress.

### 10.5. *The electrochemical switch*

Integral parts of the electrochemical switch that occurs in cancer are other switches:

- **Metabolic switch:** which goes well beyond the oversimplified idea of an OXPHOS to glycolytic switch. Actually this switch is characterized by increased glycolysis while in addition to OXPHOS and a great plasticity to reverse back to oxidative metabolism when required. Parts of the metabolic switch are also increased glutaminolysis and lipogenesis/lipolysis on demand. This is a very dynamic switch that adapts cells to environmental circumstances such as hypoxia and hypoxia/reperfusion events.
- **Enox1 to Enox2 switch:** The ENOX1 ↔ ENOX2 “switch” refers to a well-documented transition from a non-cancer, growth-related ecto-oxidase (ENOX1) to a cancer-associated, constitutively active ecto-oxidase (ENOX2). This shift is supported by multiple studies showing that ENOX2 expression rises with malignant transformation and correlates with tumor aggressiveness, while ENOX1 is characteristic of non-transformed cells <sup>[415][416][417]</sup>. Although the ENOX1 to ENOX2 switch should be considered as part of the REDOX switch, we believe that its importance grants it a different place on the grid of switches.

While ENOX1 is the normal, growth-related NADH oxidase found on the surface of non-malignant cells, ENOX2 (tNOX) is a cancer-associated isoform with altered periodicity and constitutive activity. ENOX1 participates in regulated redox oscillations and supports controlled cell enlargement, and ENOX2 supports unregulated growth, displays prion-like properties, and is strongly up-regulated in malignant cells. Therefore, ENOX2 is described as a **tumor-specific member of the ecto-NOX family**, with activities that alternate in a characteristic ultradian rhythm and support cancer cell growth. For example, ENOX2

expression increases progressively from benign nevi to primary melanoma to metastatic melanoma, demonstrating a malignancy-linked up-regulation [303].

The transition from ENOX1 to ENOX2 reflects a shift from regulated, physiological redox oscillations to a cancer-specific, growth-driving redox system. ENOX2's altered periodicity and constitutive activity provide a continuous supply of extracellular electron transport and disulfide-thiol interchange, supporting membrane dynamics, vesicle trafficking, and proliferation. ENOX2 links directly to **bioelectric signaling** and **metabolic reprogramming** because it sits at the plasma membrane as a **redox-driven electron transporter** whose oscillatory activity shapes membrane potential, proton flux, and growth-linked redox rhythms. The ENOX1→ENOX2 transition is therefore not just a marker of malignancy, it is a **bioelectric and metabolic rewiring event**.

ENOX2 is an ecto-NADH oxidase with two key activities:

- **Electron transport (ECTO-ETC):** transfers electrons from cytosolic NADH to extracellular acceptors.
- **Protein disulfide–thiol interchange:** supports membrane protein conformational cycling.

These activities occur in 22-26 min ultradian oscillations, which cancer cells use as a “growth clock”. ENOX2 activity results in stabilization of voltage-gated channel conformation, especially Kv10.1/Eag1, which is oncogenic, and maintains a pro-proliferative  $V_{mem}$ , bypassing the regulated oscillations of ENOX1. Although there is no experimental proof, we believe that ENOX2 can indirectly increase proton export (activating NHE1) creating an increased intracellular pH. ENOX2 also supports high glycolytic flux while maintaining a cytosolic redox balance favorable for rapid biomass synthesis.

Regarding the cell membrane, ENOX2 creates **local hyperpolarizing microdomains**, not a globally hyperpolarized membrane (cancer cell membrane polarization is decreased). Those microdomains are enough to drive  $Ca^{2+}$  entry and metabolic activation, even though the *overall* membrane is depolarized.

In summary:

ENOX2 drives  $V_{mem}$  hyperpolarization. This in turn increases  $Ca^{2+}$  influx, activating mitochondrial metabolism. Increased mitochondrial metabolism hyperpolarizes  $\Delta\psi_m$ , increasing ATP production, and thus supporting NHE1 and ENOX2, which maintains alkaline  $pH_i$  and cytosolic redox conditions that favor proliferation. This circuit replaces the regulated ENOX1-based system with a malignant ENOX2-driven engine.

**pH switch:** created by increased proton export through over-activity/over-expression of cell membrane channels, transporters and enzymes such as NHE1, MCT1 and 4, V-ATPase proton pumps, carbonic anhydrase IX and XII and their companion cotransporter NBC1. Cancer cells produce a considerable amount of protons in the form of lactic acid and CO<sub>2</sub> that is hydrated to carbonic acid. Extrusion of these protons through channels, transporters and enzymes creates a very acidic extracellular milieu while at the same time inducing moderate hyperkalemia inside the cell (pH gradient inversion).

**Redox switch:** The redox switch in cancer refers to reversible chemical modifications, primarily the oxidation (and reduction) of specific cysteine residues in proteins, that act like molecular "on/off" toggles in response to cellular redox state (balance of oxidants like reactive oxygen species, or ROS, versus antioxidants). These switches sense changes in ROS levels and rapidly alter protein function, stability, localization, or interactions, thereby controlling key processes in cancer initiation, progression, survival, and therapy resistance. Unlike stable genetic mutations, redox switches are dynamic and reversible, allowing cancer cells to adapt quickly to oxidative stress. Cancer cells often produce elevated ROS due to rapid metabolism, oncogene activation (e.g. KRAS), and mitochondrial dysfunction, yet they avoid death by fine-tuning these switches to keep ROS at "pro-tumor" levels, high enough to drive signaling for proliferation and metastasis, but not so high as to trigger apoptosis or ferroptosis.

ROS (especially H<sub>2</sub>O<sub>2</sub>) react with thiol groups (-SH) on cysteines, forming modifications like sulfenylation (-SOH), glutathionylation (-SSG), or disulfides. These change protein conformation or activity. Enzymes like thioredoxin or glutaredoxin can reverse them, resetting the switch. This is distinct from (but interacts with) other signals like phosphorylation. The major redox switches are:

**Keap1-Nrf2 Pathway: The Master Antioxidant Redox Switch** This is the most established "redox switch" in cancer biology. Under normal conditions, Keap1 (a sensor protein) binds Nrf2 (a transcription factor) and targets it for degradation. Oxidative stress modifies key cysteines in Keap1, causing it to release Nrf2. Nrf2 then enters the nucleus, activates antioxidant response elements (AREs), and turns on genes for antioxidants and detox enzymes (e.g. HO-1, SOD, GPX, glutathione synthesis enzymes, thioredoxin). In cancer: Early on, it suppresses tumors by preventing DNA damage from ROS. Once established (often via KEAP1 mutations common in lung, pancreatic, and other cancers), hyperactive Nrf2 keeps ROS in a pro-survival range. This promotes proliferation, metabolic reprogramming (e.g. via the pentose phosphate pathway for NADPH), evasion of cell death, metastasis, and resistance to chemo/radiotherapy. Cancer cells hijack this switch for cytoprotection [\[418\]\[419\]\[420\]\[421\]](#).

**GAPDH Redox Switch: Metabolic Adaptation for Survival** Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme, has an active-site cysteine (Cys-152) that is highly sensitive to  $H_2O_2$ . Oxidation inactivates GAPDH, slowing glycolysis and rerouting glucose-6-phosphate onto the oxidative pentose phosphate pathway (oxPPP). This generates NADPH, the cell's main "reducing power" for regenerating antioxidants (e.g. glutathione) and clearing peroxides. In tumors, this switch is crucial under oxidative stress (e.g. during anchorage-independent growth in metastasis or in the tumor microenvironment). It protects cancer cells from endogenous peroxide stress and enables spheroid formation/tumor growth in vivo. Oxidation-insensitive GAPDH mutants (engineered via CRISPR) impair this adaptation, leading to higher peroxide levels, reduced tumor growth, and synergy with pro-oxidant therapies like cisplatin or radiation [\[422\]\[423\]\[424\]\[425\]](#).

**p21 Redox Switch: Controlling Proliferation vs. Senescence** A more recently discovered switch (2025) on the CDK inhibitor p21 (a cell-cycle brake). In G2 phase, oxidation (sulfenylation) of cysteine 41 (C41) weakens p21's inhibition of CDK2, allowing CDK2 to phosphorylate and degrade p21 via the SCF-SKP2 pathway. This favors continued cell division. The reduced form of p21 stabilizes it, strengthening CDK inhibition and promoting cell-cycle exit or senescence. In cancer: proliferating tumor cells often favor the oxidized state for growth. Disrupting the switch (e.g. preventing oxidation) can drive senescence, especially under stress like radiation. This has emerging implications for therapies that exploit redox to force permanent arrest [\[426\]\[427\]\[428\]](#).

Redox switches intersect with nearly all cancer hallmarks:

- **Proliferation & survival:** Moderate ROS via switches activate MAPK, NF- $\kappa$ B, or AP-1 pathways.
- **Metastasis & invasion:** Antioxidants (via Nrf2 or GAPDH) help cells survive oxidative stress during travel.
- **Metabolic reprogramming:** Supports Warburg effect and NADPH generation.
- **Therapy resistance:** Up-regulated antioxidants blunt ROS-based treatments.
- **Tumor microenvironment:** ROS from cancer cells reshape stroma and immune cells.

Context is critical: In early/pre-cancerous cells, strong redox defenses (e.g. Nrf2) can prevent initiation; in advanced tumors, they fuel progression.

In summary, the redox switch is not a single entity but a fundamental layer of regulation that cancer exploits for adaptability. It explains why tumors thrive in stressful, oxidative environments and offers

promising, context-specific targets for new treatments. Research continues to map more switches via redox proteomics, revealing additional druggable sites.

**Mitochondrial membrane potential switch:** A mitochondrial membrane-potential “switch” in cancer refers to a reproducible transition in how tumor mitochondria generate, maintain, and use  $\Delta\Psi_m$ , shifting from a normal, demand-regulated state to a **hyperpolarized, growth-driving, apoptosis-resistant state**. This is one of the most conserved bioenergetic signatures of malignant transformation.

Cancer cells consistently display:

- **Hyperpolarized  $\Delta\Psi_m$**  (often -180 to -220 mV vs. -140 to -160 mV in non-transformed cells).
- **Reduced proton leak**, increasing coupling efficiency.
- **Increased reliance on mitochondrial  $\text{Ca}^{2+}$  uptake** to drive TCA flux.
- **Resistance to mitochondrial permeability transition (mPTP)**, enabling survival under stress.
- **ROS signaling tuned to proliferative ranges**, not apoptotic ranges.
- **Dependence on ETC Complex I and II** despite high glycolysis.
- Alkaline pH<sub>i</sub> reduces proton back-pressure on the ETC, allowing **stronger proton pumping** and  $\Delta\Psi_m$  **hyperpolarization**.
- Hypopolarized V<sub>mem</sub>
- Cell membrane microdomains where ENOX2/NHE1 activity increases cytosolic  $\text{Ca}^{2+}$ , which mitochondria import via MCU. Matrix  $\text{Ca}^{2+}$  activates: PDH, IDH, and  $\alpha$ -KGDH.
- This increases NADH/FADH<sub>2</sub> supply and **drives  $\Delta\Psi_m$  upward**.
- Down-regulation of UCPs and ANT-mediated leak increases coupling efficiency, raising  $\Delta\Psi_m$ .

This is not a passive consequence of the Warburg effect; it is an **active bioelectric reprogramming event**. The  $\Delta\Psi_m$  switch is the **mitochondrial arm** of cancer’s bioelectric program integrating: V<sub>mem</sub> (ENOX2, Eag1, hERG), pH<sub>i</sub> (NHE1, CAIX),  $\Delta\Psi_m$  (ETC,  $\text{Ca}^{2+}$ ,  $\text{Na}^+/\text{H}^+$  exchanger), Redox (NADH/NAD<sup>+</sup>, ROS) into a single, self-reinforcing proliferative state.

**Note:** there is no direct evidence of hyperpolarized V<sub>mem</sub> microdomains. However, indirect evidence shows that, being a cell-surface protein, ENOX2 is enriched in specific membrane regions of malignant cells [429][430]. ENOX2 organizes into oscillatory microdomains that support disulfide-thiol exchange and NADH oxidase activity, driving uncontrolled proliferation. Inhibition disrupts these domains, elevating cytosolic NADH, boosting ceramide, and triggering apoptosis via sphingolipid shifts [431]. Although speculative, the idea that these microdomains exist would explain some of ENOX2 effects. However,

ENOX2 has no evident effect on ion channels. ENOX2 is understood to influence cancer-cell  $\text{Ca}^{2+}$  signaling mainly *indirectly* through its effects on redox state, membrane potential, and growth-related pathways.

## Section 11. Future perspectives

Until now, the electrochemical network has been viewed as a disintegrated model. This vision led to targeting different parts of the network separately: the redox system, the pH paradigm, the mitochondrial membrane potential. The next therapeutic breakthroughs, which are already advancing swiftly, will come from an integrated approach targeting the whole network simultaneously. This will critically affect cancer cells while preserving normal cells in which the electrochemical characteristics are different. New nanomaterials, in the preclinical experimental phase, are making this simultaneous attack possible.

Finally, targeting NAMPT (nicotinamide phosphoribosyl transferase, the enzyme necessary for  $\text{NAD}^+$  biosynthesis would probably drastically alter the fifth pillar of the electrochemical network.

**Targeting the deregulated ECN:** the script is one, consistent with creating a favorable ECN for proliferation, and invasion. The manifestations of this script (pH, Eh,  $\Delta\Psi_m$ , and  $\Delta\Psi_p$ ) are mainly similar in most cancer cells, however, the performing actors can be different among tumors and patients. There is no one-size-fit-all treatments. Combinatorial therapies targeting the deregulated ECN will probably need to be adapted to each patient according to which channels or transporters are altered. There are also permanent actors, that are always on stage, for example NHE1, which means that the different therapies will have at list some central targets.

## Section 12. Conclusions

Based on the available literature we have built a physiopathologic model of cancer progression produced by a deregulated electrochemical network. We have also shown the high interdependence of the different components of the network and suggested that it can be targeted by an adequate combination of existing drugs.

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