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Cancer nanomedicines targeting tumor extracellular pH

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ABSTRACT

Tumors have been a highlight in the research of nanomedicine for decades. Despite all the efforts in the decoration of the nano systems, tumor specific targeting is still an issue due to the heterogeneous nature of tumors. Hypoxia is frequently observed in solid tumors. The consequent acidification of tumor extracellular matrices may bring new insight to tumor targeting. In this review, we present the polymeric nano systems that target tumor extracellular pH (pH_e).

As a self-derived disease, the consistent differences between normal tissue and tumor are often inadequate to facilitate the development of effective therapy. No significant change in mortal rate among patients with advanced cancer is observed over more than half a century [1,2]. Among all the thriving new technology in cancer treatment, chemotherapy remains the most widely used method. However, toxic agents that can kill cancer cells can also damage normal cells. Hence there are numerous side/adverse effects, but limited treatment outcomes. Further research revealed a difference of functional vasculature between tumor and normal tissue. Nano system formed from various polymeric carriers brought new promise to treatments. Studies of anti-cancer nanoparticles thrive in the area of enhanced permeability and retention (EPR) effect, and ligand/receptor facilitated internalization [3,4]. However, the heterogeneity among cancer cell populations as well as the heterogeneous up-regulation/expression of receptors/antigens on cancer cell membranes limits the clinical application of nanoparticles decorated with one kind of ligands [5,6].

The functional vasculature in a tumor area is often maldeveloped and insufficient to provide enough nutrition to fast dividing cells. The resulting lack of oxygen and nutrients triggers an alteration of metabolism in tumor cells as an adaptation. The anaerobic condition leads to a production of lactic acid, resulting in an acidic pH in many solid tumors. Although acidic environment causes trouble in drug permeability and facilitates tumor invasion in some cases, it also brings opportunity for anti-cancer nano systems. In this review, we present the polymeric nano systems that target tumor pH_e.

1. Tumor vasculature and angiogenesis

As fast growing masses, tumors require an extra supply of oxygen and other nutrition. This requirement of supply triggers the formation of new vasculature. In a growing tumor, the origin of vessels includes the original host vessels that run through the tumor tissue, and the neovasculature formed as a result of tumor angiogenesis factors [7–13]. The pre-existing host vessels per unit tumor mass do not increase over time, but the shape of venulesis often deformed, elongated, and often dilated [7,9]. As tumors grow, some original vessels are crushed, while remaining vessels seem to be able to adopt the change and resist the destruction brought about by tumor growth. However, tumor arterioles often lack spontaneous vasomotion, which is typical in normal vessels [7–11].

Although the vessels vary in different tumor types, sometimes even within one tumor mass, the new vessels formed in accelerated growth exhibit abnormalities both in structure and in function. For example, structurally, the vessel wall is incomplete, lack pericytes and biological receptors; the vessels are often elongated and exhibit an arteriovenous shunt; the vascular density is chaotic and the intercapillary space is expanded. Functionally, the vessels are more fragile; the speed and direction of blood flow is unstable; the vascular permeability is increased, which may result in hemoconcentration and high interstitial fluid pressure [7,14]. When increased vascular permeability is combined with the often malfunctioned lymphatic drainage,

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it results in the EPR effect, which is used in most antitumor nanosystems [15–17].

Therefore blocking neovascularization and starving tumors to death was considered beneficial to cancer patients. The vascular endothelial growth factor (VEGF) and its receptor VEGFR2 are among the most investigated. Clinically, bevacizumab (monoclonal anti-VEGF antibody), sunitinib and sorafenib (second-generation multitargeted receptor tyrosine kinase inhibitors) have given numerous patients prolonged life spans [18–24]. However, the increased span is often limited to months. Recent research revealed what seems to be contradictory – treatment with VEGF inhibitors may trigger metastasis [18,25,26], which is the primary cause of mortality in cancer patients [27]. To further understand the situation, we need to investigate the hypoxia and corresponding metabolism in tumors.

2. Hypoxia and metabolism under hypoxia

A growing tumor mass needs to meet the bioenergetic and biosynthetic demands, despite the fluctuating nutrition and oxygen supply. In blood, healthy tissue, and typical hypoxic solid tumors, the oxygen concentration ranges from 10% to 12.5%, 3% to 6% and 1% to 2%, respectively [28,29]. Hypoxia can trigger the activation of different genes, which can in turn change the metabolism in the cells [30]. For example, the hypoxia-inducible factor 1(HIF-1) is up-regulated in most malignant tumors and over 60% of metastases [29,31]. It activates the glycolytic or tumor metabolic phenotype [32,33], which results in the acidification of tumor extracellular environments. HIF consists of two subunits – α- and β-subunits. Three α-subunit isoforms are discovered so far, with HIF-1α being the most studied [34–36]. For all three isoforms, the post-translational regulation of stability is similar in mechanism. Under normoxic conditions (normal pO_2 in tissues), proline in the oxygen-dependent degradation domain (ODDD) and asparagine in the C-terminal activation domain (C-TAD) can be hydroxylated by the oxygen sensor prolyl hydroxylase domain (PHD) dioxygenasesand factor inhibiting HIF (FIH) respectively. However, under hypoxia, PHD proteins and FIH are inactive and the stability of HIF-α is preserved [36–39]. HIF-α then translocates into the nucleus, heterodimerizes with HIF-β and binds with hypoxia-response elements (HRE) in the promoter or enhancer regions of DNA. Thus a series of downstream changes are triggered, and the metabolic balance is altered [36,40–42].

The expression of glycolytic enzymes and glucose transporters (GLUT1 and GLUT3) are up-regulated by HIF [42]. As a result, glucose molecules are more efficiently caught and converted to pyruvate, which helps the cancer cells to survive and proliferate under a limited oxygen and nutrient supply. The resulting pyruvate, instead of going through a tricarboxylic acid (TCA) cycle, is converted into lactic acid directly. However, under aerobic metabolism, one molecule of glucose can produce 38 adenosine triphosphate (ATP) molecules maximum; whereas in anaerobic metabolism, one molecule of glucose can produce only 2 ATP molecules [33,36]. The phenomenon of preference for using the truncated pathway – Warburg effect – was observed on cancer cells even in the presence of oxygen [33,36], but it is still not totally clear why cancer cells use this pathway which is 19-fold less efficient in ATP production. One explanation is believed to have to do with the decrease of the downstream products that prevent/prohibit and the increase of the ones that facilitate tumorigenesis, and harnessing glucose metabolism in cancer cells may be of therapeutic benefits [33,36,43,44].

Aerobic glycolysis is one primary metabolic change often observed in cancer cells, where above 90% of pyruvate (which is produced from glucose) is converted to lactate, which is eventually transported outside the cell membrane [33,43]. H^+ ions are formed during glycolysis, ATP hydrolysis, and glutaminolysis and also transported out the cell. Large amount of H^+ ions are produced as a result of high rate of glycolysis and lactic acid production. They would normally be washed out by blood and the interstitial pH remains unchanged [45]. However, the blood flow rate in tumors is often decreased as the result of abnormalities in vasculature. For example, in Grade I astrocytoma's blood flow can be as low as 0.03 ml/g/min, compared with 0.25–0.78 ml/g/min in normal gray matter. Therefore the over-produced H^+ ions are accumulated in the tumor interstice [46]. The low tumor pH_e is then a result of over-production of lactic acid and carbonic acid. Some isoforms of H^+/lactate monocarboylate transporters (MCT) are up-regulated under hypoxia and can pump lactate outside the cells [47]. HIF also induces eco enzyme carbonic anhydrase (CA) IX or XII, which present in the cell membrane and convert carbon dioxide into carbonic acid when carbon dioxide molecules diffuse out from the membrane. HCO_3^- is subsequently taken up by the cells and H^+ is left to add on the acidity of tumor extracellular fluid [48]. The expression and activity of Na^+/H^+ exchanger 1 (NHE1) is also enhanced under hypoxia. NHE1 can substitute one molecule of extracellular Na^+ with one molecule of intracellular H^+. NHEs play an important role in maintaining the intracellular pH of cells [49,50]. The decreased tumor pH_e can facilitate the invasion of tumor, slow down the uptake of basic anticancer drugs (such as doxorubicin (DOX)) [51,52]. Thus combined with the altered metabolism, the tumor cells usually become more resistant to chemotherapy. However, it also offers an opportunity for anticancer nano-treatment. An illustration of the influence of hypoxia on tumor pH_e is shown in Fig. 1.

3. pH measurement and estimates of tumor pH_e

pH electrodes are the most frequently used measurement in tumor pH estimates [53]. The measurements by probe tips in micrometer to millimeter range mainly reflect the tumor pH_e. However, insertion of the probe into the tumor tissue may cause damage to cells and surrounding capillaries [54]. Thus, the resulting estimates may be influenced. Finer probe tips were also designed, but with a loss of sensitivity [55].

^{31}P-nuclear magnetic resonance (NMR) is a non-invasive spectroscopy based on the pH-dependent chemical shift of phosphates, $H_2PO_4^-$ and HPO_4^{2-} ($H_2PO_4^- = HPO_4^{2-} + H^+$) [56–60]. However, these inorganic phosphates are mainly distributed intracellularly. Therefore, the measurements largely reflect intracellular pH. By changing phosphates into organic phosphates, the distribution can be altered into the entire water phase or extracellular water phase [61–64]. Dimethyl methylphosphonate (DMMP) and 3-aminopropylphosphonate (3-APP) are non-toxic and chemically inert. DMMP can distribute through the entire water phase, while 3-APP is extracellular. Employment of DMMP and 3-APP in ^{31}P-NMR can measure not only the compartmental volume, but also pH_e.

Tumor pH_e estimates are generally lower than those of normal tissue (Fig. 2). Volk et al. [65] examined 268 human xenograft samples of 30 tumor cell lines on rnu/rnu rats. Glass electrodes inserted into a 25 gauge bevelled needle were used in the measurement, with a length of the sensing portion of 250 μm. The resulting average xenograft pH_e is 6.84, ranging from 6.71 to 7.01, with a variation within single tumor of 0.3 to 0.8 units, in contrast to the physiological pH of arterial blood of 7.4. Studies on human patient reveal a similar trend. A separate study [66] on 67 tumor nodules in 58 patients results in an average pH_e of 7.01 (5.66–7.78). Average pH_e of examined adenocarcinomas, soft tissue sarcomas, squamous cell carcinomas and malignant melanomas are 6.93 ± 0.08 (5 .66–7.78), 7.01 ± 0.21 (6.25–7.4),