Hypoxia and its impact on the tumour microenvironment of gastroesophageal cancers

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Abstract
The malefashion of the hypoxic tumour microenvironment (TME) in cancer progression was recognized decades ago but the exact mechanisms that augment the hallmarks of cancer and promote treatment resistance continue to be elucidated. Gastroesophageal cancers (GOCs) represent a major burden of worldwide disease, responsible for the deaths of over 1 million people annually. Disentangling the impact of hypoxia in GOCs enables a better overall understanding of the disease pathogenesis while shining a light on novel therapeutic strategies and facilitating precision treatment approaches with the ultimate goal of improving outcomes for patients with these diseases. This review discusses the underlying principles and processes of the hypoxic response and the effect of hypoxia in promoting the hallmarks of cancer in the context of GOCs. We focus on its bidirectional influence on inflammation and how it drives angiogenesis, innate and adaptive immune evasion, metastasis, and the reprogramming of cellular bioenergetics. The contribution of the hypoxic GOC TME to treatment resistance is examined and a brief overview of the pharmacodynamics of hypoxia-targeted therapeutics is given. The principal methods that are used in measuring hypoxia and how they may enhance prognostication or provide rationale for individually tailored management in the case of tumours with significant hypoxic regions are also discussed.

Key Words: Esophageal cancer; Gastric cancer; Tumor hypoxia; Tumour microenvironment; Gastroesophageal cancer

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Core Tip: Improved methods in measuring the oxygen status in the tumour microenvi-
INTRODUCTION

One of the major turning points in the study of solid tumors arose with the realization that a critical regulatory influence in the process of angiogenesis was an environmental feature; hypoxia[1,2]. Many studies have since demonstrated the oncogenic transforming power of hypoxia in the microenvironment of different tumor types and the observation that tumor oxygenation status could disrupt the anti-tumor effects of radiation therapy was published over 60 years ago[3-8]. This review will discuss the role of hypoxia in the tumor microenvironment (TME) of gastroesophageal cancers (GOCs) including gastric cancer (GC) and oesophageal cancer (OC), how it augments disease, and additionally its relevance in the setting of prognostication and therapeutic targeting.

GOC is a substantial cause of morbidity and mortality, responsible for 1.2 million deaths per year globally[9-12]. An improved understanding of the risk factors for GC has seen a steady decline in both the incidence and mortality which is in sharp contrast to the rising incidence of OC, particularly oesophageal adenocarcinoma (OAC) globally[13,14]. GOCs develop insidiously and consequently, are commonly diagnosed at an advanced stage where chemotherapy with or without radiation remains the treatment of choice in the neoadjuvant setting[15]. Treatment at this stage is rarely curative and several mechanisms account for this resistance to treatment including tumor cell-intrinsic and extrinsic mechanisms. Hypoxia is a characteristic feature of the TME and a key mediator in conferring and enhancing treatment resistance[16-18]. The TME being the complex reciprocity between both the cellular (resident and infiltrating) and non-cellular components that surround, envelop, and make up the tumour mass, the components of which are summarized in Figure 1[19-21]. The exact mechanisms underlying resistance continue to be elucidated and as such, interest in the role of hypoxia in translational oncology research has garnered increasing interest recently as shown in Figure 2.

Hypoxia mediates aggressive, metastatic, and treatment-resistant disease by augmenting the hallmarks of cancer through various cellular and physiological events including; enhanced tumour cell proliferation, survival, immune evasion, inflammation, induction of angiogenesis, and activation of invasion[16,17,22]. In large part these events are influenced or orchestrated by the relationship between oxygen availability and the genes encoding hypoxia-inducible factors (HIF) and von Hippel Lindau protein (pVHL)[23,24]. HIFs are a family of heterodimeric transcription factors consisting of a labile α subunit and a stable β subunit. There are several HIF isoatypes but the most well-studied is HIF1. HIF1-α contains domains amenable to post-translational modifications thereby mediating interactions with the molecular machinery responsible for cellular degradation[25,26]. When induced, HIF1-α associates with the constitutively expressed HIF1-β subunit and together act to bring about the transcription of a multitude of genes involved in complex signalling pathways with a diverse degree of roles. There exists a whole host of HIF target genes that are transcribed in response to hypoxia that have been implicated in driving tumour progression. The roles of these target genes range from receptors to enzymes to further transcription factors and more (Table 1), which are involved in the enhancement of inflammation, angiogenesis, immune evasion, and the other remaining hallmarks of cancer.
**Table 1** Hypoxia induces the transcription of a range of genes that mediate diverse roles in promoting the hallmarks of cancer[178-180]

| Function               | Gene                                                                 |
|------------------------|----------------------------------------------------------------------|
| Enzymes                | MMP1, MMP3, LOX, ADAMST1, ACE                                        |
| Transcription factors  | Twist1, Snail, Slug, β-Catenin, c-Myc, Oct4, NF-kB                   |
| Receptors              | CXCR4, c-Met, TLR4, Notch                                            |
| Growth factors         | VEGF, TGFβ                                                          |
| Transporters           | Glut-1, MDR1                                                        |
| Intracellular signalling| Calc1, Rac1, RhoE                                                   |
| Bioenergetics          | LDHA, PGK1, PKM2, GAPDH1, GPI, ALDOC                                 |

In the setting of normoxia, HIF1-α is regulated by two principal mechanisms; oxygen-dependent pVHL-dependent degradation, and oxygen-dependent non-pVHL-dependent inactivation (Figure 3)[25,27,28]. Hydroxylation by oxygen-dependent prolyl hydroxylase domain enzymes trigger recognition by the E3 ubiquitin ligase, pVHL, ensuring proteosomal degradation. In the non-pVHL dependent pathway, induction of factor inhibiting HIF leads to hydroxylation of an asparagine residue preventing HIF1-α from localizing with the co-activators p300 and CBP, hence disabling transcriptional activation[29].

The contribution of hypoxia to disease progression makes it an attractive therapeutic target and potential prognostic aide. However, in the setting of GOC, there are currently no agents specifically targeting hypoxia, nor are there any biomarkers that assess the extent of tumour hypoxia, to guide treatment choice or to indicate the likelihood of treatment response. In this era of precision medicine, a validated biomarker would improve the standard of care for this group of patients.

**HYPOXIA PROMOTES THE HALLMARKS OF CANCER WITHIN THE TME**

**Inflammation**

Cancer has long been described as a “wound that never heals”, in part due to inflammation, one of the enabling characteristics of cancer originally described by Hanahan and Weinberg[30-31]. Hypoxia and inflammation are intricately intertwined as illustrated through the fact that hypoxia has been shown to directly induce signalling via the inflammatory master transcription factor nuclear factor-kappa light chain enhancer of activated B cells (NF-kB), and likewise NF-kB induces HIFs[32-37]. In the context of malignancy, there exists a multitude of cancer implicated genes that are regulated by both HIFs and NF-kB, such as cyclooxygenase 2 and interleukin-6 (IL-6)[38]. This illustrates the complex crosstalk between signalling pathways and the difficulty involved in unravelling the net influence of certain factors in the network. In the setting of GOC, OAC has been described as “a model of inflammatory driven upper gastrointestinal cancer”[39,40]. The paramount importance of inflammation in the aetiology of OC is further validated by the risk reduction conferred by administration of the non-steroidal anti-inflammatory drugs such as aspirin, as demonstrated in a meta-analysis of 9 observational studies by Corley et al[41] and Farrow et al[42]. In a retrospective study of 53 patients with OAC and the metaplastic precursor lesion, Barrett’s oesophagus (BO), immunohistochemical staining of specimens revealed a significant increase in the expression of HIF1-α in OAC and BO compared to normal tissue but no further elevation between BO and OAC[43]. Furthermore, histological assessment of specimens’ inflammatory status, based on recruitment of neutrophils (reflecting acute inflammation) and monocytes (reflecting chronic inflammation) (known as the Sydney System), demonstrated a significant correlation with HIF1-α expression from normal tissue to metaplastic tissue but no association between other stages or between inflammatory status[43].

**Angiogenesis**

As previously mentioned, one of the defining discoveries involved in the study of the TME was the effect of hypoxia on angiogenesis[44-46]. This was originally demonstrated in HIF1-β deficient hepatoma cells having markedly reduced vascular endothelial growth factor (VEGF) mRNA levels when cultured under hypoxic
Figure 1 The components of the tumour microenvironment are affected by hypoxia in numerous ways. Important cellular components of the tumour microenvironment include immune cells including macrophages, dendritic cells, myeloid-derived suppressor cells, T cells, natural killer cells, as well as cancer-associated fibroblasts. Non-cellular aspects include the extracellular matrix and signalling molecules such as vascular endothelial growth factor, adenosine, and cytokines and chemokines including interleukin-6, interferon-γ, CXCL1, CXCL3, CCL28\[12-14, 40\]. CAF: Cancer associated fibroblasts; OxPhos: Oxidative phosphorylation; ROS: Reactive oxygen species; VEGF: Vascular endothelial growth factor; NFκB: Nuclear factor-kappa light chain enhancer of activated B cells; HIF: Hypoxia inducible factor; ECM: Extracellular matrix; EMT: Epithelial-mesenchymal transition.

In the setting of GOC, a study of 92 oesophageal biopsy samples found a significant increase in the expression of HIF1-α in OAC vs dysplastic and metaplastic tissues but not between normal and metaplastic tissues[48]. These findings also reflected an increase in VEGF and HIF2-α expression in OAC vs dysplastic tissue. Several studies have revealed how hypoxia appears to drive tumour cell plasticity and hence vasculogenic mimicry, a process that allows malignant cells to impersonate endothelial cells and form a network of vessels, and in a sense bypass true angiogenic activity[49-54]. In an in vitro analysis of oral squamous cell carcinoma (OSCC) cells, transfection with siRNA targeting HIF1-α was shown to inhibit both vasculogenic mimicry (through three-dimensional culture) and proliferation (as measured by MTT assay)[55]. Validation of these results in a xenograft implant model was then performed; the HIF-1α knockout mice showed a longer time to tumour formation and had smaller tumours. In an experiment conducted by Chai et al[56] of 160 OSCC tumour tissues, both HIF1-α and the degree of vasculogenic mimicry correlated negatively with overall survival (OS). In a separate study, OSCC cell lines cultured under conditions of severe hypoxia (0.5% oxygen) for 5 d secreted exosomes which
through tube formation assays, were shown to increase the angiogenic capacity of human umbilical vein endothelial cells when cultured together\[57\]. Vessel formation was significantly increased compared to umbilical vein endothelial cells cultured with exosomes obtained from OSCC cells exposed to normoxic conditions. When assessed in an in vivo implant model, findings reflected those found in the in vitro assay. As a consequence of these described phenomena, the blood vessels formed in tumours do not resemble those found in non-malignant tissues. The resulting network is disorganized and highly permeable and this limits the supply of blood and hence oxygen, nutrients, and anti-cancer drugs, further contributing to tumour hypoxia.

**Immune evasion**

The cancer-immune set point refers to the equilibrium between factors that promote or suppress the anti-cancer immune response\[58\]. This is of great interest in GOC given the yet unrealized efficacy that was predicted of immune checkpoint inhibitor drugs in treating these cancer types, which are generally characterized as having high tumour mutational burden and evident immune cell infiltration\[39\]. A hypoxic TME promotes an immunosuppressive phenotype through actions on the diverse array of cellular and non-cellular entities across innate and adaptive immune arms and thus constitutes a vital host factor that may be contributing to a high cancer-immune set point and treatment failure. For example, in the context of cancer, the recruitment of myeloid-derived suppressor cells (MDSCs) is associated with less favourable patient outcomes which are likely mediated by their potent dampening of the anti-tumour immune response\[60-62\].

MDSCs are defined as “a heterogenous population of cells of myeloid origin that consist of myeloid progenitors, immature macrophages, immature granulocytes, and immature dendritic cells” (DCs)\[63,64\]. In a murine model of OSCC, intratumoural MDSC percentages were shown to correlate with the tumour progression sequence\[65\]. The role of IL-6 was then explored in the context of MDSCs and tumour progression. In patients with OSCC compared to healthy controls, serum IL-6 was significantly increased. Also, the percentage of intratumoural MDSCs correlated with general serum IL-6 levels. Delving further into this, the murine model of OSCC was utilized with 3 cohorts; IL-6 knockout, IL-6 stimulation (via 100 ng intraperitoneal injection twice weekly for 6 wk), and normal wild type. The cohort receiving IL-6 had a significant 3-fold increase in the percentage of MDSCs compared to the IL-6 deficient cohort (15% to 5% respectively). These findings were analogous when examining tumour invasiveness. As mentioned previously, HIF has been shown to upregulate the transcription of inflammatory factors including IL-6, and overall, the results demonstrate the importance of hypoxia in driving the pro-tumour immunosup-
Regulation of hypoxia-inducible factor 1-α by oxygen levels and von Hippel Lindau protein. Hydroxylation by oxygen-dependent prolyl hydroxylase domain enzymes triggers recognition by the E3 ubiquitin ligase von Hippel Lindau, ensuring proteasomal degradation. In the non-von Hippel Lindau protein dependent pathway, induction of Factor Inhibiting hypoxia-inducible factor (FIH) leads to hydroxylation of an asparagine residue preventing HIF1-α from localizing with the co-activators p300 and CBP, hence disabling transcriptional activation[30]. The HIF pathway functions to conduct and orchestrate the cellular response to low oxygen availability[24,25]. HRE: Hypoxia response element; ARNT: Aryl hydrocarbon receptor nuclear translocator; PHD: Prolyl hydroxylase domain enzymes; VHL: Von Hippel Lindau; HIF1-α: Hypoxia-inducible factor 1-α; FIH: Factor inhibiting hypoxia-inducible factor.

MDSCs[38,66]. Others have shown the hypoxic TME to drive MDSC differentiation to tumour associated macrophages (TAMs), again in a manner that is orchestrated by HIF1-α[67].

TAMs comprise a large part of the cellular TME and as such are gaining further infamy for their role in driving tumour progression[68,69]. Studies have demonstrated how TAM recruitment and infiltration into the TME is in part mediated by the hypoxic response and HIF-driven regulation of chemoattractant including CCL2, CCL5, and receptors such as CXCR4[70-73] (Figure 4). There is strong evidence that macrophage infiltration and density are associated with worse patient outcomes in the setting of malignancy[74-76]. A meta-analysis of 16 OC cancer studies (n = 2292), found that M2-polarised pro-tumour macrophage density to be predictive of worse OS and disease stage[77,78]. In addition, in vitro evidence suggests that TAM density is significantly associated with an increase in programmed death-ligand 1 expression on OSCC cells[78]. Once infiltrated into the TME, low oxygen tension enhances the oncogenic role of TAMs via the promotion of proliferative and angiogenic growth signalling pathways[79,80]. Notably, while two studies have characterized the correlation between HIF1-α expression, TAM infiltration, and patient survival in the setting of gastric malignancy, the impact of hypoxia on the biology of TAMs could be further expanded in the context of GOC[81,82].

Signifying the potential of innate immune research in cancer, Gilead recently invested $4.8 billion for ownership of magrolimab[83], a monoclonal antibody that works through the disruption of CD47 which is expressed on cancer cells and acts to downregulate the anti-tumour phagocytic capability of macrophages. Targeting hypoxia-mediated CD47 function may also extend to cancers of the alimentary tract. Immunohistochemical staining and reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) of OSCC specimens taken from 14 patients demonstrated a significant increase in expression of CD47 while another preclinical study revealed an augmented response to immune checkpoint inhibition in
Figure 4 The effects of hypoxia on immune evasion. Hypoxia has been shown to impair antigen uptake and migration in dendritic cells while at the same time increasing vascular endothelial growth factor production thus impairing the bridge between the innate anticancer immune response and the adaptive response while also enhancing angiogenic signalling. Hypoxia-inducible factor-mediated transcription of the cytokine interleukin-6 and FoxP3 results in the subsequent recruitment of immunosuppressive myeloid derived suppressor cells and in increased proportion of protumourigenic Tregs respectively. Low oxygen status is also linked with decreased tumour expression of the natural killer (NK) cell receptor ligand MHC class I chain-related molecule A, as well as its receptor NKG2D on NK cells. Hypoxia-dependent transcription of chemokines such as CCL2 and CCL5 enhance the recruitment of tumour associated macrophages through receptors such as CXCR4. DC: Dendritic cell; MDSC: Myeloid derived suppressor cell; NK cell: Natural killer cell; TAM: Tumour associated macrophage; Treg cell: T regulatory cell; VEGF: Vascular endothelial growth factor; HIF: Hypoxia inducible factor; IL: Interleukin; MICA: MHC class I chain-related molecule A.

Natural killer (NK) cells are a type of innate lymphoid cell that are capable of recognizing tumour cells through two principal mechanisms; altered expression of self or missing-self[87,88]. For example, in the absence of cellular stress, MHC class I chain-related molecules (MICA and MICB) are not normally expressed on cells. In one study of prostate cancer cells, culture under hypoxic conditions is shown to result in the shedding of MICA hence characterizing an immune evasive phenotype[89]. Hypoxia also affects both resting and activated NK cells directly by curtailing the expression of costimulatory NKG2D and other NK cell receptors (NKp46, NKp30) which enable NK cell function[90]. Furthermore, a low oxygen environment has revealed impaired NK cell differentiation in one in vitro study[91]. The density of infiltrating NK cells has been shown to be prognostic in OSCC[92].

In human OC, NK cells that demonstrate high expression of a novel inhibitory regulator protein, T cell immunoglobulin domain and mucin domain 3 (Tim-3) are predisposed to apoptosis and hence fail to combat tumour progression[94]. Increased expression of Tim-3 in this context occurs through NF-κB signalling thus linking hypoxia to NK cell-mediated anti-tumour dysfunction. NK cells are also an important entity in GC. Tumour infiltrating NK cells expressing high levels of Tim-3 have been correlated with adverse prognosis in a study of 62 patients with the disease[95].
DCs present antigens to T cells including CD4+ T helper cells, resulting in the initiation of the adaptive anti-tumour immune response[96]. In cancer, impaired DC function is associated with defective anti-tumour immune responses and hence cancer progression[97-99]. While there are contrasting studies, the net effect of the hypoxic TME may be skewed towards a tolerogenic DC phenotype[100,101]. An in vitro study of peripheral blood mononuclear cells isolated from a healthy human cohort and cultured under hypoxic conditions (1% oxygen) showed that hypoxia impairs DC uptake of antigens and causes modulation of their cytokine expression patterns in both resting and activated states[100]. Hypoxia increased VEGF production and CXCR4 expression and lead to a reduction in DC production of tumor necrosis factor-α thereby revealing the pro-angiogenic and immunosuppressive effect of reduced oxygen tension on DCs. Lysosomal-associated membrane protein (LAMP3) is a marker of mature DCs and it has been shown to be induced by hypoxia in breast cancer both in vitro and in vivo[102]. It is thought to be implicated in metastasis[103]. RT-qPCR analysis of 157 O8CC tissues as well as immunohistochemical staining of 46 specimens reveal its expression to be correlated with poor patient outcomes, further emphasizing the tolerogenic capacity of DCs[104]. Again, in the context of OAC, co-culture with DCs has been shown to induce Treg (T regulatory) differentiation supporting the tolerogenic DC phenotype in these malignancies[105]. Given that successful activation of adaptive T cell responses is dependent on DC migration to peripheral lymphoid organs, further research and investigation of the effect of hypoxia in the TME on DCs is required to fully dissect the potential clinical impact regarding patient outcomes and treatment resistance[106].

Hypoxia-induced HIF1-α expression is also associated with the upregulation of the transcription factor Forkhead Box Protein P3 (FoxP3), highlighting the role of hypoxia in regulating the abundance and function of Treg cells, further illustrating the potential immunosuppressive effect of a hypoxic TME on anti-tumour immunity[107,108]. In a study of GC, the frequency of Treg cells was significantly higher in the tumour compared with peripheral circulation wherein, intratumoural levels of Foxp3 correlated with TNM stage[109]. In a complementary study, elevated Treg/CD8+ cell ratio was shown to be an independent predictor for worse OS in a study of 133 patients with GC. Tregs are also crucially important in OC; one study found an increased percentage of peripheral Treg cells in OC patients vs healthy controls and they further demonstrated that a higher proportion of Tregs was inversely correlated to survival[110]. The administration of an agent that disrupts Treg recruitment to a hypoxic TME may represent a potential therapeutic target capable of improving outcomes[111].

Invasion, migration, and metastasis
The activation of cancer-associated fibroblasts (CAFs) in hypoxic TMEs has been implicated in the altered deposition, remodelling and degradation of the extracellular matrix (ECM) and hence invasion, migration, and metastasis[112-114]. In a study of 183 patients with OAC, characteristic expression of CAF marker α-SMA was found to be correlated with worse OS[115]. It was initially hypothesized that increased collagen production and fibrosis would present an obstacle to tumour cell invasion and metastasis, but evidence suggests that this is a lot more complex. In one study of pancreatic carcinoma cells, collagen has been shown to increase expression of the key epithelial to mesenchymal transition (EMT) transcription factor Snail in a transforming growth factor-β-mediated manner[116]. Thus, this series of events is thought to be involved in the activation of CAFs thereby, ensuring enhanced migratory capacity, invasiveness, survival, and ECM deposition in a positive feedback loop[117,118]. In the area of GC, an in vitro assay revealed extracellular matrix metalloproteinase inducer (EMMPRIN) promoted EMT and hence invasion and migration of an OC cell line[119]. The authors followed up this study by showing, through HIF1-α interference and culture under hypoxic (1% oxygen) conditions, that EMMPRIN was regulated by HIF1-α. Further research probing the relationship between traditionally neglected components of the TME like CAFs and hypoxia in upper gastrointestinal cancers is required.

Altered energetics
Cells deprived of oxygen promote tumour proliferation and survival through reprogramming of energy metabolism[30]. The observation that neoplastic cells shift their metabolism from aerobic to anaerobic respiration was first observed nearly 100 years ago by Otto Warburg[120,121]. This shift is orchestrated by the hypoxia master regulator HIF which upregulates enzymes involved in glycolysis such as pyruvate dehydrogenase kinase 1, and ultimately the production of lactate from pyruv-
Hypoxia and gastroesophageal cancer

MEASURING HYPOXIA

Measuring tissue and tumour hypoxia is challenging. There are four principal methods for measuring oxygen levels in vivo: the Eppendorf oxygen electrode, exogenous markers, endogenous markers, and imaging techniques. The Eppendorf electrode quickly became the gold standard for measuring oxygen tension when it was introduced at the beginning of the millennium after studies confirmed that low tumour oxygenation status was associated with worse outcomes in cervical as well as head and neck cancer.[129-134] Evidence suggests that this is conferred predominantly through the release and metabolism of ATP by the surface membrane nucleotidases CD39 and CD73.[133,135,136] In one in vitro experiment, an epithelial cell line demonstrated increased CD73 expression when exposed to hypoxic conditions, and examination of the CD73 gene has identified a binding site for HIF1.[137] Subsequent binding to purinergic receptors and adenosinergic signalling is known to mediate an anti-tumour immunosuppressive phenotype through effects on Tregs, MDSCs, TAMS, and B lymphocytes across various solid tumour types including OC.[135,138-141] In the context of GOC, a gene expression study of several radiotherapy resistant OC cell lines, CD73 expression was shown to be increased in TE-2, TE-13, and KYSE170 when compared to parent cell lines.[142] Once again, given the hypoxia-driven mechanism, this highlights the pro-inflammatory, tumour-promoting effect of the adenosine axis, thereby signifying another potential method of clinically targeting hypoxia pathways in the treatment of GOC.

Also, hypoxia (oxygen of 1.5%) driven reprogramming of energetic metabolism is linked to PD-1 immune checkpoint blockade resistance.[143] In vivo treatment with metformin, decreases OCR in tumour cells, while increasing consumption in T cells resulting in reduced hypoxia. The authors further examined the effect of anti-PD-1 agents in concert with metformin administration in vivo in a melanoma tumour type that traditionally fails to respond to immune checkpoint blockade. The synergistic effect demonstrated substantially increased tumour elimination.[143] These intricately woven hypoxia-mediated effects exist in concert with one another to contribute to an aggressive phenotype characterized by treatment resistance and poor prognosis.

Immunoblot analysis of both gastric and OSCC specimens has demonstrated reductions in the expression of the β catalytic subunit of a key protein involved in oxidative phosphorylation, ATP synthase, further implicating the role of metabolic reprogramming in upper gastrointestinal malignancies.[124] It is also probable that an altered bioenergetic phenotype contributes to treatment resistance in a hypoxia-driven manner. In one study, the expression of 4 proteins involved in metabolic respiration in the setting of OAC (n = 23), were assessed prior to chemoradiation.[125] Increased levels of the oxidative phosphorylation protein ATP5B were significantly increased in those with poor response to chemoradiation as defined per tumour regression grade. This suggests that tumours that retain some sense of metabolic plasticity may predict treatment-refractory disease.

Lactate dehydrogenase is responsible for converting pyruvate to lactate under hypoxic conditions.[126] In a study of 152 patients with GC, immunohistochemical staining for lactate dehydrogenase (LDH) isoenzyme 5 demonstrated significant associations between immunoreactivity and a number of different tumour features such as tumour size, venous and lymphatic invasion, and tumour stage.[127] Inoculation of mice with LDH knock-out pancreatic cancer cells has been shown to result in reduced tumour size.[128] Furthermore, the quantity of MDSCs isolated from the LDH knock-out cancer mice both in tumour and spleen was significantly less in controls, and they demonstrated lower suppressive activity.

The effects of these processes are not restricted to neoplastic cells, as the evidence implicates hypoxia-driven metabolic shifts in other cellular components of the TME, particularly immune cells. Tissue hypoxia in cancerous or non-cancerous cells results in the build-up of the purine adenosine, extracellularly which augments a plethora of the hallmarks of cancer.[129-134] It is also probable that an altered bioenergetic phenotype contributes to treatment resistance in a hypoxia-driven manner. In one study, the expression of 4 proteins involved in metabolic respiration in the setting of OAC (n = 23), were assessed prior to chemoradiation.[125] Increased levels of the oxidative phosphorylation protein ATP5B were significantly increased in those with poor response to chemoradiation as defined per tumour regression grade. This suggests that tumours that retain some sense of metabolic plasticity may predict treatment-refractory disease.

Use of endogenous hypoxia markers in GOC is extensive and is discussed in the context of prognosis and treatment resistance.[148-152] Exogenous markers such as lactate dehydrogenase (LDH) isoenzyme 5, 5 demonstrated significant associations between immunoreactivity and a number of different tumour features such as tumour size, venous and lymphatic invasion, and tumour stage.[127] Inoculation of mice with LDH knock-out pancreatic cancer cells has been shown to result in reduced tumour size.[128] Furthermore, the quantity of MDSCs isolated from the LDH knock-out cancer mice both in tumour and spleen was significantly less in controls, and they demonstrated lower suppressive activity.

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as pimonidazole are administered to a patient and undergo chemical modification in hypoxic cells and are then amenable to visualization in specimens. A summary of the major methods used to measure tumour hypoxia and their associated advantages and disadvantages can be found in Table 2.

In the last decade, several studies have characterized gene expression signatures corresponding to oxygenation status[153-155]. Using a 15 gene expression panel derived from these studies, Ye et al[156] classified 24 cancer types from The Cancer Genome Atlas into a hypoxia score of high, low, and intermediate after adjusting for confounding factors such as sex and ethnicity. They were further able to validate this categorization with independent proteomic data where hypoxic status was known. 135/193 (70%) of GC samples had high hypoxic status while only 34/124 (27%) of OC samples fell into this category. There may be differences between OSCC and OAC but they were grouped together in this study. They further built on these findings by comparing molecular characteristics such as miRNA expression, highly mutated genes, and significant copy number alterations between the hypoxia score high and low tumours. In both OC and GC samples that had molecular signatures of high hypoxic status, a number of miRNAs that target the tumour suppressor gene tumour protein p53 inducible nuclear protein 1 (TP53INP1), were significantly downregulated[156].

TREATMENT RESISTANCE AND PROGNOSIS

Ionizing radiation generates free radicals from molecules of oxygen which then induce double-stranded DNA breaks resulting in mitotic catastrophe. This is one of the key mechanisms for radiation-induced tumour cell death and it is reliant on the presence of oxygen within the TME[144,157]. GC and OC cells cultured in vitro under hypoxic conditions (1% oxygen) were more resistant to radiation-induced cell death compared to GC and OC cells cultured under normoxic conditions, as assessed by colony formation assay[158]. The contribution of hypoxia to radiotherapy treatment resistance is relatively well established but its role in conventional chemotherapy and molecularly targeted therapy is less clear cut, particularly in GOC. Functional inactivation of HIF1-α in GC cell lines demonstrated increased susceptibility to 5-fluorouracil and cisplatin as determined by proliferation and apoptosis assays which lends support to the use of HIF1-α in predicting response to therapy[159]. Analysis of cell cycle distribution patterns following treatment with 5-fluorouracil revealed a greater proportion of senescent HIF1-α deficient cells compared with controls. Likewise, the apoptotic cell fraction as determined by caspase 3 cleavage of HIF1-α deficient cells was greatly increased. The mechanism for this is thought to be mediated by HIF1-α dependent suppression of P53 induction in response to 5-fluorouracil[159,160]. Another potential mechanism is suggested by a different study, using RT-PCR and Western blot to demonstrate the HIF1-α dependent upregulation of p-glycoprotein in GC cells incubated at 1% oxygen levels[161]. P-glycoprotein is a transporter protein that augments the efflux of drugs from cells and hence is associated with chemoresistance in GOC[162,163].

There are a large number of studies that have investigated the prognostic value of hypoxia in OC. A systematic review carried out by Peerlings et al[152] evaluated 22 studies assessing various hypoxia-related markers and established that increased expression of HIF1-α in early-stage OSCC was associated with increased resistance to chemoradiotherapy treatment. They also conclude that radiologically, the positron emission tomography (PET) marker 18F-FETNIM was significantly predictive for response to combined chemoradiation in the setting of OSCC[164]. In brief, these tracers work by diffusing into cells non-specifically. In the absence of oxygen, they undergo a chemical reaction and their resultant physicochemical properties do not allow diffusion out of the cell[165]. PET with 18F-FAZA (18F-fluoroazomycin arabinoside) has been shown to predict radiotherapy response in OAC murine xenografts[166]. Validation of the tracer 18F-FIX4 has been performed in OC but is yet to be studied as a potential prognostic factor[167]. Overall, imaging of hypoxia continues to be an attractive approach for studying the TME and subsequent patient outcomes.

The markers assessed in the systematic review by Peerlings et al[152] included HIF1-α, VEGF, carbonic anhydrase IX, GLUT1, Beclin-2, HIF2-α, as well as PET. The most common method used to assess these markers was immunohistochemical staining of surgical or biopsied specimens i.e. an invasive technique. The authors indicate that HIF1-α overexpression was associated with worse outcomes for OS and disease-free
Table 2 Techniques used in the measurement of tissue oxygenation and their associated advantages and disadvantages[10,181-183]

| Technique       | Advantages                                                                 | Disadvantages                                                                 |
|-----------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Needle Electrodes | Instrumental in establishing the link between hypoxia and treatment failure | Prone to sampling error due to poor spatial resolution                         |
|                 | Real time direct measurement                                              | Invasive and requires direct access to tumours                                |
| Exogenous Markers | More sensitive than electrodes at lower oxygen levels                     | Requires biopsy and immunohistochemistry                                       |
|                 | Reproducible                                                              |                                                                              |
|                 | Precise spatial resolution                                                |                                                                              |
| Endogenous Markers | Precise spatial resolution                                                | Requires biopsy and immunohistochemistry                                       |
|                 | Can be serological such as Osteopontin                                    |                                                                              |
|                 | Can be tissue based such as HIFs or carbonic anhydrase IX                  |                                                                              |
| Radiological    | Non-invasive                                                              | Expensive                                                                     |
|                 | Reproducible                                                              | Radiation exposure                                                            |
|                 | Precise spatial resolution                                                | Relatively less well established                                              |

HIF: Hypoxia inducible factor.

Survival in OSCC but the evidence for its association in OAC was inconclusive, mainly due to the absence of data. VEGF expression correlated with patient outcomes in OSCC but not OAC[152]. In contrast, carbonic anhydrase IX appears to be an independent predictor of survival in OAC. Carbonic anhydrase IX is a glycoprotein expressed on the cell surface and its primary function is the catalytic conversion of carbon dioxide to bicarbonate and protons[150,168]. Under the transcriptional control of HIF1-α, the metalloenzyme is thought to contribute to tumour growth and proliferation through the regulation of pH, ECM degradation, and EMT[168,169]. In the majority of studies assessing endogenous markers, the determination of what constituted “hypoxic” was based on relatively arbitrary thresholds of immunohistochemical expression, with very little in the way of standardized protocols across studies. For example, Munipalle et al[151] defined “high” HIF-1α expression as greater than 10% of OSCC cells showing positive staining. Birner et al[170] devised a score based on intensity and percentage of cells showing positive expression in a cohort of 333 OCs. Anything above the median was then considered a “high” expression while those below were considered a "low" expression.

In a more recent systematic review and meta-analysis, Luo et al[148] examined the clinical predictive value of HIF2-α. It included 40 studies with 4345 cancer cases but only 2 of these studies assessed upper gastrointestinal cancers. Of these 2 studies, 1 was solely GC (n = 127), while the other was both GC and OC (n = 177)[149,171]. Based on the Newcastle Ottawa score, the authors determined that both of these papers were of high quality. Both of these studies demonstrated a statistically significant association between HIF2-α and OS on univariate analysis but not multivariate. In the pooled analysis, the authors conclude that high HIF2-α expression was associated with a lower OS.

While there is a non-insignificant aggregate of clinical evidence denoting a statistically significant association between endogenous markers of tumour oxygenation and clinical outcomes, the heterogeneity in study methods and contrasting results ultimately indicates a need for more prospective research with greater adherence to the standardization of reporting. The REMARK recommendations for tumour marker prognostic studies published by the Equator Network lay out a checklist for researchers to improve both quality and transparency in research[172]. The wealth of data as discussed above, demonstrating the correlation between outcomes or treatment resistance and tumour hypoxia further illustrates the importance of the development and clinical implementation of new techniques in measuring tumour hypoxia such as non-invasive imaging[148,149,151,170,171].
HYPOXIA-TARGETED THERAPIES

Hypoxic areas of the TME inherently suffer from poor perfusion and disorganized vasculature and this has been one of the primary limitations to systemically administered therapeutics[173]. Nevertheless, a number of agents have been tested in clinical studies. Hypoxia-targeted therapies mainly consist of bioreductive prodrugs (hypoxia-activated prodrugs) but molecularly targeted agents that inhibit effectors in hypoxia-responsive pathways such as HIF1-α target genes or receptor tyrosine kinases like the VEGF receptor could be grouped here as well[173].

Bioreductive agents such as tirapazamine work in a similar manner to exogenous markers of hypoxia; they undergo chemical modification in hypoxic cells resulting in hypoxia-selective cytotoxicity. The bioreductive alkyllating agent apaziquone demonstrated efficacy as a first-line agent in early clinical studies of bladder cancer but in a phase II study in 20 patients with GC, there was no clinical benefit[174,175]. In a preclinical murine model of OSCC and OAC, administration of the bioreductive prodrug evofosfamide was shown to delay tumour growth in combination with radiotherapy vs radiotherapy alone[176]. This came with the added benefit of no additional toxicity. As of the time of writing, there have been no clinical trials investigating the potential use of evofosfamide or other bioreductive prodrugs in OC and although the efficacy of these agents has largely been disappointing as first-line treatment in other cancer types, they may potentially improve sensitivity when used in combination with conventional chemoradiation.

CONCLUSION

The myriad of components that comprise the TME and the effects imposed on them by oxygen deprivation ensures that researchers have yet to scratch the surface in disentangling the key processes amenable to overcoming treatment-refractory disease and prognostication. Hypoxia plays a role in promoting immunosuppressive cells and subverting anti-tumour immune responses within the TME. Hypoxia also promotes the additional hallmarks of cancer including inflammation, angiogenesis, and reprogramming of metabolism. The intricate nature of these hypoxia-mediated effects is very complex and further research is required to elucidate the mechanisms as they pertain to GOC. Standardization of methodology in hypoxia focused basic research and clinical reporting would be conducive to driving this area forward. This deeper understanding will hopefully reveal novel therapeutic targets to control disease progression in GOC but currently, this remains out of reach. However, hypoxia as a clinical marker to stratify patients into certain treatment pathways or aid prognosis is something that is firmly within our grasp.

REFERENCES

1. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992; 359: 843-845 [PMID: 1279431 DOI: 10.1038/35943a0]
2. Mazure NM, Chen CY, Yeh P, Laderoute KR, Giaccia AJ. Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. *Cancer Res* 1996; 56: 3436-3440 [PMID: 8758908]
3. Rofstad EK, Rasmussen H, Galapathi K, Mathiesen B, Nilsen K, Graff BA. Hypoxia promotes lymph node metastasis in human melanoma xenografts by up-regulating the urokinase-type plasminogen activator receptor. *Cancer Res* 2002; 62: 1847-1853 [PMID: 11912164]
4. Bedogni B, Welford SM, Cassarino DS, Nickoloff BJ, Giaccia AJ, Powell MB. The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. *Cancer Cell* 2005; 8: 443-454 [PMID: 16338658 DOI: 10.1016/j.ccr.2005.11.005]
5. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J Clin Invest* 2013; 123: 3664-3671 [PMID: 23999440 DOI: 10.1172/JCI67230]
6. Thomsen RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer* 1955; 9: 539-549 [PMID: 13304213 DOI: 10.1038/bjc.1955.55]
7. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol* 1953; 26: 638-648 [PMID: 13106296 DOI: 10.1259/0007-1285-26-312-638]
8. Kolstad P. Intercapillary distance, oxygen tension and local recurrence in cervix cancer. *Scand J Clin Lab Invest Suppl* 1968; 106: 145-157 [PMID: 5731701]
King R et al. Hypoxia and gastroesophageal cancer

9 Rubenstein JH, Shaheen NJ. Epidemiology, Diagnosis, and Management of Esophageal Adenocarcinoma. *Gastroenterology* 2015; 149: 302-17. [PMID: 25957861 DOI: 10.1053/j.gastro.2015.04.053]

10 Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. *Gut* 2015; 64: 381-387 [PMID: 25320104 DOI: 10.1136/gutjnl-2014-308124]

11 Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends—An Update. *Cancer Epidemiol Biomarkers Prev* 2016; 25: 16-27 [PMID: 26667886 DOI: 10.1158/1055-9965.EPI-15-0578]

12 Sitarz R, Skierucha M, Miłko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. *Cancer Manag Res* 2018; 10: 239-248 [PMID: 29445300 DOI: 10.2147/CMAR.S149619]

13 Bosetti C, Bertuccio P, Malvezzi M, Levi F, Chetanoùt L, Negri E, La Vecchia C. Cancer mortality in Europe, 2005-2009, and an overview of trends since 1980. *Ann Oncol* 2013; 24: 2657-2671 [PMID: 23921790 DOI: 10.1093/annonc/mdt301]

14 Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiol Biomarkers Prev* 2014; 23: 700-713 [PMID: 24618998 DOI: 10.1158/1055-9965.EPI-13-1057]

15 Rice TW, Blackstone EH, Rusch VW. 7th edition of the AJCC Cancer Staging Manual: esophagus and esophagogastric junction. *Ann Surg Oncol* 2010; 17: 1721-1724 [PMID: 20336299 DOI: 10.1245/s10434-010-1024-1]

16 Ruan K, Song G, Ouyang G. Role of hypoxia in the hallmarks of human cancer. *J Cell Biochem* 2009; 107: 1053-1062 [PMID: 19479945 DOI: 10.1002/jcb.22214]

17 Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl)* 2015; 3: 83-92 [PMID: 27774485 DOI: 10.2147/HP.S93413]

18 Vausel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* 2007; 26: 225-239 [PMID: 17440684 DOI: 10.1007/s10555-007-9055-1]

19 Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, Gong Z, Zhang S, Zhou J, Cao K, Li X, Xiong W, Li G, Zeng Z, Guo C. Role of tumor microenvironment in tumorigenesis. *J Cancer* 2017; 8: 761-773 [PMID: 28382138 DOI: 10.7150/jca.17648]

20 Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G, Sun Y. New horizons in tumor microenvironment biology: challenges and opportunities. *BMC Med* 2015; 13: 45 [PMID: 25857315 DOI: 10.1186/s12916-015-0278-7]

21 Belli C, Trpani D, Viale G, D’Amico P, Dusso BA, Della Vigna P, Orsi F, Curigliano G. Targeting the microenvironment in solid tumors. *Cancer Treat Rev* 2018; 65: 22-32 [PMID: 29502037 DOI: 10.1016/j.ctrv.2018.02.004]

22 Gillies RJ, Gatenby RA. Hypoxia and adaptive landscapes in the evolution of carcinogenesis. *Cancer Metastasis Rev* 2007; 26: 311-317 [PMID: 17404691 DOI: 10.1007/s10555-007-9065-z]

23 Wang GL, Jiang BH, Rae EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci USA* 1995; 92: 5510-5514 [PMID: 7539918 DOI: 10.1073/pnas.92.12.5510]

24 Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 1996; 16: 4604-4613 [PMID: 8756616 DOI: 10.1128/mcb.16.9.4604]

25 Semenza GL. HIF-1: upstream and downstream of cancer metabolism. *Curr Opin Genet Dev* 2010; 20: 51-56 [PMID: 19942427 DOI: 10.1016/j.gde.2009.10.009]

26 Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 2008; 30: 393-402 [PMID: 18498744 DOI: 10.1016/j.molcel.2008.04.009]

27 Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylases. *Science* 2001; 292: 468-472 [PMID: 11292861 DOI: 10.1126/science.1057986]

28 Majumdar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. *Mol Cell* 2010; 40: 294-309 [PMID: 20965423 DOI: 10.1016/j.molcel.2010.09.022]

29 Masoud GN, Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. *Acta Pharm Sin B* 2015; 5: 378-389 [PMID: 26579469 DOI: 10.1016/j.apsb.2015.05.007]

30 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

31 Dvorak HF. Tumors: wounds that do not heal-redux. *Cancer Immunol Res* 2015; 3: 1-11 [PMID: 25568067 DOI: 10.1158/2326-6066.CIR-14-0209]

32 Koong AC, Chen EY, Giaccia AJ. Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. *Cancer Res* 1994; 54: 1425-1430 [PMID: 8137243]

33 Culver C, Sundqvist A, Mudie S, Melvin A, Xirodimas D, Rocha S. Mechanism of hypoxia-induced NF-kappaB. *Mol Cell Biol* 2010; 30: 4901-4921 [PMID: 20696840 DOI: 10.1128/MCB.00409-10]

34 van Uden P, Kenneth NS, Rocha S. Regulation of hypoxia-inducible factor-lalpha by NF-kappaB. *Biochem J* 2008; 412: 477-484 [PMID: 18393939 DOI: 10.1042/BJ20080476]

35 van Uden P, Kenneth NS, Webster R, Müller H, Mudie S, Rocha S. Evolutionary conserved
regulation of HIF-1α by NF-κB. PLoS Genet 2011; 7: e1001285 [PMID: 21298084 DOI: 10.1371/journal.pgen.1001285]

Eltzschig HK, Carmeliet P. Hypoxia and inflammation. N Engl J Med 2011; 364: 656-665 [PMID: 21323543 DOI: 10.1056/NEJMra0910283]

Bartels K, Grenz A, Eltzschig HK. Hypoxia and inflammation are two sides of the same coin. Proc Natl Acad Sci USA 2013; 110: 18351-18352 [PMID: 24187149 DOI: 10.1073/pnas.1318345110]

Balasubraman K. HIF-1 at the crossroads of hypoxia, inflammation, and cancer. Int J Cancer 2016; 138: 1058-1066 [PMID: 25784597 DOI: 10.1002/ijc.29519]

Picardo SL, Maher SG, O'Sullivan JN, Reynolds JV. Barrett's to oesophageal cancer sequence: a model of inflammatory-driven upper gastrointestinal cancer. Dig Surg 2012; 29: 251-260 [PMID: 22868386 DOI: 10.1159/000341498]

O'Sullivan KE, Phelan JJ, O'Hanlon C, Lysaght J, O'Sullivan JN, Reynolds JV. The role of inflammation in cancer of the esophagus. Expert Rev Gastroenterol Hepatol 2014; 8: 79-760 [PMID: 24857183 DOI: 10.1586/17474124.2014.913478]

Corley DA, Kerlikowsk K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology 2003; 124: 47-56 [PMID: 12512029 DOI: 10.1053/gast.2003.50008]

Farrow DC, Vaughn TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Ling FC, Khochfar J, Balduis SE, Brabender J, Drebber U, Mattusch C, Khochfar S, Baldus SE, Brabender J, Drebber U, Bollschweiler E, Hoelscher AH, Schneider PM. HIF-1alpha protein expression is associated with the environmental inflammatory reaction in Barrett's metaplasia. Dis Esophagus 2009; 22: 694-699 [PMID: 19302222 DOI: 10.1111/j.1442-2050.2009.00957.x]

Witz IP. The tumor microenvironment: the making of a paradigm. Cancer Microenviron 2009; 2 Suppl 1: 9-17 [PMID: 19701697 DOI: 10.1007/s12307-009-0025-8]

Otrock ZK, Holahan KJ,螫 C, Kim WH, Dibrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Balamurugan K, Griffiths EA, King R, Farrow DC, Vaughn TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Witz IP. The tumor microenvironment: the making of a paradigm. Cancer Microenviron 2009; 2 Suppl 1: 9-17 [PMID: 19701697 DOI: 10.1007/s12307-009-0025-8]

Otrock ZK, Holahan KJ, Chen Y, Cobleigh M, Zalut M, Lamirande E, Dai S, Agostini M, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Balamurugan K, Griffiths EA, King R, Farrow DC, Vaughn TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Witz IP. The tumor microenvironment: the making of a paradigm. Cancer Microenviron 2009; 2 Suppl 1: 9-17 [PMID: 19701697 DOI: 10.1007/s12307-009-0025-8]

Otrock ZK, Holahan KJ, Chen Y, Cobleigh M, Zalut M, Lamirande E, Dai S, Agostini M, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Balamurugan K, Griffiths EA, King R, Farrow DC, Vaughn TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Witz IP. The tumor microenvironment: the making of a paradigm. Cancer Microenviron 2009; 2 Suppl 1: 9-17 [PMID: 19701697 DOI: 10.1007/s12307-009-0025-8]

Otrock ZK, Holahan KJ, Chen Y, Cobleigh M, Zalut M, Lamirande E, Dai S, Agostini M, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Balamurugan K, Griffiths EA, King R, Farrow DC, Vaughn TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Witz IP. The tumor microenvironment: the making of a paradigm. Cancer Microenviron 2009; 2 Suppl 1: 9-17 [PMID: 19701697 DOI: 10.1007/s12307-009-0025-8]

Otrock ZK, Holahan KJ, Chen Y, Cobleigh M, Zalut M, Lamirande E, Dai S, Agostini M, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]

Balamurugan K, Griffiths EA, King R, Farrow DC, Vaughn TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoonberg JB, West AB, Rotterdam H, Fraumeni JF Jr, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7: 97-102 [PMID: 9488582]
King R et al. Hypoxia and gastrointestinal cancer

38: 389 [PMID: 31488217 DOI: 10.1186/s13046-019-1384-8]

58 Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017; 541: 321-330 [PMID: 28102259 DOI: 10.1038/nature21349]

59 Power R, Lowery MA, Reynolds JV, Dunne MR. The Cancer-Immune Set Point in Oesophageal Cancer. Front Oncol 2020; 10: 891 [PMID: 32582555 DOI: 10.3389/fonc.2020.00891]

60 Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 2009; 58: 49-59 [PMID: 18446337 DOI: 10.1007/s00262-008-0523-4]

61 Condamine T, Ramachandran I, Youn JJ, Gabrilovich DI. Regulation of tumor metastasis by myeloid-derived suppressor cells. Annu Rev Med 2015; 66: 97-110 [PMID: 25341012 DOI: 10.1146/annurev-med-051013-052304]

62 Gabitass RF, Annels NE, Stocken DD, Pandha HA, Middleton GW. Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. Cancer Immunol Immunother 2011; 60: 1419-1430 [PMID: 21644036 DOI: 10.1007/s00262-011-1028-0]

63 Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009; 9: 162-174 [PMID: 19197294 DOI: 10.1038/nri2506]

64 Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer 2013; 13: 739-752 [PMID: 24060865 DOI: 10.1038/nrc3581]

65 Chen MF, Kuan FC, Yen TC, Lu MS, Lin PY, Chung YH, Chen WC, Lee KD. IL-6-stimulated CD11b+ CD14+ HLA-DR- myeloid-derived suppressor cells, are associated with progression and poor prognosis in squamous cell carcinoma of the esophagus. Oncotarget 2014; 5: 8716-8728 [PMID: 25238263 DOI: 10.18632/oncotarget.2368]

66 Ambler DR, Fletcher NM, Diamond MP, Saed GM. Effects of hypoxia on the expression of inflammatory markers IL-6 and TNF-a in human normal peritoneal and adhesion fibroblasts. Syst Biol Reprod Med 2012; 58: 324-329 [PMID: 23043632 DOI: 10.3109/19396368.2012.713439]

67 Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JJ, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, McCaffrey TV, McCaffrey JC, Gabrilovich DI. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the microenvironment. J Exp Med 2010; 207: 2439-2453 [PMID: 20876310 DOI: 10.1084/jem.20100587]

68 Poh AR, Ernst M. Targeting Macrophages in Cancer: From Bench to Bedside. Cell 2015; 162: 239-248 [PMID: 26446180 DOI: 10.1016/j.cell.2015.06.011]

69 Murdock C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. Blood 2004; 104: 2224-2234 [PMID: 15231578 DOI: 10.1182/blood-2004-03-1109]

70 Murdock C, Lewis CE. Macrophage migration and gene expression in response to tumor hypoxia. Int J Cancer 2005; 117: 701-708 [PMID: 16106399 DOI: 10.1002/ijc.21422]

71 Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, Saccani S, Nebuloni M, Vago L, Mantovani A, Melillo G, Sica A. Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med 2003; 198: 1391-1402 [PMID: 14597738 DOI: 10.1084/jem.20030267]

72 Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002; 196: 254-265 [PMID: 11857487 DOI: 10.1002/path.1027]

73 Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010; 141: 39-51 [PMID: 20371344 DOI: 10.1016/j.cell.2010.03.014]

74 Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, Delaney A, Jones SJ, Iqbal J, Weisenburger DD, Bast MA, Rosenwald A, Muller-Hermelink HK, Rimsza LM, Campo E, Delabie J, Bziel RM, Cook JR, Tubbs RR, Jaffe ES, Lenz G, Connors JM, Staudt LM, Chan WC, Gascoyne RD. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med 2010; 362: 875-885 [PMID: 20220182 DOI: 10.1056/NEJMoa0905680]

75 Li J, Xie Y, Wang X, Li F, Li S, Li M, Peng H, Yang L, Liu C, Peng L, Zou H, Zhao J, Qi Y, Cao Y, Hu J. Prognostic impact of tumor-associated macrophage infiltration in esophageal cancer: a meta-analysis. Future Oncol 2019; 15: 2303-2317 [PMID: 31227146 DOI: 10.2217/fon-2018-0669]

76 Yagi T, Baba Y, Okadome K, Kiyozumi Y, Hiyoshi Y, Ishimoto T, Iwatsuki M, Miyamoto M, Yoshida N, Watanabe M, Komohara Y, Baba H. Tumour-associated macrophages are associated with poor prognosis and programmed death ligand 1 expression in oesophageal cancer. Eur J Cancer 2019; 111: 38-49 [PMID: 30822683 DOI: 10.1016/j.ejca.2019.01.018]

77 Harney JH, Dimitriadis E, Kay E, Redmond HP, Boucher-Hayes D. Regulation of macrophage production of vascular endothelial growth factor (VEGF) by hypoxia and transforming growth factor beta-1. Ann Surg Oncol 1998; 5: 271-278 [PMID: 9607631 DOI: 10.1007/BF02303785]

78 Kuwabara K, Ogawa S, Matsumoto M, Koga S, Claus M, Pinksy DJ, Lenny P, Leavy J, Witte L, Joseph-Silverstein J. Hypoxia-mediated induction of acidic/basic fibroblast growth factor and...
platelet-derived growth factor in mononuclear phagocytes stimulates growth of hypoxic endothelial cells. *Proc Natl Acad Sci USA* 1995; 92: 4606-4610 [PMID: 7538678 DOI: 10.1073/pnas.92.10.4606]

81 Zhang WJ, Chen C, Zhou ZH, Gao ST, Tee TJ, Yang LQ, Xu YY, Pang TH, Xu XY, Sun Q, Feng M, Wang H, Lu CL, Wu GZ, Wu S, Guan WX, Xu GF. Hypoxia-inducible factor-1 alpha Correlates with Tumor-Associated Macrophages Infiltration, Influences Survival of Gastric Cancer Patients. *J Cancer* 2017; 8: 1818-1825 [PMID: 28819379 DOI: 10.7150/jca.19057]

82 Osinsky S, Bushovskaya L, Ganusevich I, Kovel'skaya A, Gumenyuk L, Olijnichenko G, Merzetskov S. Hypoxia, tumour-associated macrophages, microvesSEL density, VEGF and matrix metalloproteinases in human gastric cancer: interaction and impact on survival. *Clin Transl Oncol* 2011; 13: 133-138 [PMID: 21324802 DOI: 10.1007/s12094-011-0630-0]

87 Seven to Gilead: "Eat me". *Nat Biotechnol* 2020; 38: 389 [PMID: 32265557 DOI: 10.1038/s41587-020-0496-1]

88 Zhao CL, Yu S, Wang SH, Li SG, Wang JJ, Han SN. Characterization of cluster of differentiation 47 expression and its potential as a therapeutic target in esophageal squamous cell cancer. *Onco Lett* 2018; 15: 2017-2023 [PMID: 29399202 DOI: 10.3892/onl.2017.7447]

92 Tao H, Qian P, Wang F, Yu H, Guo Y. Targeting CD47 Enhances the Efficacy of Anti-PD-1 and CTLA-4 in an Esophageal Squamous Cell Cancer Preclinical Model. *Onco Res* 2017; 25: 1579-1587 [PMID: 28357964 DOI: 10.3727/096550417X1490050520895]

97 Suzuki S, Yokobori T, Tanaka N, Sakai M, Sano A, Inose T, Sodha M, Nakajima M, Miyazaki T, Kato H, Kuwano H. CD47 expression regulated by the miR-133a tumor suppressor is a novel prognostic marker in esophageal squamous cell carcinoma. *Oncol Rep* 2012; 28: 465-472 [PMID: 22641226 DOI: 10.3892/or.2012.1831]

102 Caligiuri MA. Human natural killer cells. *Blood* 2008; 112: 461-469 [PMID: 18650461 DOI: 10.1182/blood-2007-09-077438]

90 Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; 9: 503-510 [PMID: 18425107 DOI: 10.1038/ni1582]

95 Siemers DR, Hu N, Sheikh AK, Chung E, Frederiksen J, Pross H, Graham CH. Hypoxia increases tumor cell shedding of MHC class I chain-related molecule: role of nitric oxide. *Cancer Res* 2008; 68: 4746-4753 [PMID: 18559521 DOI: 10.1158/0008-5472.CAN-08-0054]

99 Balsamo M, Manzini C, Pietra G, Raggi F, Blengio F, Mingari MC, Varesio L, Moretta L, Bosco MC, Vitale M. Hypoxia downregulates the expression of activating receptors involved in NK-cell-mediated target cell killing without affecting ADCC. *Eur J Immunol* 2013; 43: 2756-2764 [PMID: 23913266 DOI: 10.1002/eji.201334448]

107 Yun S, Lee SH, Yoon SR, Myung PK, Choi I. Oxygen tension regulates NK cells differentiation from hematopoietic stem cells in vitro. *Immunol Lett* 2011; 137: 70-77 [PMID: 21354208 DOI: 10.1016/j.imlet.2011.02.020]

112 Xu B, Chen L, Li J, Zheng X, Shi L, Wu C, Jiang J. Prognostic value of tumor infiltrating NK cells and macrophages in stage II/III esophageal cancer patients. *Oncotarget* 2016; 7: 74904-74916 [PMID: 27736796 DOI: 10.18632/oncotarget.12484]

117 Liu Y, Cheng Y, Xu Y, Wang Z, Du X, Li C, Peng J, Gao L, Liang X, Ma C. Increased expression of programmed cell death protein 1 on NK cells inhibits NK-cell-mediated anti-tumor function and indicates poor prognosis in digestive cancers. *Oncogene* 2017; 36: 6143-6153 [PMID: 28692048 DOI: 10.1038/onc.2017.209]

122 Zheng Y, Li Y, Liu J, Yang H, Li F, Zhao S, Qi Y, Zhang Y, Huang L. TNF-a-induced Tim-3 expression marks the dysfunction of infiltrating natural killer cells in human esophageal cancer. *J Transl Med* 2019; 17: 165 [PMID: 31093941 DOI: 10.1186/s12967-019-1917-0]

127 Wang Z, Zhu J, Gu H, Yuan Y, Zhang B, Zha D, Zhou J, Zhu Y, Chen W. The Clinical Significance of Abnormal Tim-3 Expression on NK Cells from Patients with Gastric Cancer. *Immune Cell* 2015; 44: 578-589 [PMID: 26214042 DOI: 10.3109/08820139.2015.1052145]

132 Ma Y, Sharin GV, Peiyuan Z, Sharin MR. Dendritic cells in the cancer microenvironment. *J Cancer* 2013; 4: 36-44 [PMID: 23366903 DOI: 10.7150/jca.5046]

137 Janikashvili N, Bonnotte B, Katsanis E, Larmonier N. The dendritic cell-regulatory T lymphocyte crosstalk contributes to tumor-induced tolerance. *Clin Dev Immunol* 2011; 2011: 430394 [PMID: 22110524 DOI: 10.1155/2011/430394]

142 Manicassamy S, Pulendran B. Dendritic cell control of tolerogenic responses. *Immunol Rev* 2011; 241: 206-227 [PMID: 2148899 DOI: 10.1111/j.1600-065X.2011.01015.x]

147 Gabrilovich DI, Cierpik IF, Carbone DP. Dendritic cells in antitumor immune responses. I. Defective antigen presentation in tumor-bearing hosts. *Cell Immunol* 1996; 170: 101-110 [PMID: 8655590 DOI: 10.1006/cimm.1996.0139]

152 Elia AR, Cappello P, Puppo M, Fraone T, Vanni C, Eva A, Musso T, Novelli F, Varesio L, Giovarelli M. Human dendritic cells differentiated in hypoxia down-modulate antigen uptake and change their chemokine expression profile. *J Leukoc Biol* 2008; 84: 1472-1482 [PMID: 18725395 DOI: 10.1189/jlb.0808208]

157 Mancinco A, Schioppa T, Larghi P, Pasqualini F, Nebuloni M, Chen IH, Sozzani S, Austyn JM, Mantovani A, Sica A. Divergent effects of hypoxia on dendritic cell functions. *Blood* 2008; 112: 3723-3734 [PMID: 18694997 DOI: 10.1182/blood-2008-02-142091]

162 Nagelkerke A, Bussink J, Mucic H, Wouters BG, Lehmann S, Sweep FC, Span PN. Hypoxia stimulates migration of breast cancer cells via the PERK/ATF4/LAMP3-arm of the unfolded protein
Hypoxia and gastroesophageal cancer

King R et al. Hypoxia and gastroesophageal cancer

response. Breast Cancer Res 2013; 15: R2 [PMID: 23294542 DOI: 10.1186/bcr3373]

Mujic H, Nagelkerke A, Rouschop KM, Chung S, Chaudary N, Span PN, Clarke B, Milosevic M, Sykes J, Hill RP, Koritzinisky M, Wouters BG. Hypoxic activation of the PERK/eIF2alpha arm of the unfolded protein response promotes metastasis through induction of LAMP3. Clin Cancer Res 2013; 19: 6126-6137 [PMID: 24043183 DOI: 10.1158/1078-0432.CCR-13-0526]

Liao X, Chen Y, Liu D, Li F, Li X, Jia W. High Expression of LAMP3 Is a Novel Biomarker of Poor Prognosis in Patients with Esophageal Squamous Cell Carcinoma. Int J Mol Sci 2015; 16: 17655-17667 [PMID: 26226391 DOI: 10.3390/ijms16081765]

Somja J, Demoulin S, Roncarati P, Herfs M, Bletard N, Delvenne P, Hubert P. Dendritic cells in Barrett's esophagus carcinogenesis: an inadequate microenvironment for antitumor immunity? Am J Pathol 2010; 182: 2168-2179 [PMID: 23619476 DOI: 10.1016/j.ajpath.2013.02.036]

Martin-Fontecha A, Sebastiani S, Hopken UE, Ugazzioni M, Lipp M, Lanzavecchia A, Sallusto F. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and pruning. J Exp Med 2003; 198: 615-621 [PMID: 12925677 DOI: 10.1084/jem.20030448]

Ben-Shoshan J, Mayssal-Aslaudler S, Mor A, Keren G, George J. Hypoxia controls CD4+CD25+ regulatory T-cell homeostasis via hypoxia-inducible factor-1alpha. Eur J Immunol 2008; 38: 2412-2418 [PMID: 18792019 DOI: 10.1002/eji.200838318]

Clambey ET, McNamee EN, Westrich JA, Glover LE, Campbell EL, Jedlicka P, de Zoeten EF, Cambier JC, Stenmark KR, Colgan SP, Eltzschig HK. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. Proc Natl Acad Sci USA 2012; 109: E2784-E2793 [PMID: 22988108 DOI: 10.1073/pnas.1202366109]

Yuan XL, Chen L, Li MX, Dong P, Xue J, Wang J, Zhang TT, Wang XA, Zhang FM, Ge HL, Shen LS, Xu D. Elevated expression of FoxP3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner. Clin Immunol 2010; 134: 277-288 [PMID: 19900843 DOI: 10.1016/j.clinim.2009.10.005]

Kono K, Kawaiha H, Takahashi A, Sugai H, Mimura K, Miyagawa N, Onnata H, Fuji H. CD4(+)CD25high regulatory T cells increase with tumor stage in patients with gastric and esophageal cancers. Cancer Immunol Immunother 2006; 55: 1064-1071 [PMID: 16328385 DOI: 10.1007/s00262-006-092-8]

Shen Z, Zhou S, Wang Y, Li RL, Zhong C, Liang C, Sun Y. Higher intratumoral infiltrated Foxp3+ Treg numbers and Foxp3+/CD8+ ratio are associated with adverse prognosis in resectable gastric adenocarcinoma. J Cancer Res Clin Oncol 2010; 136: 1585-1595 [PMID: 20221835 DOI: 10.1007/s00432-010-0816-9]

Cirri P, Chiarugi P. Cancer associated fibroblasts: the dark side of the coin. Am J Cancer Res 2011; 1: 482-497 [PMID: 21984967]

Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. Front Biosci (Landmark Ed) 2010; 15: 166-179 [PMID: 20036813 DOI: 10.2741/3613]

Petrova V, Amnicchiario-Petruzzelli M, Melino G, Amelio I. The hypoxic tumour microenvironment. Oncogenesis 2014; 3: 10 [PMID: 23962402 DOI: 10.3389/onc.2014.00011-9]

Underwood TJ, Hayden AL, Derozet M, Garcia E, Noble F, White MJ, Thrirdborough S, Mead A, Hayden AL, Derouet M, Garcia E, Noble F, White MJ, Thrirdborough S, Mead A, Clemons N, Mellone M, Uzoh C, Primrose IN, Blaydes JP, Thomas GP. Cancer-associated fibroblasts predict poor outcome and promote peristin-dependent invasion in esophageal adenocarcinoma. J Pathol 2015; 235: 466-477 [PMID: 25345775 DOI: 10.1002/path.4467]

Shields MA, Dangi-Garimella S, Krantz SB, Bentrem DJ, Munshi HG. Pancreatic cancer cells respond to type I collagen by inducing sial expression to promote membrane type 1 matrix metalloproteinase-dependent collagen invasion. J Biol Chem 2011; 286: 10495-10504 [PMID: 21288898 DOI: 10.1074/jbc.M110.195628]

Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res 2007; 67: 10123-10128 [PMID: 17979453 DOI: 10.1158/0008-5472.CAN-07-3127]

Radisky DC, Kenny PA, Bissell MJ. Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? J Cell Biochem 2007; 101: 830-839 [PMID: 17218138 DOI: 10.1002/jcb.21186]

Wu X, Qiao B, Liu Q, Zhang W. Upregulation of extracellular matrix metalloproteinase inducer promotes hypoxia-induced epithelial-mesenchymal transition in esophageal cancer. Mol Med Rep 2015; 12: 7419-7424 [PMID: 26458866 DOI: 10.3892/mmr.2015.4410]

Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029-1033 [PMID: 19469938 DOI: 10.1126/science.1160809]

Nobel Prize Outreach. The Nobel Prize in Physiology or Medicine 1931. [cited 24 February 2021]; In: Nobel Prize Outreach [Internet]. Available from: https://www.nobelprize.org/prizes/medicine/1931/summary/

Eales KL, Hollinshead KE, Tennant DA. Hypoxia and metabolic adaptation of cancer cells. Oncogenesis 2016; 5: e190 [PMID: 26807645 DOI: 10.1038/oncscis.2015.50]

Semenza GL. Tumor metabolism: cancer cells give and take lactate. J Clin Invest 2008; 118: 3835-3837 [PMID: 19033652 DOI: 10.1172/JCI37373]

Isidoro A, Martinez M, Fernández PL, Ortega AD, Santamaría G, Chamorro M, Reed JC, Cuevra JM. Alteration of the bioenergetic phenotype of mitochondria is a hallmark of breast, gastric, lung
and oesophageal cancer. Biochem J 2004; 378: 17-20 [PMID: 14683524 DOI: 10.1042/BJ20031541]

125 Lynam-Lennon N, Maher SG, Maguire A, Phelan J, Muldoon C, Reynolds JV, O'Sullivan J. Altered mitochondrial function and energy metabolism is associated with a radioresistant phenotype in oesophageal adenocarcinoma. PloS One 2014; 9: e100738 [PMID: 24968221 DOI: 10.1371/journal.pone.0100738]

126 Doberty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Invest 2013; 123: 3685-3692 [PMID: 23994463 DOI: 10.1172/JCI69741]

127 Kolev Y, Uetake H, Takagi V, Sugihara K. Lactate dehydrogenase-5 (LDH-5) expression in human gastric cancer: association with hypoxia-inducible factor (HIF-1alpha) pathway, angiogenic factors production and poor prognosis. Ann Surg Oncol 2008; 15: 2336-2344 [PMID: 18521687 DOI: 10.1245/s10434-008-9955-5]

128 Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. J Immunol 2013; 191: 1486-1495 [PMID: 23817426 DOI: 10.4049/jimmunol.1202702]

129 Hagberg H, Andersson P, Lacombe W, Jacobson I, Butcher S, Sandberg M. Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. J Neurochem 1987; 49: 227-231 [PMID: 3585332 DOI: 10.1111/j.1471-4159.1987.tb03419.x]

130 Ballarin M, Fredholm BB, Ambrosio S, Mahy N. Extracellular levels of adenosine and its metabolites in the striatum of awake rats: inhibition of uptake and metabolism. Acta Physiol Scand 1991; 142: 97-103 [PMID: 1877368 DOI: 10.1111/j.1748-1716.1991.tb09133.x]

131 Zetterström T, Vernert L, Ungersdott U, Tossman U, Jonzon B, Fredholm BB. Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci Lett 1982; 29: 111-115 [PMID: 7088412 DOI: 10.1016/0304-3940(82)90338-x]

132 Eltzschig HK, Sitkovsky MV, Robson SC. Purinergic signaling during inflammation. N Engl J Med 2012; 367: 2322-2333 [PMID: 23234515 DOI: 10.1056/NEJMra1205750]

133 Blay J, White TD, Hookin DW. The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. Cancer Res 1997; 57: 2602-2605 [PMID: 9205063]

134 VaupeL P, Mayer A. Hypoxia-Driven Adenosine Accumulation: A Crucial Microenvironmental Factor Promoting Tumor Progression. Adv Exp Med Biol 2016; 876: 177-183 [PMID: 26782210 DOI: 10.1007/978-1-4939-3023-4_22]

135 Ohta A. A Metabolic Immune Checkpoint: Adenosine in Tumor Microenvironment. Front Immunol 2016; 7: 109 [PMID: 27066002 DOI: 10.3389/fimmu.2016.00109]

136 Chambers AM, Matosevic S. Immuno-metabolic Dysfunction of Natural Killer Cells Mediated by the Hypoxia-CD73 Axis in Solid Tumors. Front Immunol 2017; 8: 367 [PMID: 29039764 DOI: 10.3389/fimmu.2017.00109]

137 Young A, Mittal D, Stagg J, Smyth MJ. Targeting cancer-derived adenosine: new therapeutic approaches. Cancer Discov 2014; 4: 879-888 [PMID: 25035124 DOI: 10.1158/2159-8290.CD-14-0341]

138 Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. Nat Rev Cancer 2017; 17: 709-724 [PMID: 29059149 DOI: 10.1038/nrc.2017.86]

139 Hatfield SM, Kjaergaard J, Lukashev D, Schreiber TH, Belikoff B, Abbott R, Sethumadhavan S, Philbrook P, Ko K, Cannici R, Thayer M, Rodig S, Kutok JL, Jackson EK, Karger B, Podack ER, Ohta A, Sitkovsky MV. Immunological mechanisms of the antitumor effects of supplemental oxygenation. Sci Transl Med 2015; 7: 277ra30 [PMID: 25739764 DOI: 10.1126/scitranslmed.aab2600]

140 Wang MX, Ren LM, Shan BE. Inhibitory effects of extracellular adenosine triphosphate on growth of esophageal carcinoma cells. World J Gastroenterol 2005; 11: 5915-5919 [PMID: 16273599 DOI: 10.3748/wjg.v11.i38.5915]

141 Fukuda K, Sakakura C, Miyagawa K, Kurita Y, Kin S, Nakase Y, Hagiwara A, Mitsufuji S, Okazaki Y, Harashizaki Y, Yamagishi H. Differential gene expression profiles of radioresistant oesophageal cancer cell lines established by continuous fractionated irradiation. Br J Cancer 2004; 91: 1543-1550 [PMID: 15365572 DOI: 10.1038/sj.bjc.6602187]

142 Scharping NE, Menk AV, Whetstone RD, Zeng X, Del戈ffie GM. Efficacy of PD-1 Blockade Is Potentiated by Metformin-Induced Reduction of Tumor Hypoxia. Cancer Immunol Res 2017; 5: 9-16 [PMID: 27941003 DOI: 10.1158/2326-6066.CIR-16-0103]

143 Fyles AW, Milosevic M, Wong R, Kavanagh MC, Pintilie M, Sun A, Chapman W, Levin W, Manchul L, Keane TJ, Hill RP. Oxygenation predicts radiation response and survival in patients with cervix cancer. Radiother Oncol 1998; 48: 149-156 [PMID: 9938886 DOI: 10.1016/s0167-8140(98)00044-5]

144 Nordmark M, Bentzen SM, Rudat V, Brizel D, Lartigau E, Stadler P, Becker A, Adam M, Molles M, Dunst J, Terriis DJ, Overgaard J. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 2005; 77: 18-24 [PMID: 16096619 DOI: 10.1016/j.radonc.2005.06.038]
De Wever O, Ceelen W, Pattyn P. Hypoxia imaging with Fleming IN.

Shi WJ. Inducible factor-1 alpha contributes to hypoxia-induced chemoresistance in gastric cancer.

Liu L. P53 vs. 5-fluorouracil resistance in gastric and esophageal cancer: A systematic review.

Hammond EM, Hu Q, Chen H, Liang K, Yuan Y, Xiang Y, Ruan H, Zhang Z, Song A, Zhang H, Liu L, Diao L, Lou Y, Zhou B, Wang L, Zhou S, Gao J, Jonasch E, Lin SH, Xia Y, Lin C, Yang L, Mills GB, Fox NS. Tumour Hypoxia.

Buffa FM, Harris AL, West CM, Miller CJ. Large meta-analysis of multiple cancers reveals a common, compact and highly prognostic hypoxia metagene.

Luo D. The Clinicopathologic and Prognostic Value of Hypoxia-Inducible Factor-2α in Cancer Patients: A Systematic Review and Meta-Analysis.

Hasegawa T, Matsuzaki T, Sawada T, Ohira M, Hirakawa K. Effects of acute and chronic hypoxia on the radiosensitivity of gastric and esophageal cancer cells.

Kato Y. Effects of acute and chronic hypoxia on the radiosensitivity of gastric and esophageal cancer cells.

Buffa FM, Harris AL, West CM, Miller CJ. Gene Expression Signatures as Biomarkers of Tumour Hypoxia.

Luo D, Tong GH, Chen XX, Zheng HC, Wang YZ. HIF2α is associated with poor prognosis and affects the expression levels of survivin and cyclin D1 in gastric carcinoma. Dis Esophagus 2011; 24: 177-181 [PMID: 21073615 DOI: 10.1111/j.1442-2050.2010.01122.x]
167 Klaassen R, Bennink RJ, van Tienhoven G, Bijlsma MF, Besseling MG, van Berge Henegouwen MI, Wilminck JW, Nederveen AJ, Windhorst AD, Huishof MC, van Laarhoven HW. Feasibility and repeatability of PET with the hypoxia tracer [(18)F]HX4 in oesophageal and pancreatic cancer. Radiother Oncol 2015; 116: 94-99 [PMID: 26049919 DOI: 10.1016/j.radonc.2015.05.009]

168 Pastorekova S, Gillies RJ. The role of carbonic anhydrase IX in cancer development: links to hypoxia, acidosis, and beyond. Cancer Metastasis Rev 2019; 38: 65-77 [PMID: 31076951 DOI: 10.1007/s10555-019-09799-0]

169 Chiche J, Ile K, Laferrère J, Trottier E, Dayan F, Mazure NM, Brahim-Horn MC, Pouyssegur J. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. Cancer Res 2009; 69: 358-368 [PMID: 1918021 DOI: 10.1158/0008-5472.CAN-08-2470]

170 Birner P, Jesch B, Friedrich J, Riegler M, Zacherl J, Hejna M, Wrba F, Schultheis A, Schoppmann SF. Carbonic anhydrase IX overexpression is associated with diminished prognosis in esophageal cancer and correlates with Her-2 expression. Ann Surg Oncol 2011; 18: 3330-3337 [PMID: 21519917 DOI: 10.1245/s10434-011-1730-3]

171 Griffths EA, Pritchard SA, Valentine HR, Whitchelo N, Bishop PW, Ebert MP, Price PM, Welch IM, West CM. Hypoxia-inducible factor-1alpha expression in the gastric carcinogenesis sequence and its prognostic role in gastric and gastro-oesophageal adenocarcinomas. Br J Cancer 2007; 96: 95-103 [PMID: 1719985 DOI: 10.1038/sj.bjc.6603524]

172 Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An Abridged Explanation and Elaboration. J Natl Cancer Inst 2018; 110: 803-811 [PMID: 29873743 DOI: 10.1093/jnci/djy088]

173 Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011; 11: 393-410 [PMID: 21606941 DOI: 10.1038/nrc3064]

174 Phillips RM, Hendriks HR, Sweezy JB, Reddy G, Peters GJ. Efficacy, pharmacokinetic and pharmacodynamic evaluation of apaziquone in the treatment of non-muscle invasive bladder cancer. Expert Opin Drug Metab Toxicol 2017; 13: 783-791 [PMID: 28637573 DOI: 10.1080/17425255.2017.1341490]

175 Dirix LY, Tommesen F, Cassidy J, Epelbaum R, ten Balkel Huinink WW, Pavlidis N, Sorio R, Gamucci T, Wolf J, Te Velde A, Lan J, Verweij J. EO9 phase II study in advanced breast, gastric, pancreatic and colorectal carcinoma by the EORTC Early Clinical Studies Group. Eur J Cancer 1996; 32A: 2019-2022 [PMID: 8943690 DOI: 10.1016/0959-8049(96)00226-2]

176 Spiegelberg L, van Hoof SJ, Biemans R, Lieuwes NG, Marcus D, Niemans R, Theys J, Yaromina A, Lambin P, Verhaegen F, Dubois LJ. Evofosfamide sensitizes esophageal carcinomas to radiation without increasing normal tissue toxicity. Radiother Oncol 2019; 141: 247-255 [PMID: 31431383 DOI: 10.1016/j.radonc.2019.06.034]

177 R Core Team. R: A Language and Environment for Statistical Computing. [cited 24 February 2021]. In: The R Foundation [Internet]. 2019; Available from: https://www.r-project.org/

178 Tsai YP, Wu KJ. Hypoxia-regulated target genes implicated in tumor metastasis. J Biomed Sci 2012; 19: 102 [PMID: 23241400 DOI: 10.1186/1423-0127-19-102]

179 Gordon JD, Bertout JA, Hu CJ, Diehl JA, Simon MC. HIF-2alpha promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. Cancer Cell 2007; 11: 335-347 [PMID: 17418440 DOI: 10.1016/j.ccr.2007.02.006]

180 Benita Y, Kikuchi H, Smith AD, Zhang MQ, Chung DC, Xavier RJ. An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. Nucleic Acids Res 2009; 37: 4587-4602 [PMID: 19491311 DOI: 10.1093/nar/gkp425]

181 Hammond EM, Asselin MC, Forster D, O’Connor JP, Senra JM, Williams KJ. The meaning, measurement and modification of hypoxia in the laboratory and the clinic. Clin Oncol (R Coll Radiol) 2014; 26: 277-288 [PMID: 24602562 DOI: 10.1016/j.clon.2014.02.002]

182 Rademakers SE, Span PN, Kaanders JH, Sweep FC, van der Kogel AJ, Bussink J. Molecular aspects of tumour hypoxia. Mol Oncol 2008; 2: 41-53 [PMID: 19383328 DOI: 10.1016/j.molonc.2008.03.006]

183 Le QT, Courter D. Clinical biomarkers for hypoxia targeting. Cancer Metastasis Rev 2008; 27: 351-362 [PMID: 18483785 DOI: 10.1007/s10555-008-9144-9]
