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Hypoxia-induced therapy resistance: Available hypoxia-targeting strategies and current advances in head and neck cancer

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ABSTRACT

Most solid tumors, such as head and neck cancers, feature a hypoxic microenvironment due to angiogenic dysregulation and the consequent disruption of their vascular network. Such nutrient-deprived environment can induce genomic changes in several tumor cell populations, conferring survival and proliferative advantages to cancer cells through immunosuppression, metabolic switches and enhanced invasiveness. These transcriptional changes, together with the selective pressure hypoxia exerts on cancer cells, leads to the propagation of more aggressive and stress-resistant subpopulations increasing therapy resistance and worsening patient outcomes. Although extensive preclinical and clinical studies involving hypoxia-targeted drugs have been performed, most of these drugs have failed late-stage clinical trials and only a few have managed to be implemented in clinical practice. Here, we provide an overview of three main strategies to target tumor hypoxia: HIF-inhibitors, hypoxia-activated prodrugs and anti-angiogenic agents; summarizing the clinical advances that have been made over the last decade. Given that most hypoxia-targeted drugs seem to fail clinical trials because of insufficient drug delivery, combination with anti-angiogenic agents is proposed for the improvement of therapy response via vascular normalization and enhanced drug delivery. Furthermore, we suggest that using novel nanoparticle delivery strategies might further improve the selectivity and efficiency of hypoxia-targeted therapies and should therefore be taken into consideration for future therapeutic design. Lastly, recent findings point out the relevance that hypoxia-targeted therapy is likely to have in head and neck cancer as a chemo/radiotherapy sensitizer for treatment efficiency improvement.

Introduction

It is a well-known fact that many solid tumors feature an abnormal vasculature, characterized by poorly organized, leaky and irregular vessels [1]. The main cause of this chaotic web is the pro-angiogenic switch the tumor permanently turns on by releasing factors such as tumour growth factor-β (TGF-β), platelet-derived growth factor-β (PDGF-β) and vascular endothelial growth factor (VEGF) [2]. This tumor neovascularization is enhanced in an attempt to make nutrient supply catch up with its rapid growth and expansion, but often results in an opposite effect [3]. As a consequence of inefficient vascular network, blood can pool into tissues increasing the interstitial fluid pressures, which impedes the efficient delivery of oxygen and nutrients to some tumor regions [2,4]. Whilst oxygen levels in normal tissues usually range from 3% to 7% O_2 (20–74 mmHg), the disrupted tumor vasculature brings these numbers down to 0.3–4.2% O_2 (2–32 mmHg) with the majority of tumors presenting median oxygen levels below 2% (13 mmHg), a condition referred to as hypoxia [5]. Paradoxically, even though fast growth impairs their nutrient supply, tumors are able to continue expanding under hypoxic conditions [2,6]. The answer to this contradiction resides in the genomic changes induced by hypoxia, enabling tumor cells to acclimatize and survive under such nutrient-deprived environment [5,7]. Additionally, prolonged hypoxia exposure will exert a selective pressure leading to the viability and propagation of the most stress resistant and aggressive subpopulations of tumor cells [8].

The classic hypoxia signalling pathway is triggered by the activation of hypoxia-inducible factors (HIFs) composed of two subunits: an alpha (HIF-1α, HIF-2α, or HIF-3α) and a beta (HIF-1β) [1,8,9]. In the presence of oxygen, two proline residues of the HIF-α subunit are subject to hydroxylation by prolyl hydroxylase domain proteins (PHDs), which allows the interaction between HIF-α and the von Hippel-Lindau protein (pVHL) [10]. As a result of this interaction, pVHL recruits an E3 ubiquitin ligase complex that ubiquitinates HIF-α, targeting it for proteasomal degradation [11]. However, when oxygen levels drop below 2% O_2 the inhibitory hydroxylation of HIF-α is majorly reduced, preventing its degradation [12]. Consequently, the HIF-α subunit, which most frequently is HIF-1α or HIF-2α, will now be free to translocate into the nucleus and associate with the constitutively expressed HIF-1β, forming the heterodimeric transcription factor complex HIF1 or HIF2, respectively [13]. The HIF-α/β complex, in interaction with its coactivator p300/CBP, will bind to hypoxia response elements (HREs)

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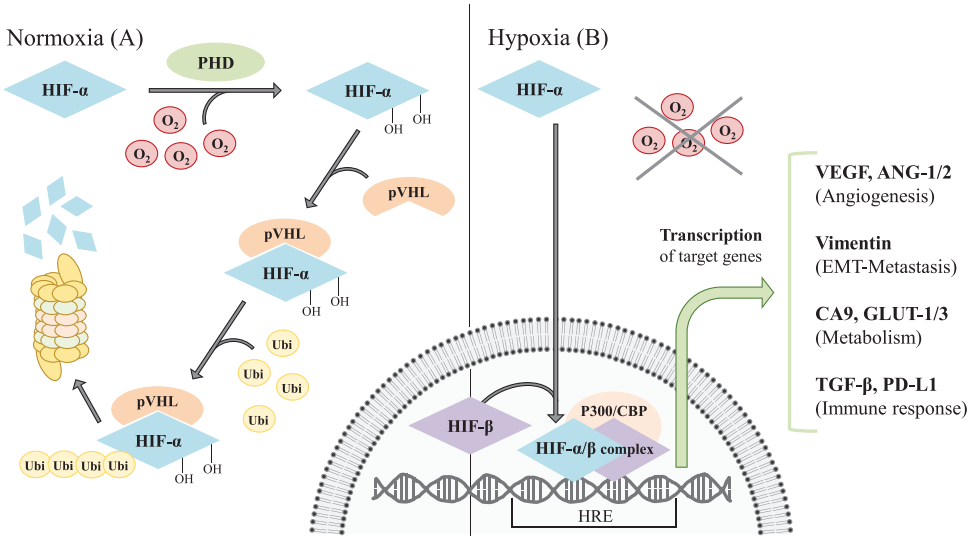

Fig. 1. Hypoxia signalling pathway: (A) Under normoxic conditions HIF-α is hydroxylated by prolyl hydroxylase domain proteins (PHDs), allowing the interaction between HIF-α and the von Hippel-Lindau protein (pVHL). Consequently, pVHL will recruit an E3 ubiquitin ligase complex that will target HIF-α for proteasomal degradation. (B) Under hypoxic conditions however, the hydroxylation reaction does not take place and HIF-α is now free to translocate into the nucleus and associate with HIF-1β and coactivator p300/CBP, forming a heterodimeric transcription complex. Binding of this HIF-α/β complex to hypoxia response elements (HREs) will result in the upregulation of HIF target genes, regulating various cellular processes such as angiogenesis (VEGF and ANG-1/2), invasiveness (Vimentin), metabolism (CA9 and GLUT-1/3) and immunity (TGF-β and PD-L1).

upregulating the expression of HIF target genes (Fig. 1) [14]. More than 100 genes are regulated by HIF, influencing a large range of cellular functions such as angiogenesis, metabolism, cell proliferation, immunity, invasion/metastasis and apoptosis [1,7,15].

Downstream effects of hypoxia

One of the main downstream effects of hypoxia is neovascularization, induced in an attempt to improve oxygen and nutrient supply to the tumor [6]. Both HIF-1α and HIF-2α lead to the upregulation of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), angiopoietin 1 and 2 (ANG-1/2) and angiopoietin 1 receptor (TIE-2), all of which play key roles in neovascularization [16]. Moreover, HIF-1α has been shown to upregulate the expression of stem cell factor (SCF) and several microRNAs, which also play their role in neoangiogenesis [7,17].

Concurrently, tumor cells are capable of recruiting, through chemoattraction, other cell types such as tumor-associated macrophages (TAMs), to further enhance angiogenesis and invasion [14,16]. Other target genes affected by the hypoxia signalling pathway include transcription factors such as Snail, Slug and Twist1, which regulate the expression of the EMT modulators like E-cadherin, vimentin, and N-cadherin [18–20]. Additionally, hypoxia further enhances invasiveness through the upregulation of promigratory factors such as the hepatocyte growth factor (HGF) and stromal cell-derived factor 1 (SDF-1a) [6,21]. Given the impaired tumor vasculature, these invasion and migration processes will be further facilitated by the leakiness of tumoral vessels, which will allow metastatic cancer cells to escape through their walls [2,14].

Another mechanism induced by HIF-1α is the potentiation of glycolysis, to supply the tumor with an energy resource for proliferation and expansion even at low oxygen concentrations [7,22]. This is achieved by triggering the overexpression of key components of the glycolytic pathway, such as the carbonic anhydrase-9 (CA-9); glucose transporter-1,3 (GLUT-1,3); glyceraldehyde phosphate dehydrogenase (GAPDH); lactate dehydrogenase-A (LDHA); pyruvate dehydrogenase kinase 1 (PDK1); phosphofructokinase L (PFKL) and phosphoglycerate kinase 1 (PGK1) [7,23]. The final product of this glycolytic pathway is pyruvate, which in hypoxic conditions will be metabolized to lactic acid, the accumulation of which will lower the intracellular pH [24]. As previously mentioned, in this hypoxic and acidic tumor microenvironment a higher infiltration of tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) is observed [14]. However, tumor-derived cytokines and growth factors that are present in the microenvironment are able to transform these macrophages into immunosuppressive (or M2) TAMs [6,25]. Consequently, immunosuppressive TAMs release factors such as interleukin (IL)-10 and the transforming growth factor β (TGF-β), which are able to downregulate the expression of human leukocyte antigens (HLAs) and convert cytotoxic T lymphocytes into regulatory/immunosuppressive T cells [26]. Furthermore, HIF-1α directly enhances the expression of programmed death-ligand 1 (PD-L1) in tumor cells as well as TAMs and dendritic cells, further supporting immunosuppression in the hypoxic microenvironment [1,14,26,27].

Taken together, the hypoxic tumor microenvironment does not only enhance proliferation and invasiveness of tumor cells, but also allows them to evade the immune system, conferring them a survival privilege. Apart from the impunity that hypoxia confers to tumoral cells, the dysfunctional vasculature of this niche will also impair drug delivery, further increasing immunotherapy and chemotherapy resistance [6,28]. Since most chemotherapeutic agents perform its cytotoxic function by oxidizing free radicals and ROS, poor delivery and low oxygen levels significantly decrease their efficiency [14]. Similarly, radiation induces the formation of unstable free radicals in the DNA which react with oxygen molecules causing irreversible damage. However, in the absence of sufficient oxygen radiation-induced DNA damage can be reversed, leading to radiotherapy resistance [1,29]. Altogether, it comes as no surprise that hypoxia would be positively correlated with worse prognoses across all types of cancer, and therefore targeting it has undoubtedly therapeutic benefits [14,30].

Hypoxia and head and neck cancer

Head and neck squamous cell carcinoma (HNSCC), which represents over 95% of all head and neck cancers, is an umbrella term that comprises diverse carcinomas developed along the mucous membranes of the oral cavity, oropharynx, larynx and hypopharynx [31]. Globally, HNSCC is the sixth most common malignancy and mortality rates still remain at around 40–50% [31,32]. The main risk factors for HNSCC are tobacco and alcohol abuse, as well as high-risk human papillomaviruses (HPV), particularly type HPV-16 [33]. Given that the epidermal growth factor receptor (EGFR) is overexpressed in more than 80% of these malignancies, a monoclonal antibody against EGFR named Cetuximab was approved by FDA in 2006 for the treatment of HNSCC, but unfortunately its efficacy has proved to be limited [32,34].

Nowadays, the standard of care for HNSCC is radiotherapy with or without chemotherapeutic agents such as cisplatin and 5-fluorouracil

Table 1
Classification and specific targets of four different groups of hypoxia-targeted drugs.

Group	Drug	Classification	Target	Refs.
HIF-α inhibitors	PT2385	Allosteric modulator	HIF-2α	[37]
	Acriflavine	Topical antiseptic	HIF-1 dimerization	[38]
	BAY 87–2243	Mitochondrial Complex I inhibitor	HIF-1α/2α	[39]
	Digoxin	Cardiac Glycoside	HIF-1α	[40]
	Digitoxin	Cardiac Glycoside	Topoisomerases and MAPK	[40]
	EZN-2208	PEGylated SN38	Topoisomerase I	[41]
	BEZ-235	Imidazoquinoline derivative	PI3K/Akt/mTOR	[42]
	Metformin	Type-II diabetes drug	mTOR	[43]
	Rapamycin	Macrolide compound	mTOR	[44]
	Minnelide	Diterpenoid triepoxide prodrug	p300, Hsp70	[45]
Anti-angiogenic drugs	Bevacizumab	Monoclonal Ab	VEGF	[46]
	Ramucirumab	Monoclonal Ab	VEGFR-2	[47]
	Axitinib	Tyrosine kinase inhibitor	VEGFR-1/2/3	[48]
	Sunitinib	Tyrosine kinase inhibitor	VEGFR-1/2, KIT and PDGFRα/β	[49]
	Imatinib	Tyrosine kinase inhibitor	PDGFRα and c-KIT	[50]
Hypoxia- activated prodrugs	Apaziquone	Indolequinone EO9	DNA (single-strand breaks)	[51]
	Tirapazamine	Benzotriazine di-N-oxide	DNA (single/double-strand breaks)	[52]
	Nimorazole	Nitroimidazole anti-infective	DNA (single/double-strand breaks)	[53]
	TH-302	5-nitroimidazole compound	DNA (crosslinker)	[54]
	TH-4000	Tyrosine kinase inhibitor	EGFR	[55]

[35]. Additionally, an anti-PD-1 monoclonal antibody called pembrolizumab has recently been approved for the treatment of recurrent/metastatic HNSCC as monotherapy or in combination with chemotherapy [36]. Despite the recent advances in targeted therapy, patient outcomes and survival rates of HNSCC have not improved substantially over the last years which, added to its high incidence, raises the urgency to find more effective treatment alternatives [31–33]. Radiotherapy not only plays a key role in primary care but is also preferable for organ conservation [35]. Unfortunately, and as previously mentioned, tumor hypoxia significantly decreases radiotherapy efficacy, enhancing therapy resistance and cancer recurrence.

With that in mind, this review reinforces the idea that targeting and reversing hypoxia could be the key to improve therapy response in solid tumors such as HNSCC. For that matter, it brings together diverse available strategies that target tumor hypoxia and its current advances, in order to examine their therapeutic potential alone or in combination. Additionally, it points out the major setbacks faced in clinical trials and suggests alternatives for future studies that could help pave the road to hypoxia-targeted therapy.

Available strategies to target tumor hypoxia

Tumor hypoxia can be targeted by various signalling pathways and at several different levels. Therefore, the list of hypoxia-targeted drugs that will be reviewed has been divided into three main groups: HIF-α inhibitors, anti-angiogenic drugs and hypoxia-activated prodrugs (Table 1).

Inhibitors of hypoxia-inducible factors

HIF-1α and HIF-2α are overexpressed in the majority of human cancers and, together with HIF-1β, they play a key role in tumor malignancy and therapy resistance [14]. Additionally, as well as its main role as a transcription factor subunit, HIF-2α can form a translation initiation complex, involving RNA-binding protein RBM4 and cap-binding protein eIF4E2, which enhances translation of several target mRNAs under hypoxic conditions [56]. Therefore, targeting HIF-α activity could prove useful for the disruption and reversal of adverse hypoxia-induced effects.

There are several different strategies to target HIF-α activity, depending on the signalling level that is being targeted. As an example of a direct HIF-α inhibition, PT2385 is an allosteric modulator that binds to HIF-2α and inhibits its dimerization with HIF-1β, preventing the formation of the HIF-2 transcriptional complex and its downstream effects [37]. Given its specificity for HIF-2α, it is of particular interest for cancer types in which HIF-2α plays a key role, such as Glioblastoma or Clear Cell Renal Cell Carcinoma for which clinical trials are currently ongoing (NCT03216499 and NCT02293980, respectively). A similar mechanism of action is used by acriflavine, an FDA-approved antibacterial that has been shown to inhibit HIF-1 dimerization and has recently obtained encouraging results in preclinical models of brain cancer [38]. Moreover, acriflavine has been demonstrated to also inhibit EMT and the unfolded protein response, helping to re-sensitize cancer cells to antineoplastic drugs [57]. Using a different mechanism, BAY 87-2243 is a potent small molecule inhibitor of mitochondrial complex I which blocks reactive oxygen species (ROS) production thus reducing hypoxia-induced HIF-1 activity [58]. So far, it has been demonstrated that BAY 87-2243 is capable of reducing tumor hypoxia in head and neck cancer xenografts and, when administered prior to radiotherapy, improving local tumor control due to radio sensitization, opening the doors for new upcoming clinical trials in head and neck cancer patients [39].

Interestingly, another repurposed FDA-approved drug, in this case a cardiac glycoside for heart failure named digoxin (Lanoxin), was shown to inhibit HIF-1α in preclinical studies in mice [59]. Given the used doses in preclinical experiments were greatly above the circulating therapeutic doses of digoxin, translation into clinical trials were expected to result in severe toxicity and poor outcomes [40]. In fact, a completed phase II clinical trial of digoxin in patients with recurrent prostate cancer found that using inferior circulating therapeutic doses of digoxin, in order to avoid toxicity, was not enough to produce a significant improvement in the patients (NCT01162135) [60]. Alternatively, a similar cardiac glycoside called digitoxin (Digitaline) seems to inhibit expression of HIF-α via targeting of topoisomerases and the MAPK signalling pathway, and has demonstrated promising results in several types of cancer at clinically achievable doses [40,61]. Other similar drugs that downregulate the expression of HIF-α via topoisomerase inhibition include EZN-2208, a new version of irinotecan that consists of its active metabolite, SN38, attached to a polyethylene glycol (PEG) to improve its solubility [41]. EZN-2208 has been studied in different solid tumor types and so far obtained encouraging results in preclinical studies and a phase I clinical trial for chronic lymphocytic leukaemia and neuroblastoma [62].

Given that one of the downstream effects of the PI3K/AKT/mTOR signalling pathway is translation of HIF-α mRNA, inhibitors of one or several components of this pathway result as well in indirect downregulation of HIF-α [63]. Amongst this group of inhibitors, we can distinguish between multi-targeted drugs such as BEZ-235 and metformin, and selective inhibitors like rapamycin [42,43,64]. Unfortunately,

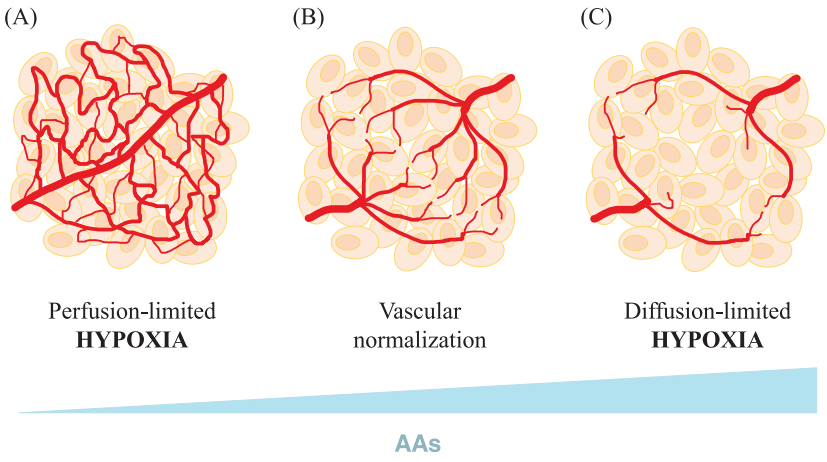

Fig. 2. Anti-angiogenic drugs for vascular normalization: (A) The tumor-released pro-angiogenic factors disrupt the angiogenic homeostasis and lead to an aberrant and impaired vasculature which is not able to properly deliver oxygen to the cells, causing perfusion-limited hypoxia. (B) Anti-angiogenic drugs (AAs) given at low doses are able to revert the aberrant neovascularization and normalize the vasculature, re-perfusing tumor cells. (C) Anti-angiogenic drugs given at high doses destroy the vascular network and leave tumor cells without nearby vessels, causing diffusion-limited hypoxia. Figure inspired by Meaney et al., 2020.

BEZ-235 (Dactolisib) showed high toxicity levels in preclinical studies and the completed clinical trials so far have not obtained any benefits [65]. On the contrary, metformin (Glucophage), which indirectly inhibits mTORC1 through activation of the adenosine monophosphate protein kinase (AMPK) [66], has obtained promising results and is currently ongoing several clinical trials in breast cancer (NCT01101438), endometrial cancer (NCT01697566), colorectal cancer (NCT02614339), prostate cancer (NCT01864096) and oral cancer (NCT03685409 and NCT02581137). Differently to metformin, rapamycin (Rapamune) is an allosteric inhibitor that directly inhibits mTOR by binding to its FKBP-rapamycin-binding (FRB) domain and so far, encouraging results have been obtained in preclinical and clinical studies in prostate cancer patients [44,67]. Lastly, minnelide is a prodrug that turns into the active metabolite triptolide, which targets p300 and the heat shock protein 70, inhibiting the transcriptional activity of HIF-1 [56,68]. Currently, minnelide is undergoing a phase II clinical trial in patients with refractory pancreatic cancer, the results of which should be expected soon (NCT03117920).

Anti-angiogenic drugs

Apart from inhibiting the pro-angiogenic signalling that hypoxia induces, anti-angiogenic drugs also enable us to tackle the root cause of hypoxia: the abnormal tumor vasculature. Even though it may seem contradictory, it has been shown that anti-angiogenic drugs are capable of normalizing the tumor vasculature by preventing the formation of the new unfunctional vessels (Fig. 2) [69]. If successful, this approach could re-perfuse the tumor not only decreasing hypoxia, but increasing drug delivery as well, improving therapy efficiency [6,70]. However, it is important to keep in mind that, when used at high doses, anti-angiogenic agents may lead to the opposite effect, worsening tumor hypoxia [6].

Most of these anti-angiogenic agents are targeted towards VEGFR and PDGFR, the main receptors involved in neovascularization [71], and can be divided into monoclonal antibodies and tyrosine kinase inhibitors (TKIs). Differently to the others, bevacizumab (Avastin) is an FDA-approved monoclonal antibody that targets VEGF instead of inhibiting its receptor [46,72]. So far, bevacizumab has been approved in combination with chemotherapy for the treatment of advanced non-small cell lung cancer [73] and advanced colorectal cancer [74]; and in combination with immunotherapy for the treatment of advanced renal cell cancer [75]. Additionally, since access to bevacizumab is limited in some regions or circumstances, more affordable biosimilars have been developed some of which have already been approved for advanced non-small cell lung cancer and metastatic colorectal cancer (Bevacizumab Updates – Big Molecule Watch) [76]. Other monoclonal antibodies with anti-angiogenic properties include ramucirumab (Cyramza), which targets VEGFR-2 and has been approved for the treatment of metastatic gastric cancer and hepatocellular cancer as monotherapy, and for non-small cell lung cancer, colorectal cancer and gastric cancer in combination with chemotherapy [47,77,78].

Next in order, there are several anti-angiogenic TKIs which also inhibit VEGFR and/or PDGFR and have obtained promising results in recent clinical trials. Axitinib (Inlyta), which inhibits VEGFR-1/2/3, has recently completed two phase III clinical trials in which, combined with immunotherapy, managed to improve patient outcomes in advanced renal cell cancer (NCT02853331 and NCT02684006). The multitargeted TKI sunitinib (Sutent), which inhibits both VEGFR-1/2 and PDGFRα/β, has been approved by the FDA for the treatment of metastatic renal cell cancer [79]. Similarly, imatinib (Gleevec) is an inhibitor of PDGFRα and c-KIT that got approved by the FDA in 2001 for the treatment of chronic myeloid leukemia (CML) [80].

Hypoxia-activated prodrugs

Hypoxia-activated prodrugs (HAPs) are inactive bioreductive components that get activated by one or two electron reductions performed by endogenous oxidoreductases under hypoxic conditions (Fig. 3) [28,81]. This hypoxia selectivity is achieved through reduction reactions that can normally be reversed in the presence of oxygen [82]. Therefore, instead of targeting hypoxia-induced signalling like HIF-α inhibitors and anti-angiogenic drugs, HAPs use hypoxia as an advantage to selectively induce anti-neoplastic effects in hypoxic areas, which are normally the most aggressive and therapy resistant tumour regions. These anti-neoplastic agents that result from HAP activation normally consist in DNA-reactive cytotoxins that cause irreversible DNA damage leading to cellular death [82,83].

As an example, apaziquone (EO9/Qapzola) is activated through a 2-electron reduction, performed by reductases such as DT diaphorase (DTD) and NAD(P)H Quinone Dehydrogenase 1 (NQO1), transforming into a DNA damaging species that will induce DNA single-strand breaks [51,84]. Given that bladder tumors normally feature elevated DTD levels, apaziquone was found to be effective for this specific cancer type in preclinical studies [84]. However, a phase III clinical trial evaluating the efficacy and safety of apaziquone in non-muscle invasive bladder cancer patients was unfortunately discontinued (NCT01469221) [85].

Tirapazamine (Tirazone) is another prodrug that after activation via reductases is capable of inducing DNA strand breaks [52]. Similarly to apaziquone, even though promising results were obtained in preclinical and clinical studies in in head and neck cancer, tirapazamine in combination with chemoradiotherapy failed to obtain any clinical benefits in phase III trials (NCT00174837) [52,85,86]. Alternatively, an oxygen mimetic prodrug named nimorazole (Nagoxin) has been demonstrated to replace oxygen in the chemical reactions induced by radiotherapy acting as a radiosensitizer [87]. Nimorazole together with radiotherapy has been approved in Denmark and Norway for the treatment of HPV-negative HNSCC, and recent studies have successfully included

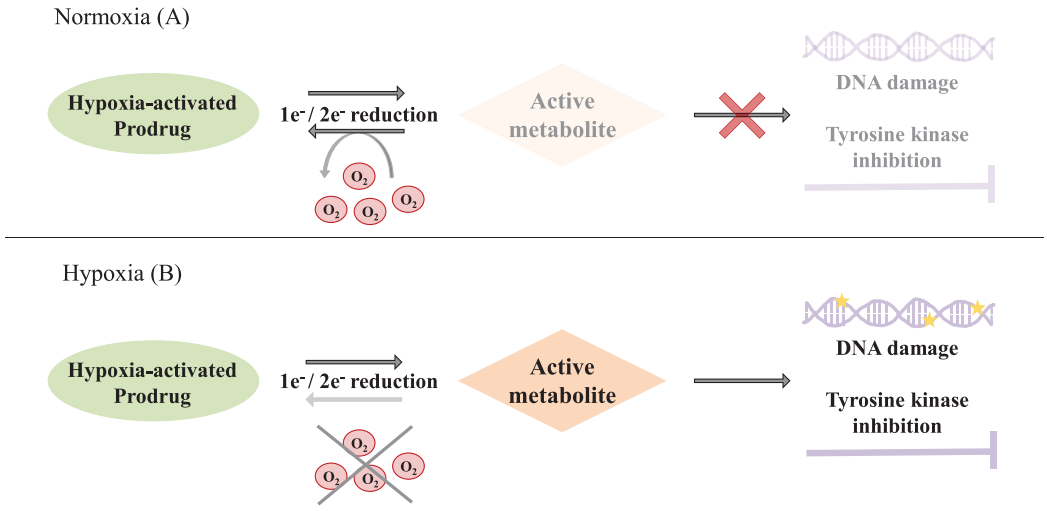

Fig. 3. Hypoxia-activated prodrugs: (A) Under normoxic conditions hypoxia-activated prodrugs undergo 1 or 2 electron reductions performed by endogenous oxidoreductases, which can be immediately reversed by available oxygen inactivating the drug. (B) Under hypoxic conditions however, reduction of hypoxia-activated prodrugs cannot be reversed due to the lack of available oxygen, leading to the stabilization of cytotoxic metabolites which normally induce cell death through DNA damage or inhibition of tyrosine kinase receptors.

chemotherapy in this therapeutic regimen as well [53,88]. Furthermore, a phase III clinical trial called NIMRAD using nimorazole alongside radiotherapy in patients with Recurrent/Metastatic-HNSCC is currently ongoing in the UK (NCT01950689). This accomplishment represents a major landmark in hypoxia-targeted therapy for HNSCC, especially considering that HPV-negative HNSCC feature a lower response to radiotherapy when compared to HPV-positive HNSCC [35]. This differential radiotherapy response is mainly due to HPV-positive HNSCC having smaller primary tumors, increased cell arrest in the G2 phase, defects in DNA damage repair and better immune response, amongst other features [89]. These recent advances may be pointing that combinatorial approaches including radiotherapy and hypoxia-targeted strategies could increase radiosensitivity and treatment response in HPV-negative HNSCC. Unfortunately, even though HPV-positive patients generally present higher radiotherapy responses it has been reported that under hypoxic conditions, tumor cells become radioresistant as well and in their case hypoxia-targeted strategies have not been shown to be effective nor improve the situation so far [90].

Additional HAPs that are currently being studied include TH-302 (Evofosfamide), a 2-nitroimidazole prodrug of the cytotoxin bromoisophosphoramide, which is capable of DNA alkylation and crosslinking once active [54,91]. Unfortunately, two phase III clinical trials testing TH-302 plus chemotherapy in metastatic pancreatic adenocarcinoma and soft tissue sarcoma were discontinued after not meeting their primary endpoint (NCT01746979 and NCT01440088, respectively) [85]. Consequently, an ongoing phase III clinical trial testing TH-302 plus chemotherapy in esophageal cancer has been withdrawn (NCT02598687). Nevertheless, a phase I/II clinical trial of TH-302 with or without Bortezomib in patients with relapsed/refractory multiple myeloma has recently obtained encouraging results [92]. Another prodrug that is being tested is TH-4000 (Tarloxotinib) which, differently to most HAPs, is not a DNA damaging species but an EGFR inhibitor [93]. Unfortunately, despite obtaining encouraging results in preclinical studies, a phase II trial in patients with EGFR mutant non-small cell lung cancer was terminated due to lack of efficacy (NCT02454842) [94].

With the exception of nimorazole, the majority of HAPs have failed late-stage clinical trials and having poor tissue penetration has been suggested as the most common cause of clinical failure [82,95]. In other specific cases, such as tirapazamine, activation/reduction of HAPs is not properly inhibited in the presence of oxygen which leads to higher non-specificity and toxicity, limiting dose intensification [95]. More importantly, knowing hypoxia does not only serve as a prognostic biomarker but can as well be used as a predictive biomarker for treatment efficacy, one of the major setbacks to consider in clinical trials is the lack of patient stratification based on the hypoxia-status of the tumor [82]. One of the available strategies to measure tumor hypoxia is direct pO_2 measurements using electrodes, a highly invasive technique lacking reliability and reproducibility. For that matter, other non-invasive methods such as PET/CT (positron emission tomography/computed tomography) and PET/MRI (positron emission tomography/magnetic resonance imaging) using glucose analogues and/or hypoxia-selective PET tracers are being used to delineate tumor regions and their hypoxia levels [82,96]. In fact, several studies have already demonstrated that using PET hypoxia imaging before and during radiochemotherapy helps stratify treatment response and potentially decrease tumor recurrence [96,97]. Therefore, combining these strategies with radiochemotherapy and/or hypoxia-targeted drugs might allow us to target the most hypoxic and therapy resistant tumor regions, improving treatment efficacy.

Focusing back on the poor tissue penetration setback, one could have predicted that drug delivery would likely be disrupted by the malfunctioning vasculature of hypoxic tumors. For that matter, novel delivery strategies together with alternative drug combinations are currently being suggested and tested, some of which will be reviewed in the next chapter.

Hypoxia-targeted combinatorial therapy via nanoparticles

One of the most recent and revolutionary strategies that have been suggested to improve treatment response is nanoparticles containing combination of HIF-targeted drugs [98,99], anti-angiogenic agents [100] and/or hypoxia-activated prodrugs [2,101,102]. Considering most hypoxia-activated prodrugs have failed clinical trials, likely due to insufficient drug delivery, a recent in silico analysis attempted to evaluate the outcomes of lipidic nanoparticles containing both anti-angiogenic agents (AAs) and hypoxia-activated prodrugs (HAPs), in an attempt to improve HAPs delivery and efficiency. The vascular normalization effect that low-dose AAs induce is time-restricted given that tumor cells keep the pro-angiogenic switch on, inducing a subsequent re-neovascularization when the drug effects fade away [6]. This 'normalization window' has been studied during the last few years and according to these studies, precise timing and dosing are essential when administering anti-angiogenic drugs in combination therapies. The combination of AAs and HAPs is more complicated than it may seem because if they are both administered simultaneously, the re-perfusion that AAs

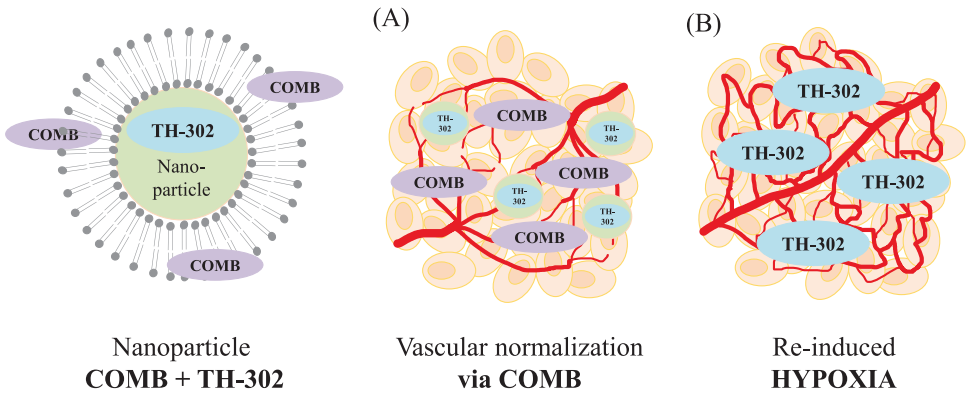

Fig. 4. Nanoparticles combining AAs (COMB) + HAPs (TH-302): (A) Initial release of the anti-angiogenic COMB reverses neovascularization, normalizing tumor vasculature thus allowing efficient delivery of nanoparticles containing TH-302. (B) Delayed release of the hypoxia-activated prodrug TH-302 allows to trap it in the required hypoxic tumor environment, where its activity will be enhanced by low oxygen concentration.

would trigger would reduce HAPs activity given the parallel increase in oxygen levels. Therefore, this study tries to come up with a solution by combining several mathematical models based on glioblastomas, to predict the feasibility of this combination treatment using nanocells as drug delivery vehicles.

The mathematical data used was based on a HAP called TH-302 (Evofosfamide) and an AA called COMB (Combretastatin) that targets VEGF. What they propose is binding COMB to the external layer of the nanoparticle instead of inside, where TH-302 lies, allowing a prior release of COMB that would therapeutically benefit in two different ways: Firstly, the preparatory vascular normalization effect would enhance nanoparticle delivery inside the tumor; and secondly, the delayed release of TH-302, which should optimally occur once neovascularization has been retriggered, could trap TH-302 in a hypoxic region enhancing its activity and efficacy (Fig. 4) [2].

An additional advantage of using nanoparticles, apart from time-controlled release and synergistic effect, is the optional addition of external tumor-specific antibodies to further increase tumor selectivity [99]. Other studies using similar nanoparticle strategies include one that attempted to combine two HIF-α inhibitors, called YC-1 and irinotecan, and observed as a result a 5.7-fold increase in efficiency in lung cancer cells [98]. Furthermore, experiments involving nanodelivery have also combined hypoxia-targeted therapies with other chemotherapeutic agents or targeted drugs as well. One of these experiments managed to co-deliver an antineoplastic agent (doxorubicin) and a HIF-1 inhibitor drug (triptolide) and the results pointed out that triptolide enhanced the uptake of doxorubicin, improving efficacy in oral squamous cell carcinoma cells [103]. A similar experiment, which synthesized nanoparticles containing cisplatin and metformin, also proved to increase drug efficiency and decrease toxicity in HNSCC models [99].

Conclusion and perspectives

Taken together, hypoxia is irrefutably responsible for the aberrant neovascularization, invasiveness, metabolic switch, immunosuppression and therapy resistance that characterizes solid tumors; and therefore, hypoxia-targeted therapies have important potential in alleviating aggressive tumor behaviour and improving therapy response in cancer patients. However, taking into account that tumor vasculature in most solid tumors is dysfunctional because of hypoxia, a combinatorial approach using anti-angiogenic drugs could enhance drug delivery and consequently treatment response. Furthermore, using more selective and efficient delivery strategies such as nanoparticles for the administration of hypoxia-targeted drugs alone or in combination are important considerations in designing future hypoxia-targeted therapies.

Focusing on head and neck cancer, the oxygen-mimetic HAP called nimorazole has already proved to be useful for radio-sensitization in HNSCC, being part of its standard of care in some countries and potentially extending its use across Europe after the result release of currently ongoing clinical trials. Similarly, metformin is currently ongoing two clinical trials in oral carcinomas, the results of which are also eagerly awaited. It has also been recently demonstrated that combining HIF-inhibitors, such as triptolide or metformin, with chemotherapeutic agents, like cisplatin and doxorubicin, enhances cytotoxicity and efficiency thus improving treatment response. These recent results point out that similarly to nimorazole being administered before radiation in order to sensitize cells and improve radiotherapy efficacy, other hypoxia-targeted drugs such as triptolide or metformin could be used prior to chemotherapy regimens in order to increase treatment response. Therefore, the main interest of hypoxia-targeted drugs may not reside in its individual activity but instead its sensitization role for other standard of care and targeted therapies.

Author contributions

MT and VC formulated the topic of the review. VC wrote the manuscript and prepared the table and figures. MT critically revised the manuscript and the figures.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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