Manipulating extracellular tumour pH: an effective target for cancer therapy

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The pH in tumour cells and the tumour microenvironment has played important roles in cancer development and treatment. It was thought that both the extracellular and intracellular pH values in tumours are acidic and lower than in normal cells. However, recent progress in the measurement of pH in tumour tissue has disclosed that the intracellular pH (pHi) of cancer cells is neutral or even mildly alkaline compared to normal tissue cells. This review article has summarized the recent advancement in the measurement pHi and extracellular pH (pHe) in cancer cells, and the effect of pHi and pHe on proliferation, migration and biological functions of cancer cells. This paper has also elaborated recent treatment strategies to manipulate pHi and pHe for cancer treatment. Based on the recent progress in pHi and pHe manipulation in cancer treatment, we have proposed potential nanoparticle-based strategies to manipulate pHi and pHe to effectively treat cancer.

1. Introduction

Cancer is one of the most severe diseases in the world. According to statistics, in total 8.8 million people died from cancer in 2015, accounting for 17% of the total deaths.1,2 Researchers have made great efforts to understand the pathogenesis and properties of cancer in order to develop effective treatments for clinic application. As known, extracellular and intracellular pHs in tissues affect the function of the cells and play an important role in cancer development and treatment. As reported, the extracellular pH (pHe) affects the proliferation of human T cells and the expression of the interleukin-2 receptor.3 It is widely accepted that the pHe of cancer cells is more acidic than normal cells.4–6 Generally, pHe values of the normal tissues (brain tissues, subcutaneous tissues, etc.) are in the rage of 7.2–7.5. However, pHe of tumour cells is mildly acidic in the range of 6.4–7.0. Since Warburg et al. first reported the abnormal anaerobic glycolysis in tumour cells, they measured the glucose and lactic acid in tumor veins and found more lactic acid and less glucose on the tumour tissue than on the normal tissue due to the fermentation process in the tumour side, which may affect pHe and pHi in tumour.7 Consequently, it has

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been assumed that pH_e and pH_i in cancer cells should be more acidic than those in normal cells during 1930s to 1980s.\textsuperscript{7,8} With the progress on sensing technologies, several techniques have been developed to measure pH_i and pH_e in cancer cells including pH-sensitive nuclear magnetic resonance spectroscopy (MRS), positron emission tomography (PET) radiotracers, magnetic resonance imaging (MRI) and optical imaging (Optics).\textsuperscript{9} It has been found that pH_i in cancer cells is actually mildly alkaline or near neutral, similar to normal cells.\textsuperscript{10,11} These new findings subvert the traditional assumption that the pH_i in cancer cells is more acidic than normal cells. More extracellular acidity and more intracellular alkalinity means a smaller ratio of pH_e/pH_i.

Subsequently, researchers have investigated the mechanisms of pH controls in cancer cells and microenvironments. Numerous membrane transporters across tumour cells have been found for pH homeostasis in cancer cells, and further been used to manipulate pH_e and pH_i.\textsuperscript{2,13–17} These novel strategies have been developed to control the pH_e/pH_i ratio in cancer microenvironments and cells to induce apoptosis of cancer cells, improving the treatment efficiency.\textsuperscript{18}

In this review, we have summarized the recent progress on the studies of pH_e and pH_i in tumour tissues and their corresponding normal tissues. Then, we have further outlined the mechanisms of pH_e/pH_i maintenance in cancer cells and the developed therapeutics to manipulate the pH_e/pH_i in cancer tissues. In the outlook, the potentials of new strategies using state-of-art nanotechnology to manipulate the pH_e/pH_i in cancer tissues have been proposed for cancer treatment.

### 2. pH_e/pH_i in tumour tissues versus normal tissues

#### 2.1. Technologies for \textit{in vivo} pH measurement and their accuracies

Several approaches for the measurement of pH_e and pH_i in tumour have been developed including pH-sensitive electrodes (POT), chemical exchange saturation transfer MRI (CEST-MRI), MRS, PET, MRI, and Optics.\textsuperscript{5,6,19–31} Table 1 summarized some basic information of four major technologies for \textit{in vivo} pH measurement.

Although several novel MRI and optical imaging agents (probes) have been developed and applied for \textit{in vivo} pH measurement, there is not adequate data of the pH values measured by the same MRI or Optics method for comparable analysis. Thus, for the consistency of the comparison, the pH measured by POT or MRS were collected and compared in Section 2.2 and 2.3.

#### 2.2. Extracellular pH (pH_e)

According to the literature reports, pH_e of eight types of tumour tissues and the corresponding normal tissues has been summarized in Fig. 1. These data were selected based on the measurements using pH-sensitive electrodes.\textsuperscript{5,6,14–39}

As shown in Fig. 1, pH_e of cancer cells is 0.3–0.7 pH unit lower than that of corresponding normal cells. For example, malignant melanoma tissues have an average pH_e of 6.96 while the average pH_e in normal skin cells is 7.39,\textsuperscript{4} which is 0.43 difference. The average pH_e in vulvar tumours is 7.26, 0.7 pH unit less than in normal vulvar tumours (with an average pH_e of 7.96).\textsuperscript{6} Uterine tumour tissues also have a lower average pH_e (6.92) than normal uterus, whose average pH_e is 7.64.\textsuperscript{6} Although the average pH_e of two kinds of brain tumours is slightly different, they are both more acidic than normal brain tissues.\textsuperscript{39} Similar results have also been observed in other tissues, such as lung,\textsuperscript{34} breast,\textsuperscript{39} and skeletal muscle.\textsuperscript{35–37} Thus, it is very clear that most cancer cells usually have a more acidic pH_e than their corresponding normal cells, and the differences vary from 0.3–0.7.

Warburg \textit{et al.} proposed that tumour cells used glycolysis rather than oxidative phosphorylation to acquire energy, even in the presence of oxygen.\textsuperscript{7} Excess anaerobic glycolysis has been considered as the major reason for the extracellular acidity of tumour tissues.\textsuperscript{36,39} For most animal cells, there are two different pathways for glucose metabolism, \textit{i.e.} aerobic and anaerobic glycolysis. The detailed processes of glucose metabolism in the cells have been briefly outlined in Fig. 2. There are two possible pathways for glucose metabolism in the cells: aerobic and anaerobic pathway. Generally, one glucose molecule is metabolized to two pyruvate molecules, producing two ATP molecules as the energy. In the aerobic pathway, two pyruvate molecules react with CoA-SH and form acetyl-CoA by releasing CO_2. Subsequently, the produced acetyl-CoA undergoes the citric acid cycle, finally degrading into CO_2 and producing 30 ATP molecules. In the anaerobic process, two pyruvate molecules transfer into two lactate molecules with the assistance of lactate dehydrogenase, but this transfer only produce 2 ATP. The overall reactions of these two ways are briefly expressed as follows:

\[
C_6H_{12}O_6 (d\text{-glucose}) \rightarrow 6CO_2 + 6H_2O + 38ATP \text{ (aerobic)}
\]

\[
C_6H_{12}O_6 (d\text{-glucose}) \rightarrow 2C_3H_6O_3 (lactate) + 2ATP \text{ (anaerobic)}
\]
In the normal cells, most glucose is fully metabolized to produce carbon dioxide, water and the energy via the aerobic pathway. However, in the tumour cells, the glucose is mostly metabolized through the anaerobic pathway, which produces a large amount of lactate and releases limited energy due to a high level of pyruvate and hypoxia in the tumour environment. During the process, the tumour growth requires a large amount of energy compared to the normal tissue, which produces more CO₂ and lactic ions in tumour. The produced CO₂ was excreted extracellularly, resulting in the acidic condition in the tumour microenvironment, i.e. 0.3–0.7 pH units lower than the average pHₑ of normal tissues.

### 2.3. Intracellular pHᵢ: acidic or not?

Interestingly, pHᵢ of cancer cells is not acidic, not as postulated previously. Since the 1980’s, more research outcomes have demonstrated that pHᵢ of cancer cells is around neutral and even mildly alkaline.¹⁰,¹¹ Fig. 3 has displayed the pHᵢ of six kinds of tumour tissues and their corresponding normal tissues collected from MRS method.¹³,¹⁴–⁴⁵

Very surprisingly, the average pHᵢ of these tumour cells is slightly higher than that in their corresponding normal cells, although the difference is less than 0.1 pH unit and not significant. For example, the average pHᵢ of brain tumours is 7.31, slightly higher than normal brain cells (7.24).¹¹,⁴²,⁴⁶ Redmond et al. reported that the intracellular environment of osteosarcoma cells is also mildly more alkaline than in normal cells.¹³ Furthermore, this weak alkalinity of the intracellular environment in tumour cells has also been discovered in many other types of tumours, such as hepatoblastoma¹¹ and squamous cell carcinoma.⁴⁴ These evidences thus clearly indicate that pHᵢ of tumour cells is near neutron or even more alkaline. Thus, the discrepancy of pHₑ and pHᵢ in tumour cells is much larger than in normal tissues.

### 2.4. How cancer cells maintain their unbalanced pHₑ/pHᵢ ratio?

For most cells, the maintenance of neutral (or mild alkaline) pHᵢ is achieved by transporting respiratory end-products (such as CO₂ and lactate) across the cell membrane. When the extracellular concentration of acidic respiratory end-products is lower than intracellular, the excess CO₂ can passively across the

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**Table 1** The technologies for in vivo pH measurement

| Technology | First use | Accuracy (pH unit) | Mechanism | Major Advantage | Reference |
|------------|-----------|--------------------|-----------|-----------------|-----------|
| POT | 1950s | ±0.1–0.2 pH | Use of pH-sensitive electrodes with tip diameters ranging from 0.5 pm to 2 mm | Electrodes can be directly controlled by hand and the results can be easily read | 4 and 6 |
| PET | 1970s | ±0.08 pH | Based on the presence of pH-dependent biologically active molecule | High sensitivity (nM–pM level detected) | 23 and 24 |
| MRS | 1980s | ±0.06 pH | Based on the pH-dependent chemical shift of the resonance frequency | Real-time observation of multiple metabolites | 25 and 26 |
| MRI | 1990s | ±0.1 pH | Based on the pH-dependent relaxation agent, hyperpolarized ¹³C-labelled agent, and/or proton-electron double resonance imaging | Visible, concentration-independent | 19–22 and 27–29 |
| Optics | 2000s | ±1.5% (±0.1 pH) | Based on the specificity of fluorescence probes and pH sensitivity of their emission lifetime | Non-invasive, Independent to the concentration of agent and intensity of the excitation light | 31 and 32 |
| CEST-MRI | 2000s | ±0.01 pH | Based on the agents that are capable of exchanging protons with the surrounding water molecules, lead to the continuous buildup of magnetic saturation of water, resulting in extremely enhanced sensitivity | Very low concentration extremely high sensitivity | 21 and 33 |

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**Fig. 1** The comparison of average extracellular pH values of different tumours with normal tissues. Blue dots refer to the average extracellular pH of some cancer tissues, while black dots the average extracellular pH of corresponding normal tissues. All dots (average pHₑ ± SEM) referred to the average extracellular pH of a specific kind of cancer or normal tissues listed. Data were taken from several different sources (ref. 4, 6 and 34–39), which were given in the text correspondingly.

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(For more detailed content, please refer to the original article.)
cell membrane by diffusion. However, in most cases, the CO₂ and lactate generated from glucose metabolism is accumulated in extracellular tumour site due to low blood flow rate, resulting in development of acidic microenvironments in tumour. In this situation, the release of CO₂ and lactate in microenvironments mainly relies on numerous special membrane proteins, such as carbonic anhydrase enzymes (CA2, CA9 and CA12). More relevant pH regulators are listed in Table 2 and discussed in Section 3. Overall, the maintenance of pH₄ and pH₅ is based on passive diffusion and active membrane transporters. Table 2 briefly summarizes some major pH regulators in tumours and their main functions, including anion exchangers (SLC4A1, SLC4A2, and SLC4A3), proton transporter vacuolar ATPase (V-ATPase), mono-carboxylate transporters (MCT1, MCT2, MCT3, and MCT4), sodium ion based chloride/bicarbonate exchanger (SLC4A8) and Na⁺/H⁺ exchanger 1 (SLC9A1).

In the last two decades, several complicated mechanisms have been revealed about how cancer cells maintain the alkaline pHₑ and acidic pHᵢ. Among them, the mechanism for the import of weak bases (e.g. bicarbonate) and the extrusion of weak acids (e.g. CO₂, H₂CO₃, and lactate) with the assistance of proteins in tumour cell membrane has been clearly demonstrated. Apart from this, the intracellular protons have been pumped out of tumour cells in three different ways, including direct discharge from the cells, exchange with other extracellular cations (e.g. Na⁺), and extrusion by the vacuolar ATPase.

3. The effect of pHₑ and pHᵢ on tumour activity

As discussed above, the difference of pHₑ and pHᵢ in tumour cells is much larger than in normal cells. The maintenance of pHₑ and pHᵢ in the tumour mainly relies on some specific proton pumps and intracellular buffer systems. For instance, the balance of HCO₃⁻/CO₂⁻ buffer system in tumour is administrated by carbonic anhydrase enzymes CA2, CA9 and CA12. Besides, the Na⁺/H⁺ buffer system is manipulated by Na⁺/H⁺ exchangers, such as SLC9A1. The regulation of pHₑ and pHᵢ depends on the synergic effect of all of these pumps and buffer systems.

It is known that even the little change of pHₑ/pHᵢ ratio may severely affect many biological and chemical processes in the cells, and eventually result in the proliferation and aggressiveness of cancer cells. For example, the incubation of melanoma in the acidic environment can significantly enhance its metastasis, aggressiveness and migratory activity in vitro. Martinez-Zaguilan reported that C8161 and A375P cells were cultured in acidic medium (pH 6.8) for 3 weeks and then transferred to the membrane invasion culture system (MICS) chambers. They found that C8161 cells and A375P cells treated in acidic
medium have significantly enhanced migration and invasion, as shown in Fig. 4. Moellering et al. also reported that acidic-treated C8161 cells cultured in normal medium (pH 7.4) showed higher aggressiveness than those cultured in acidic environment (low pH group) and control (native group), as shown in Fig. 5. The C8161 cells cultured in lower pH medium (6.7) has shown the inhibition of the cell invasion, indicating less aggressiveness. These results have demonstrated that the regulation of pH_e and pH_i ratio in the tumour is highly important for metastasis, aggressiveness and migratory activity. Fine control of pH_e and pH_i in tumour may improve the cancer treatment.

Table 2  The summary of some major pH regulators in cancer cells and their main functions in manipulating the ratio of extracellular pH and intracellular pH in tumour cells

| Name     | Description                                         | Function                                                                 | Reference |
|----------|-----------------------------------------------------|--------------------------------------------------------------------------|-----------|
| SLC4A1   | Anion exchangers                                    | Transport HCO_3^- out of cancer cells                                    | 53 and 54 |
| SLC4A2   |                                                     |                                                                          |           |
| SLC4A3   |                                                     |                                                                          |           |
| SLC4A7   | Sodium bicarbonate cotransporters                  | Mediate the coupled movement of sodium and bicarbonate ions across the plasma membrane | 55        |
| SLC4A8   | Sodium ion-based chloride/bicarbonate               | Transport Cl^- out of tumour cells and simultaneously import HCO_3^- into cancer cells powered by Na^+ | 56        |
| SLC9A1   | Na^+/H^+ exchanger 1                               | Transport intracellular produced H^- to the extracellular environment, and import Na^+ at the same time | 56        |
| MCT1     | Monocarboxylate transporters                        | Transport (both inside to outside and outside to inside) the products of glycolysis (such lactic acid and other monocarboxylates) | 57 and 58 |
| MCT2     |                                                     |                                                                          |           |
| MCT3     |                                                     |                                                                          |           |
| MCT4     |                                                     |                                                                          |           |
| V-ATPase | Proton transporter vacuolar ATPase                 | A proton pump on the membrane of tumour cells, responsible for the stransportation of H^+ between intracellular and extracellular plasma | 59        |

Fig. 4  Comparison of the migration and invasion of C8161 and A375P cells treated in standard and acid media. The migration and invasion properties of cells treated in acidic medium (pH 6.8) were drawn in white bar, while black bar referred to the value of cells cultured in standard medium (pH 7.4). Data analysis was performed using Student’s t-test: *P < 0.01; **P < 0.005; ***P < 0.001. This figure is adapted from ref. 73 with permission from Kluwer Academic Publishers.

Fig. 5  Invasion of different C8161 phenotypes. Representative invasion assay results for C8161 phenotypes assayed in their respective media. Native group meant the cells were incubated in normal medium. Low pH group represented the cells cultured in acidic medium (pH 6.7). LH group meant the cells were cultured in acidic medium for 1 month and then transferred into normal medium before the experiment. This figure is reproduced from ref. 74 with permission from Springer Netherlands.
Furthermore, the slight change of pH_e and pH_i may also disorder the function of some proteins (such as tenascin and fibronectin), particularly in cancer cells.\textsuperscript{75,76} For example, mild change of environmental pH by 0.7 pH unit dramatically affected the RNA alternative slicing. The major expression of tenascin-C (TN-C) isoforms was 8 kb TN mRNA in human skin fibroblasts at pH 7.4, while 6 kb TN mRNA isoform was the majority of TN-C expression at pH 6.7 (see Fig. 6).

Tumour microenvironment triggers the tumour heterogeneity during the cancer development. It is well known that acidic condition and hypoxia are important characteristics in the tumour microenvironment. The homeostasis of pH_e and pH_i is very important for all kinds of cells. As discussed above, compared with normal cells, cancer cells have a more acidic pH_e and more alkaline pH_i, suggesting that the pH homeostasis regulation of tumour tissues may be more complex and involve in more proteins and buffer systems. The pH environment may influence the growth and function of the cells in two main ways. On the one hand, the 0.1 alteration in the ratio of pH_e/pH_i may affect many essential biochemical processes in the cell metabolism system, such as ATP synthesis, cell proliferation, aggressiveness, migration and diffusion, and the function of some membrane proteins.\textsuperscript{40} On the other hand, the tiny disturbance of pH_i may activate the mechanism of alternative splicing of constituents in extracellular matrix to produce isoform of tenascin and fibronectin, which specifically occur in cancer cells rather than in normal cells.\textsuperscript{75,76} Although the isoforms of these alternatively spliced proteins do not involve in the manipulation of tumour’s pH_e/pH_i ratio, they may provide binding sites for antigen-based cancer therapy.\textsuperscript{15}

4. Strategies to manipulate the pH_e/pH_i ratio

As discussed above, the small change in pH_e/pH_i ratio of tumour cells may disturb many biological functions, including proliferation, aggressiveness, and migration. This relationship demonstrates that adjusting the pH_e/pH_i ratio in the tumour tissues may halt cancer progress or even completely inhibit cancer growth. In recent years, several approaches have been developed to manipulate pH_e/pH_i ratio for cancer treatment. These approaches can be classified as direct manipulation and indirect manipulation. Direct manipulation is to regulate the pH_e/pH_i ratio of tumour cells by using acidic/alkaline drugs and indirect manipulation is based on operating the pH regulators of tumour cells.

4.1. Direct manipulation using small molecule drugs

The drugs for direct manipulation are mainly small molecular substances (such as bicarbonates). This approach is to directly increase pH_e of tumour tissues to the normal level (0.3–0.7 pH unit). It can be achieved by oral administration of alkaline agents or even by simple adjustment of diet habit.

The alkaline agents include sodium bicarbonate and trisodium citrate.\textsuperscript{77} In practice, it seems difficult to maintain the mildly alkaline microenvironment near tumour tissues via oral administration, as a high dose and continuous intake of the alkaline substrate is required. Based on the breast cancer study, White et al. investigated the exact daily dose of sodium bicarbonate needed for breast cancer treatment.\textsuperscript{78} The calculated daily dose for a normal human (with 70 kg weight) would be 31.75 g sodium carbonate or 32.5 g trisodium citrate.\textsuperscript{79} Another example is the Tris-base buffer to inhibit tumour progression and metastasis.\textsuperscript{80} The size of the pancreatic tumour in the mouse model was significantly decreased after 200 mM of Tris-buffer treatment. Based on their data, the daily dose for the mice can be calculated as 18.2 g of Tris-base buffer per kg, equivalents to 31 g of Tris-base intake per day for an adult (70 kg). Although it is possible for a cancer patient to intake more than 30 g alkaline agents (such as sodium carbonate or trisodium citrate) with daily drinking water, it would be more efficient to deliver alkaline agent to the tumour tissues rather than to the whole body. A recent non-randomized controlled study investigated the efficacy of local infusion of alkaline agent.\textsuperscript{81} Researchers found that there was a 6.4-fold difference of geometric mean of viable tumour residues (VTR) when the hepatocellular carcinoma patients were treated with transarterial chemoembolization (TACE) accompanied with or without locally infusing bicarbonate (LIB) into tumour (Table 3). Such a local administration may be a better strategy for anticancer therapy.

The adjusted diet could be low in protein but high in potassium and/or magnesium.\textsuperscript{82-84} It has been proved that potassium can effectively neutralize mineral acidity and even mildly alkaline pH of urine via KHCO_3 generation or glutamine sparing.\textsuperscript{85} The pH_i may be altered by a large change of the intake of potassium due to its fundamental physiologic and metabolic
The pHe/pHi ratio may increase. The abnormal proton transportation and change of lines may promote the programmed cell death in some tumour cell membranes. Most of these proton pumps on the tumour cell membrane have a few specific isoforms that do not exist on the normal cell surface. Thus these isoforms may provide some specific target sites for cancer therapy. Once these functional proton pumps are inhibited, the pH balancing system of tumour cells may be disordered and the pHe/pHi ratio may decrease and pHi increase. Proton pumps (or more exactly Na+/H+ exchangers) directly exchange intracellular H+ with extracellular Na+ and then lead to the induction of apoptosis in many types of gastric cancer cells, which involves in the regulation of tumour pH. Besides, the inhibition of proton extrusion by Na+/H+ exchanger inhibitors or V-ATPase inhibitors may make cancer cells susceptible or vulnerable. Now a few proton pump inhibitor drugs have been used in the clinical stage. Table 4 lists some inhibitors and their target proton pumps. As seen in Table 4, the current inhibitor drugs mainly focus on two major pH regulators (V-ATPase and SLC9A1) and only one of these drugs, cariporide, has been successfully developed to phase III clinical trial.

### 4.2. Indirect manipulation: proton pump inhibitors

The second alternative strategy to administrate the pHe/pHi ratio is to inhibit the functional proton pumps. It is well known that the maintenance of high pHe/pHi ratio in tumour tissues relies on many proton regulators (pumps) on the cell membrane. Most of these proton pumps on the tumour cell membrane have a few specific isoforms that do not exist on the normal cell surface. Thus these isoforms may provide some specific target sites for cancer therapy. Once these functional proton pumps are inhibited, the pH balancing system of tumour cells may be disordered and the pHe/pHi ratio may increase. The abnormal proton transportation and change of the pHe/pHi ratio may affect the behaviour of tumour cells. Recent research reports have demonstrated that the inhibition of proton regulators have suppressed the proliferation and promoted the programmed cell death in some tumour cell lines. For example, treatment with proton pump inhibitors led to the induction of apoptosis in many types of gastric cancer.

| Inhibitors drugs | Identification site | Function & description | Reference |
|------------------|---------------------|------------------------|-----------|
| Omeprazole, esomeprazole | V-ATPase | Can be activated in the slightly acidic environment, and then inhibit V-ATPase via covalent interaction. Work on V-ATPase at high dose | 94 and 95 |
| Bafilomycin | V-ATPase | Commonly inhibits V-ATPase (not selective for tumour cells) with high cell toxicity | 96 and 97 |
| Diuretic amiloride | SLC9A1 | Inhibits NHE-1 with unacceptable high concentration | 98 |
| EIPA (derivative of amiloride) | SLC9A1 | 200 times stronger than amiloride, has not used in clinical trial yet | 98 and 99 |
| Cariporide | SLC9A1 | Decrease the intracellular pH of cancer cells. Has been developed to the third stage of clinical trial | 90 and 100–102 |
cells is to promote cancer glycolysis to the utmost extent by maximizing the glucose supplement. The extremely high rate of glycolysis may break the capacity of proton pumps in tumour cells, which means that tumour cells cannot timely transport acidic metabolites (such as $\text{H}^+$, $\text{H}_2\text{CO}_3$, lactate etc.) outside and hence decreases $\text{pHi}$. For example, a very high glycolysis rate was observed in human melanoma cells (cultured in the medium containing high amount of glucose) when DNP, an uncoupling agent, was added.\(^{106}\)

The programmed cell death can also be activated by the sharp reduction of $\text{pHi}$, finally leading to cell death.\(^{102}\) This may result from several different mechanisms, one of which is the reduction of glycolysis metabolism.\(^{107}\) For example, the enzymatic functions of hexokinase, one of the vital enzymes for the maintenance of the high level of glycolysis metabolism in tumours, was strongly inhibited (activity decreased from $82 \pm 3.2\%$ to $31.2 \pm 5.7\%$) with the sharp decrease (from 8 to 6 respectively) of $\text{pHi}$ in SNB-19 glioma cells.\(^{106}\)

The tumour glycolysis can be promoted by inhibiting the production of mitochondrial ATP, which requires some specific inhibitors. meta-Iodo benzylguanidine is one of the inhibitors of mitochondrial complex 1, acting as proton extrusion inhibitors (or hyperglycemia) and then decreasing $\text{pHi}$ in cancer cells.\(^{106,109−111}\) However, this drug is normally used as a radioiodine therapy agent, and the dose used for radioiodine therapy is not high enough to perform a strong inhibition on proton transportation. Dinitrophenol (DNP), a new type of chemotherapeutic drugs, has also shown a remarkable enhancement in glycolysis with the increase of blood pressure at a low dose. It has been reported that mM-level DNP can inhibit the proliferation of cancer cells and lead to apoptosis in the human pulmonary adenocarcinoma Calu-6 cell line.\(^{112}\)

Overall, even though there are some drugs (such as metaiodobenzylguanidine and DNP) that have shown their ability to decrease $\text{pHi}$ by boosting the glycolysis rate in tumour cells, the hyperglycemia-reliable mechanism restricts the feasibility of this cancer therapy strategy.

5. Conclusions and future prospective

In this review, $\text{pHe}$ and $\text{pHi}$ in tumour cells have been summarized and the ways to manipulate cellular pH in cancer cells have been discussed. It is clear that tumour cells have a more acidic $\text{pHe}$ (0.3–0.7 lower) than normal cells, and $\text{pHi}$ in tumour cells is neutral or even more alkaline than that in normal cells. The abnormally high ratio of $\text{pHe}/\text{pHi}$ in tumour cells is due to the high rate of glycolysis in tumour cells, which produces numerous acidic products (such as $\text{H}_2\text{CO}_3$ and $\text{CO}_2$). The maintenance of $\text{pHe}/\text{pHi}$ relies on several special proton pumps on tumour cell membranes, such as SLC9A1 and V-ATPase. Then the mechanisms of these proton pumps are
discussed and two potential pH manipulating strategies are presented, including direct manipulation by delivering small molecule drugs and indirect manipulation sing proton pump inhibitors.

It has been demonstrated that treatment of cancers (halting its proliferation, aggressiveness and even inducing programmed cell death) is very possible by manipulating pH_e/pHi ratio in tumour. A future potential method is to combine 2 or 3 inhibitors so that pH_e/pHi can be well controlled, which may significantly enhance the efficacy of the cancer treatment.

The other potential way to direct pH manipulation can be achieved by target delivery of alkaline nanoparticles to the tumour tissues by virtue of EPR effect. Of course, inhibitor-loaded nanoparticles can be further functionalized with target ligands, which may enhance the accumulation in the tumour tissues and manipulate the pH_e/pHi ratio.

Another potential way to direct PH manipulation can be achieved by target delivery of alkaline nanoparticles to the tumour tissues by virtue of EPR effect. Thus, accumulated alkaline nanoparticles neutralize the extracellular acids and efficiently increase pH_e. Moreover, some alkaline nanoparticles can be modified as a carrier for delivering anticancer drugs to more efficiently treat cancers.114,115

Conflicts of interest
There are no conflicts to declare.

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