Causes, Consequences, and Combat of pH Dysregulation in Cancer

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Abstract

Dysregulated pH is recognized as a hallmark of cancer. Many cancer-associated cellular processes are altered by changes in pH, including proliferation, apoptosis evasion, metabolic adaptation, cell migration, and tumorigenesis. We describe how both intracellular pH (pHi) and extracellular pH (pHe) are altered in cancer progression due to dysregulation of membrane transport proteins. pH-sensitive proteins, known as pH sensors, then modify their behavior in response to this altered pH microenvironment of cancer, further exacerbating the cancerous phenotype. Obtaining a mechanistic understanding of how this pH change is driven is critical for designing anti-cancer therapeutics. These therapeutics include drugs that are more effective in an altered pH microenvironment, and drugs that combat further pH dysregulation and restore normal pH to the tumor.

Introduction

In recent years, an overwhelming amount of interest in the pH dysregulation of cancer cells has surfaced. While the term 'cancer' defines a wide variety of diseases, it is recognized that all cancers share basic adaptive characteristics that differentiate them from normal cells and contribute to the cancerous phenotype, such as an increase in glycolytic metabolism(Gatenby and Gillies, 2004) and a decrease in oxidative phosphorylation(Dey and Moraes, 2000; Grippo and Maker, 2017). This metabolic shift in cancer, termed the "Warburg Effect", is often accompanied by cellular changes in redox(Jorgenson et al., 2013; Moreira et al., 2016), osmolarity(Counillon et al., 2016), mitochondrial membrane potential(Dey and Moraes, 2000), and dysregulated pH(Schwartz et al., 2017; Webb et al., 2011). Specifically, the pH gradient between the cytosol and the extracellular space is reversed in cancers, with cancer cells having an increased pHi (7.3-7.6 versus 7.2 in normal cells) and decreased pHe (6.8-7.0 versus 7.4 in normal cells)(White et al., 2017). This pH gradient reversal is now recognized as a critically important hallmark of cancer.

In cancer cells, the pHi increases while the pHe decreases as a result of upregulation of ion transporters such as the Na^+-H^+ exchanger NHE1(Counillon et al., 2016; McLean et al., 2000; Chiang et al., 2007) and monocarboxylate-H^+ efflux cotransporters(Pinheiro et al., 2008; Chiche et al., 2011; Damaghi et al., 2013), which pump protons from the cytosol into the extracellular space (figure 1). Carbonic anhydrases that hydrolyze CO_2 into HCO_3^- and H^+ in the extracellular space also contribute to the pH gradient reversal(Swietach et al., 2009; Pastorek et al., 2008). These pH changes have been found to promote cancerous behaviors, including proliferation, apoptosis evasion, metabolic adaptation, cell migration, and tumorigenesis.

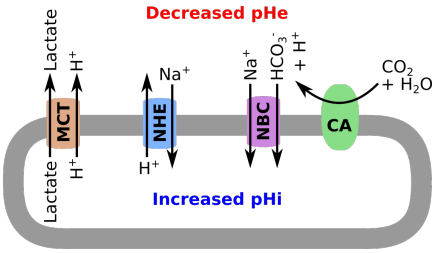

Figure 1: Cellular membrane/transport proteins that contribute to pH dysregulation in cancer.

These cellular processes are upregulated in the altered pH microenvironment of cancers due to the presence of specific pH sensors. A pH sensor is a protein whose structure and/or function is altered in response to a change in pH(Schönichen et al., 2013). In the altered microenvironment of cancers, the pH changes can make a pH sensor constitutively activated or repressed, leading to the upregulation of cancerous cellular activities(White et al., 2017).

This review is split into three main sections: Causes,

Consequences, and Combat. We will first examine the membrane transporters whose increased activities lead to a reversed pH gradient in cancer (Causes), followed by the cell's responses to that pH change (Consequences), and how they are driven at the molecular level via altered activity of pH sensors. Finally, different therapeutic strategies to increase drug efficacy and restore normal pH to cancers (Combat) will be discussed.

Causes of pH Dysregulation in Cancer

Some of the most notable changes in protein expression and activity in cancers are found in proteins that alter the pH environment. These proteins are often membrane transport proteins, and many have attracted interest as drug targets. This section will highlight a variety of proteins that regulate both pHi and pHe, and how they contribute to cancer progression.

MCT1 and MCT4 are two major monocarboxylate-H^+ cotransporters associated with cancers(Counillon et al., 2016). MCT1 facilitates the export of lactate and protons produced by glycolysis, preventing intracellular acidification under normal conditions. Inhibition and silencing of MCT1 has been demonstrated to drastically decrease pHi, most likely due to inability to excrete excess lactate generated from glycolysis(Mathupala et al., 2004). The pHi in MCT1 knockouts can be rescued by expression of MCT4, exemplifying the importance and redundancy of these transporters in pH maintenance(Mathupala et al., 2004). From a mechanistic standpoint, it is not clear whether MCT upregulation is a driver of cancer or a result of cancer, but it is clear that MCTs are drivers of pH change. When expressed in Ras-transformed fibroblasts, MCT4 caused alkaline intracellular pH(Counillon et al., 2016). MCTs are also implicated in cell migration, a critical process for metastasis, as they are localized to the leading edge of migrating cells, though their exact role in these processes is unknown(Gallagher et al., 2009).

Carbonic anhydrases (CAs) are another important class of enzymes commonly upregulated in cancers, and have been found to associate with MCTs in the plasma membrane. Their enzymatic activity catalyzes the conversion of CO_2 derived from oxidative phosphorylation into HCO_3^- in the extracellular matrix, acidifying the pHe. Bicarbonate is then often transported back into the cells, which causes an increase in pHi (see next section). CAIX and CAXII are membrane bound CAs that are commonly overexpressed in aggressive cancers and have been shown to be drivers of pH changes(Wang et al., 2015; Zheng et al., 2015). It seems paradoxical that CAs would be upregulated in cancers, which have decreased oxidative phosphorylation and therefore generate less CO_2. In 2015, some insight into this peculiarity was presented by Jamali et al, demonstrating that in breast cancer cells, non-catalytic proton transport activity of the CAIX augments activity of MCT1(Jamali et al., 2015), showcasing the interplay between different classes of pH regulatory proteins. This process increases pHi, driving glycolysis and proliferation. CAIX has also been suggested to promote migration and invasion(Tafreshi et al., 2011).

When CAIX converts CO_2 into HCO_3^- and H^+, HCO_3^- is driven down its concentration gradient back into the cell, leading to alkalization of the intracellular environment in addition to acidification of the extracellular space. This process is mainly facilitated by Na^+/HCO_3^- cotransporters (NBCs)(Parks and Pouyssegur, 2015). The transport of HCO_3^- and regulation of NBCs play a role in a variety of cancers(Jamali et al., 2015; Parks and Pouyssegur, 2015). It may also be interesting to consider the role of NBCe1 in the context of a whole tumor. One study showed that NBCe1 is regulated by hypoxia(Sedlakova, 2014), which is significant because cells inside a tumor are in a hypoxic environment, while cells on the exterior are more oxygenated. HCO_3^- produced by more oxygenated cells from higher oxidative phosphorylation could be taken up by glycolytic cells on the interior of the tumor to help increase their pHe. Conversly, lactate from glycolytic cells on the interior of a tumor may be used as a carbon source in oxidative phosphorylation in oxygenated cells on the exterior(Nakajima and Houten, 2012). The hypothesis that carbon symbiosis occurs between normoxic and hypoxic regions of tumors has yet to be thoroughly investigated(Nakajima and Houten, 2012). This hypothesis would suggest that NBCs are not necessarily drivers of cancer, but are drivers of pH dysregulation after a tumor has already formed.

The ion transporter that is perhaps the most widely accepted as a driver of cancer is the Na^+/H^+ exchanger NHE1. NHE1 is recognized as a major regulator of cellular pH in an abundance of cancer types(Stock and Pedersen, 2017). NHE1 exchanges an extracellular Na^+ for an intracellular H^+ at the plasma membrane, thus directly decreasing the pHe and increasing the pHi. NHE1 normally helps prevent cytosolic acidification, thereby promoting glycolysis and proliferation and driving cancer(Stock and Pedersen, 2017). Furthermore, in migrating cells, NHE1 localizes to pseudopodia and invadopodia(Damaghi et al., 2013), and also associates with actin and cytoskeletal re-

organization kinases(Stock and Pedersen, 2017). Taken together, it is clear that NHE1 plays an important role in cancer migration and invasion, though its exact mechanism in these processes is unknown.

Consequences of pH Dysregulation in Cancer

All proteins have an optimal pH range for activity. However, pH sensors are unique in that they change their activity as a direct result of a proton binding to a critical residue. They are usually sensitive within the narrow range of physiological pH fluctuations. Interestingly, 15% of somatic mutations in cancer involve histidine substitutions, which is the amino acid most likely to be implicated in pH sensing and regulation(Kan et al., 2010) due to its near-physiological pKa value of 6.5. Characterizing and determining the nature of these mutations is becoming a new frontier in cancer biology. pH sensors are found in a variety of cancer-related cellular processes, some of which are described below.

NHE1 begs to be mentioned again, as it also functions as a pH sensor in addition to its ion channel activity. NHE1 is highly upregulated by increased intracellular protons, which would occur in glycolytic cancer cells. Though the mechanism of binding and transport of protons is complex and cooperative, it is known that there are four conserved histidine residues in the C-terminal domain whose protonation facilitates binding to a phospholipid, regulating activity of the transporter(Webb et al., 2016).

Perhaps the most significant pH sensors are glycolytic proteins. Glycolysis is regulated via a pH associated negative feedback loop. Glycolysis generates lactic acids and protons, but shuts down if the environment is too acidic to prevent detrimental acidosis of the cell. Two glycolytic enzymes are known pH sensors, lactate dehydrogenase (LDHA) and phosphofructokinase-1 (PFK-1)(Damaghi et al., 2013; Reshkin et al., 2014). The molecular mechanisms for their pH sensitivity were predicted using software called "pHinder", though their mechanisms have never been experimentally determined(Isom et al., 2013). LDHA is upregulated at higher pHi, consistent with increased glycolysis in cancer(Structural basis for altered activity of M- and H-isozyme forms of human lactate dehydrogenase, 2001). There exists a plethora of conflicting data for PFK-1, however. While PFK-1 and its activators are upregulated in cancer, so is its glycosylation, which inhibits function(Yi et al., 2012). The precise regulation of PFK-1 in cancer has yet to be determined.

Yet another cellular behavior regulated by pH is actin cytoskeleton remodeling. This is especially important for cancers as cell migration, and therefore metastasis, are impossible without actin remodeling. The enzymes cofilin and talin, which are involved in actin remodeling, are known to increase activity at higher pHi(Frantz et al., 2008). Cofilin mediates actin polymerization and membrane protrusion, while talin binds to actin and regulates adhesion. The mechanisms of pH sensing are experimentally characterized(White et al., 2017). Both are regulated by protonation states of histidine residues. Talin undergoes a conformational change when deprotonated, and cofilin changes its binding affinity upon deprotonation(White et al., 2017). These proteins may be interesting therapeutic targets, considering their mechanisms are well studied and they are so critical for cancer cell migration.

In addition to pH being important for the regulation of ion transporters, glycolysis, and actin remodeling, pH also contributes to cellular signaling. There is a family of GPRCs that are activated by acidic pHe, including GPR4, 65, and 68. Their activation is important for a variety of signaling pathways pertinent to cancer proliferation and metastasis, including MAPK, PIP3K, and cAMP related pathways(Justus et al., 2013). Some cancers even have activating mutations of GPCR. For example, human luteinizing hormone receptor in three case studies of testicular cancer was found to have an activating mutation of Asp578 to a histidine(Yang et al., 2015), yet the effects of pH on this mutant have yet to be explored.

Such pH-sensitive mutations that consistently arise in cancers are of increasing interest in the pH dysregulation in cancer field(White et al., 2017). These mutations are often His->Arg or Arg->His mutations(White et al., 2017), as these mutations can alter the protein's ability to modify its structure or function in response to a change in pH. The Arg273His mutation in p53 is one such recurrent mutation. The positive charge on Arg273 is responsible for the protein's ability to bind to the DNA backbone. When this residue is mutated to a histidine, which can be neutrally charged, especially at higher pH, the protein's ability to bind DNA decreases drastically(Joerger et al., 2005). While His->Arg or Arg->His mutations are over-represented in cancers, it is currently unclear which of these mutations are drivers of cancer, and which arise later on in cancer progression. An interesting hypothesis is that these mutations are adaptive to the altered pH microenvironments of cancer, and are thus more likely to arise in an environment with increased pH. This modified evolutionary landscape in the tumor microenvironment is

currently poorly understood, but has potential to greatly increase our understanding of how mutations arise in cancer and exacerbate the cancerous phenotype.

Combating pH Dysregulation in Cancer

The pH gradient reversal found in the microenvironment of cancers is often responsible for decreased efficacy of a wide variety of cancer drugs, leading to drug resistance for many chemotherapies and radiotherapies(Daniel et al., 2013). Many cancer drugs are weak bases, and must pass through the cell membrane to reach their intracellular target protein. As the pHe around cancer cells is very acidic, the ratio of positively charged drug/neutral drug increases, decreasing the drugs ability to enter the cancer cell and reach its therapeutic target (figure 2). Even if the drug passes through the cell membrane, it is then most likely to be sequestered and trapped in a more acidic organelle of the cell, such as the lysosome, where it will be degraded(Alfarouk et al., 2015; Raghunand et al., 1999). This relationship between the acidity or basicity of a drug and the pH microenvironment of the cancer cell implies that weakly acidic drugs would be ionized to a smaller degree and be able to pass through the cell membrane more easily. This has been shown to be the case for chlorambucil, which exhibits increased cytotoxicity when the pHe is more acidic(Parkins et al., 1996).

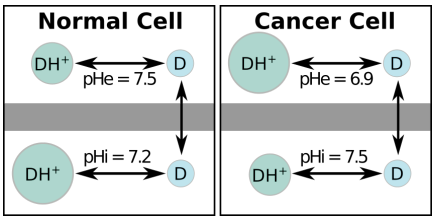

Figure 2: In normal cells, drugs are more likely to cross the cell membrane and become protonated inside the cell (left). Drugs that are protonated at lower pH are less likely to cross the membrane to reach their target in cancer cells (right).

While designing cancer drugs as weak acids instead of weak bases may increase their efficacy in the altered pH microenvironment of the cancer cell, drug resistance is still certainly possible due to the presence of drug efflux pumps. The efflux pump Breast Cancer Resistance Protein (ABCG2) has been shown to more efficiently pump cancer drugs out of the cell at a lower pHe(Breedveld et al., 2006). Additionally, the efflux pump P-glycoprotein exhibits pH-dependent activity(Breedveld et al., 2006), although the precise effects of its activity on the neutral and charged forms of weakly acidic and basic drugs is still unclear. A potential strategy to circumvent this is the co-treatment of cancer drugs with an efflux pump inhibitor.

Instead of designing cancer drugs that are more active in the pH-dysregulated environment of cancer, another increasingly popular strategy is to design drugs that actively try to restore the regular pH properties of cells. Knockout, knockdown and/or inhibition studies have been performed on a variety of the pH regulating proteins discussed above, including Na^+/H^+ exchangers (NHE1)(Amith et al., 2014), monocarboxylate-H^+ transporters (MCT1/2)(Marchiq and Pouysségur, 2015), and carbonic anhydrases (CAIX)(McDonald et al., 2016), many of which hold promising therapeutic potential. As discussed, CAIX and MCT1 cooperate to increase proton and lactate flux(Jamali et al., 2015), illustrating the importance of understanding not only structural data of drug targets, but their mechanistic modes of action as well.

A more non-targeted approach toward restoring normal pH levels has also been considered. This approach is known as buffer therapy, and involves ingestion of bicarbonate or similar buffers, which helps increase pHe by sequestering protons(Silva et al., 2009). This approach has been shown to effectively increase pHe and prevent metastases(Robey et al., 2009), and is particularly promising considering that redundancy of pH regulatory proteins may decrease the effectiveness of more targeted approaches.

No two cancers are completely identical, highlighting the importance of different therapeutic strategies. While problems in the above therapeutic strategies still exist, recent advancements indicate that they hold great potential for restoring normal pH levels in cancers. A combination of the above approaches, in tandem with current anticancer drugs, may prove to be very effective in fighting cancers.

Conclusion

pH is critical for cellular processes, as every enzyme's activity is a function of pH. As such, the dysregulation of pH is expected to lead to suboptimal cellular behavior, as described above in cancer. In recent years, the field of pH dysregulation in cancer has greatly improved our understanding of this critical hallmark of cancer, particularly with the characterization of pH sensors, yet there remains

a lot to be discovered. Moving forward, it will be important to consider how redox state is altered in the cancer environment in addition to pH, as it is known that redox potential and pH are closely intertwined(Jamieson et al., 2015; Jamieson et al., 2016). To date, their combined, and possibly synergistic, effect in cancer has been largely ignored. Additionally, the field would benefit greatly from the generation of improved tools to detect pH changes over short and long spatial and temporal scales. The prospect of saving lives and halting disease in its tracks will continue to fuel the field of pH regulation in cancer as we work to broaden our understanding from fundamentals to clinical trials.

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